# TABLE OF CONTENTS

Consolidated Minutes of the 95th Annual Meeting of the South Dakota Academy of Science................................................................................... 1

Presidential Address—It’s Just A Theory: Science and the Pursuit of Truth. David Bergmann................................................................. 11

**Special Symposium: Science at the Sanford Underground Laboratory**
**Presented at the 95th Annual Meeting of the South Dakota Academy of Science**

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eukaryotic Microbial Diversity Within Biofilm and Seep-Water Samples Isolated from the Former Homestake Gold Mine, Lead, South Dakota. C. M. Anderson and S.S. Bang</td>
<td>19</td>
</tr>
<tr>
<td>Site Investigations and Geotechnical Assessment at DUSEL. Zbigniew J. Hladysz</td>
<td>20</td>
</tr>
<tr>
<td>Molecular Survey and Bioprospecting of the Microbial Communities in Homestake DUSEL. Sookie S. Bang and Cynthia M. Anderson</td>
<td>21</td>
</tr>
<tr>
<td>Plans and Developments for the Deep Underground Science and Engineering Laboratory (DUSEL). W. M. Roggenthen</td>
<td>22</td>
</tr>
<tr>
<td>Center for Ultra-Low Background Experiments at DUSEL (Cubed). Barbara Szczepinska, Drew Alton, Xinhau Bai, Dan Durben, Jaret Heise, Haiping Hong, Stan Howard, Chaoyang Jiang, Kara Keeter, Christina Keller, Robert Metcalf, Dana Medline, Dongming Mei, Andre Petukhov, Joel Rauber, Bill Roggenthen, Jason Spaans, Yongchen Sun, Keenan Thomas, Michael Zehfus, and Chao Zhang</td>
<td>23</td>
</tr>
<tr>
<td>Long Baseline Neutrino Experiment: Physics and Project Status. Milind Diwan</td>
<td>24</td>
</tr>
<tr>
<td>Opportunities and Challenges: Experiences in Performing Research at the Sanford Underground Laboratory at Homestake. Jason Van Beek, Jaret Heise, William Roggenthen, and Larry Stetler</td>
<td>25</td>
</tr>
<tr>
<td>Slow Ground Motion Studies at Homestake DUSEL. Larry D. Stetler, James T. Volk and Jason K. VanBeek</td>
<td>29</td>
</tr>
</tbody>
</table>

## Complete Senior Research Papers
**Presented at the 95th Annual Meeting of the South Dakota Academy of Science**

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Comprehensive Herbarium Database of Vascular Plants from Northeastern Wyoming and Western South Dakota. Curtis Card, Grace Kostel and Mark Gabel</td>
<td>77</td>
</tr>
<tr>
<td>Antimicrobial Screening on Fourteen Traditional American Indian Medicinal Plants. J. L. Jacobs, J. Dixson, M. Gabel, D. Bergmann, J. Decory, and C. Geffre</td>
<td>85</td>
</tr>
</tbody>
</table>
Model for Classifying and Monitoring Hackberry
(Celtis occidentalis L.)–Shrub Ecological Type in Sand Hills Prairie
Ecosystem. Daniel W. Uresk, Daryl E. Mergen, and Jody Javersak .................. 105

Ecological Model for Classifying and Monitoring Boxelder in
Northwestern Nebraska. Daniel W. Uresk, Daryl Mergen,
and Jody Javersak .......................................................................................... 121

Landscape Content in Relation to Bird Species Occurrence in Sagebrush
Habitats of North and South Dakota. Amy R. Lewis
and Kenneth F. Higgins .................................................................................. 129

Suggestions for A New Approach for Use in Assessing Indicated Breeding
Pair Populations of Ducks During Spring in North America.
Kenneth F. Higgins, Kent C. Jensen, and Kristel K. Bakker .......................... 139

A Temporal Reassessment of Waterfowl Populations and Habitat
Conditions on Five Historic Field Study Areas in Canada and the

Waterfowl in the Prehistory of South Dakota. David C. Parris
and Kenneth F. Higgins .................................................................................. 165

“Rock-Chewing Dogs”: Cretaceous Pierre Shale Group Concretions
Gnawed by Cynomys ludovicianus (Rodentia, Sciuridae).
James E. Martin and Kenneth Brown ................................................................ 175

Culture of Advanced-Sized Largemouth Bass for Stocking into South
Dakota Impoundments. Matthew J. Ward, Michael L. Brown
and Isak J. Csargo ............................................................................................ 181

Off-Season Spawning Behaviors in Northern Largemouth Bass.
Daniel E. Spengler and Michael L. Brown ...................................................... 193

Fitting Circles for Phase Space Analysis.
Michael Janes, Edward Corwin, and Antonette Logar ................................. 207

Passive Microwave Devices Made Using Resistive Loads Fabricated by
Direct-Write Fabrication. Thomas P. Montoya and Andrew W. Downs .......... 215

Integrated Pest Management in Yards and Gardens: Comparing South
Dakota and Regional Master Gardeners. Rhoda L. Burrows .......................... 227

Abstracts of Senior Research Papers
Presented at the 95th Annual Meeting of the
South Dakota Academy of Science

A Preliminary Study on Essentiality of Dihydroxyacetone Kinase in
Methicillin-Resistant Staphylococcus aureus. Jordan Foos, Chun Wu
and Adhar Manna ............................................................................................ 239

Enzymatic Identification of the Phosphoryl Group Donor in A
Hypothetic Protein, MRSA Dihydroxyacetone Kinase.
Kelly Schilling and Chun Wu .......................................................................... 240

Innate Immune Responses of Porcine Intestinal Epithelial Cells to
Bacteria-Associated Molecular Patterns. C. Sreenivasan and
R. S. Kaushik .................................................................................................... 241

Differential Expression of Candida albicans Genes in Response to Silver
Compounds. Polly A. Hall, Pankaj Mehrotra and Cynthia Anderson ............ 242

Expression of Two Nitrosomonas europaea Proteins, Hydroxylamine
Oxidoreductase and NE0961, in Escherichia coli BL21 (DE3).
Pankaj Mehrotra and David Bergmann ........................................................... 243

Real-Time T-P Knot Algorithm for Baseline Wander Noise Removal and
ECG-Derived Respiration (EDR) Signal Estimation from the
Electrocardiogram (ECG) for Respiratory Rate.
S. P. Arunachalam and L. F. Brown ............................................................. 244
A New Paleontological Facility for Education, Research, and Reposition at the Museum of Geology, South Dakota School of Mines and Technology, Rapid City, South Dakota. James E. Martin

The Earliest Record of Proboscidea in South Dakota from the Middle Miocene (Barstovian) Fort Randall Formation. D. C. Pagnac

The Earliest Record of Proboscidea in South Dakota from the Middle Miocene (Barstovian) Fort Randall Formation. D. C. Pagnac


The Tyrant King of Mud Butte: Notes on a Tyrannosaurus rex Specimen from Butte County, South Dakota. M. M. Pinsdorf

Paleozoic Fish from the Permian Minnekahta Limestone of the Black Hills. N. Dierks and D. Pagnac

Experimental Adipocere Formation: Implications for Concretion Formation on Vertebrate Fossils. Randolph J. Moses

An Ecological Comparison of the Green River and Florissant Insects. L. J. Clarke

Sample Analysis of Archaeotherium (Artiodactyla: Entelodontidae) from the Conata Picnic Ground “Big Pig Dig”, Badlands National Park, South Dakota. Matthew T. Miller

A New Stratigraphic Occurrence of the Leptaucheniine Oreodont Sespia in the Upper Brule Formation of South Dakota. E. Welsh

How Faberge Eggs Are Made in the Badlands: Unique Preservation Observed in Fossil Eggshells. E. Welsh and H. Minkler

The Final Closing of the Big Pig Dig: an Unusual Dilemma Encountered at the Unique Site in Badlands National Park. W. A. Thompson and M. W. Weiler

Retroactively Geo-Referencing Herbarium Specimens of Phragmites australis. K. I. Miller and C. A. Johnston

Diagnosis and Appraisal of Bacterial Leaf Streak Disease Severity in Wheat. Y. R. Kandel, L. E. Osborne, K. D. Glover, and C. A. Tande


Genetic Characterization of Two Chromosomal Segments Associated with Seed Dormancy and Plant Height in Rice (Oryza sativa L.). Heng Ye, Chase R. Mowry and Xing-you Gu

Determination of the Genetic Basis of Pseudoflower Formation in Boechera holboellii. Riston H. Haugen, David H. Siemens, and Cynthia Anderson

Fine-Mapping A Genomic Region Containing a Seed Dormancy QTL, A Semi-Dwarf Gene, and the Viviparous-1 Locus on Chromosome 1 of Rice (Oryza sativa L.). Matt Josephson, Heng Ye and Xingyou Gu

Anti-Microbial Properties of Monarda fistulosa. C. Geffre, J. Jacobs, J. Dixson, M. Gabel, D. Bergmann, and J. Decory

Antimicrobial Activity of Native South Dakota Plant Extracts on Escherichia coli. Gitanjali Nandakafle and Neil Reese

The Antibiosis of Leaves of Specific Plants on Selected Bacteria. R. V. Jensen and D. Hazelwood

Nitrogen Transport and Its Regulation in the Arbuscular Mycorrhizal Symbiosis. H. Bücking, E. W. Gachomo, S. Choudhari, and Y. Beesetty
Effects of Mycorrhizae on Growth of Corn. Suzanne Swett and Donna Hazelwood ................................................................. 267
Identification and Characterization of Arbuscular Mycorrhizal Fungal Communities Associated With Prairie Cordgrass. E. Liepold, C. Fellbaum, J. L. Gonzalez-Hernandez, and H. Bücking ................................................................. 268
Salt Tolerance Variability in South Dakota Cordgrass (Spartina pectinata Bosc Ex Link) Selections. Alma Meza, Seth Schmoll and R. Neil Reese ................................................................. 269
Effect of Nodulation on Drought Response in Alfalfa. Peizhi Yang, Michael Peel, Bing Li, Bart Weimer, Dong Chen, Tianming Hu, and Yajun Wu ........................................................................ 270
Vegetation Characterization Within Select Prairie Dog Colonies in Southwestern South Dakota. E. D. Boyda, J. Butler, and L. Xu ................................................................. 271
Effects of Prescribed Burning on Species Diversity and Biomass in Smooth Brome Infested Rangeland of the Northern Great Plains. B. Arlt, C. Grewing, and L. Xu ........................................................................ 273
2-D Profiling of Total Proteome Changes in Cereals and Oil Crops Under Abiotic Stresses. Ansuman Roy and Jai S. Rohila ........................................................................ 275
The Switchgrass Moth, Blastobasis repartella. P. J. Johnson, A. Boe and D. Adamski ........................................................................ 277
Effects of Initial Egg Extrusion Techniques and Fertilization in Ovarian Fluid During Landlocked Fall Chinook Salmon Spawning. Matt Wipf, Dan J. Durben, and Michael E. Barnes ........................................................................ 278
The Effect of Male Landlocked Fall Chinook Salmon on Embryo Survival. Matt Wipf, Dan J. Durben, and Michael E. Barnes ........................................................................ 279
Feeding Habits of Game Fish in Mille Lacs Lake from Spring to Late Summer 2009. Brian Bolin and Donna Hazelwood ........................................................................ 280
Comparative Anatomy and Evolution of the Shovelnose Catfish Group Sorubim (Bleeker) (Pimelodidae). Uriel Angel Buitrago-Suarez ........................................................................ 281
Queen Number and Population Structure in the Ant Camponotus modoc. John H. Duvall-jisha and Garth M. Spellman ........................................................................ 282
The South Dakota Spider Survey (SDSS): Inventorying the Distribution and Diversity of Key Predators. L. B. Patrick ........................................................................ 285
Glacial Isolation and Postglacial Colonization Enhance Genetic Diversity in A Neotropical Migrant Passerine. G. M. Spellman, K. J. Burns, S. E. Cameron, J. Hudon, and J. Klicka ........................................................................ 286

A Multilocus Study of Phylogeography in the Bushtit (*Psaltriparus minimus*). Laura Kramer and Garth Spellman.......................... 288


Absence of *Haemonchus contortus* and Other Abomasal Nematodes in Eastern South Dakota Whitetail Deer During the 2010 Winter Season, A. A. Eljaki, J. A. Knutsen, and M. B. Hildreth.......................... 294


Investigation of the Potential Energy Surface for the CCO + N₂ Reaction, Maximilian Rude and Justin Meyer .......................................................... 296

Development of Silver Nanoparticle Based Conductive Inks for Printed Solar Cell Applications. Ravi Shankar, Keith W. Whites and Jon J. Kellar .......................................................... 297

Examination of the Rule of Mixtures for Two Polymeric Systems: Glass Transition Temperature (T_g) and Specific Refractive Index Increment (dn/dc). Shady Awwad, Tsvetanka Filipova and David A. Boyles.......................... 298

Influence of Carbonate Units on the Chemistry of Bisphenol A Polycarbonate: Synthesis of Poly(Ethercarbonate)s Containing Precisely Controlled Monomers of Variable Length. Mohammad S. Alomar and David A. Boyles.......................... 299

Design and Synthesis of a High Polarity Monomer for Elaboration Into A Field Responsive Material for Wound Film Capacitors. Hammad Younes and David A. Boyles.......................... 300

Spectroscopic Studies of Iodine-doped Polycarbonates. M. R. Barth, D. A. Boyles, and T. S. Filipova.......................... 301

Effects of Structure on Sorption of Selected Tetrachlorobiphenyl Congeners by Natural Soluble Organic Matter. N. K. Kadukuntla and H. V. Mott.......................... 302
Synthesis and Characterization of Mesoporous Manganese-Doped Ferrite for Potential Application in High Temperature Thermochemical Hydrogen Production. Michael Opoku, Rajesh Shende, and Jan A. Puszynski .................................................................................................................. 303
Resistance to Airflow of Porous Membranes Used in Photobioreactors. Gary A. Anderson, Anil Kommareddy, Zhengrong Gu and Joanne Puetz Anderson .......................................................................................... 305
Using the Methods of Science to Determine How to Teach Science. Andy Johnson .................................................................................................................. 306
Bringing Authentic Research Into the General Chemistry Laboratory Curriculum: Northern Plains Undergraduate Research Center. Mary Berry, Miles Koppang, Joe Vitt, Krisma Dewitt, Gary Earl, Duane Weisshaar, Paul Weber, and Carl Fictorie .......................................................................................... 307
The Horse Before the Cart and Big Kids Teach Little Kids: An Alternative Approach for Science and Math Education in South Dakota. Paul Kuhlman, Darwin Daugaard, Cathy Ezrailson, Tina Keller, Miles Koppang .......................................................................................... 308
The South Dakota School of Mines and Technology’s Science Education Outreach Program: Exposing Young, Inquisitive Minds to the Wonder of Science. Rachel Brown, Natalie Toth, and Sally Shelton .......................................................................................... 309
Bridging the Gap: Expanding Science Classroom Experiences Using New and Web-based Technologies. Cathy Ezrailson .......................................................................................... 310
Modeling Physics: A Research-Based Method That Supports Learning. Rose Emanuel and Roberta Traxinger .......................................................................................... 311
From Theory to Hypothesis to Test: Applying Science to Teaching Biology. Robert Tatina .......................................................................................... 312
CONSOLIDATED MINUTES OF THE
95TH ANNUAL MEETING OF THE SOUTH DAKOTA
ACADEMY OF SCIENCE
APRIL 9-10, 2010
HOSTED BY BLACK HILLS STATE UNIVERSITY
HOLIDAY INN CONVENTION CENTER
SPEARFISH, SD

EXECUTIVE COUNCIL

The Executive Council met at 11:00 am Friday 9 April 2010 for a final check
of plans for the symposium and the meeting.

Reports

President Krisma DeWitt opened the executive committee meeting and noted
that a quorum was present. The Secretary’s report, Treasurer’s report, Auditor’s
Report, and Proceedings Editor’s Report were given by Donna Hazelwood, Pat
Tille, Mark Gabel and Audrey Gabel, and Bob Tatina, respectively.

Proceedings Editor, Bob Tatina, noted that the library at USD no longer houses
the Proceedings Collection. After contacting state and private university libraries
in South Dakota by e-mail, he distributed copies which had been requested, and
the extras were destroyed. Therefore, copies of back issues are no longer available. He plans to digitize the set, but because of the time involved in the process
will look into alternatives including hiring students and investigating services
to accomplish this. To resolve issues on page charges for printing abstracts for
symposium presenters, Bob moved and Mike Barnes seconded that a waiver for
page charges be implemented for printing in the Proceedings the abstract and
titles for invited symposium speakers. The motion passed by voice vote.

On a related issue, Bob noted that the original estimate of the cost for page
charges at the SDSU facility for color figures in printed Proceedings did not
equal the exact cost. Because pages printed in black and white are copied on a
black and white copier, pages with color had to be copied on a color printer and
inserted by hand. The current costs for printing for reprints are $15.00 for 50
reprints and the bid estimate for printing the 2010 proceedings at the SDSU fa-
cility are $80.00 for 50 reprints. Bob discussed discouraging paper copies of the
Proceedings and consensus was to provide electronic distribution via the website
and printing paper copies for distribution only to libraries.

On the question of publishing abstracts in the Proceedings, the executive
council was in consensus that for abstracts to be published, either a paper or
poster must have been presented at the annual meeting. On the other hand,
whole papers have been published in the Proceeding but have not been presented
at the meeting.
Old Business

President Krisma DeWitt extended a warm thank you to the local arrangements committee, Dave Bergman, John Dixson, Cynthia Anderson, graduate and honors students in Biology and BHSU for hosting the annual meeting, and to the Holiday Inn staff. She also thanked members of the executive board and symposium presenters and attendees.

Krisma brought everyone’s attention to the status of the policy and procedures manual to be passed President-to-President. Apparently, the intended outcome has not occurred, and the process needs to be restarted. Bob mentioned that forwarding a handbook of procedures from the current local arrangements committee to the next committee would be useful.

Following up on collaboration with other scientific societies in South Dakota, Cindy Anderson visited with the Dakota Chapter of Fisheries. Mike Barnes indicated that increased awareness that the Proceedings is a viable publication outlet. He also mentioned that the best way to achieve collaboration is to have face-to-face meetings with members and leaders of other societies.

Krisma acknowledged the work of Neil Reese, webmaster of the SDAS website. She mentioned that the graphic arts project which originally was to be undertaken by a faculty member at MMC will not occur because the faculty member will be leaving MMC to pursue a graduate degree. Neil inquired about having a commercial company take-over the website design portion.

The collaboration among the Presidents of institutions of higher learning in South Dakota is currently in hiatus because one of the organizers, the President of MMC, has resigned his position.

From EBSCO host a royalty check for the Proceedings in the amount of $28.61 has been issued, but because of late delivery the check was void. The check will be reissued and sent to in care of the Proceedings Editor, Bob.

New Business

Fellows

A slate of Fellows has been nominated with nominators in parenthesis including Maureen Diggins (Mike Wanous), H.L. Hutcheson (Gary Larson and Arvid Boe), Gary Earl (Duane Weisshaar), and Waldimar “Wally” Klawiter Jr. (Pat Tille). Mike Barnes moved and Bob Tatina seconded that the members nominated as Fellows be recommended to the membership at the business meeting. The motion passed.

Nominations

Because last year the membership voted to increase the number of Members-at-Large from three to four for 2009-2011 terms, the executive council will propose to the membership increase from three to four for 2010-2012 terms. The Nominating committee, Miles and Mike Barnes will solicit nominations for Members-at-Large and for Second Vice President.

Resolutions

Gary Larson will be in charge of resolutions to be presented at the business meeting.
The Role of the Academy in Science Fairs

Kris mentioned that with the loss of the regional science fair at Timberlake, the number of regional science fairs in South Dakota has decreased from five to four. Apparently the role of the South Dakota Academy of Science in providing eight awards in the amount of $25.00 each, for a total of $200.00, to each of the regional science fairs is not currently being acknowledged. The question was raised concerning uniformity among standards for judging was raised. Pat Tille suggested that awards and certificates be sent to the teacher after the event and then the teacher would present the award. Cindy suggested that rubrics for judging awards be sent to the Executive Committee. Pat will solicit rubrics. She also has a list of the guidance counselors for each high school in SD. It was noted that the last Junior Academy was hosted by DSU. The question was raised about paying for the winner of each of the four regional science fairs to present at the Academy. The Science Fairs will be an item of discussion for the Fall Executive Council Meeting.

South Dakota Content Standards for Science

A lively discussion on the topic noted that the statement is ambiguous. In the past the South Dakota Academy of Science has passed resolutions, but this has not been effective. Suggestions included making personal contact with representatives in sequential order of potential effectiveness: 1) face-to-face, 2) phone, and 3) email/letter.

Miles introduced a concern about registration fees for high school teachers who were giving presentations at the Annual Meeting. The outcome was to not charge the teachers in question to attend the meeting. Pat Tille expressed frustration with preregistration and either non-payment and the question of generating numbers for attendance at the banquet and lunch. Finally, Bob mentioned that faculty at SDSM&T requested to bring a class to attend a specific session. The Executive Council made an Executive Decision in agreement with this request.

BUSINESS MEETING
Saturday 10 April 2010

President Krisma DeWitt introduced incoming President, Dave Bergman who gave the well received Presidential Address “It’s Just a Theory: Science in the Pursuit of Truth.”

Krisma opened the annual meeting. The Secretary’s report and the Treasurer’s report were given by Donna Hazelwood and Pat Tille, respectively. The Auditing Committee, Audrey Gabel and Mark Gabel, reported that everything was in order. The Proceedings Report was provided by Bob Tatina.

Old Business

On behalf of the academy, Krisma offered thanks to Black Hills State University for hosting the annual meeting and to the local arrangements committee, Dave Bergman, Cynthia Anderson, and John Dickson, and to the Holiday Inn
staff for their support of the meeting, and members of the local arrangements committee and the symposium presenters.

Update on collaboration of state of South Dakota scientific organizations. Toward the goal of generating coordination among the science societies in South Dakota, Cynthia Anderson met with the South Dakota chapter of the American Fisheries Society during the business meeting and invited members from the society to present at the annual meeting of the SDAS. She encouraged the membership to act as an ambassador for the Academy and attend the annual meeting of science societies in South Dakota.

New Business

The following individuals have been nominated as Fellows of the South Dakota Academy of Science:

Maureen Diggins, Augustana College, nominated by Mike Wanous
H.L. Hutcheson, SDSU, nominated by Gary Larson and Arvid Boe
Gary Earl, Augustana College, nominated by Duane Weisshaar
Waldimar “Wally” Klawiter Jr, University of Sioux Falls, nominated by Pat Tille

Krisma recommended that all of the individuals nominated be elected as Fellows. Mike Wanous moved and Audrey Gabel seconded the motion. The motion passed by acclimation. Formal notification will be sent by incoming President Dave Bergman.

The fee structure for payment of registration fees for the annual meeting was discussed. Miles Koppang moved and Tim Mullican seconded a motion to allow inclusion of high school students, faculty and students from tribal schools, student guests, unless presenters, to be charged the student rate. A friendly amendment by Cynthia Anderson stipulated that all other requests be considered by the local arrangements committee. The motion passed by acclimation.

A concern about a resolution passed by the South Dakota joint legislature 2010 on Global Warming was discussed by Bob Tatina. The petition forwarding this resolution to the legislature included signatures of many SDAS members, but in the absence of knowledge or consent of the individuals so listed. He noted that it would be beneficial for members to read such proposed legislation and have more direct exposure to the legislators to help avoid passage of such frivolous measures. Suggestions were made to visit with local legislators face to face, send letters directly to legislators, make phone calls, and send e-mail messages. Bob noted that locating joint resolutions can be done by accessing the SD Legislature web site and clicking on joint resolutions.

Items for discussion fall 2010 Executive Council meeting 11:00 am Sat 11 Sept. Al’s Oasis Oacoma, SD. Location of future meetings. Payment structure for high school students and high school teachers, students undergraduate, graduate, K-12, Website, SDAS presence on social networking sites such as Facebook and Twitter.

Krisma DeWitt pointed out that to allow for increased representation the number of member-at-large positions was increased from three to four for the
2009-2011 terms. The Executive Counsel proposes to increase the number of member-at-large positions from three to four for the 2010-2012 term. Bob Tatina and Mark Gabel moved to increase the number of member-at-large positions from three to four for the 2010-2012 term. The motion passed by acclamation.

The following slate was presented by the Nominating Committee:

**Second Vice President**
Chun Wu  Mount Marty College

**Two year terms 2010-2012**
Uriel Buitrago  Mount Marty College
Tim Mullican  Dakota Wesleyan University
George Mwangi  University of Sioux Falls
Jeff Palmer  Dakota State University

**One year terms 2010-2011**
Audrey Gabel  Black Hills State University
Tom Montoya  SDSM&T

Pat Tille moved and Bob Tatina seconded acceptance of the slate of nominees. The motion passed by acclamation.

President Krisma DeWitt passed the gavel to incoming President Dave Bergman.

**New Business**

President Dave Bergmann announced the AAAS undergraduate poster award winners. They are:

**Undergraduate female awardee (underlined)**

**Undergraduate male awardee (underlined)**

Discussion followed on the topic of inviting the award winners of the Science Fairs to present at the SDAS annual meeting.

Gary Larson presented the following **Resolutions of the South Dakota Academy of Science**: The South Dakota Academy of Science wishes to congratulate and thank David Bergman, Cynthia Anderson, and others on the Planning Committee at Black Hills State University for making the local arrangements that insured a successful 2010 meeting in Spearfish. Special recognition and thanks to the Spearfish Holiday Inn for excellent food, service, and accommodations for the meeting.

We thank Krisma DeWitt for her able leadership as our president over the past year, and thanks also to Bob Tatina for arranging the program for 2010 and
for his steadfast commitment and expertise as editor of the SDAS Proceedings. Thanks to David Bergman for his presidential address entitled “It’s Just a Theory: Science in the Pursuit of Truth”. We are grateful to Donna Hazelwood for her continued service as secretary and thanks also to Pat Tille for her service as treasurer; and we also thank Neil Reese for his ability and willingness to continue as our webmaster. The resolutions were passed by acclamation.

Announcements

The Executive Council will meet 11:00 am Saturday 11 Sept. 2010 at Al’s Oasis, Oacoma.
Forward announcements to Webmaster Neil Reese.
The 96th SDAS annual meeting will be hosted by DSU at Cedar Shore resort.
The 97th SDAS annual meeting will be hosted by USD.

Respectfully submitted,
Donna Hazelwood
Secretary

SOUTH DAKOTA ACADEMY OF SCIENCE
TREASURER’S REPORT

<table>
<thead>
<tr>
<th>Deposits</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning Statement 3-14-2009</td>
<td>7525.88</td>
</tr>
<tr>
<td>Deposit SDAS Mtg/Memberships 2009</td>
<td>5320.00</td>
</tr>
<tr>
<td>Science Fair Awards</td>
<td>(775.00)</td>
</tr>
<tr>
<td>SDAS Mtg (Aramark-NSU)</td>
<td>(1958.34)</td>
</tr>
<tr>
<td>Secretary of State</td>
<td>(10.00)</td>
</tr>
<tr>
<td>Speakers Annual Mtg</td>
<td>(1500.00)</td>
</tr>
<tr>
<td>SDAS Plaques</td>
<td>(72.32)</td>
</tr>
<tr>
<td>National Association Dues</td>
<td>(50.00)</td>
</tr>
<tr>
<td>Board Mtg Lunch (Al’s Oasis)</td>
<td>(128.03)</td>
</tr>
<tr>
<td>Checking Account Balance 2-15-2010</td>
<td>8352.19</td>
</tr>
<tr>
<td>Cash on Hand (SDAS Mtg-Cash Box)</td>
<td>300.00</td>
</tr>
<tr>
<td>SDAS 2010 Mtg Deposits to Date (4-6-2010)</td>
<td>1060.00</td>
</tr>
<tr>
<td>Certificate of Deposit</td>
<td>8076.26</td>
</tr>
<tr>
<td>Total assets</td>
<td>$17,788.45</td>
</tr>
</tbody>
</table>

Respectfully Submitted
Pat Tille
SDAS Treasurer
SOUTH DAKOTA ACADEMY OF SCIENCE
PROCEEDINGS EDITOR’S REPORT

This is my report of the year March 31, 2009 to March 31, 2010.

1. At and after last year’s annual meeting, all members were given their 2009 Proceedings. Those that were not in attendance at the annual meeting were mailed a copy.

2. The following libraries were mailed a complimentary copy: AC, BHSU, DSU, DWU, MMC, NSU, OLC, SDSMT, SDSU, UND, UND, Am. Mus. Nat. Hist., U of WA and the Booth Fish Hatchery (Spearfish)

3. The following indexing/abstracting services and institutions were mailed complimentary copies:
   Chemical Abstract Services
   AcadSci., Inc.
   NISC USA (Fish)
   Cambridge Scientific Abstracts
   GeoRef Library
   NISC CO (Wildlife)
   Baywood Publishing (Anthropology)
   Thompson Scientific (Biosis)
   EBSCO Host (pdfs)

4. The following non-member institution purchased copies:
   Linda Hall Library (Kansas City MO)
   Curran and Assoc.

5. Because the library at USD no long had room to house extra copies of past issues of the Proceedings, a note was sent to all libraries in the state asking them if they could house these copies. Since there were no positive responses, I offered to send a complete set to each library and/or to send copies to complete their holdings of the Proceedings. Copies or sets were sent to DWU, USF, Oakwood Lakes Outdoor Laboratory, Fish Hatchery in Spearfish and BHSU.

6. I also hired a student to convert paper copies of back volumes to pdfs with the intent to have these uploaded to the Academy website. The 1995 issue has been converted with 53 additional volumes left. However, the cost, about $150.00/volume, was higher than I anticipated, so I have put this project on hold.

7. For the 2009 Proceedings:
   a. All members received pdfs of all article of the entire volume
   b. No. of paper copies printed = 110
   c. No. of full papers published = 13
   d. No. of abstracts published = 62
   e. No. of titles only published = 0
   f. No. distributed to members = 69
   g. No. distributed to libraries, abstracting services, etc.: 32
   h. Electronic files of were provided to Neil Reese, Webmaster, for uploading to the SDAS website and to EBSCOhost to fulfill our contract with them.
8. First call for papers sent out early February; second call sent out 1 March.
9. 2010 Schedule produced and distributed
   a. For the 2010 annual meeting:
      i. No. of oral presentations = 59
      ii. No. of posters = 46
10. Motions to bring to the Executive Committee:
    a. Waiver of page charges for publishing abstracts and titles of invited sym-
       posium presenters. I feel that these individuals, some of whom have not
       been members should be rewarded for the service that they provide the
       Academy. The waiver would not apply to symposium presenters who
       choose to publish a full paper.
    b. Electronic copy of the Proceedings shall mean pdfs sent by email and
       not CDs or other media that would have to be sent by USPS mail.
    c. Does publishing require presenting? Over the two years that I have been
       editor I have allowed several full meritorious papers to be published even
       though their authors, who were members of the Academy, did not present
       their paper as a poster or orally. This year I have one abstract and
       possible two full papers, the authors of which have indicated that these
       will not be presented at the annual meeting.
11. The Proceedings account at SDSU has been closed and all monies transferred to
    the SDAS account at First National Bank in Mitchell.
12. 2009 Fiscal Report  (for the FY 1 April 2009 to 31 March 2010

    Formatting  1461.00
    Printing    4734.52
    Digitizing Proceedings  146.25
    Misc. Printing    0.00
    Supplies, postage, etc.  520.01
    Total expenses  7169.82
    Total Invoiced  5940.00
    Payments Received  5850.00
    Profit/(loss)  (1319.82)

    **Balance 12/21/09**  9832.91
    **Balance 4/04/10**  8513.09
    Proportion Paid  69/72

*Submitted by
Robert Tatina
Editor*
SOUTH DAKOTA ACADEMY OF SCIENCE
FELLOWS OF THE ACADEMY (1998-2011)

Kenneth F. Higgins 1998
Chuck Estee 1999
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
Nominated 2010 for 2011
Maureen Diggins 2011
Gary Earle 2011
H.L. Hutchinson 2011
Waldimar “Wally” Klawiter Jr. 2011
SOUTH DAKOTA ACADEMY OF SCIENCE
2010-2011 EXECUTIVE COMMITTEE

President  Dave Bergmann, BHSU, Biology, 652-2420, DaveBergmann@bhsu.edu
President-Elect  Anthony Cole, DWU, Biology 995-2708, ancole@dwu.edu
First Vice-President  Gary Larson, SDSU Biology, 688-4552, Gary.Larson@sdstate.edu
Second Vice-President  Chun Wu, MMC, Chemistry, 688-1381, cwu@mtmc.edu
First Past President  Krisma DeWitt, MMC Chemistry 668-1530, kdewitt@mtmc.edu
Second Past President  Nels H. Troelstrup, Jr. SDSU, Biology and Microbiology, 688-5503, Nels.Troelstrup@sdstate.edu
Secretary  Donna Hazelwood, DSU, Biology, 256-5187, donna.hazelwood@dsu.edu
Treasurer  Patricia Tille, SDSU, Prog. Dir. Clinical Lab. Sci., 605-688-6016, pat.tille@sdstate.edu
Proceedings Editor  Robert Tatina, DWU, Biology (retired) 996-7670, rotatina@dwu.edu
Publicity  Andrew Detwiler, SDSM&T, IAS, 394-1995, Andrew.Detwiler@sdsmt.edu
Publicity  James Sorenson, MMC, Biology, 668-1581, jsorenson@mtmc.edu
Publicity  Robert Tatina, DWU, Biology (retired) 996-7670, rotatina@dwu.edu
Webmaster  Neil Reece. SDSU, Biology 688-4568, neil.reece@sdstate.edu

Members-at-Large
2009-2011  Arvid Boe, SDSU, Plant Science 688-4759, Arvid.Boe@sdstate.edu
2009-2011  Miles Koppang, USD, Chemistry 677-5693, Miles.Koppang@usd.edu
2010-2011  Audrey Gabel, BHSU, 642-6251, Audrey.Gabel@bhsu.edu
2010-2011  Thomas Montoya, SDSM&T, EE, CENG 394-2459, Thomas.Montoya@sdsmt.edu
2010-2012  Uriel Buitrago-Suarez, MMC, Biology 668-1516, uriel.buitrago@mtmc.edu
2010-2012  Tim Mullican, DWU, biology 995-2715, timullic@dwu.edu
2010-2012  George Mwangi, USF, Chemistry 575-2069, George.mwangi@usiouxfalls.edu
2010-2012  Jeffrey Palmer, DSU, Mathematics 256-5190, Jeff.Palmer@dsu.edu
PRESIDENTIAL ADDRESS

It’s Just A Theory: Science And The Pursuit Of Truth

Address to the South Dakota Academy of Science
Spearfish Holiday Inn and Convention Center, Spearfish, SD
April 10, 2010

Presented by David Bergmann
Black Hills State University
Spearfish, SD 57799

Scientific knowledge continues to advance at a dizzying pace. Dr. Carol Lushbough’s talk yesterday on bioinformatics illustrated the immense amount of data resulting from genome sequencing projects—so great an amount of data that a new branch of science, bioinformatics—has arisen from the interface of two very different disciplines—molecular biology and computer science. The far-sighted acquisition by the state of the old Homestake mine in Lead and its conversion into the Deep Underground Science and Engineering Laboratory (DUSEL) has generated great excitement among diverse scientists here and worldwide; from the groundbreaking experiments on neutrinos and other aspects of subatomic physics described by Dr. Kara Keeter and others to experiments to characterize the geology, hydrology, and biology of the deep subsurface environment, a window into a unique environment has been opened. The physics experiments at DUSEL will likely shed light on such questions as the nature of matter and amount of matter in the universe. In addition, DUSEL provides access to deep aquifers possibly isolated from the surface for millennia, which may contain microbial ecosystems not dependent on photosynthesis as their energy source, or which may contain microbes adapted to extreme conditions, such as high temperatures.

Despite the increasingly rapid acquisition of knowledge about the physical world, there are problems with the relationship between the community of scientists and the larger society we live in. There has been considerable concern that there is a lack of “scientific literacy” in our nation, in that a large proportion of our population has poor understanding of such concepts as geological history and the structure of matter (National Science Board, 2004). Perhaps even more troubling than the lack of knowledge about specific scientific theories is a real confusion about what constitutes science. There is certainly a lack of understanding about what scientific theories are, and how they relate to our search for truth about the physical world.

We all have heard about controversies regarding the teaching of Intelligent Design in schools as an alternative to evolution—one main objection to the evolution by natural selection being that it is a “theory” and not a “fact”. The South Dakota Academy of Science passed a resolution in 1998 specifying that evolution be required in the curriculum for preparing high school biology teachers in our state, but the legislature has not yet approved this requirement.
The lack of understanding about the nature of science was even more forcefully illustrated recently in a resolution (House 1009) recently passed by the South Dakota State Legislature, which would mandate a “balanced view” in environmental science instruction: the theory of global warming due to human combustion of fossil fuels (accepted by the majority of scientists), and the view (advanced primarily by political groups), that global warming is not occurring would both be presented to students. The resolution states that “global warming is a scientific theory rather than a proven fact” which implies that scientific theories are mere speculations, not supported by repeated experimentation or systematic collection of observations.

I do not believe that even the best theory in the physical sciences can ever achieve the level of absolute certainty as, for example, a mathematical theorem. Our theories are always subject revision or even refutation based on new and compelling data. In my opinion, it is our duty to teach the children of our communities the best theories we have, without regard to political correctness. To do otherwise risks either not adequately preparing our citizens for challenges which they will face in health and environmental issues, or equally dangerous, foster the cynical idea among youth that truth does not exist, but is defined merely be one’s political viewpoint. The Academy should consider how we can best educate and inform the people of our state about the nature of science, and consider how we can uphold the integrity of science education.

The confusion about scientific theories versus what people commonly call “facts” is part of a wider misunderstanding about what science is, how scientific knowledge is acquired, and what scientific knowledge can tell us. As Dr. Andy Johnson indicated to me in a recent conversation at Black Hills State University, the question of what scientific knowledge is constitutes a rather deep question of epistemology. Truth is often perceived by people as a complete and changeless entity which is passed, in the form of a rigid, dogmatic body of knowledge, from a figure of authority to a learner, who accepts this body of knowledge uncritically. All too often, this occurs in our institutions of learning, in which a wide array of “facts” are passed from instructor to students with little critical thought being elicited in the students, so that an appreciation for what the scientific method is and a desire to learn more is sacrificed to an acquisition of the largest number of “facts” which are considered useful by the educational system. The hierarchical nature of our institutions of learning and the pronounced one-sidedness in the instructor-student relationship tend to reinforce this rather dogmatic view of scientific knowledge among students. As Jay Lemke (1995) commented “The structure of our institutions conveys a more powerful message than their content.”

Lynn White, Jr. (1968) stated that, to a scientist, “truth is not a citadel of certainty to be defended against error; it is a shady spot where one eats lunch before tramping on.” This view may be a bit overstated; nevertheless, it illustrates that scientific theories evolve and are not rigidly fixed, static entities. Some “constructivist” philosophers would go so far as to say that truth about the physical universe does not exist apart from those ideas we construct as a framework to explain experimental observations and that theories are to be evaluated not in terms of being accurate representations of an unknown truth but rather in terms
of their viability, or coherence and ability to explain observations (Von Glaserfeld 1997). Max Jammer (1999) proposed that science consists of a somewhat abstract set of concepts which can be related to physical observations, such as the data from the senses, by a series of rules of interpretation, and notes that it is impossible for science to photographically reproduce nature, because our concepts of science are essentially products of our own minds.

It should also be borne in mind that progress in science is often not always a constant, direct progression towards greater knowledge; progress may consist of long periods of relative inactivity interspersed with revolutionary breakthroughs. There may even be occasional false starts. Thomas Kuhn (1962) emphasized this when he noted that paradigms (such as accepted concepts) in science and other fields may persist unaltered for extended times before being overthrown. In the field of molecular biology I am often amazed that, for many years after its discovery by Friedrich Miescher in 1869, DNA was generally not considered to be the genetic material of cells—rather proteins were accorded this honor. The fragments of DNA which could be characterized seemed too small and too simple, composed of only four kinds of nucleotides, to convey genetic information, while proteins, composed of 20 possible amino acids and very diverse in size and composition seemed much better candidates for genes. It took a number of well-designed experiments, including those of Frederick Griffith, Oswald Avery, Alfred Hershey and Martha Chase, and Edwin Chargaff, over a span of 24 years, to cause the paradigm shift that made molecular biology possible (Becker et al. 2006).

One might also remember that the same year in which James Watson and Francis Crick (1953) published their double-helical structure of DNA, Linus Pauling (1953), a more experienced structural biologist who had pioneered many aspects of the structural biology of proteins, also published a paper describing the structure of DNA, which he described as a triple helix.

Despite the uneven progress of science and the inexact correspondence between the conceptual models of science and the physical world, I would not go this far to say that truth does not exist, as some of the more radical constructivists propose, even if our understanding of reality is fragmentary and sometimes inaccurate, and if our views of physical reality must change as more data are gained.

In addition to the common misconception of scientific knowledge as an unchanging body of facts passed down from authority figures, such as teachers to (often reluctant) pupils is the perception among many that science and religion are incompatible. If people feel that they are forced choose between science and religion, science is often rejected because it does not provide answers to the ultimate questions regarding the meaning of human life. The flames of conflict have been fanned by those in the scientific community, such as Richard Dawkins (1986), who argue that science leads us to conclude that God does not exist and the universe is without meaning and fundamentalist Christians on the other, who hold that Scripture, including the account of the creation of world and humanity in Genesis (1:1-2:4) must be interpreted literally. While it must be conceded that the theory of evolution by natural selection cannot be reconciled with a literal interpretation of Genesis, I do believe that region and science
can, and should coexist. Dr. Michael Wanous spoke at length on this subject during his 2007 presidential address to the Academy on how these two entities can productively co-exist. Among those scientists favoring a new dialogue between biology and science is Francis Collins, director of the Human Genome Sequencing Project (2003).

I believe that it is wise for us as scientists to make no claims that science can tell us anything other than the physical universe. As Stephan Jay Gould has suggested, science and religion have largely different domains. Referring to the domains of science and religion which have been termed “magisteria” he stated (1997) “I believe, with all my heart, in a respectful, even loving concordat between our magisteria—the NOMA (non-overlapping magisteria) solution. NOMA represents a principled position on moral and intellectual grounds, not a mere diplomatic stance. NOMA also cuts both ways. If religion can no longer dictate the nature of factual conclusions properly under the magisterium of science, then scientists cannot claim higher insight into moral truth from any superior knowledge of the world’s empirical constitution. This mutual humility has important practical consequences in a world of such diverse passions.” I find Gould’s statement particularly forceful because I understand that he was an agnostic. I also believe religion can contribute valuable insights into morality and the meaning of human life- insights which are critical to the proper application of the fruits of scientific knowledge in the world. If, for example, human lives are saved or human suffering is mitigated through the application of medical technology or if a plant or animal species is rescued from extinction, it may well be because, as a result of our religious beliefs, we consider them sacred in some sense, either as reflecting the nature of God in the case of humans or the product of God’s work through evolution in the case of other life. Perhaps, as we address students about the nature of science in our lectures, or address members of the communities we belong to about science, that we acknowledge the contributions of religion, while remaining committed to teach the theories we consider to be the most descriptive and best verified by experimental evidence, such as the theory of evolution by natural selection. In this way, science, and the concept of evolution in particular, may be perceived as less threatening and more compatible with religious beliefs.

The South Dakota Academy of Science has taken an active role in the promoting science education, as evidenced by the papers presented this morning on this topic. Certainly, improving science education is one solution to the lack of understanding as to what constitutes science. In his 2005 presidential address to the Academy, Robert Tatina outlined some of the challenges to science education in our region: low wages for K-12 teachers, extremely heavy teacher workloads which leave little, if any, time for innovation; poor teacher preparation in the area of science with too few science courses required for teacher education; an over-reliance on textbooks, which excessively drive course content; the relative isolation of science instructors in many schools (one person teaching all science and math courses) and perhaps most importantly, a lack of hands-on, open ended inquiry in science laboratories.

One solution he suggested for improving the content of science courses, particularly biology, was to concentrate less on the findings of science but more on the process of conducting scientific research by learning in detail about key
experiments and how they changed ideas. Another suggestion was to remove excessive preoccupation with numerous details and concentrate more on the unifying concepts of biology, such as DNA and information flow, cell theory, and evolution by natural selection. By focusing on the process of science in classrooms, students would also be better prepared for more inquiry-based laboratory courses.

Perhaps one key to fostering an appreciation of what science truly is and how science is done is to improve laboratory experiences for science students, not only in K-12 education but also for higher education (Smith et al. 2005). For example Dr. Cyndi Anderson and Lisbeth Fayer have proposed revising some of the high-enrollment, biology survey laboratory sessions to make them more inquiry-based. Students would form semester-long groups to test a particular hypothesis of interest to them. Students would research hypotheses of their own creation, decide appropriate methods of testing them, interpret data, and re-evaluate their hypothesis. By conducting their own experiments, the process of science becomes less foreign, and students are empowered to learn about the physical world on their own, trusting their own observations, rather than accepting dogmatically ideas which have been handed to them (or cynically rejecting the idea the ideas of anyone else). I think that learning the scientific method in this fashion would benefit not only science majors but also non-scientists as they develop valuable critical thinking skills and more open minds. It should be noted, however, that considerable care must be used in creating course structures which can help students adapt to this learning environment.

We should also consider what the Academy can do to fill the void left by the termination of the “Science on the Move” program- a state-sponsored, mobile laboratory which brought laboratory projects, including some involving molecular biology, to remote school districts. It would be good to think of new ways to introduce inquiry-based lab experiences to these schools.

As an Academy I would encourage us to find ways in which our organization can foster a more open, less dogmatic, more experiment-based approach to science education at all levels. Some of this can be done through papers and symposia at our annual meetings, during which faculty involved with science education in our state, who share common challenges such as isolation, limited time, and scarce resources, can share productive instructional strategies.

While at the University of Minnesota as a postdoctoral researcher, I remember many productive, informal conversations with colleagues involved in projects not directly related to mine, yet facing some common problems, which I had simply by walking down the hall and knocking on a door. In some of the institutions in our state, unfortunately, it seems like that next door may be in Denver or Minneapolis. I think it possible that the Academy can do much to decrease the feelings of isolation many of us involved in scientific research and education may experience.

Perhaps our website can include links of use for science education, in particular for revising science laboratory courses. We can assist with workshops on science education.

I believe the Academy’s participation in regional science fairs is of great value, as I have observed considerable enthusiasm for science among middle school
students and often considerable sophistication in their grasp of the scientific method. Not only do students learn about the nature of science, but they may well teach their parents, who may assist them with their projects.

I would like to see the Academy do more to foster more interest in science at the high school level, and increase participation of these students in research projects. This may be a useful way of maintaining high enrollments in science and engineering programs in state universities.

In conclusion, let us consider what we can do, especially within the sphere of science education, to foster a more accurate view of science in the community, and, in so doing, open people’s minds.

LITERATURE CITED


Kuhn, T.S. 1962. The Structure of Scientific Revolutions, 1st. ed. Univ. of Chicago Press, Chicago, IL


Special Symposium: Science At The Sanford Underground Laboratory

presented at The 95th Annual Meeting of the South Dakota Academy of Science
EUKARYOTIC MICROBIAL DIVERSITY
WITHIN BIOFILM AND SEEP-WATER SAMPLES
ISOLATED FROM THE FORMER HOMESTAKE
GOLD MINE, LEAD, SOUTH DAKOTA

C. M. Anderson*1 and S.S. Bang2
1Center for the Conservation of Biological Resources
Black Hills State University
Spearfish, SD 57799
2Dept. of Chemical and Biological Engineering
South Dakota School of Mines and Technology
Rapid City, SD 57701
*Cynthia.Anderson@bhsu.edu

ABSTRACT

A metagenomic approach was used to obtain an early assessment of the eukaryotic diversity in biofilm and water samples taken from the former Homestake mine during initial re-entry activities in preparation for its conversion to a Deep Underground Science and Engineering Laboratory (DUSEL). The samples consisted of a biofilm scraping taken from a depth of 610-meters at the Ross Shaft, and DNA extracted from water taken from a seep running from ~200 to 914 meters along the mineshaft. The ITS1-5.8S-ITS2 region of the rDNA locus was amplified, cloned and sequenced. The resulting sequence data were screened for chimeras, and aligned to existing sequences in GenBank in order to identify the taxa within the samples. We identified unique sequences, classifying to either Protista or Fungi, with a low percent identity to previously sequenced taxa, with some exhibiting low similarity even at the taxonomic level of Division. Not surprisingly, a number of the sequences classify to Fungi or Protista commonly found in terrestrial ecosystems and waters. This is likely the result of introduction of microbes to the subsurface during mining operations. Further analysis of deeper, pristine regions of the DUSEL will provide new knowledge of the diversity of the eukaryotic microorganisms capable of surviving in the deep subsurface. Likewise, further metagenomic research into the eukaryotic diversity of the DUSEL will lead to an understanding of how introduced surface–dwelling microbes evolve to adapt to life in the subsurface, and how their introduction affects endemic subsurface microbial populations.
SITE INVESTIGATIONS AND GEOTECHNICAL ASSESSMENT AT DUSEL

Zbigniew J. Hladysz
DUSEL Project Office
South Dakota School of Mines and Technology
Rapid City, SD 57701
Zbigniew.Hladysz@sdsmt.edu

ABSTRACT

The Deep Underground Science and Engineering Laboratory (DUSEL) being planned at the site of the former Homestake Gold Mine in Lead, SD, will host physics experiments which require shielding from cosmogenic radiation. Therefore, current plans include the construction of rooms for these experiments at depths of 1.48 km (4850 ft) and 2.25 km (7400 ft) below the surface. The standard laboratory modules will consist of excavations between 50 and 100 m in length and 20 m in width. The long baseline experiment, however, will involve sending a beam of neutrinos through a chord of the Earth from northern Illinois to intercept a large detector at the 4850 ft level of the laboratory in Lead, SD. If it is determined that this will be a water detector, it will require one or more large cavities to be excavated that may be in the form of a right cylinder with a diameter of 55 m and a height of 60 m. These detectors would constitute the largest previous excavations at this depth by far. Construction of the cavities required the development of a geotechnical characterization program including preliminary drilling and coring, site mapping, in-situ stress measurements, and laboratory characterization of rock mechanics properties as well as preliminary geotechnical assessment of the site. The goals of the geotechnical work include delineation of prospective sites for the cavities and the provision of geotechnical input for the designer. The rock quality at the currently identified site is good and the plans for the excavations are proceeding on schedule.
MOLECULAR SURVEY AND BIOPROSPECTING OF THE MICROBIAL COMMUNITIES IN HOMESTAKE DUSEL

Sookie S. Bang* and Cynthia M. Anderson  
1South Dakota School of Mines and Technology  
Rapid City, SD 57701  
2CCBR/WestCore  
Black Hills State University  
Spearfish, SD 57799  
*sookie.bang@sdsmt.edu

ABSTRACT

In accordance with the current activities of developing the former Homestake mine as a Deep Underground Science and Engineering Laboratory (DUSEL), we focused on identification of microbial community structures and metabolic diversity using culture-independent and culture-dependent methods. Molecular characterization of microbial communities in DUSEL water and weathered soil–like samples was carried out by 16S rDNA sequence analysis. Geochemical analyses indicated that both samples were high in sulfur, rich in nitrogen and salt, but with significantly different metal concentrations, high in the soil and low in the water. 16S rDNA sequence analysis showed that phylogenetic diversity of archaeal communities was low and novel, whereas that of bacterial communities was high. In particular, bacteria operational taxonomic units (OTUs) containing the highest percentage of sequences classified to the same order as sulfur oxidizing soil bacteria such as *Thiobacillus plumbophilus* and *Thiohalomonas nitratireducens*. Community members of bacteria from individual Homestake ecosystems were heterogeneous and distinctive to each community with several unique phylotypes identified from the water sample. As part of bioprospecting for novel extremophiles, we identified thermophilic *Geobacillus* strains isolated from weathered soil-like samples to produce high levels of cellulases and hemicellulases. These findings offer critical information on the microbial community compositions of the drifts and water from upper layers of the mine, the deeper part of which is currently submerged. Archiving this information is critical as the current dewatering program proceeds and the ecological conditions in the mine continue to change, thus affecting subsequent changes in the microbial diversity.
ABSTRACT

The former Homestake Gold Mine in Lead, SD, is rapidly making the transition to becoming a significant influence in the world of underground scientific investigations. The site is being maintained and improved in anticipation of becoming a deep underground science and engineering laboratory that will be unparalleled in the world as a multidisciplinary laboratory with a primary focus on detectors for particle physics. South Dakota, through the South Dakota Science and Technology Authority, and the DUSEL Project, funded by the NSF, are cooperating closely to preserve the site and to produce the necessary planning documents to lead to a Major Research Equipment and Facilities Construction project (MREFC), which would fund the large underground laboratory construction. Substantial work has been performed in the underground including geotechnical characterization, infrastructure assessment, and laboratory design which will lead to a Preliminary Design Report within the coming year. Similarly, the surface assessment of existing buildings and initial planning for new buildings are well underway. This work has resulted in the preparation of plans for the main campuses at the 4850 level and the 7400 level (feet below the surface), as well as provisions for more localized experiments at intermediate levels.
CENTER FOR ULTRA-LOW BACKGROUND EXPERIMENTS AT DUSEL (CUBED)

Barbara Szczerbinska1*, Drew Alton2, Xinhau Bai3, Dan Durben4, Jaret Heise5, Haiping Hong3, Stan Howard3, Chaoyang Jiang6, Kara Keeter4, Christina Keller6, Robert McTaggart7, Dana Medlinc, Dongming Mei6, Andre Petukhov3, Joel Rauber7, Bill Roggenthen1, Jason Spaans6, Yongchen Sun6, Keenan Thomas6, Michael Zehfus4, and Chao Zhang6

1Dakota State University, Madison, SD 57042
2Augustana College, Sioux Falls, SD 57197
3South Dakota School of Mines and Technology, Rapid City, SD 57701
4Black Hills State University, Spearfish, SD 57799
5Sanford Underground Laboratory, Lead, SD 57754
6University of South Dakota, Vermillion, SD 57069
7South Dakota State University, Brookings, SD 57007

*Barbara.szczerbinska@dsu.edu

ABSTRACT

The selection of Homestake Mine in Lead, South Dakota, by the National Science Foundation (NSF) as the site for the Deep Underground Science and Engineering Laboratory (DUSEL) opened new research opportunities to the faculty and students in the state of South Dakota. One of many efforts allowing these scientists a significant participation in the activities planned at DUSEL was the creation of a 2010 Research Center focused on ultra-low background or a Center for Ultra-low Background Experiments at DUSEL (CUBED). The main objectives of this research center are: 1) to bring together the current South Dakota faculty to develop a critical mass of expertise necessary for South Dakota’s full participation in the large-scale collaboration at DUSEL; 2) to increase the number of research faculty members in South Dakota to complement and supplement existing expertise in nuclear physics and materials sciences; 3) to train and educate graduate and undergraduate students as a way to develop the scientific workforce of the state. The main research focus of CUBED is aimed at experiments searching for rare and difficult to detect phenomena such as neutrinoless double-beta decay and dark matter. Major scientific activities proposed by CUBED include low a background counting facility driven by physics, biomedical research and homeland security, an underground crystal growth lab, a purification/depletion facility on noble gases, and an electroforming copper underground facility. We will discuss the current status of the center and provide detailed information of research activities at CUBED.
LONG BASELINE NEUTRINO EXPERIMENT: PHYSICS AND PROJECT STATUS

Milind Diwan
Physics Department
Brookhaven National Laboratory
Upton, NY 11973
diwan@bnl.gov

ABSTRACT

I will describe the science and technical progress on the Long Baseline Neutrino Experiment (LBNE) at DUSEL. LBNE consists of a very large sensitive detector at the Homestake site at a depth of 4850 ft and an intense beam of neutrinos from Fermi National Accelerator Laboratory, 1300 km away. The detector will record neutrino events from the beam and measure the total energy and particle type. The recorded energy spectrum after travel over the long distance will allow precise measurements of neutrino parameters related to quantum mechanical mixing and mass differences. The measurements will provide further hints about quarks--lepton unification and the origins of matter. The same detector can be used to find decays of protons, which are also predicted to take place if the idea of unification is correct. LBNE now has a strong collaboration of 46 institutions and 220 scientists. The project has funding to create the detailed engineering design and cost estimate for a timely execution.
OPPORTUNITIES AND CHALLENGES: EXPERIENCES IN PERFORMING RESEARCH AT THE SANFORD UNDERGROUND LABORATORY AT HOMESTAKE

Jason Van Beek1,2*, Jaret Heise2, William Roggenthen1, and Larry Stetler1

1Geology and Geological Engineering Department
South Dakota School of Mines and Technology
501 East Saint Joseph Street
Rapid City, SD 57701
2Sanford Underground Laboratory at Homestake
630 East Summit Street
Lead, SD 57754
*Vanbeek.jason@gmail.com

ABSTRACT

The former Homestake mine in Lead, SD, has been chosen by the NSF as the location for the proposed Deep Underground Science and Engineering Laboratory (DUSEL). Prior to the establishment of the federally-funded facility, an interim early science program is being facilitated by the South Dakota Science and Technology Authority, which currently operates the Sanford Laboratory. Many unique opportunities are associated with early access to the Lab site as well as a number of challenges, including access to services such as power and communications and reconciling desired experiment locations with the approved safely-accessible laboratory areas. Experience gained from observing and participating in research has led to an understanding of best practices for safely and effectively utilizing the Sanford Laboratory area and resources.

Keywords

DUSEL, Homestake, South Dakota Science and Technology Authority, Sanford Laboratory

INTRODUCTION

The art and science of gold mining were studied by geologists and miners for 125 years at the Homestake mine in Lead, SD, until the mine was generously transferred by Barrick Gold Corporation to the state of South Dakota for the development of an underground laboratory. The recent conversion of the mine into a deep underground science laboratory has brought a new generation of miners to Lead—physicists, engineers, biologists, and geologists—seeking to uncover details about the nature of the universe, the earth, and the limits of life.
The South Dakota Science and Technology Authority (SDSTA) provides oversight to researchers so they are able to safely conduct experiments and collect data within the mine. Currently, approximately 18 groups are actively conducting science experiments on the surface and at 11 separate levels as part of the Sanford Laboratory Early Science Program. At the present time, the DUSEL effort supported by the National Science Foundation is directed toward preparing the engineering design for the larger federally-funded project, which is the ultimate goal for the facility.

**Early Science Access**—Groups interested in conducting research at the Sanford Laboratory first contact the Science Liaison Director, who collects information about the proposed experiments, the equipment to be used and the associated Laboratory infrastructure requirements. Science Liaison department staff then use that information to identify suitable candidate locations, relying on existing maps, past experience, and dedicated underground investigations. In some cases a site-selection visit with the research team is necessary to ensure a particular location is acceptable. Advice from other Laboratory departments such as Engineering and Environment, Health & Safety may also influence the choice of location.

Once a site (or sites) is identified, the process of planning an underground trip to perform work begins. As part of the planning process, the research team provides documents detailing activities, personnel and procedures, work planning steps as well as an analysis of the job hazards. Depending on the nature of the work, certain training may be required in addition to the standard site-specific training. Those researchers expecting to be on-site for more than 40 hours per year are currently required to take a 10-hour OSHA training class, scheduled sessions of which are offered for free by the Laboratory.

As soon as possible in the planning process, paperwork is circulated internally alerting Laboratory personnel of the proposed trip to determine if there will be any scheduling conflicts. Afterward, an “Action Plan” is submitted on behalf of the research group that provides specific details about the trip, including the start time and expected duration as well as listing the required personal protective equipment and any other relevant considerations such as contact information.

**Performing Early Science**—Early science research at the Sanford Laboratory takes several forms. Some groups install instruments in defined areas while other groups, such as those performing biological and hydrological experiments, require more flexibility to collect samples from multiple locations within the accessible underground volume. Studies requiring fixed installations must provide greater detail in planning to ensure safe execution of the work and to ensure that data can be gathered effectively.

Ground motion and water level monitoring in the mine requires specialty installation, power, network and occasional access. Equipment installation, fine tuning, and troubleshooting can take days or weeks. The lifetime of each installation is expected to be months to years. For example, an ideal location for a ground-motion monitoring site selected by one of the groups was located at a distance from sources of anthropogenic noise (shafts, pumps, ventilation drifts,
walkways, other experiments) on a main level 1250 meters (4100 feet) below the surface. However the location fell well outside the safety footprint of the laboratory and was not equipped with 110V power or fiber-optic network connectivity. Although a review by safety personnel and installation of ventilation, ground control, power, and network connectivity could have made this site usable, this location would greatly increase the financial and time costs associated with the experiment. A second location was selected by focusing on areas within the safety footprint and within 100 meters of existing utilities. Noise levels were sacrificed in order to benefit from a more reasonable completion schedule and budget. After the site had been selected and confirmed by the SDSTA, a site visit was conducted to plan the installation and coordinate with SDSTA personnel on requirements and expectations. A second installation trip was planned and scheduled, and the necessary paperwork and safety training was completed. The installation was accomplished without any major problems.

**On-site Visit**—A first-time researcher visiting the Sanford Laboratory to go underground first registers with reception at the main Administration building and then is directed to their contact person or the Safety Department for the required safety training. Completion of the safety training is documented and archived on-site for future visits. Before going underground the visitor is equipped with personal protective equipment and transported to the headframe to board the “cage”. The cage is used as primary access and egress to the underground for equipment and personnel. The current Laboratory access policy calls for each research team to be escorted by qualified Laboratory staff (the ratio of Lab staff to scientists depending on the destination). Laboratory staff members are there to ensure the safety of the science team and help teams with logistics and coordination. Upon completion of the underground activities, the team returns to the Administration building to return personal protective equipment and generate a shift report detailing the events of the trip.

**Early Science Challenges and Opportunities**—Installations, experiments, and sample collections that go smoothly have several attributes in common: communication, adequate preparation, well-understood action plans, and backup plans. Active research groups require constant communication with the Safety and Science departments of the SDSTA. Installations and underground visits typically require initial communication >1 month before the intended visitation date with occasional communication thereafter to establish expectations and fulfill safety obligations. Job hazard analyses and other paperwork typically require several revisions and are expected to be in place before the visit date. It is important for science groups to realize that a significant amount of time and effort is required both on- and off-site prior to any installation of equipment underground. When planning for a relatively complex experiment, some groups have found it beneficial to send a subset of the entire team in advance to assemble equipment and supplies, scout the location of interest, and prepare for installation.

A document database is currently hosting information associated with Sanford Laboratory early science, DUSEL design and materials relevant to contractors. To enhance the effectiveness of early science at the Sanford Lab, we will add
to a publicly-accessible server previous research data, archived Homestake mine documents, safety procedures and forms, geologic maps, and diamond drill core archive information. Moreover, new data generated from early science will be added to a public database, the availability of which will make the planning process more effective and efficient. New underground infrastructure will improve water removal, ground control, ventilation, networking, and power to increase the safety footprint of the Sanford Underground Laboratory.
SLOW GROUND MOTION STUDIES
AT HOMESTAKE DUSEL

Larry D. Stetler*, James T Volk and Jason K. VanBeek

1Department of Geology and Geological Engineering
SD School of Mines & Technology, Rapid City, SD
501 E. Saint Joseph St.
Rapid City, SD 57701
2Fermi National Accelerator Laboratory
Batavia, Illinois 60510
*larry.stetler@sdsmt.edu

ABSTRACT

Instruments have been installed on the 2000-ft level in the former Homestake gold mine in Lead, South Dakota, to measure natural and seismic-induced ground motions. Additional instrumentation in the water column has provided correlation between ground motion and changes in the water table elevation. Together, these data have provided indications of changes occurring to the in-situ stress field and relative ground positions. Two types of hydrostatic water level sensors have been utilized, Tevatron-style and Budker sensors. In addition, high resolution Geomechanics tiltmeters have been installed. The Tevatron-style and Budker sensors utilize water-filled tubing to connect the sensors along a length of drift. An upper air-filled line allows water to transfer between sensors and to self-level, i.e., to provide a measure of tilt, as ground motion occurs. Simultaneous measurements of water levels have been collected using a pressure transducer submerged into the water column. Results indicate that as water mass was removed, the apparent collapse of pore space resulted in a net lowering of the surface shown as a general tilt of the floor toward the water pool. Thus far, the tiltmeters have recorded three types of signals, 1) passage of earth tides, 2) changes in relative positions of ground points associated with the net lowering of the surface toward the water pool, and 3) seismic activity associated with high magnitude earthquakes. Analytical methods have been developed to remove the low frequency signal associated with earth tides and to resolve the directional component of seismic waves. These techniques allow net tilt magnitude to be estimated, and for resolution between local and global seismic events that will become important to development of large underground cavities at DUSEL.

Keywords

Slow Ground Motion, Homestake DUSEL, Tiltmeters
INTRODUCTION

The Homestake gold mine in Lead, SD, operated continuously from 1877 to 2003. Over the life of the mine, 63 underground levels were developed from surface to 2484 m depth associated with ~560 km of drifts. Groundwater was intersected early in the development of the underground workings, and pumping water from the lower levels was a continuous operation until June 2003 when the pumps were shut off. At about the same time it was announced the mine would cease operations, the scientific community began to propose the facility be used to host a new national laboratory. After a lengthy down-select process by the National Science Foundation, the former Homestake mine was officially selected to house the Deep Underground Science and Engineering Laboratory, or DUSEL, in mid-2007. The shafts were re-opened in spring of 2008, and by the time the pumping system had been restarted, the water had risen to the 1380 m-level, a total rise of 1104 m. By summer 2010, the water level had been reduced to the ~1540 m-level and a new deep-water pump was installed to complete the water reduction to the bottom of the mine.

Since the facility was developed as a mine, removal of rock induced a change to the natural in-situ stress conditions in the underground. Subsequent re-filling of the large void spaces, calculated at $2.13 \times 10^7 \text{ m}^3$ (Rahn and Roggenthen 2002), by in-flowing water between 2003-2008 changed the stress conditions again. Currently, as the water is again removed, another change to the stress condition can be expected. Understanding these changing stress conditions may be important physical parameters to ensure stability of the large cavities created at the 1478 m-level and for alignment of the experiments in these cavities.

The physics of the stress changes are related to the distribution of the total stress at a point of depth, which is described as:

$$\sigma_T = \sigma_k + \sigma_p$$  \hfill (Equation 1)

where:

- $\sigma_T$ = total stress
- $\sigma_k$ = skeletal (rock) stress
- $\sigma_p$ = pore water stress

Thus, as water either flowed into, or has been removed from the underground, the stress condition of the rock and pore water has also changed. Conversely, removal of the skeletal matrix (rock excavation) will also induce a stress change. Instrumentation currently used at the site has been successful in detecting results of the changes to the stress regime, but not to the individual components of stress.

The objective of this research was to record any observable changes in the stress-field and measure the resulting changes in ground position. Thus far, two components of slow ground motion have been detected: 1) passage of earth tidal forces including seismic wave activity; and 2) that associated with the removal of water mass from the underground spaces and the resulting subsidence of the
ground. These forces change the stress conditions at the short-, to long-term scale, respectively. The short-term response represents a rise and fall of the land due to passage of earth tides, but essentially contains no net elevation change. Seismic wave activity provides a similar short-term response. The long-term response accounts for semi-permanent changes in ground elevation due to adjustments in the stresses described in Equ. 1. These results will be used to more fully understand the phenomenon of ground position changes and deformation as a function of force-field changes (Shiltsev 2010, 1995).

**METHODS**

Data utilized to parameterize the research objective included water table drawdown and slow ground motion data. Drawdown data were recorded from the No. 6 winze (Figure 1) beginning in Fall 2008. These data also contained basic meteorological parameters (air and water temperatures, barometric pressure, and specific conductance of the water) that were recorded from inside the water column and at the underground instrumentation site. Ground motion data have

![Figure 1. Schematic diagram of the dewatering instrumentation inside No. 6 winze. Instruments were suspended below the water table and recorded by datalogger at the levels indicated.](image_url)
been collected from the 610 m-level using three types of ground motion sensors. These stations have been active since late 2008.

**Dewatering Instrumentation and Methods**—Water reduction measurements have been recorded between October 2008 and February 2010 in No. 6 winze from the 1387 and the 1478 m-levels. Depth of water table below the land surface has been collected using a pressure transducer suspended on a 107 m cable initially from the No. 6 winze hoist room on the 1387 m-level. When the level of the water had been reduced, the instrumentation setup was transferred to the No. 6 winze station on the 1478 m-level. These data have been collected on a 2-minute increment and have been considered as a high resolution dataset. Air temperature at the datalogger and water and air temperature from the winze were also collected. A barometric pressure gage at the datalogger also recorded air pressure at the depth of the instrumentation. Current depth of the pressure transducer is the 1582 m-level. Since summer 2009, the water level has been maintained roughly between the 1540-1555 m-levels as the pumping system has been refit to allow for complete removal of the water to the total depth of the mine.

Slow Ground Motion Instrumentation and Methods: Long-term changes in relative positions of subsurface points may reflect induced changes to skeletal or fluid stresses and are most likely due to changes in pressure forces. Short-term changes to relative positions of subsurface points are mostly due to earth tides and seismic wave activity. Monitoring to quantify results of changes to the stress-field and point position responses has been made using three types of tiltmeters. These systems are located on the 610 m-level (Figure 2) and consist of 1) ultra-high resolution (UHR) Geomechanics tiltmeters (0.5 nano-radian resolution); high resolution (HR) Budker Institute (Russia) tiltmeters (1 micron resolution); and low resolution (LR) Tevatron-style (Fermi National Accelerator Laboratory) tiltmeters (5 micron resolution). The UHR tiltmeters measure tilt between two ends of a small beam internal to the sensor. These instruments were mounted on concrete pillars connected to solid rock. The HR and LR instruments are water level systems and have connecting pipes filled with air (upper line) and water (lower line) that essentially operate as a horizontal manometer (Figure 2). Differences in the water level between two individual sensors provide a measure of ground tilt (Figure 3). These have been deployed as arrays of six sensors each that consist of two legs, one oriented in a mostly north-south direction and one set in a more east-west orientation. Arrays A and B (LR sensors) were separated by about 300 m, each array being 300 m in length with a sensor spacing of 61 m. Array C (HR sensors) was installed on top of array A but had a sensor spacing of 30 m. Array’s A and B were installed on concrete pillars connected to solid bedrock. Array C was installed using plastic wood platforms attached to rock bolts drilled and epoxied 2.4 m into the rib. Each array contained air temperature, relative humidity, and barometric pressure sensors and a centrally located computer that was connected to the internet and configured for remote access and operation. Thus, system operations and data collection were controlled remotely. Detailed descriptions and operation of the HR and LR sensors have been given by Volk et al. (2006; 2008).
Figure 2. Tiltmeter array (six sensors each) locations on the 610 m-level. Each array (A, B, and C) consists of two legs oriented in different directions. Grey line work represents drifts on the 610 m-level.

Figure 3. Schematic diagram of one leg (three instruments) of a tiltmeter array. Water seeks a neutral level such that ground motion results in varying distances from the sensor to water level (h1, h2, and h3) in each pot. Differences in measurements between any two pots result in a measure of ground tilt, or motion. Shaded area represents the water-filled portion of the system.
RESULTS

The following results represent the initial 18 months of long-term experiments that are continuing into the future. Expansion of the network will include arrays of HR and LR sensors to other deeper levels and additional UHR tiltmeters. Current results are divided into two parts: 1) Dewatering and Stress and 2) Slow Ground Motion.

Dewatering and Stress: Infilling of the mine workings by surface and groundwater (and resulting water quality) was of great concern during the years prior to the closure of the mine (Nelson, 2003a, 2003b; Rahn and Roggenthen, 2002). An inflow model developed by Zahn (2002) was utilized here to predict flooding. Inflow was shown as a simple water balance as a function of the pre-closure deep groundwater inflow rate and average monthly precipitation:

\[ I = I_{surf} + I_{gwat} = 30.36 \times Pptn_{mo} + 627.61 \times \frac{(X-300)}{(8150-300)} \]  

(Equation 2)

where:

- \( I \) = total inflow
- \( I_{surf} \) = surface water inflow as a function of monthly precipitation (inch)
- \( I_{gwat} \) = groundwater inflow as a function of depth below the water table (feet)

Inflow measurements collected at the mine were used to confirm the closeness of the predicted inflow model as a function of days after the pumps were shut off (Figure 4). The water reduction model, a reversal of Equ (2) that included a pumping rate term, was not able to be confirmed due to inconsistent pumping rates during the dewatering process. Thus, the drawdown curve predicted by the model, and that actually occurring, varied (Figure 5).

Barometric pressure data were collected simultaneously with the water pressure data. Analysis of these data from the static pumping periods has revealed effects due to changes in the stress regime as the rock-water matrix was altered as a function of atmospheric pressure. Figure 6 shows an identical and instantaneous behavior in both curves.

Slow Ground Motion: Tiltmeters are instruments that record the relative position of one point in regard to another. As the solid earth moves beneath one or more points being monitored, the relative difference in the magnitude of the movement is detected. The changes of position might occur at both the short- (minutes to hours) to long-term (days to years) time-frame. Examples of each type of movement are earth tides or seismic waves and a stress change in the rock, respectively. Both types of ground motion have been detected with the installed sensors.

Earth tides are the same as ocean tides except that they occur in continental rocks that are pulled outward in response to the lunar gravity pull. All ground is affected by these low frequency forces. At Homestake DUSEL, all three tiltmeter types have detected the diurnal signature of this tide (Figure 7). Fast Fourier Transforms (FFTs) have been used to determine the frequencies associated with these phenomena (Figure 8) and as indicated on the inset chart, the frequencies
Figure 4. Actual vs. modeled inflow using the precipitation-groundwater model described in the text. Modeled data were in cumulative gallons (after Rahn and Roggenthen, 2002) which filled the modeled void spaces as a function of depth.

Figure 5. Model vs. actual measured drawdown. Model assumed a constant pumping rate of 1500 gpm which would have reached the 1615 m-level after 127 days. Actual drawdown reached 1545 m in 442 days. Model data were based on cumulative gallons pumped for each level after Rahn and Roggenthen (2002). Fluctuation in the actual drawdown represents changes in the pumping rate.
of the waves are within a few percent of the actual known tidal periods, thus, confirming the source of this waveform.

High frequency seismic waves typically are measured using high frequency instrumentation. Tiltmeter analysis has shown the detection of strong earthquake seismicity at DUSEL where the differing resolution sensors have successfully recorded seismic activity but at varying levels of detail. Figure 9A shows data for the 7.0M Haiti earthquake of January 12, 2010 from the UHR sensors. The LR sensors did not record this wave even though data acquisition was 5 seconds compared to 1 minute for the UHR sensors. This earthquake occurred at 14:53 MST and was detected at Homestake at 15:10 MST, a delay of 17 minutes. The plot clearly shows oscillations occurred for a period of 40 minutes. By comparison, Figure 9B shows the 8.8M Chilean earthquake of February 26, 2010 from the UHR sensors. This earthquake occurred at 23:34 MST and was recorded at 23:50 MST, a delay of 16 minutes. This instrument recorded wave activity for 3 hours due to multi-paths of travel by the waves. Figure 9C shows the same earthquake recorded by the HR sensors. The first appearance was at 00:03 on February 27, a delay of 29 minutes. These sensors show activity for a total of 23 minutes due to their lower sensitivity.

One objective of setting the HR and LR sensors in arrays with two differently oriented legs (Figure 2) was to reduce directionality of the ground motion signals. Initial attempts to accomplish this involved isolation of the seismic waves.

Figure 6. Barometric pressure plotted with water level showing an instantaneous water table response to changes in pressure.
Figure 7. Ground motion detected on LR tiltmeters for January 2010. Daily fluctuations are evident as well as lunar cycle deformation (~14 days). Net change in position (thick lines on plot) between one lunar cycle is ~20 µm and is due to ground subsidence toward the south.

Figure 8. Fast Fourier Transform of tiltmeter data that reveals recurrent frequencies. Inset table shows the details of the analysis for each peak and the error from the known earth tides.
by removing the low frequency signals utilizing a high-pass filtering technique. This spreadsheet model involved constructing a time-averaged high-pass filter equal to the original sample minus the sample average value for a specific time period. This filter was moved sequentially through the data and was successful in removing the earth tide component and isolating the high frequency seismic signals. The next step involved isolating the seismic waves in the direction the wave would travel from its source to where it would intersect the plane of tiltmeters at DUSEL. However, due to variations in the properties of crustal rocks, the arrival direction of these seismic waves might not be exactly linear from the source, i.e., they do not travel along a straight line. Each array leg was transformed into vectors and a vector normal to those two vectors was computed. This was then projected onto the two separate axes to determine the best estimate of arrival direction for the Chilean earthquake. This was determined to be 192.25°.

Figure 10 shows the results of these analyses. All earth tides have been successfully removed, and the arrival and subsequent seismic activity from this 8.8M event are evident for up to 4 hours. G1-G5 show the wave arrival from various directions as the signal circled the earth multiple times. The short-term effects of these waves are confirmed in that all data prior to and after the seismic activity return to the zero line, i.e., there is no net ground deformation resulting from this earthquake.
The other component of ground motion consists of the long-term changes in elevation. If this has occurred, the beginning and ending points from a short-term event (such as the tidal cycle) will most likely not be the same. Figure 7 clearly shows the earth tides with the ~12 hour cycle readily observed. However, there is a net change in the position of the points through time which is seen as the upward-sloping nature to the data. This indicates that the relative position of the sensor itself has changed.

**DISCUSSION**

*Dewatering and Stress*—Hydrologists have established that water levels in wells respond to increases and decreases in the pressure exerted on a subsurface matrix (rock and fluid). This is referred to as barometric efficiency, a term that is a ratio between the pressure changes in the water column and aquifer to the unit weight of water (Gonthier, 2007). Physically, the water level in a well fluctuates in response to changes in the pressure regime depending on the hydrologic system. A shallow unconfined well subjected to an increase in barometric pressure normally responds by a slight fall in the water level in the well and often shows a time-lag effect due to the non-uniform matrix properties and air movement through the vadose zone (Rasmussen and Crawford, 1997). In effect, the full barometric pressure is exerted on the water surface but only partially in the ma-
trix, thus, water is pushed back into the formation (Sara, 2003) and, after a time lag, is equalized in the matrix material. Conversely, in a confined aquifer, an increase in barometric pressure has an immediate response in the water table due to pressure effects applied instantaneously to both the well and confined aquifer system. Water level rises in direct response to increased barometric pressures.

Equation 1 shows that at a point, two components of stress exist to make up the total stress. If either of the two components change (increase or decrease), the other must adjust to make up for the difference. The effect is that as atmospheric pressure increases aerially across a land surface, increased stress to the skeletal component of the confined aquifer system ejects fluid into the wellbore. In essence, the pore space is reduced due to increased compressive stress on the matrix resulting in a net rise in the water level observed in the well. The opposite occurs from a decreased atmospheric pressure.

At Homestake DUSEL, several periods of pumping have existed since summer 2009 during which the pumping rate matched the inflow rate, thus, providing a static water table. However, as Figure 6 indicates, during these static periods the water table has actually exhibited fluctuations of more than 0.3 m closely tied to changes in atmospheric pressure. The response is immediate and no lag in the pressure and water curves has been observed. Further analysis of these data will result in estimations of the system compressibility, a required parameter for aquifer and system characterization.

**Slow Ground Motion:** All sensor arrays have been successful in detecting earth tides and net tilt. The directional components of the tiltmeter data indicate the floor is tilting generally toward the south. The most likely explanation is that removal of water mass from the lower levels (below and south of the tiltmeter locations) is causing a decrease in pore water pressure as fluid is drained into the shafts and pumped from the mine. This causes an increase in the skeletal stress which decreases pore space resulting in a net compression of the rock and a lowering of the ground surface. These data are not providing the magnitude of stress changes, but are recording the affects of changes to the stress regime. Addition of instrumentation to record the components of stress will allow for more accurate reduction of the tilt data in the future.

Attempts to remove the tidal component from the signal have been successful. The signal can then be more readily evaluated for net changes in ground position between specified time periods. In the same way, the reduction of directional wave fronts will become a useful tool to determine local vs. regional or global seismic events. It is anticipated that as excavation of large cavities begins at the deeper levels of the mine, net fluctuations in ground position will be readily determined. These data will provide basic rock characterization for assessing long-term stability of the cavities.

**ACKNOWLEDGEMENTS**

Instruments utilized to measure drawdown and slow ground motion data were supplied by awards NSF-EAR-0757883 and a South Dakota School of Mines.
and Technology Nelson Research Grant to the senior author. Fermi National Accelerator Laboratory provided tiltmeters and expertise.

LITERATURE CITED


Complete Senior Research Papers

presented at

The 95th Annual Meeting

of the

South Dakota Academy of Science
A SURVEY OF MYXOMYCETES FROM THE BLACK HILLS OF SOUTH DAKOTA AND THE BEAR LODGE MOUNTAINS OF WYOMING

A. Gabel*, E. Ebbert¹, M. Gabel¹ and L. Zierer²

¹Biology Department
Black Hills State University
1200 University
Spearfish, South Dakota 57799
²Deceased
*audrey.gabel@bhsu.edu

ABSTRACT

Fruiting bodies of slime molds were collected intermittently from the field from 1990-2009. During the summers of 2007-2009 slime molds also were cultured in moist chambers from downed, decaying wood, dried deciduous leaf litter, and dried grass litter that was gathered three times each summer from four areas at each of the following six sites. Alabaugh Canyon and Boles/Redbird Canyons were dry, prairie/woodlands in Fall River and Custer Counties in the southern Black Hills. Pony Gulch and Spring Creek, in the central hills were moist, broad gulches in Pennington County, and the northern hills sites were Botany Bay and Grigg’s Gulch, narrow, moist canyons in Lawrence County. Contents of a total of 648 cultures were examined. Presence of a species in a culture was considered to be a collection and some cultures contained more than one species. Eighty-two species from both field and culture surveys were identified, and 62 are considered as new records for the Black Hills, and 61 for western South Dakota. Six hundred fifty-seven collections representing 65 species developed in culture included species from all orders of Myxomycetes. Only five species occurred at a relative abundance > 5% and 20 occurred only once. Four hundred eighty-three collections representing 54 species occurred on wood, 115 collections representing 29 species occurred on leaf litter, and 59 collections representing 10 species were on grass litter. Several species occurred on more than one substratum. The highest number, 178 collections and 39 species, occurred on substrata from the Boles/Redbird Canyon site, the southwest dry, open prairie/woodland. The lowest number, 74 collections and 19 species, occurred on substrata from Grigg’s Gulch, a northern, moist site with a high canopy cover. Fuligo megaspora was frequently collected from southern, dry sites.

Keywords

Black Hills, Fuligo megaspora, Myxomycetes, South Dakota
INTRODUCTION

Plasmodial slime molds (Myxomycetes) are unique organisms with two distinctly different growth forms. The free-living, multinucleate plasmodium resembles an amoeboid-organism which feeds on bacteria and other microorganisms. When environmental conditions are appropriate, a reproductive phase develops and fruiting bodies that resemble fungi are formed which contain spores for dispersal and survival. In addition to spores, microcysts (encysted myxamoebiae and swarm cells) and sclerotia (encysted plasmodia) serve as resting stages and are capable of surviving unfavorable environmental conditions. Slime molds occur on a variety of substrata including the bark of living trees and woody vines, decayed logs on the ground, herbaceous plants and grasses, and litter of herbaceous plants and grasses. Herbivore dung and soils also support growth (Kilgore et al. 2009, Keller and Braun 1999, Martin et al. 1983, Martin and Alexopolous 1969). In the past slime molds were classified as fungi and studied by mycologists, but because of the amoeboid stage most taxonomists currently place them with the protists (Keller and Everhart 2010, Kilgore et al. 2009, Spiegel et al. 2004, Ing 1994, Frederick 1990) accommodating both the amoeboid stage and the fruiting bodies.


Little attention has been directed toward surveying the Great Plains in the mid-continental United States. This area as described by Kaul (1986) includes northwestern Texas, eastern New Mexico, western Oklahoma, eastern Colorado, Kansas, Nebraska, eastern Wyoming, South Dakota, eastern Montana and North Dakota. Keller and Schoknecht (1989) reported *Fuligo megaspora* from Colorado and northwestern Nebraska. Keller and Brooks (1971), Kramer (1956), Brooks (1941, 1942), and Schaffner (1904) collected in Kansas, and Seaver (1908) collected in North Dakota. Rollins (2007) initiated surveys in mid-continental grasslands and has reported collections from the Thunder Basin National Grassland in Wyoming.

Even fewer reports focus on the Black Hills in South Dakota and Bear Lodge Mountains in Wyoming. In 1898, Macbride published a list in the Proceedings of the Iowa Academy of Science of 30 species he collected during one August visit in the Black Hills of South Dakota. Later, Macbride (1922) listed an additional 3 species from the Black Hills, and 6 more species were located by staff in collections at the National Fungal Collection at Beltsville, Maryland.

The Black Hills is a unique area where several biomes meet and overlap (Froiland 1990). The Nature Conservancy’s Ecoregions of the United States of America (1999) identifies the Black Hills as a unique ecoregion (Fig. 1). Annual average precipitation for the Black Hills area from 1961-1990 was from less than 40 cm near the periphery to 60 cm in the Lead/Deadwood area (Trimarchi et al. 1998). The area is located along the western border of South Dakota and northeastern Wyoming, extending approximately 166 km north to south and 108 km east to west. It is approximately 480 km from the Rocky Mountains and even more isolated from the boreal and eastern deciduous forests. However, the flora includes representatives from each of these biomes, and the mid-continent grasslands.

Geologically, it is an area characterized by an uplifted dome with a Precambrian core. Some of the older rocks date to 2.5 billion years before the present. Several episodes of uplift and erosion have occurred, accompanied by sedimentation. Near the end of the Cretaceous the last of the epicontinental seas retreated and more uplifts occurred extending as late as the Eocene. A final minor uplift occurred in the Pleistocene, producing essentially the modern appearance of the area (Froiland 1990).

The primary goal of this survey was to extend our knowledge of the occurrence of plasmodial slime molds in the Black Hills of South Dakota and adjacent Wyoming, and western South Dakota, and to contribute to the ongoing world survey of Myxomycetes. The present survey includes specimens collected randomly and intermittently from the field for several years and specimens fruiting in culture on substrata that were collected from six selected sites in the Black Hills from 2007-2009. For the culture studies we hypothesized that more species and specimens would occur from wetter sites with higher canopy covers, and high plant species diversity/hectare in the northern hills compared to drier, more open sites with less plant species diversity/hectare in the southern hills (Gabel and Gabel 2007). We also hypothesized that more species and specimens would be recovered from woody substrata than on deciduous leaf and grass litter substrata.
METHODS

Field Collections—From 1990-2009 fruiting bodies of plasmodial slime molds were collected intermittently and randomly from western South Dakota and adjacent eastern Wyoming. Specimens were air dried, placed in boxes with label information and placed in the fungal collection located in the Black Hills State University Herbarium (BHSC). Labels included: order, species, habitat, elevation, substrate, date collected, location (latitude/longitude or township, range and section), county, collection number, accession number, collector and identifier. Latitudes and longitudes are listed as the centroid for the site unless a reading was taken for a particular collection.

Moist Chamber Cultures—The 6 sites in the Black Hills from which substrata were collected are described in Table 1 and located in Figure 1. Sites included 2 in the southern hills (Alabaugh Canyon and Boles/Redbird Canyons), 2 in the central hills (Pony Gulch and Spring Creek), and 2 in the northern hills (Botany Bay and Grigg’s Gulch). Downed, decaying wood, and dead deciduous leaf and grass litter retrieved from the ground were collected during the summers of 2007-2009. An intensely hot forest fire occurred at the Alabaugh Canyon site after the July 2007 collection. Although litter was mostly destroyed and wood substrata were burned we continued to collect at this site following our regular schedule. Each of the 6 sites was visited in June, July and August in the same time sequence each of the 3 years. The 3 substrata were collected, each from 4 different areas at each site during each visit. Substrata were placed in clean paper sacks, brought to the laboratory, dried overnight and placed in moist chambers the following day. Substrata were not weighed. Aseptic techniques were followed when preparing moist chamber cultures. Wood from each area of a site was placed on 2 layers of paper towels in a 5 cm x 7 cm x 3 cm washed and surface sterilized plastic box. Leaf
litter and grass litter from each area of a site were placed on 2 layers of #1 Whatman filter paper in plastic petri dishes (two dishes/area/substrate) that provided approximately the same surface area for observation between the plastic boxes and petri dishes. Twelve cultures for each of the 6 sites/collection day were prepared and 3 collection days each season for 3 years resulted in 648 cultures for the 3-year study. All substrata were immersed in deionized water for 20-24 hrs after which excess water was decanted. Moist chamber cultures were incubated at room temperature (15º C to 18º C) under ambient light in a normal day/night cycle. Relative humidity was not monitored. Cultures were observed for fruiting bodies weekly for 4 weeks in 2007, for 6 weeks in 2008, and for 8 weeks in 2009. When possible in 2008 and 2009, wood was classified as coniferous or deciduous, and when possible during the entire study substrata were identified to genus and species. Culture procedures generally followed Keller et al. (2004). Specimens were identified following Keller and Braun (1999), Stephenson and Stempen (1994), Nannenga-Bremekamp (1991), Martin et al. (1983), Farr (1981), and Martin and Alexopoulos (1969). Nomenclature generally followed Martin and Alexopoulos (1969). Exsiccatae were prepared similarly to field collections for inclusion in the fungal collection. Although Frederick (1990) does not include the genus Ceratiomyxa with the myxomycetes, we are following Tran


<table>
<thead>
<tr>
<th>Site Name</th>
<th>AL</th>
<th>BB</th>
<th>BR</th>
<th>GG</th>
<th>PG</th>
<th>SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site Description</td>
<td>prairie</td>
<td>narrow</td>
<td>prairie</td>
<td>narrow</td>
<td>broad</td>
<td>broad</td>
</tr>
<tr>
<td>woodland</td>
<td>canyon</td>
<td>woodland</td>
<td>canyon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latitude</td>
<td>43</td>
<td>44</td>
<td>43</td>
<td>44</td>
<td>44</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>20.533N</td>
<td>25.283N</td>
<td>47.439N</td>
<td>26.633N</td>
<td>04.946N</td>
<td>51.839N</td>
</tr>
<tr>
<td>Longitude</td>
<td>103</td>
<td>103</td>
<td>104</td>
<td>103</td>
<td>103</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>33.35W</td>
<td>52.883W</td>
<td>01.805W</td>
<td>57.117W</td>
<td>38.804W</td>
<td>38.479W</td>
</tr>
<tr>
<td>Twn/Rng/Sec</td>
<td>T8S/R5E/S20</td>
<td>T5N/R2E/S4</td>
<td>T3S/R1E/S3</td>
<td>T6N/R1E/S26</td>
<td>T2N/R4E/S31-33</td>
<td>T2S/R4E/S20,21</td>
</tr>
<tr>
<td>Elevation (m)</td>
<td>977</td>
<td>1258</td>
<td>1273</td>
<td>1236</td>
<td>1564</td>
<td>1629</td>
</tr>
<tr>
<td>County</td>
<td>Fall River</td>
<td>Lawrence</td>
<td>Custer</td>
<td>Lawrence</td>
<td>Pennington</td>
<td>Pennington</td>
</tr>
<tr>
<td>Canopy Cover (%)</td>
<td>30</td>
<td>81</td>
<td>20</td>
<td>63</td>
<td>49</td>
<td>43</td>
</tr>
<tr>
<td>Understory</td>
<td>JC/RA</td>
<td>RL/PV/SR</td>
<td>CM/RA</td>
<td>JC/SO</td>
<td>PA/PV</td>
<td>PA/PV</td>
</tr>
<tr>
<td>Ave. Precip. *</td>
<td>34.6</td>
<td>44.1</td>
<td>36.8</td>
<td>44.1</td>
<td>41.3</td>
<td>45.4</td>
</tr>
</tbody>
</table>
et al. (2008) and Stephenson et al. (2008), and including our collections of Ceratiomyxa fruticulosa in this report. One species from the culture study, Dianema corticatum, has been designated cf, considered similar to C. fruticulosa, but based on poor quality material. Genera that were not identified to species and species for which we have no vouchers were not included in this study.

A collection listed in the results is defined as the presence of 1 or more fruiting bodies of a species in a culture. Plasmodia were not counted. Relative abundance index of a species was calculated based on the number of collections of a species in relation to the total number of collections (657) in the 3-year study. Percentages above 5% were considered abundant, 3.0–5.0% as common, 1.5–2.9% as occasional, 0.5–1.4% as uncommon and less than 0.5% as rare. Canopy cover was estimated from 4 readings from each of 4 randomly selected reference sites using a spherical densiometer, and readings were averaged (Lemmon 1957). Cluster analysis was used to compare the collection sites using slime mold species as variables. NTSYSpc (Rohlf 2005) was used to standardize the data, compute distance coefficients, and complete a cluster of the distance matrix.

RESULTS

A total of 82 species from field and moist chamber culture was gathered from western South Dakota, and the Black Hills of South Dakota and Bear Lodge Mountains of Wyoming. This study lists 62 species considered as new records for the Black Hills region and 61 for western South Dakota.

Field—Table 2 lists order, genus, species, site, county, latitude/longitude and habitat for collections. One hundred forty-six collections representing 30 species and 19 genera from 18 habitats were collected. One hundred-ten collections representing 9 species were aethalioid or pseudoaethelioid, which represents 75% of all field collected specimens. Five orders excluding the Echinosteliales are represented. All specimens in the Ceratiomyxales, Liceales, Stemonitales and Trichiales occurred on wood. Species in the Physarales occurred on wood, deciduous leaves, grass, soil and stems. Collections of Badhamia gracilis occurred on stems of Opuntia polyacantha Haw. and Yucca glauca Nutt. Species of Diderma occurred on leaf litter. Diderma crustaceum and Physarum bitectum that occurred on both leaf and grass litter were collected at the Big Hill sites in late April and early May near retreating snow at an altitude of 1529 m, in a mixed deciduous/coniferous habitat of Betula papyrifera, Picea glauca, Pinus ponderosa and Populus tremuloides. One specimen of Fuligo intermedia was collected on wood, F. septica occurred on wood, leaves, grass and soil, and Mucilago crustacea was collected on wood, leaves and soil. Fuligo megaspora, the most commonly collected field species was collected on conifer wood, Juniperus virginiana leaves, grass and soil. It was collected only from southwestern Custer, southern Pennington, and southern Fall River County. All were dry prairie/woodlands dominated by grasses with Pinus ponderosa and Juniperus virginiana the common trees.

Moist Chamber Cultures—Numbers of collections increased from 190 in 2007, to 202 in 2008, to 265 in 2009. Numbers of species occurring increased from 35 in 2007, to 41 in 2008 and to 43 in 2009. Eighteen species occurred in
Table 2. Species of slime molds collected from the field in western South Dakota and adjacent Wyoming from 2000 to 2009. All counties are in western South Dakota except Crook County, which is in Wyoming. Co. Abb. = county abbreviation, CR = Crook, CU = Custer, FA = Fall River, HA = Harding, LA = Lawrence, ME = Meade, PE = Pennington, and PR = Perkins. Dominant vegetation of habitats: 1 = Acer negundo L., Fraxinus pennsylvanica Marsh., Pinus ponderosa; 2 = Betula glandulosa, Picea glauca, Salix sp.; 3 = Betula papyrifera, Corylus cornuta Marsh., Picea glauca; 4 = Betula papyrifera, Ostrya virginiana, Pinus ponderosa, Quercus macrocarpa; 5 = Betula papyrifera, Pinus ponderosa; 6 = Cerocarpus montanus, Juniperus virginiana, Pinus ponderosa; 7 = Fraxinus pennsylvanica, Pinus ponderosa; 8 = Fraxinus pennsylvanica, Pinus ponderosa; 9 = Juniperus virginiana, Pinus ponderosa; 10 = Ostrya virginiana, Picea glauca, Pinus ponderosa; 11 = Picea glauca, Pinus ponderosa; 12 = Betula papyrifera, Picea glauca, Pinus ponderosa, Populus tremuloides; 13 = Physocarpus monogynous (Torr.) Coult., Pinus ponderosa; 14 = Pinus ponderosa; 15 = Pinus ponderosa, Populus tremuloides; 16 = Pinus ponderosa, Quercus macrocarpa; 17 = prairie; 18 = Populus tremuloides; 19 = urban. * = new record for the Black Hills.

<table>
<thead>
<tr>
<th>Orders and Species</th>
<th>Site Name</th>
<th>Co. Abb.</th>
<th>Lat./Long.</th>
<th>Habitat No.</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CERATIOMYXALES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Ceratiomyxa fruticulosa</td>
<td>Botany Bay</td>
<td>LA</td>
<td>44 25.200, 103 52.860</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>(Mull.) Macbr.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Ceratiomyxa fruticulosa</td>
<td>Rochford USFS</td>
<td>PE</td>
<td>44 7.062, 103 44.022</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Macbr.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LICEALES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Cribraria macrocarpa</td>
<td>Botany Bay</td>
<td>LA</td>
<td>44 25.200, 103 52.860</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Schrad.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* C. purpurea Schrad.</td>
<td>Botany Bay</td>
<td>LA</td>
<td>44 25.200, 103 52.860</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Dictydium cancellatum</td>
<td>Mirror Lake</td>
<td>LA</td>
<td>44 33.534, 104 0.582</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>(Batsch) Macbr.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lycogala epidendrum (L.) Fr.</td>
<td>Grigg's Gulch</td>
<td>LA</td>
<td>44 27.498, 103 57.042</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Alabaugh Canyon</td>
<td>Spearfish Canyon</td>
<td>LA</td>
<td>44 22.788, 103 55.254</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>FA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lycogala epidendrum (L.) Fr.</td>
<td>Big Hill</td>
<td>LA</td>
<td>44 24.528, 103 56.460</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>LA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lycogala epidendrum (L.) Fr.</td>
<td>Black Fox</td>
<td>LA</td>
<td>44 8.832, 103 50.622</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Botany Bay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burno Gulch</td>
<td></td>
<td>LA</td>
<td>44 25.392, 103 50.424</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>CR</td>
<td>Cranberry Spr. 2</td>
<td>CR</td>
<td>44 25.164, 104 8.892</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Cranberry Spr. 1</td>
<td></td>
<td>CR</td>
<td>44 25.164, 104 8.892</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Orders and Species</td>
<td>Site Name</td>
<td>Co. Abb.</td>
<td>Lat./ Long.</td>
<td>Habitat No.</td>
<td>Total No.</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------------------</td>
<td>----------</td>
<td>-----------------------------</td>
<td>-------------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td>Eleventh Hr. Gulch</td>
<td>LA</td>
<td>44 24.528, 103 56.460</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Grigg’s Gulch</td>
<td>LA</td>
<td>44 27.498, 103 57.042</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Horsethief Lake</td>
<td>PE</td>
<td>43 55.388, 103 29.220</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Moon</td>
<td>PE</td>
<td>43 59.460, 103 59.172</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Redbird Canyon</td>
<td>PE</td>
<td>43 47.041, 104 00.985</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Rochford USFS</td>
<td>PE</td>
<td>44 7.062, 103 44.022</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Cave Hills</td>
<td>HA</td>
<td>45 44.640, 103 32.400</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Swede Gulch</td>
<td>LA</td>
<td>44 10.632, 103 48.198</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Englewood Spring</td>
<td>LA</td>
<td>44 16.656, 103 47.016</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pony Gulch</td>
<td>PE</td>
<td>44 04.942, 103 38.845</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Rochford Cem.</td>
<td>PE</td>
<td>44 6.702, 103 44.832</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Puckeroo Spring</td>
<td>HA</td>
<td>45 33.120, 103 10.200</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Rochford USFS</td>
<td>PE</td>
<td>44 7.062, 103 44.022</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Spearfish</td>
<td>LA</td>
<td>44 29.832, 103 52.266</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Botany Bay</td>
<td>LA</td>
<td>44 25.200, 103 52.860</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Grigg’s Gulch</td>
<td>LA</td>
<td>44 27.498, 103 57.042</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Rochford USFS</td>
<td>PE</td>
<td>44 7.062, 103 44.022</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>* Reticularia lycoperdon Bull.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* R. olivacea (Ehrenb.) Fr.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* R. splendens Morgan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Tubifera ferruginosa (Batsch) J. F. Gmel.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHYSARALEs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Badhamia gracilis (Macbr.) Macbr.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Diderma crustaceum Peck</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* D. effusum (Schwein.) Morgan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* D. squamulosum (Alb. &amp; Schwein.) Fr.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- Asterisks indicate species that are new to science for South Dakota.
- Co. Abb. refers to the county abbreviation.
- Lat./Long. represent the latitude and longitude coordinates.
- Habitat No. and Total No. columns indicate the number of sites and total occurrences, respectively.
<table>
<thead>
<tr>
<th>Orders and Species</th>
<th>Site Name</th>
<th>Co. Abb.</th>
<th>Lat./Long.</th>
<th>Habitat No.</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Fuligo intermedia T. Macbr.</td>
<td>Rochford USFS</td>
<td>PE</td>
<td>44 7.062, 103 44.022</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>* F. megaspora Sturgis</td>
<td>Alabaugh Canyon</td>
<td>FA</td>
<td>43 20.436, 103 32.964</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Boles Canyon</td>
<td>CU</td>
<td>43 48.192, 104 1.566</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Boles Canyon</td>
<td>PE</td>
<td>43 51.660, 104 1.566</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Redbird Canyon</td>
<td>CU</td>
<td>43 47.108, 104 00.745</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Roby Canyon</td>
<td>CU</td>
<td>42 44.724, 104 1.566</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>* Fuligo septica (L.) F. H. Wigg</td>
<td>Alabaugh Canyon</td>
<td>FA</td>
<td>43 20.436, 103 32.964</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Big Hill - C</td>
<td>LA</td>
<td>44 24.96, 103 53.436</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Black Fox</td>
<td>LA</td>
<td>44 8.832, 103 50.622</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Boles Canyon</td>
<td>CU</td>
<td>43 48.624, 104 2.766</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Botany Bay</td>
<td>LA</td>
<td>44 25.200, 103 52.860</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Boulder Hill</td>
<td>PE</td>
<td>43 57.714, 103 23.898</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Craven Canyon</td>
<td>FA</td>
<td>43 23.034, 103 46.866</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>East Roubaix</td>
<td>LA</td>
<td>44 16.656, 103 36.186</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Eleventh Hr. Gulch</td>
<td>LA</td>
<td>44 24.528, 103 56.460</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Galena</td>
<td>LA</td>
<td>44 20.136, 103 38.592</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Gunderson Ranch</td>
<td>HA</td>
<td>45 52.210, 103 46.136</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>McKenna Ranch</td>
<td>PR</td>
<td>45 09.349, 102 51.007</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Redbird Canyon</td>
<td>CU</td>
<td>43 47.108, 104 99.745</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Rochford USFS</td>
<td>PE</td>
<td>44 7.062, 103 44.022</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>So. Dak. G, F &amp; P</td>
<td>LA</td>
<td>44 27.412, 103 49.410</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Zierer Ranch</td>
<td>ME</td>
<td>44 28.538, 103 05.558</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Orders and Species</td>
<td>Site Name</td>
<td>Co. Abb.</td>
<td>Lat./Long.</td>
<td>Habitat No.</td>
<td>Total No.</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-------------------------</td>
<td>----------</td>
<td>-------------------------</td>
<td>-------------</td>
<td>-----------</td>
</tr>
<tr>
<td><em>Mucilago crustacea</em> Wiggers</td>
<td>Alabaugh Canyon</td>
<td>FA</td>
<td>43 20.436, 103 32.964</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Boles Canyon</td>
<td>CU</td>
<td>43 48.624, 104 2.766</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Canyon City</td>
<td>LA</td>
<td>44 50.052, 103 36.234</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Eleventh Hr. Gulch</td>
<td>LA</td>
<td>44 24.528, 103 56.460</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>No. Slim Butte</td>
<td>HA</td>
<td>45 32.314, 103 01.893</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Picnic Spring</td>
<td>HA</td>
<td>45 52.442, 103 29.272</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td><em>Physarum bitectum</em> G. Lister</td>
<td>Big Hill - A</td>
<td>LA</td>
<td>44 24.528, 103 56.460</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Big Hill - C</td>
<td>LA</td>
<td>44 23.658, 103 55.254</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td><strong>STEMONITALES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comatricha nigra (Pers.) Schroet.</td>
<td>Grigg’s Gulch</td>
<td>LA</td>
<td>44 27.498, 103 57.042</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><em>Enerthenema berkeleyanum</em> Rostaf.</td>
<td>Grigg’s Gulch</td>
<td>LA</td>
<td>44 27.498, 103 57.042</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Stemonitis axifera (Bull.) Macbr.</td>
<td>Brownsville</td>
<td>LA</td>
<td>44 16.518, 103 41.664</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Silver City</td>
<td>PE</td>
<td>44 4.452, 103 35.148</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>S. fusca Roth</td>
<td>Botany Bay</td>
<td>LA</td>
<td>44 25.200, 103 52.860</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Cranberry Spr. 1</td>
<td>CR</td>
<td>44 25.164, 104 8.892</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Rochford USFS</td>
<td>PE</td>
<td>44 7.062, 103 44.022</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td><em>S. herbatica</em> Peck</td>
<td>Warren Peak</td>
<td>CR</td>
<td>44 28.614, 104 27.294</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>S. splendens Rost.</td>
<td>Spearfish</td>
<td>LA</td>
<td>44 22.788, 103 51.564</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td><strong>TRICHIALES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Arcyria cinerea</em> (Bull.) Pers.</td>
<td>Grigg’s Gulch</td>
<td>LA</td>
<td>44 27.498, 103 57.042</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><em>A. denudata</em> (L.) Wettst.</td>
<td>Botany Bay</td>
<td>LA</td>
<td>44 25.200, 103 52.860</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>A. mutans (Bull.) Grev.</td>
<td>Botany Bay</td>
<td>LA</td>
<td>44 25.200, 103 52.860</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>
2008 that were not present in 2007, and 12 species occurred in 2009 that were
not present in 2007 or 2008 (Table 3).

A total of 657 collections representing 65 species and 20 genera gave a species
to genus ratio of 3.3. More than 1 species occurred from several of the 648 moist
chambers (Table 3). Species are listed, sites where they occurred, the years they
were present and the substrata utilized by each. Only 5 species (8% of all spe-
cies) occurred at a relative abundance above 5.0%. These were: Arcyria cinerea
(13%), Comatricha nigra (13%), Ceratiomyxa fruticulosa (6%), Perichaena corticalis
(6%), and Perichaena minor var. pardina (6%). These 5 species accounted
for 43% of the collections. Six species (9% of all species) were classified as com-
mon (3-5%). These species were: Comatricha pulchella, Cribraria microcarpa,
C. minutissima, Echinostelium minutum, Enerthenema papillatum, and Perichaena
vermicularis. The 6 species (9% of species) listed as occasional (1.5-2.9%) were:
Dictydium cancellatum, Diderma hemisphaericum, Didymium dubium, Licea
minima, Licea variabilis, and Perichaena chrysosperma. The 13 species (20%
of species) that were classified as uncommon (0.5-1.4%) were: Arcyria carnea,
A. nutans, Comatricha laxa, C. subcaespitosa, Cribraria atrofusca, C. lepida, Di-
dymium clavus, D. difforme, Lamproderma arcyronema, L. scintillans, Physarum
auriscalpium, P. nutans, and Perichaena pedata. The majority of species occurred
less than 5 times (< 0.5%) and were classified as rare. Twenty species occurred
only once during the 3-year study. Species listed as abundant that occurred at 6
sites all 3 years were: Arcyria cinerea, Ceratiomyxa fruticulosa, Comatricha nigra,
C. pulchella, Cribraria minutissima, Echinostelium minutum, Enerthenema papil-
latum, and Perichaena corticalis. Perichaena minor var. pardina and P. vermicularis
occurred at 5 sites all 3 years.

Of the 108 moist chambers prepared for each site, 179 collections occurred from
Boles/Redbird Canyon substrata representing 39 species. Substrata from
Spring Creek had the second highest number of collections of 143, representing 37 species. Eighty-two collections representing 26 species, 104 collections representing 29 species and 75 collections representing 23 species occurred on substrata from Botany Bay, Pony Gulch, and Alabaugh, respectively. Only 74 collections and 19 species occurred on substrata from Grigg’s Gulch. Botany Bay had the highest number of species/collection of 0.32. Alabaugh Canyon, the burned site had a species/collection of 0.31, Pony Gulch 0.28, Spring Creek 0.26, Grigg’s Gulch 0.26 and Boles/Redbird Canyons 0.22.

Table 1 describes the 6 sites and a cluster analysis (Fig.2) indicates the relation-
ship among them based on the species of slime molds collected. Boles/Red-

<table>
<thead>
<tr>
<th>Order and Species</th>
<th>Sites</th>
<th>Years</th>
<th>Substrates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AL</td>
<td>BB</td>
<td>BR</td>
<td>GG</td>
</tr>
<tr>
<td>CERATIOMYXALES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Ceratiomyxa fruticulosa (Mull.) Macbr.</td>
<td>1</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>ECHINOSTELIALES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Echinostelium minutum de Bary</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>LICEALES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Cribraria atrofusca G. W. Martin &amp; Lovejoy</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>* C. cancellata var. fusca (List. Nann.-Bremek)</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. elegans Berk. &amp; M. A. Curtis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>* C. leptida Meylan</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>* C. macrocarpa Schrad.</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>* C. microcarpa (Schrad.) Pers.</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>C. minutissima Schwein.</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>*C. splendens (Schrad.) Pers.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>* C. violacea Rex</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dicydium cancellatum (Batsch) Macbr.</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>*Licea kleistobolus G. W. Martin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>* L. minima Fr.</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>L. variabilis Schrad.</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>PHYSARALES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Badhamia gracilis (Macbr.) Macbr.</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>* Diderma hemisphaerium (Bull.) Hornem.</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>* Didymium anellus Morgan</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>* D. clavus (Alb. &amp; Schwein.) Rabenh.</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>* D. difforme (Pers.) Gray</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Order and Species</td>
<td>Sites</td>
<td>Years</td>
<td>Substrates</td>
<td>Total</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-------</td>
<td>-------</td>
<td>------------</td>
<td>-------</td>
</tr>
<tr>
<td>* D. dubium Rostaf.</td>
<td>AL 8</td>
<td>BB 0</td>
<td>BR 8</td>
<td>GG 0</td>
</tr>
<tr>
<td>* D. ochroidesum G. Lister</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 1</td>
</tr>
<tr>
<td>* D. quitense (Pat.) Torrend</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>* D. serpula Fr.</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>* D. squamulosum (Alb. &amp; Schwein.) Fr.</td>
<td>AL 3</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>* Physarum auriscalpium Cooke</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>* P. bivalve Pers.</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>P. cinereum (Batsch) Pers.</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>P. compressum Alb. &amp; Schwein.</td>
<td>AL 1</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>* P. didermoides (Pers.) Rostaf.</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>* P. globuliferum (Bull.) Pers.</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>* P. leucophaeum Fr.</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>P. nutans Pers.</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>* P. pusillum (Berk. &amp; M. A. Curtis) G. Lister</td>
<td>AL 1</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>* P. viridae (Bull.) Pers.</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>STEMONITAELES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Comatricha laxa Rostaf.</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 2</td>
<td>GG 0</td>
</tr>
<tr>
<td>C. nigra (Pers. ex J. F. Gmel.) J. Schrot</td>
<td>AL 14</td>
<td>BB 5</td>
<td>BR 26</td>
<td>GG 10</td>
</tr>
<tr>
<td>* C. pulchella (C. Bab.) Rostaf.</td>
<td>AL 2</td>
<td>BB 1</td>
<td>BR 7</td>
<td>GG 4</td>
</tr>
<tr>
<td>* Comatricha subcaespitosa Peck</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>Comatricha typhoides (Bull.) Rost.</td>
<td>AL 1</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>* Enerthenema papillatum (Pers.) Rostaf.</td>
<td>AL 4</td>
<td>BB 1</td>
<td>BR 2</td>
<td>GG 3</td>
</tr>
<tr>
<td>* Lamproderma arcyriofonema Rost.</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>* L. scintillans (Berk. &amp; Broome) Morgan</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 2</td>
<td>GG 2</td>
</tr>
<tr>
<td>Stemonitis axifera (Bull.) T. Macbr.</td>
<td>AL 0</td>
<td>BB 0</td>
<td>BR 0</td>
<td>GG 0</td>
</tr>
<tr>
<td>Order and Species</td>
<td>Sites</td>
<td>Years</td>
<td>Substrates</td>
<td>Total</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------</td>
<td>-------</td>
<td>------------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>AL</td>
<td>BB</td>
<td>BR</td>
<td>GG</td>
</tr>
<tr>
<td>S. fusca Roth</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>* S. nigrescens Rex</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TRICHIALES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Arcyria carnea (G. Lister) G. Lister</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>* A. cinerea (Bull.) Pers.</td>
<td>3</td>
<td>14</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>* A. denudata (L.) Wettst.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>A. incarnata (Pers. ex J. F. Gmel.) Pers.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>* A. insignis Kalchbr. &amp; Cooke</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. nutans (Bull.) Grev.</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>* A. occidentalis (T. Macbr.) G. Lister</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>* Dianema cf. corticatum Lister</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>* Hemitrichia abietina (Wigand) G. Lister</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>* Metatrichia vesparium (Batsch) Nann.-Bremek.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>* Perichaena chrysosperma (Curr.) Lister</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>P. corticalis (Batsch) Rostaf.</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>* P. depressa Lib.</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>* Perichaena minor var. parodina (G. Lister) Minak.</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>* P. pedata (Lister &amp; G. Lister) Lister ex E. Jahn</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>* P. vermicularis (Schwein.) Rostaf.</td>
<td>2</td>
<td>0</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>* Trichia botrytis (J. F. Gmel.) Pers.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T. contorta (Ditmar) Rostaf.</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>* T. varia (Pers. ex J. F. Gmel.) Pers.</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total Number of Collections</td>
<td>75</td>
<td>82</td>
<td>179</td>
<td>74</td>
</tr>
</tbody>
</table>
bird Canyons and Alabaugh Canyon have similar vegetation, canopy cover and precipitation. However, a forest fire in July 2007, removed nearly all of the litter composition at Alabaugh Canyon. The cluster analysis using the summarized data indicates different species were collected and Alabaugh Canyon is significantly different from all other sites. Botany Bay and Grigg’s Gulch have similar vegetation, canopy cover and altitude and precipitation and similar species of slime molds, clustering close together. Pony Gulch and Spring Creek, the central hills sites are not as similar as Botany Bay and Grigg’s Gulch and the cluster analysis indicates this dissimilarity.

Four hundred eighty-three collections (74%) representing 54 species (83%) occurred on wood. The following species, all rated as abundant or common, occurred exclusively or most frequently on wood: *Arcyria cinerea*, *Ceratiomyxa fruticulosa*, *Comatricha nigra*, *C. pulchella*, *Cribaria microcarpa*, *C. minutissima*, *Echinostelium minutum*, *Enerthenema papillatum*, and *Perichaena corticalis*. One hundred fifteen collections (18%) representing 29 species (45%) occurred on leaf litter and *Perichaena vermicularis*, rated as abundant, occurred most frequently on leaf litter. Fifty-nine collections (9%) representing 10 species (15%) occurred on grass litter. *Perichaena minor* var. *pardina*, rated as abundant, occurred most frequently on grass litter.

All 38 collections of *Ceratiomyxa fruticulosa* and 107 collections of 13 species in the Liceales occurred on wood. Fifty-two percent of *Echinostelium minutum* collections occurred on wood and the remainder occurred on leaf litter (19%).

![Figure 2. A cluster analysis comparing collection sites for the moist chamber study using slime mold species as variables. AL = Alabaugh Canyon, BR = Boles/Redbird Canyons, SC = Spring Creek, PG = Pony Gulch, BB = Botany Bay and GG = Grigg’s Gulch.](image-url)
and grass litter (29%). Ninety-six percent of the 171 collections (11 species) in the Stemonitales occurred on wood with only 4% on leaf litter and none utilizing grass litter as a substratum. The order Trichiales contained 226 collections (19 species) of which 59% occurred on wood, 24% on leaf litter and 17% on grass litter. Only the 84 collections in the Physarales representing 20 species had a majority of 57% occurring on leaf litter, 30% occurring on wood and 13% on grass litter (Table 3).

Data did not show seasonality for numbers of collections. In 2007, 30%, 40% and 29% of collections occurred in June, July and August. Thirty-six percent, 32% and 32% of collections occurred in June, July and August in 2008. In 2009, 26%, 34% and 40% of collections occurred in June, July and August, respectively. We considered numbers of species less than abundant as too infrequent to make any conclusions regarding their seasonality. Only three of the species that ranked as abundant, *Arcyria cinerea*, *Ceratiomyxa fruticulosa*, and *Comatricha nigra*, occurred each month of each year of the study. The remaining two abundant species, *Perichaena corticais* and *P. minor* var. *pardina* occurred in June, July and August during the survey but not each year (Table 3).

Tabulating time of development of fruiting bodies in moist chambers for 2008 and 2009 showed that 56% and 30% of species occurred after the first week, and 86% and 74% occurred by the third week reading, respectively. Fruiting bodies increased at the 4-week observation period to 90% and 87% in 2008 and 2009. *Didymium ochroideum*, *D. quitense* and *Physarum globuliferum* were observed after 6 weeks in 2008, and *D. ochroideum*, *D. squamulosum* and *P. bivalve* were observed after 8 weeks in 2009.

The devastating fire at the Alabaugh Canyon site after the June and July 2007 collections provided a unique opportunity to follow post-burn colonization of slime molds. Two collections were conducted prior to the fire and 7 after the fire. Table 4 lists pre- and post burn species. Three species, *Arcyria cinerea*, *Comatricha nigra* and *Perichaena minor* var. *pardina*, were collected before and after the burn, and *P. minor* var. *pardina* on grass litter was identified from the August 2007 collection, 1 month after the burn. Thirteen species were collected prior to the burn and 14 after the burn. Of the 75 collections from Alabaugh Canyon, 37 (49%) representing 9 species (39%) were in the Physarales. Three and 7 species occurred pre and post burn, respectively (Tables 3 & 4).

**DISCUSSION**

The University of Arkansas Eumycetozoa Research Project lists 12 species from the Black Hills (Custer County) and 39 species that were collected throughout South Dakota prior to this study. After nomenclatural updates and removing duplication between Macbride’s (1898, 1922) species and species at the National Fungal Collection in Beltsville, Maryland, and between the field and moist chamber current collections, we list 62 species as new records for the Black Hills. All species in the present study were collected in South Dakota, except for 1 which was only collected in the Black Hills of Wyoming (Crook County).
Since the Black Hills is a unique ecoregion (The Nature Conservancy 1999, Froiland 1990) with flora from the Great Plains, eastern deciduous forest, southwestern United States, Rocky Mountains and boreal forest, it is difficult to compare collections in this survey with other surveys. However, many of the species collected in this study are similar to those reported from the eastern deciduous forest (Stephenson 1988, 1989 and Stephenson et al. 2001). Species from the southern and southwestern sites in this study are similar to many collections from temperate grasslands (Ing 1994). Rollins (2007) listed *Badhamia gracilis* and *Didymium difforme*, 2 species collected in this study also, as occurring in the Thunder Basin Grasslands in Wyoming. Blackwell and Gilbertson (1980, 1984) reported species from the Sonoran Desert, several of which were collected in this study. Sixty-four species reported from the Chisos Mountains in Big Bend

---

**Table 4. List of pre- and post-burn species and dates collected at Alabaugh Canyon for 2007-2009.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Pre burn</th>
<th>Post burn</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arcyria cinerea</em></td>
<td>6-07, 7-07</td>
<td>7-09</td>
</tr>
<tr>
<td><em>Arcyria nutans</em></td>
<td>7-07</td>
<td></td>
</tr>
<tr>
<td><em>Badhamia gracilis</em></td>
<td>6-09, 7-09, 8-09</td>
<td></td>
</tr>
<tr>
<td><em>Ceratiomyxa fruticulosa</em></td>
<td>6-07</td>
<td></td>
</tr>
<tr>
<td><em>Comatricha nigra</em></td>
<td>6-07, 7-07</td>
<td>7-09, 8-09</td>
</tr>
<tr>
<td><em>Comatricha pulchella</em></td>
<td>7-07</td>
<td></td>
</tr>
<tr>
<td><em>Cribraria minutissima</em></td>
<td>6-07</td>
<td></td>
</tr>
<tr>
<td><em>Cribraria violacea</em></td>
<td>7-07</td>
<td></td>
</tr>
<tr>
<td><em>Didymium clavus</em></td>
<td>8-08</td>
<td></td>
</tr>
<tr>
<td><em>Didymium difforme</em></td>
<td>6-08, 6-09, 8-09</td>
<td></td>
</tr>
<tr>
<td><em>Didymium dubium</em></td>
<td>7-09, 8-09</td>
<td></td>
</tr>
<tr>
<td><em>Didymium squamulosum</em></td>
<td>6-09, 7-09</td>
<td></td>
</tr>
<tr>
<td><em>Echinostelium minutum</em></td>
<td>8-09</td>
<td></td>
</tr>
<tr>
<td><em>Euterthenema papillatum</em></td>
<td>6-07, 7-07</td>
<td></td>
</tr>
<tr>
<td><em>Hemitrichia abietina</em></td>
<td>6-07</td>
<td></td>
</tr>
<tr>
<td><em>Perichaena corticalis</em></td>
<td></td>
<td>7-09</td>
</tr>
<tr>
<td><em>Perichaena depressa</em></td>
<td></td>
<td>8-09</td>
</tr>
<tr>
<td><em>Perichaena minor var. parodina</em></td>
<td>7-07</td>
<td>8-07</td>
</tr>
<tr>
<td><em>Perichaena vermicularis</em></td>
<td></td>
<td>8-09</td>
</tr>
<tr>
<td><em>Physarum cinereum</em></td>
<td>7-07</td>
<td>8-08</td>
</tr>
<tr>
<td><em>Physarum nutans</em></td>
<td></td>
<td>8-09</td>
</tr>
<tr>
<td><em>Physarum pusillum</em></td>
<td></td>
<td>7-07</td>
</tr>
</tbody>
</table>
National Park (Tiffany and Knaphus 2001) included 35 species collected in this study. Morris (1954) collected in the central and western mountains of Wyoming and not in the eastern grasslands. However the present survey includes 25 of the 54 species listed in that publication. Of the 40 species reported by Sturgis (1913) from central and western Colorado, 18 were collected in this study. Collections in the Great Plains from Kansas included 3 of 15 species reported by Kramer (1956) and 18 of the 30 species reported by Schaffner (1904). Seaver (1908) reported 16 species from North Dakota of which 10 were collected in the present study. Many of the same species from the literature listed above occur in several ecoregions.

The small increase in numbers of collections and species from 2007 to 2008 and from 2008 to 2009 indicates the survey may have reached the optimum point on a species accumulation curve. However, of the 35 species collected in 2007, 18 were different in 2008 and 12 were different in 2009 from either previous year. The longer observation periods in 2008 and 2009 may account for the different species. Some species on substrata collected in 2008 and 2009 were different from those collected in 2007. The species/genus ratio of 3.3 in this study compares with the overall ratio of 3.6 in a study of biodiversity of the Colorado Plateau, which includes some vegetation similar to that in this study (Novozhilov et al. 2003). In the present study, 5 species accounted for 43% of the collections, which compares with a study of myxomycetes in western Kazakhstan, in which 3 species accounted for 52% of all records (Schnittler 2001).

Data showing higher numbers of collections and species at the Boles Canyon/Redbird Canyon site did not support our hypothesis that wetter, northern hills sites with greater plant species diversity/hectare and higher canopy cover would be the most productive. Even after the fire at Alabaugh Canyon, the number of species there were higher than at Grigg’s Gulch. Northern, wetter sites (Botany Bay with 81 collections representing 25 species and Grigg’s Gulch with 74 collections representing 19 species) with higher canopy cover were less productive than predicted (Table 1). Stephenson et al. (2004) listed a decrease in myxomycetes with an increase in rainfall in an Ecuadoran cloud forest and considered that the constant high humidity promoted colonization by myxomyceticous fungi. Tran et al. (2008) reported much lower percentages of myxomycetes in moist chambers during the rainy season in a Thailand study. Keller et al. (2009) pointed out that open habitats such as the prairie/woodlands in this study could allow for better dispersal of spores.

Data supported our hypothesis that wood would support more collections and species. We did not dry and weigh substrates, but observed approximately the same surface areas of wood, leaves and grass. However, the higher moisture-holding capability of wood may explain why it supported better survival of plasmodia and development of fruiting bodies. Species collected from wood in this study were listed on wood by Martin and Alexopoulos (1969). Wood was the most common substrate in a survey in a temperate forest in southwestern Virginia (Stephenson 1988). Rotting wood was listed by Spiegel et al. (2004) as a good substratum for slime molds. All substrates for the species collected in this study agree with those listed in Martin and Alexopoulos (1969).
Fuligo megaspora was collected only at Alabaugh Canyon, the Boles/Redbird Canyons and extreme southwestern Pennington County. These sites are all dry, open prairie/woodlands. It was frequently collected under Juniperus virginiana at these sites. Keller and Schoknecht (1989) reported Fuligo megaspora near Colorado Springs on bark of dead gymnosperms, from northwestern Nebraska on decaying leaves, on hot desert sands, and on trunks of Juniperus monosperma (Engelm.) Sarg. in New Mexico. Tiffany and Knaphus (2001) reported it as the most commonly collected species in Big Bend National Park and noted that Macbride (1922) listed it from Colorado.

Badhamia gracilis was collected almost exclusively at the dry, prairie southern sites on stems of Opuntia polyacantha Haw. and Yucca glauca Nutt. Martin and Alexopoulos (1969) listed collections from Nebraska, Colorado, Texas and New Mexico, and included these hosts as substrata. Blackwell and Gilbertson (1980, 1984) included collections from cacti in Arizona. Ing (1994) mentioned that stems of cacti and succulent leaves of agaves as major substrata in desert habitats.

Ronikier and Ronikier (2009) evaluated the literature of slime molds near snow-melt and determined that the term nivicolous is more appropriate than alpine when describing these slime molds because they occur in montane, subalpine and alpine habitats. In this study Diderma crustaceum and Physarum bitectum were collected early in the season next to melting snow in montane habitats.

Stemonitis was the only genus that was commonly collected in the field and in moist chambers. The aethalioid and pseudoaethalioid species, Fuligo intermedia, F. septica, F. megaspora, Lycogala epidendrum, Mucilago crustacea, Reticularia olivacea, R. splendens, and Tubifera ferruginosa, were collected only in the field. It is presumed that plasmodia for these species are massive and collection of small portions of downed wood, deciduous leaf litter or grass litter disrupted the plasmodia interrupting the formation of fruiting bodies. The high number of collections of these large and easily seen fruiting bodies is an artifact of collecting.

A few species collected in this study are rare or uncommon. The single collection of Enerthenema berkelyanum is listed by Macbride and Martin (1934) as rare, and by Farr (1981) and Martin and Alexopoulos (1969) as uncommon. Although there were several collections of Licea variabilis in this study, and Macbride (1898) collected it, the 1922 Macbride and 1934 Macbride and Martin publications listed it as rare. Nannenga-Bremekamp (1991) listed Perichaena pedata as rare. Uncommon species and the number of new records from this study indicate more work needs to be done in the Black Hills to achieve a reasonable documentation of the species of Myxomycetes present.

ACKNOWLEDGEMENTS

This manuscript is dedicated to Elaine Ebbert and Linda Zierer. Elaine worked tirelessly and faithfully in the laboratory for a great portion of two summers identifying specimens and preparing collections. She was an inspiration to us. Linda was able to work a short time on the project, but her enthusiasm
and contributions of collections are memorable. A. Gabel and M. Gabel wish to thank Black Hills State University for use of the facilities and for a Faculty Research Grant, which helped support student assistance. We appreciate the valuable comments and suggestions of our reviewers, Harold W. Keller and Maren Klich that improved this paper. The assistance of the staff at the National Fungal Collection in Beltsville, Maryland was very helpful in locating T. H. Macbride's Black Hills collections.

LITERATURE CITED

Alexopoulos, C. J. 1965. First list of myxomycetes from Texas, with notes on some rarely reported species. Southwestern Naturalist 10(4): 221-226.
Frederick, L. 1990. Phylum plasmodial slime molds class Myxomycota. p. 467-483 In L. Margulis et al. (eds.) Handbook of Protoctista. Jones and Barlett, Boston, MA.


University of Arkansas Eumycetozoan Research Project; (14 April 2010). Available at http://slimemold.urk.edu/
EARLY VASCULAR PLANT COLLECTIONS FROM EASTERN WYOMING AND WESTERN SOUTH DAKOTA FROM 1800-1940

M. Gabel,¹ D. Ode,² G. Kostel,¹ and C. Card³

¹Herbarium, Black Hills State University
Spearfish, SD 57799

²Department of Game, Fish and Parks, S.D. Natural Heritage Program
Pierre, SD 57501

³Department of Mathematics, Black Hills State University
Spearfish, SD 57799

*mark.gabel@bhsu.edu

ABSTRACT

The earliest scientific plant collections in the study area were probably made by members of the 1804-1806 Lewis and Clark Expedition. They were followed in 1811 by Nuttall and Bradbury in an expedition of “Overland Astorians.” Geyer and Hayden collected in the next decades. In the 1870s Donaldson and Jenney briefly explored the Black Hills. The first expedition dedicated to scientific collection of plants was accomplished by Rydberg in the 1890s who was said to have identified 700 species from the Black Hills. Hayward from Milwaukee collected over 2000 specimens in 1926 and 1927. Eventually, South Dakota collectors including Visher, McIntosh, Over and Bennett (1908-1940) added greatly to our collections and to knowledge of the flora of the region. Information on numbers and disposition of collections was obtained from a database of vascular plant collections from the area. Collections were sporadic at best prior to 1890. During the time period represented by this study, the decade of the 1920s was the time of the largest number of collections (>8000) with the major contributions by Hayward, McIntosh and Over.

Keywords

botany, plant specimens, South Dakota, Wyoming, historical collections

INTRODUCTION

In the past, knowledge of historical collections and collectors from any particular region was largely housed in the memories of old botanists. While some literature reports are available for specific collectors or collections, there has not been a reasonable way to enumerate historical collections in the study area. The advent of regional databases has made it possible to quantify dates, numbers of species and specimens, and distributions. It is the purpose of this paper to list
METHODS

The study area covered by this presentation includes all counties in South Dakota west of the Missouri River and Crook and Weston Counties in eastern Wyoming. Included are major plant collections made in the study area between the years 1800 and 1940.

This manuscript is organized by placing the early collectors into three categories. The first includes collectors who journeyed and collected specimens on or adjacent to the Missouri River. The second category includes collectors who traveled well beyond the Missouri River but were in South Dakota or Wyoming only seasonally. The third group includes people who were residents of South Dakota or Wyoming and collected and housed their collections in regional herbaria. Herbarium acronyms (Table 1) used in this report were taken from Thiers (2010). Data for most collections/collectors were extracted from a consolidated database (available at http://herbarium.bhsu.edu/) including data for the study area from 16 herbaria.

Collections enumerated in this manuscript were chosen because of historical importance, the size of the collection, or because of the novel nature of the collector and/or collection. A more complete list of collections/collectors is provided in Table 2.

RESULTS

Collections Along the Missouri River—The first scientific collections of vascular plants in our study area were probably made by members of the Lewis and Clark Expedition (1804-1806). Reveal et al. (1999) noted that of 232 known specimens from the pioneering journey, 226 are housed at PH. Others are housed at K or GH. Many of the Lewis and Clark collections were taken to England for study by Pursh, who wrote the landmark work *Flora Americae Septentrionalis* (Pursh 1813). Many of these were later purchased and returned to Philadelphia (Reveal et al. 1999).

Thomas Nuttall was an Englishman who worked briefly as a printer in St. Louis. He went up the Missouri River in the spring of 1811 with the “Overland Astorians” who were headed to Oregon for the Pacific Fur Company, a subsidiary of the American Fur Company, and sponsored by John Jacob Astor. The expedition was led by Wilson Price Hunt. Nuttall apparently did not keep a journal and the exact route taken for his plant collections is unclear, although he apparently did not venture long distances from the Missouri River. Nuttall traveled to the “Arikara villages in northern South Dakota and then, in company with the fur trader Manuel Lisa proceeded to the country of the Mandan Indians in North Dakota” (McKelvey 1955 p. 142). Nuttall returned with his plants to St. Louis in October of 1811 and learned of the potential war between
England and the United States. To the distress of his employer, Benjamin Smith Barton, he went to New Orleans and sailed with his plants for England where he showed some of his collections to Pursh who was working on the Lewis and Clark collection at the time. Disagreements ensued which are beyond the scope of this paper, but Nuttall returned to the United States in 1814 and published the *Genera of North American Plants* in 1818.

On the same expedition was John Bradbury, another Englishman, who had some training as a botanist, became a fellow of the Linnean Society in 1792, and was corresponding secretary of the Liverpool Philosophical Society (McKelvey 1955). Unlike Nuttall, Bradbury did keep a journal of his travels on this expedition. From the Arikara villages Bradbury made a trip about 200 miles farther north with Ramsay Crooks to the Mandan villages. Upon returning to the main body of the Hunt expedition, still at the Arikara villages, Bradbury left the group on 17 July 1811 and returned to St. Louis. Bradbury apparently became ill in St. Louis and did not leave for New Orleans until December. He apparently was in transit December 15th –21st (1811) and experienced the series of severe earthquakes along the New Madrid fault. He later was delayed in New York during the war of 1812, however, his plants arrived in England. When he finally returned to England, he found that Pursh (1813) had already studied his plants (McKelvey 1955). Bradbury’s plant specimens are distributed in PH, K and LIV. Fortunately, during WWII approximately 250 Bradbury specimens were sent for storage to Wales, and consequently were saved from destruction of the museum in Liverpool. Bradbury is most noted for his travelogue (1817).

Karl A. Geyer was a German who came to the United States in 1835 and a few years later accompanied the Nicollet Expedition (1838 and 1839). The purpose of the expedition was to “undertake the full exploration of the Mississippi River from its mouth to its sources” (Nicollet 1843, p. 3). Nicollet paid personally for the services of Geyer to act as “practical botanist.” The 1838 expedition explored the Minnesota River and into eastern South Dakota, and in 1839, Nicollet and Geyer extended the expedition from St. Louis to Ft. Pierre. In Geyer’s words to Hooker “…we surveyed the Missouri as high up as the Little Missouri and almost the whole of that immense country …between the Missouri and Mississippi” (Geyer 1845). Geyer somehow lost about half of the collections on the return trip. Nicollet’s (1843) report of the trip included the catalog of plants identified by John Torrey in his Appendix B. Remaining collections from this expedition are housed primarily at NY and US.

**Collections Beyond the Missouri River**—Ferdinand Vandeveer Hayden was known primarily for his expertise in geology, especially in the Rocky Mountain west. He also served as a surgeon during the Civil War. Hayden was well-educated with degrees from Oberlin College in 1850 and Albany Medical College in 1853. In 1855, 1856 and 1857 Hayden accompanied G.K. Warren’s expeditions in the region between the Platte River and Missouri River and west to the Rocky Mountains. Hayden’s report (1875) stated that 1500 specimens of plants were collected and listed 593 species, mostly identified by George Engelmann, some by John Torrey, and some by Hayden himself. Collections of the Hayden specimens are at MO and NY.
Aris B. Donaldson was hired as one of the original faculty members of the University of Minnesota where he taught rhetoric and English literature. He apparently resigned his position to serve as a botanist on the G.A. Custer expedition to the Black Hills in 1874 (Krause and Olson 1974). On the expedition, he also served as a correspondent for the St. Paul Pioneer. There are numerous reports about the abundance of wildflowers (Krause and Olson 1974, Progulske and Shideler 1984), but for some reason Donaldson collected a limited number of specimens. Coulter (1874) published a list of 74 herbaceous species collected by Donaldson. The following year Winchell published a list of 22 trees and shrubs “seen in the Black Hills” (p. 67 Winchell 1875). A second list of 18 species of trees and shrubs (many duplicates of the first list) was the result of “a reconnaissance towards the Bad Lands, … noted along the lower slopes of the Black Hills in the valley of French Creek” (p. 68 Winchell 1875). The list of plant specimens identified by Coulter was republished (Winchell 1875) with some changes. Approximately 40 specimens of the Donaldson collection have been located at NY (Masson 1994). More recently 11 specimens have been located at PUL (Shaner and Harby 2008), leaving about 23 specimens of unknown location.

W. P. Jenney collected plants on the 1875 expedition to the Black Hills (Newton and Jenney 1880). The plants were identified by Asa Gray and a list including 175 species was published as a chapter (pp. 529-537) of the 1880 report. At least some of the plant specimens are at GH, including the type specimens of *Oxytropis monticola* A. Gray.

Per Axel Rydberg was born in Sweden and came to the United States in 1882. He studied botany at the University of Nebraska under the guidance of C. E. Bessey and obtained his B.S. in 1891. In 1892 he was employed by the U.S. Department of Agriculture to study the flora of the Black Hills. According to McIntosh (1931), Rydberg’s study of the Black Hills flora was the first with botanical exploration as its primary goal. In Rydberg’s *Flora of the Black Hills of South Dakota* (1896) he enumerated “a little over 700 Phaeogams (sic) and Fernworts” (p. 478). Many of Rydberg’s specimens are at NY, but there are also significant numbers at SDC and RM. Rydberg later became the first curator of the New York Botanical Garden Herbarium.

H. E. Hayward was a Wisconsin resident, but did extensive collections in the Black Hills in the summers of 1926 and 1927. He published his findings (Hayward 1928) that included 765 species. A complete collection of specimens (>2000 specimens) from this study is currently housed at the Milwaukee Public Museum.

**Resident Botanists**—Beginning in the early 20th Century botanists from South Dakota and Wyoming began to make significant collections in the study area. S. S. Visher worked for the South Dakota Geological Survey from 1910-1914. He conducted surveys of the northwestern portion of the study area using his motorcycle for collections of plants (Ode 2006). Visher is perhaps best known for his 1914 publication on the biology of Harding County. The largest collection of Visher’s plants is housed at SDU, with smaller collections at BHSC and RM.
A. C. McIntosh was a botanist at the South Dakota School of Mines. He collected in the Black Hills from 1924-1930. His collections of over 1000 specimens are primarily at SDU. His checklist of plants (McIntosh 1931) is supplemented by a brief history of collections in the Black Hills and descriptions of habitats in the Black Hills.

W. H. Over was a homesteader in Perkins County, SD and had strong interests in nature, fossils and archaeology. Over was largely self-taught but employed by the University of South Dakota as assistant curator in 1912. He later became curator of the museum there. He published the *Flora of South Dakota* in 1932. Most of his plant collections are at SDU, with smaller numbers at US, GH, BHSC and RM.

F. L. Bennett was curator of the Herbarium at BHSC from the 1920s into the 1940s, and an avid plant collector. He had numerous short publications on the flora of the region. The largest collection of his plants is housed at BHSC with additional specimens at SDU, SDC and RM.

DISCUSSION

Today with modern field tools such as GPS and readily available transportation it is difficult to envision many of the challenges faced by the early collectors. Existing collections are evidence of their efforts and dedication. Early collections provide us with rare windows on history and opportunities to observe changes over periods greater than one lifetime.

We embarked upon this study to complete an ongoing regional database of vascular plant specimens that includes the relatively unknown and less accessible specimens collected from 1800-1940. While it was relatively easy to locate and enter data from recent collections, we wanted to incorporate the older collections presented here to make the database more valuable and more versatile. The database indicates that the early collections from 1800 to 1940 reached an apex in the decade of the 1920s with >7500 collections, most of which were collected with the coincidence of Hayward, McIntosh, and Over. It was not until the decade of the 1970s that the number of collections exceeded the number of collections in the 1920s. We hope that this study and continued additions to the database will facilitate future work on the flora of the region, aspects of climate change, historical research, and practical applications by land managers.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the curators of collections for allowing us to use data from their collections. They are: Craig Beckner, Rachel Benton, Cissie Buckert, Beth Burkhart, Jack Butler, Megan Cherry, Tom Farrell, Craig Freeman, Ron Hartman, Gary Larson, Jill Larson, Dan Licht, Neil T. Luebke, Tim Mullican, Ernie Nelson, Molly Nepokroeff, Greg Shaner, Bob Tatina, Carl Taylor, Dan Uresk, Bruce Weismann and Katherine Zacharkevics. We are grateful to A. Gabel, G. Larson and D. Wolff for thoughtful reviews of this manuscript. We
also wish to thank the National Wildlife Foundation and the National Science Foundation (BRC #0545102) for their generous financial support.

LITERATURE CITED

Bradbury, J. 1817. Travels in the interior of America, in the years 1809, 1810, 1811; including a description of upper Louisiana, together with the states of Ohio, Kentucky, Indiana, and Tennessee, with the Illinois and western territories, and containing remarks and observations useful to persons emigrating to those countries. Sherwood, Neely and Jones, Liverpool.


<table>
<thead>
<tr>
<th>Herbarium Acronym</th>
<th>Herbarium Name</th>
<th>City</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHSC</td>
<td>Black Hills State University</td>
<td>Spearfish</td>
</tr>
<tr>
<td>BM</td>
<td>Natural History Museum</td>
<td>London</td>
</tr>
<tr>
<td>GH</td>
<td>Gray Herbarium (Harvard)</td>
<td>Cambridge</td>
</tr>
<tr>
<td>K</td>
<td>Royal Botanic Garden</td>
<td>Kew</td>
</tr>
<tr>
<td>LIV</td>
<td>World Museum</td>
<td>Liverpool</td>
</tr>
<tr>
<td>MO</td>
<td>Missouri Botanical Garden</td>
<td>St. Louis</td>
</tr>
<tr>
<td>MIL</td>
<td>Milwaukee Public Museum</td>
<td>Milwaukee</td>
</tr>
<tr>
<td>NEB</td>
<td>University of Nebraska State Museum</td>
<td>Lincoln</td>
</tr>
<tr>
<td>NY</td>
<td>New York Botanical Garden</td>
<td>Bronx</td>
</tr>
<tr>
<td>PH</td>
<td>Academy of Natural Sciences</td>
<td>Philadelphia</td>
</tr>
<tr>
<td>PUL</td>
<td>Purdue University</td>
<td>West Lafayette</td>
</tr>
<tr>
<td>RM</td>
<td>University of Wyoming</td>
<td>Laramie</td>
</tr>
<tr>
<td>SDC</td>
<td>South Dakota State University</td>
<td>Brookings</td>
</tr>
<tr>
<td>SDU</td>
<td>University of South Dakota</td>
<td>Vermillion</td>
</tr>
<tr>
<td>US</td>
<td>Smithsonian Institution</td>
<td>Washington, D.C.</td>
</tr>
</tbody>
</table>

Table 1. Herbarium acronyms, herbarium names and cities (from Thiers 2010).
Table 2. Collectors in chronological order by years of active collection in the study area. Categories are described in the text. Locations are herbaria where plants are located.

<table>
<thead>
<tr>
<th>Collector</th>
<th>Years</th>
<th>Category</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis, M. &amp; W. Clark</td>
<td>1804-1806</td>
<td>Missouri River</td>
<td>PH, BM, K, NY, GH, LIV</td>
</tr>
<tr>
<td>Nuttall, T.</td>
<td>1811</td>
<td>Missouri River</td>
<td>PH, BM</td>
</tr>
<tr>
<td>Bradbury, J.</td>
<td>1811</td>
<td>Missouri River</td>
<td>NY</td>
</tr>
<tr>
<td>Geyer, C. A.</td>
<td>1839</td>
<td>Missouri River</td>
<td>NY</td>
</tr>
<tr>
<td>Hayden, F. V.</td>
<td>1850s</td>
<td>Beyond</td>
<td>MO</td>
</tr>
<tr>
<td>Donaldson, A.B.</td>
<td>1874</td>
<td>Beyond</td>
<td>NY, PUL</td>
</tr>
<tr>
<td>Jenney, W.P.</td>
<td>1875</td>
<td>Beyond</td>
<td>GH</td>
</tr>
<tr>
<td>Forwood, W. H.</td>
<td>1880s</td>
<td>Beyond</td>
<td>GH, US</td>
</tr>
<tr>
<td>Canby, W.M.</td>
<td>1882</td>
<td>Beyond</td>
<td>NY</td>
</tr>
<tr>
<td>Rusby, W. S.</td>
<td>1887</td>
<td>Beyond</td>
<td>NY</td>
</tr>
<tr>
<td>Williams, T.A.</td>
<td>1890s</td>
<td>Beyond</td>
<td>SDC, RM, NY, US</td>
</tr>
<tr>
<td>Pratt, A.D.</td>
<td>1890s</td>
<td>Beyond</td>
<td>RM, NY</td>
</tr>
<tr>
<td>Wallace, E.J.</td>
<td>1890-1911</td>
<td>Beyond</td>
<td>SDC, RM, NY, NY</td>
</tr>
<tr>
<td>Griffiths, D.</td>
<td>1892-1904</td>
<td>Beyond</td>
<td>RM</td>
</tr>
<tr>
<td>Carter, L.W.</td>
<td>1892-1897</td>
<td>Beyond</td>
<td>SDC, RM, NY, BHSC</td>
</tr>
<tr>
<td>Rydberg, P.A.</td>
<td>1892-1913</td>
<td>Beyond</td>
<td>SDC, RM, NY, GH</td>
</tr>
<tr>
<td>Hapeman, H.</td>
<td>1892-1939</td>
<td>Beyond</td>
<td>RM, NY</td>
</tr>
<tr>
<td>Bessey, C.E.</td>
<td>1898</td>
<td>Beyond</td>
<td>NEB</td>
</tr>
<tr>
<td>Murdoch, John Jr.</td>
<td>1908-1912</td>
<td>Beyond</td>
<td>RM, NY, BHSC</td>
</tr>
<tr>
<td>Moyer, L.R.</td>
<td>1909</td>
<td>Beyond</td>
<td>RM, NY</td>
</tr>
<tr>
<td>Nelson, A.</td>
<td>1910-1915</td>
<td>Beyond</td>
<td>RM</td>
</tr>
<tr>
<td>Carr, W.P.</td>
<td>1911-1914</td>
<td>Beyond</td>
<td>SDU, RM, SDC, BHSC, GH, NY</td>
</tr>
<tr>
<td>Miller, L.R.</td>
<td>1912</td>
<td>Beyond</td>
<td>NY</td>
</tr>
<tr>
<td>Vischer, S.S.</td>
<td>1912-1914</td>
<td>Resident</td>
<td>SDU, RM, BHSC, NY</td>
</tr>
<tr>
<td>Pleass, W.</td>
<td>1913</td>
<td>Beyond</td>
<td>NY</td>
</tr>
<tr>
<td>Hitchcock, A.S.</td>
<td>1913-1927</td>
<td>Beyond</td>
<td>SDC, SDU, RM</td>
</tr>
<tr>
<td>Ball, C.R.</td>
<td>1915-1925</td>
<td>Beyond</td>
<td>BHSC</td>
</tr>
<tr>
<td>Pennell, F. W.</td>
<td>1915-1930s</td>
<td>Beyond</td>
<td>NY</td>
</tr>
<tr>
<td>Carter, E.E.</td>
<td>1916</td>
<td>Beyond</td>
<td>NY</td>
</tr>
<tr>
<td>Buechel, E.</td>
<td>1917-1923</td>
<td>Resident</td>
<td>Buechel Memorial Lakota Museum, St. Francis, SD</td>
</tr>
<tr>
<td>McIntosh, A.C.</td>
<td>1920s</td>
<td>Resident</td>
<td>SDU, RM, BHSC, NY</td>
</tr>
<tr>
<td>Over, W. H.</td>
<td>1920s</td>
<td>Resident</td>
<td>SDU, RM, BHSC, NY, SDC</td>
</tr>
<tr>
<td>Lee, H.E.</td>
<td>1920s-1940s</td>
<td>Resident</td>
<td>SDU, RM, BHSC, SDC</td>
</tr>
<tr>
<td>Hayward, H.E.</td>
<td>1926-1927</td>
<td>Beyond</td>
<td>MIL, RM, SDC, NY</td>
</tr>
<tr>
<td>Palmer, E.J.</td>
<td>1929</td>
<td>Beyond</td>
<td>NY, GH, MIL</td>
</tr>
<tr>
<td>Taylor, L. E.</td>
<td>1937</td>
<td>Beyond</td>
<td>NY</td>
</tr>
<tr>
<td>Fassett, N. C.</td>
<td>1939</td>
<td>Beyond</td>
<td>WIS, NY</td>
</tr>
<tr>
<td>Bennett, F. L.</td>
<td>1928-1940+</td>
<td>Resident</td>
<td>BHSC, SDU, SDC, RM</td>
</tr>
</tbody>
</table>
A COMPREHENSIVE HERBARIUM DATABASE OF VASCULAR PLANTS FROM NORTHEASTERN WYOMING AND WESTERN SOUTH DAKOTA

Curtis Card¹, Grace Kostel² and Mark Gabel²*

¹Department of Mathematics
²Herbarium
Black Hills State University
Spearfish, SD 57799
*mark.gabel@bhsu.edu

ABSTRACT

We report on the construction of a database of essentially all vascular plant specimens from northeast Wyoming and South Dakota west of the Missouri River. The database is the result of a compilation of label information from 16 herbaria and over 96,000 plant specimens representing 1725 species and 2088 taxa. The database includes information from the 22 counties west of the Missouri River in South Dakota and two adjacent counties in northeastern Wyoming. The county represented by the most specimens and the greatest specimen density is Lawrence with >8 specimens•km⁻², while the counties with the fewest specimens and lowest specimen density are Dewey and Ziebach with 0.02 specimens•km⁻². The month in which most specimens were collected is July. The 1980s is the decade in which the greatest number of collections were made. The database is constructed using Specify software and is available via the Black Hills State Herbarium website (http://herbarium.bhsu.edu) for this continuing project.

Keywords
flora, Great Plains, grassland, Black Hills, vegetation

INTRODUCTION

The flora of western South Dakota and northeastern Wyoming is not well studied. Kaul (1986) noted “(b)asic floristic information is still lacking for large parts of our area, most notably the grasslands of Montana, Wyoming, Colorado and the Dakotas” (p. 10). Parts of the region, especially the Black Hills, have a high density of globally rare species (Ostlie et al. 1997). A major obstacle to the study of the flora of western South Dakota and northeastern Wyoming is the distribution of plant specimens. While the Rocky Mountain Herbarium (RM) at the University of Wyoming is relatively well known and has the largest number of specimens from the study area, additional specimens comprising a significant
portion of the flora of the area are distributed in a number of locations in smaller herbaria.

Western South Dakota and eastern Wyoming are included in several floras (e.g. Dorn 2001, Great Plains Flora Association 1986, Van Bruggen 1996). Unfortunately, the printed floras are static and do not include new findings (e.g. Gabel and Tackett 2008, Kostel 2009, Kostel and Hetlet 2009) or taxonomic revisions (e.g. Rothrock et al. 2009). It is the purpose of the work described here to describe the construction of a web accessible database that can be used to access data about plant specimens collected in the study area.

**METHODS**

The study area includes all 22 counties west of the Missouri River in South Dakota and Crook and Weston Counties in Wyoming. Label data from vascular plant specimens collected in the study area from and housed in 16 herbaria (Table 1) were entered into a database using Specify data management software, a relational database (Specify-6 2010). Students were employed at the University of South Dakota, South Dakota State University, the University of Wyoming and the University of Kansas to collect label data from specimens under supervision of the herbarium curators. Personnel from BHSU visited the remaining collections, annotated and entered data from those institutions. Where possible, georeferencing was completed using a variety of paper maps as well as electronic resources such as Biogeomancer (2010), GEO Locate (2010), GeoNames (2010).

**Table 1. Herbaria contributing data to the comprehensive database with acronyms.**

<table>
<thead>
<tr>
<th>Herbaria</th>
<th>Acronym</th>
</tr>
</thead>
<tbody>
<tr>
<td>Badlands National Park</td>
<td>BADL</td>
</tr>
<tr>
<td>Black Hills State University</td>
<td>BHSC</td>
</tr>
<tr>
<td>Dakota Wesleyan University</td>
<td>DWU</td>
</tr>
<tr>
<td>Devil’s Tower National Monument</td>
<td>DETO</td>
</tr>
<tr>
<td>Jewel Cave National Monument</td>
<td>JECA</td>
</tr>
<tr>
<td>Milwaukee Public Museum</td>
<td>MIL</td>
</tr>
<tr>
<td>Mount Rushmore National Memorial</td>
<td>MORU</td>
</tr>
<tr>
<td>South Dakota State University, Brookings</td>
<td>SDC</td>
</tr>
<tr>
<td>University of Kansas, Lawrence</td>
<td>KANU</td>
</tr>
<tr>
<td>University of South Dakota, Vermillion</td>
<td>SDU</td>
</tr>
<tr>
<td>University of Wyoming, Laramie</td>
<td>RM</td>
</tr>
<tr>
<td>USDA Forest Service Custer, SD</td>
<td>FSCU</td>
</tr>
<tr>
<td>USDA Forest Service Newcastle, WY</td>
<td>FSN</td>
</tr>
<tr>
<td>USDA Forest Service Spearfish, SD</td>
<td>FSS</td>
</tr>
<tr>
<td>USDA Forest Service Rocky Mountain Research Station</td>
<td>FSRC</td>
</tr>
<tr>
<td>Wind Cave National Park</td>
<td>WICA</td>
</tr>
</tbody>
</table>
and Acme Mapper (2010). Nomenclature used in the database follows USDA NRCS Plants (2010).

The BHSU Herbarium database is currently running in a virtual environment using multiple VMware ESX hosts connected to SAN storage. With VMware ESX running on multiple hosts, BHSU is able to provide reliability and redundancy to the virtual machines if any of the hosts fail. If a host were to fail, a virtual machine would continue to run but it would pull resources needed from another host until the failed host is back online. Each host can handle several virtual machines depending on the hardware resources available on the host.

We use Specify 5.2 software that accesses an SQL database. Maintenance and additions to the database are made using the Specify software. The public website is being hosted on an Apache web server and an Apache Tomcat web server. The Apache web server processes requests to the website for general information about the Herbarium using HTML and PHP. Accessing the SQL database through the internet and the Apache Tomcat server and web pages requires the use of Java software. The Apache server is unable to process SQL database requests and forwards them to the Apache Tomcat server to access (search) the database. An upgrade to Specify 6 software is planned for August 2010 and will allow mapping of specimen distributions and the continued addition of more specimens.

RESULTS

The total number of specimens included in the database is 96,107, which includes 2088 taxa and 1725 species from the study area. Approximately 81% of the specimens are georeferenced. Unfortunately, many of the older specimens do not have sufficient label data to determine meaningful longitude and latitude coordinates. The decade in which the largest number of specimens was collected is the 1980s (Figure 1). The month with the greatest number of collections is July (Table 2), while the month with the fewest collections, unsurprisingly is January.

The plant family with the most species is the Asteraceae with 274, represented by 14,860 specimens. The Poaceae of the region include 232 species, but include 17,072 specimens. Other plant families with large numbers of species or specimens include the Fabaceae (110 species and 7447 specimens), the Cyperaceae (114 species and 4260 specimens) the Rosaceae and Brassicaceae with 71 species each and 3662 and 3752 specimens, respectively.

Current numbers of specimens by county are shown in Figure 2. Notable are the greater specimen numbers and densities collected from the Black Hills (Crook, Weston, Lawrence, Pennington, Custer and Fall River Counties). Equally notable are the fewer numbers of specimens and lesser densities of specimens from Dewey, Ziebach, Shannon, Haakon, Tripp, Stanley, Lyman and Mellette Counties.

The elevation ranges of specimens from the 120,363 km² study area are shown in Figure 3. Only small areas of Crook, Weston, Lawrence, Pennington and Custer counties are above 1800 m, with more area between 900 m and 1370
Figure 1. Number of specimens by decade collected from the study area as compiled from 16 herbaria.

Figure 2. The study area with counties labeled. Numeral above county name is total current specimens from the county. Numeral below the county name is the density of specimens collected from that county (specimens•km\(^{-2}\)).
m. In contrast, only small areas along the eastern edge of the study area, adjacent to the Missouri River, are below 600 m. The majority of the land area is between 600 and 1370 m.

**DISCUSSION**

The database is a work in progress. The numbers reported here are current as of June 2010, but due to continued additions of specimens to the database and nomenclatural changes, the numbers will be continually changing.

There are greater numbers of plants from some areas of the region (e.g. Black Hills), but there is a lack of representation from counties in the eastern edge of the study area (Figure 3). It is critical that all areas of the project area, especially the eastern portion of the study area, be studied and additional specimens collected to arrive at a better understanding of the flora of the region. The authors hope that the web-accessible database will encourage additional floristic research within the study area. It is anticipated that more researchers will use specimens from the region in monographic or systematic works. This resource on plants of the study area in an easily accessible database should encourage more study of the region by researchers and land managers.

**ACKNOWLEDGEMENTS**

The authors wish to acknowledge the curators of collections for allowing us to use data from their collections. They are: Craig Beckner, Rachel Benton, Cissie Buckert, Beth Burkhart, Jack Butler, Megan Cherry, Tom Farrell, Craig Freeman, Ron Hartman, Gary Larson, Jill Larson, Dan Licht, Neil T. Luebke, Tim
We are grateful to A. Gabel, D. Ode and D. Mergen for thoughtful reviews of this manuscript. We also wish to thank the National Wildlife Foundation and the National Science Foundation (BRC #0545102) for their generous financial support.

LITERATURE CITED


ANTIMICROBIAL SCREENING ON
FOURTEEN TRADITIONAL
AMERICAN INDIAN MEDICINAL PLANTS

J. L. Jacobs*, J. Dixson, M. Gabel, D. Bergmann, J. DeCory, and C. Geffre
College of Arts and Sciences
Black Hills State University
Spearfish, SD 57799
*jay.jacobs@yellowjackets.bhsu.edu

ABSTRACT

Fourteen plant species traditionally utilized by American Indians as medicines were investigated for antimicrobial activity. The plant material, native to western South Dakota, was collected and dried, and voucher specimens were deposited in the BHSU Herbarium. A sequential gradient extraction procedure was refined to fractionate compounds with solvents in a sequential order from non-polar to polar: hexane, carbon tetrachloride, methylene chloride, and 1-butanol. A broth tube bacterial screening method was used to identify crude plant fractions with inhibitory properties—lack of turbidity indicating microbial inhibition. Four bacteria (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae) and one yeast culture (Candida albicans) were utilized in the initial screening method. Antimicrobial activity was found in seven 1-butanol, three methylene chloride, two carbon tetrachloride, and three hexane plant extract fractions. To identify active compounds, we chromatographed active crude fractions and screened for activity. Subsequently, one hexane, one carbon tetrachloride, one methylene chloride, and five 1-butanol plant fractions from five plants were subjected to flash chromatography on silica gel using a gradient solvent system. The initial solvent ratios were determined by thin layered chromatography. To conserve the limited quantity of material after chromatography, we adapted a “high throughput” 96-well micro-dilution assay. Screening was also limited to S. aureus, E. coli, and C. albicans. As a result, activity was maintained for all chromatographed crude fractions. Active compounds have been isolated from Artemisia ludoviciana and Monarda fistulosa.

Keywords
ethnobotany, South Dakota, antimicrobial screening, medicinal plants.

INTRODUCTION

Plant natural products have long served as a rich source for medicines, e.g. aspirin, paclitaxel and digoxin (Saklani and Kutty 2008). Natural products have also provided leads for new drugs, although ultimately requiring structural
modification by synthetic chemists to address pharmacodynamic and pharma-
cokinetic properties before being developed (Saklani and Kutty 2008). Many
examples of plant derived compounds serving as a source for drug design exist:
physostigmine the lead for rivastigmine which is used to treat symptoms of
Alzheimer’s disease, and ∆9-tetrahydrocannabinol the lead for nabilone used to
treat nausea from chemotherapy.

Often a biologically active molecule may bind to more than one target (i.e. re-
ceptor or enzyme) and elicit more than one response. For example, aspirin has a
number of biological effects: anti-inflammatory, anti-coagulation, analgesic and
anti-pyretic (Vane and Botting 2003). Aspirin has been shown to inhibit the
enzyme cyclooxygenase-2 which is the key enzyme in the production of prosta-
glandins (e.g. PGE$_2$ and thromboxane A$_2$), and accounts for the anti-inflammatory
and thrombus prevention properties but not the analgesic and antipyretic
effects. Research on the analgesic properties is pointing to the inhibition of the
vanilloid receptor (Davis et al. 2000). The antipyretic effect continues to be
investigated, but appears to be related to the ability of aspirin to prevent oxygen
radical formation which is involved in the induction of fever (Vane and Botting
2003). Thus, aspirin has multiple effects as a result of interactions with multiple
target sites. Since molecules with biological activity will often have more than
one biological target, our aim was to test plants that American Indians use as
medicines and initially screen for antimicrobial activity.

METHODS

Plant selection and collection—Fourteen plant species (Table 1) known to
be used for a variety of medicinal purposes by the Lakota and other American
Indian tribes of the northern plains were selected (Moerman 1998; Larson and
Johnson 1999;, Kindscher, 1992). Plant material was dried and voucher speci-
mens were deposited in the Black Hills State University (BHSU) Herbarium.
Prior to extraction, leaves and stems were cut into ~5-7 cm lengths and homog-
enized with a blender or spice (coffee) grinder.

Extraction and Chromatography—A sequential gradient extraction procedure
outlined by Riguera was refined to fractionate compounds based on their polarity
(Riguera 1997). Within this procedure, extraction was completed in a sequential
order starting with a non-polar solvent (hexanes). The polarity was increased
stepwise to carbon tetrachloride (CCl$_4$), then to methylene chloride (CH$_2$Cl$_2$)
and ultimately to 1-butanol (1-BuOH) (Figure 1). The plant material was frac-
tionated by percolating at room temperature for ~24 hours in each solvent (the
amount of plant material and solvents are listed in Table 2). The extract-solvent
solution was vacuum filtered and the solvent was removed from the crude extract
by rotary evaporation. The final extracts were labeled and stored in vials.

Following the initial antimicrobial screening, crude extract fractions from five
plants—Yucca glauca, Artemisia ludoviciana, Grindelia squarrosa, Mahonia repens,
and Monarda fistulosa—were selected for chromatography based on antimicro-
bial activity and the quantity of crude extract. Extracts were adsorbed onto silica
gel and packed into a pre-column with a fritted disc. The adsorbed extract was
then subjected to flash chromatography via a medium pressure (~10 psi) liquid chromatography system (MPLC). As a guideline, a 10:1 silica gel to crude extract ratio was used to select column size. The samples were chromatographed on either RediSep® or Biotage silica gel columns. Chromatography solvents were determined by thin layer chromatography (TLC). Following flash chromatography, the eluted material was evaluated by TLC and separated into major fractions. Samples were then concentrated, dissolved in dimethyl sulfoxide (DMSO), and re-screened in a 96-well micro-dilution assay. Chromatography and screening for activity were repeated until a single compound was noted by TLC or until the amount of material limited further work.

Table 1. Plants selected to test for antimicrobial compounds.

<table>
<thead>
<tr>
<th>Genus &amp; Species</th>
<th>Family</th>
<th>Common Name</th>
<th>Lakota Name</th>
<th>Lakota Uses*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amorpha canescens</td>
<td>Fabaceae</td>
<td>Lead Plant</td>
<td>Zitka’ tacan’</td>
<td>Stomachache</td>
</tr>
<tr>
<td>Arctostaphylos uva-ursi</td>
<td>Ericaceae</td>
<td>Bearberry</td>
<td>Wahpe’ canli’</td>
<td>Tobacco additive</td>
</tr>
<tr>
<td>Artemesia frigid</td>
<td>Asteraceae</td>
<td>Woman’s sage</td>
<td>Winyan ta pejuta</td>
<td>Irregular menstruation</td>
</tr>
<tr>
<td>Artemisia ludoviciana</td>
<td>Asteraceae</td>
<td>White sage</td>
<td>Peji’ hota</td>
<td>Dermatological aid</td>
</tr>
<tr>
<td>Dalea purpurea</td>
<td>Fabaceae</td>
<td>Purple clover</td>
<td>Tokala tapejuta hu winyula</td>
<td>general ailments</td>
</tr>
<tr>
<td>Grindelia squarrosa</td>
<td>Asteraceae</td>
<td>Gum weed</td>
<td>Pte ichi yuha</td>
<td>Poison ivy or rashes</td>
</tr>
<tr>
<td>Heuchera richardsonii</td>
<td>Saxifragaceae</td>
<td>Alum</td>
<td>Canhlo’ hnasna’la</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Ipomoea leptophylla</td>
<td>Convolvulaceae</td>
<td>Bush morning glory</td>
<td>Pejuta nige tanka</td>
<td>Stomachache</td>
</tr>
<tr>
<td>Liatris punctata</td>
<td>Asteraceae</td>
<td>Dotted gay feather</td>
<td>Tat e’ cannuga</td>
<td>Improve appetite</td>
</tr>
<tr>
<td>Mahonia repens</td>
<td>Berberidaceae</td>
<td>Oregon grape</td>
<td>Canpazi</td>
<td>Unspecified use of root (dye)</td>
</tr>
<tr>
<td>Monarda fistulosa</td>
<td>Lamiaceae</td>
<td>Wild bergamot</td>
<td>Wahpe’ wastemna</td>
<td>Upper respiratory or colds</td>
</tr>
<tr>
<td>Pulsatilla patens</td>
<td>Ranunculaceae</td>
<td>Pasque flower</td>
<td>Hoksi’ cekpa</td>
<td>speed childbirth</td>
</tr>
<tr>
<td>Ratibida columnifera</td>
<td>Asteraceae</td>
<td>Prairie cone-flower</td>
<td>Napo’stan</td>
<td>Stomachache or sideache</td>
</tr>
<tr>
<td>Yucca glauca</td>
<td>Agavaceae</td>
<td>Yucca</td>
<td>Hupestola</td>
<td>Use root for soap</td>
</tr>
</tbody>
</table>

*Within a tribe, medicinal usages are passed down from generation to generation and the passing of this knowledge may reflect that of a specific lineage whereby other medicinal uses or preparation may be performed differently within the same tribe by different healers.
Microbial cultures—Three bacteria species (Escherichia coli B ATCC 11303, Staphylococcus aureus and Pseudomonas aeruginosa PAO-Lacl) were selected from existing cultures maintained at BHSU and two additional microbial cultures (Klebsiella pneumonia ATCC 13883 and Candida albicans ATCC 10231) were purchased from Presque Isle Cultures, Erie, PA. All cultures were maintained on tryptic soy agar (TSA) slants. The species obtained from BHSU stock were plated on species specific agar for verification (e.g. manitol salt agar for Staphy-

![Flow diagram](image)

Figure 1. A flow diagram illustrates the collection, extraction, and screening process, as well as future work such as determining the structure of an active compound.
To determine the concentration of solvent that would not kill or inhibit the microbial species upon screening, we used a serial broth tube dilution method with DMSO for all bacteria and yeast species. This method was also conducted using 1-butanol (1-BuOH) since removing this solvent from the extract proved difficult without losing product in the process. Consequently, it was determined that levels of 5% (e.g. 200 µL/4 mL total volume) DMSO and 0.03% (e.g. 12.5 µL / 4 mL total volume) 1-BuOH were tolerated by all microbe species without affecting the results of the screening. Inoculum for the screening assay was then prepared for each microbial culture with tryptic soy broth (TSB) and incubated in a Lab-Line Orbit Enviro-Shaker for 24 hours at 30 °C and 250 rpm.

**Microbiological assays**—A broth tube turbidity method (BTTM) was utilized to screen for or identify crude extracts with antimicrobial activity (Berghe and Vlietinck 1991). A sample from each plant extract was weighed and dissolved in DMSO to make a 200 mg/mL plant extract solution. The solution was filtered through a sterile 0.45 µm vinyl filter into a sterilized Eppendorf tube. A 200 µL sample (40 mg plant extract) was pipetted from the Eppendorf tube into each screening test tube. Screening tubes and a control tube containing 3.75 mL of sterile TSB were inoculated with 50 µL of microbial culture (TSB inoculum) and incubated in a Lab-Line Orbit Enviro-Shaker for 24 hours at 30 °C and 250 rpm. The tubes containing extract were photographed and visually compared with control tubes for turbidity—lack of turbidity indicating positive inhibitory or antimicrobial activity.

A 96-well micro-dilution (turbidity) assay was modified (Stubbings et al. 2004; Gruppo et al. 2006; Pfaller et al. 2004) to detect antimicrobial activity and avoid

### Table 2. Quantity of plant material and solvent utilized for the initial extraction.

<table>
<thead>
<tr>
<th>Genus &amp; Species</th>
<th>Quantity of extract (g)</th>
<th>Quantity of solvent (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amorpha canescens</td>
<td>116.7</td>
<td>300</td>
</tr>
<tr>
<td>Arctostaphylos uva-ursi</td>
<td>111.2</td>
<td>250</td>
</tr>
<tr>
<td>Artemisia frigida</td>
<td>50.5</td>
<td>200</td>
</tr>
<tr>
<td>Artemisia ludoviciana</td>
<td>22.1</td>
<td>200</td>
</tr>
<tr>
<td>Dalea purpurea</td>
<td>83.4</td>
<td>300</td>
</tr>
<tr>
<td>Grindelia squarrosa</td>
<td>120.4</td>
<td>300</td>
</tr>
<tr>
<td>Heuchera richardsonii</td>
<td>86.4</td>
<td>200</td>
</tr>
<tr>
<td>Ipomoea leptophylla</td>
<td>31.3</td>
<td>200</td>
</tr>
<tr>
<td>Liatris punctata</td>
<td>45.0</td>
<td>200</td>
</tr>
<tr>
<td>Mahonia repens</td>
<td>80.1</td>
<td>300</td>
</tr>
<tr>
<td>Monarda fistulosa</td>
<td>46.7</td>
<td>200</td>
</tr>
<tr>
<td>Pulsatilla patens</td>
<td>87.2</td>
<td>300</td>
</tr>
<tr>
<td>Ratibida columnifera</td>
<td>98.9</td>
<td>300</td>
</tr>
<tr>
<td>Yucca glauca</td>
<td>70.1</td>
<td>350</td>
</tr>
</tbody>
</table>
consuming large quantities of chromatography extracts. Clear flat-bottomed 96-well microplates (Nunc) and a Bio-Rad Benchmark Plus microplate reader were utilized for this assay. Test cultures were prepared using $10^6$ colony forming units (CFU/ml) *S. aureus* in Mueller-Hinton broth (MHB), $10^4$ CFU/ml *E. coli* in MHB, and $10^6$ CFU/ml *C. albicans* in Mueller-Hinton broth with 2% glucose (MHGB) for a total volume of 200 µL per well. Given that 400 mg of extract was effectively dissolved into 2 mL of DMSO for the initial screen, samples were prepared by saturating small volumes (e.g., 100 µL) of DMSO with plant extract so that the saturation dosage would approximate 200 µg/µL or greater. A positive control (e.g., 2 µL extract and 198 µL sterile medium) and a negative control (e.g., 2 µL DMSO and 198 µL inoculated medium) were prepared for each chromatographed extract fraction. The positive control provided a visual comparison as well as verified that bacterial contamination from the extract did not influence the assay. All samples and controls were run in triplicate. Once loaded, the microplate was shaken lightly with a small lab shaker for 60 seconds and then incubated for 24 hours at 37 ºC.

To measure sample turbidity, we read optical density (OD) at 600 nm before and after incubation. Prior to measurement, proper suspension of extract and inoculums was ensured by mechanically agitating the plate for 30 seconds on the low setting. The data were exported to Microsoft Excel for analysis and compared with visual inspection of the microplate(s) under standard fluorescent light.

**RESULTS**

The sequential gradient extraction procedure resulted in adequate yields of crude extract from as little as 20 g of plant material that could be screened with the BTTM. Only twice did activity occur across two sequential crude extract fractions for the same species, and the remaining plants had activity restricted to one or no fractions. From the screening of extract fractions, microbial inhibitory activity was found in seven 1-butanol, three methylene chloride, two carbon tetrachloride, and three hexane plant extract fractions equating to fifteen extract fractions from ten plants (Table 3).

**DISCUSSION**

The BTTM screening provided a quick objective method to test plant extracts for antimicrobial activity thereby allowing us to focus on those specific extract fractions. Coloration and consistency of the plant extract rarely interfered with the procedure in comparison with the 96-well assay. In eight out of fourteen CCl$_4$ fractions where turbidity was questioned due to milky appearance or a heavy extract precipitate, the results were qualified by mechanically shaking the samples or by preparing slides and microscopically inspecting for bacteria or yeast cells. The heavy extract precipitate did not suspend as easily as would microbial cells.
Early in the project it became evident following flash chromatography on two active extracts that the amount of sample remaining in ninety percent of the fractions would have been exhausted by using the BTTM. Therefore, a modified 96-well micro-dilution assay was incorporated to overcome the issue of limited extract material. In addition to visualizing activity by holding the plate up to light (similar to test tubes), we used optical density to quantitatively measure and determine activity.

Initially, antimicrobial activity from chromatography fractions of *Y. glauca* and *A. ludoviciana* were reproduced by using the 96-well micro-dilution assay for both *S. aureus* and *C. albicans*. Subsequent assays also reproduced microbial inhibitory activity for *M. fistulosa*. Consequently, this assay was then utilized for screening all chromatography fractions.

Performance of the 96-well micro-dilution assay was limited by pigmentation, precipitation, and / or oily compounds affecting the optical density in a number of fractions. Clarifying activity on many of these fractions was resolved by recording a zero time OD reading of the microplate and comparing this to the 24 hour reading. In cases where comparing the zero time and 24 hr OD left uncertainty, we dissolved ~40 mg of extract in methanol, and the solution was then eluted through activated charcoal into an Eppendorf tube. The sample was then air dried, dissolved in DMSO, and then re-analyzed using the 96-well micro-dilution assay. If uncertainty remained after the above methods, samples were either plated out and microbial growth was assessed, or microscopy was

### Table 3: A comprehensive list of species and extract fractions with microbial inhibitory activity.

<table>
<thead>
<tr>
<th>Species</th>
<th>Solvent</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
<th>K. pneumoniae</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. canescens</em></td>
<td>C₂H₄</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>A. canescens</em></td>
<td>CCl₄</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>A. ludoviciana</em></td>
<td>C₂H₄</td>
<td>+</td>
<td>+</td>
<td>*</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>A. ludoviciana</em></td>
<td>1-BuOH</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>A. uva-ursi</em></td>
<td>1-BuOH</td>
<td>*</td>
<td>*</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td><em>D. purpurea</em></td>
<td>C₂H₄</td>
<td>*</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>G. squarrosa</em></td>
<td>CCl₄</td>
<td>*</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>G. squarrosa</em></td>
<td>1-BuOH</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>I. leptophylla</em></td>
<td>CH₂Cl₂</td>
<td>*</td>
<td>*</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td><em>L. punctata</em></td>
<td>CH₂Cl₂</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>L. punctata</em></td>
<td>1-BuOH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>M. fistulosa</em></td>
<td>CH₂Cl₂</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>*</td>
<td>+</td>
</tr>
<tr>
<td><em>M. fistulosa</em></td>
<td>1-BuOH</td>
<td>-</td>
<td>*</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>M. repens</em></td>
<td>1-BuOH</td>
<td>+</td>
<td>+</td>
<td>*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Y. glauca</em></td>
<td>1-BuOH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Inhibition or antimicrobial activity (clear)
- No inhibition (turbidity)
* Partial inhibition (partially clear solution)
used to assess the type and quantity of bacterial cells (if present). In such cases, it may be prudent to attempt smaller test tube turbidity screens such as using one milliliter total volume, especially if OD can be measured horizontally instead of vertically as is done with the microplate reader. Horizontal OD readings eliminate any obstruction of extract which precipitates and settles to the bottom of the tube. Similarly, a disk diffusion method may be useful if the compounds could be diffused into an aqueous based medium. However, this method would also require a considerable amount of material as did the BTTM and may negate any benefit.

Once activity is detected, scale up is generally necessary to produce enough material to identify structure and test for inhibitory and bactericidal properties. Our current focus is devoted to scaling up and purifying active compounds found in A. ludoviciana, Y. glauca, and M. fistulosa. Larger quantities of A. ludoviciana, Y. glauca, and M. fistulosa have been collected, extracted, and active fractions have been further refined by flash chromatography. Based on TLC, five compounds have been isolated thus far including two active compounds: one from A. ludoviciana and the other from M. fistulosa. Further research, beyond the scope of this initial project, will be necessary to identify the precise structure of these active compounds as well as potency and mode of action. Likewise, further research will be required to identify the active compounds from other plant species, such as G. squarrosa and M. repens.

ACKNOWLEDGEMENT

Funding was provided by NIH Grant Number 2 P20 RR016479 from the INBRE Program of the National Center for Research Resources. The contents of this document are solely the responsibility of the authors and do not necessarily represent the official views of NIH.

LITERATURE CITED


INFLUENCE OF ENVIRONMENT AND CULTIVAR ON FRUIT QUALITY IN NEWLY ESTABLISHED JUNEBERRY (AMELANCHIER ALNIFOLIA (NUTT.) NUTT. EX M. ROEM.) ORCHARDS ON THE FORT BERTHOLD RESERVATION

Brittany Poelaert¹, Ferdinand Bergh¹, Kerry Hartman², and R. Neil Reese¹*

¹Biology & Microbiology Department
South Dakota State University
Brookings, SD 57007
²Science Department
Fort Berthold Community College
New Town, ND 58763
*neil.reese@sdstate.edu

ABSTRACT

Juneberry (Amelanchier alnifolia) has long been treasured as a native prairie fruit. Historically, the hardy shrub was widely used by many North American Indian tribes. Berries were eaten fresh, steamed, mashed, or dried to a brick-like consistency for reconstitution at a later time. The Fort Berthold Reservation had many wild Juneberry bushes growing along the Missouri River bottom, which were lost when Lake Sakakawea was formed by damming the Missouri River. Three Juneberry cultivars, Honeywood, Martin and Smoky, were planted in 3 locations on the Fort Berthold Reservation in 2004. Fruits collected in 2009 were analyzed for their total phenolic (TP) and total monomeric anthocyanin (TMA) contents. Antioxidant capacity was determined by hydrogen atom transfer (ABTS), ferric reducing antioxidant power (FRAP) and radical scavenging (DPPH) assays. Total phenolic and anthocyanin contents were measured using the Folin-Ciocalteu reagent and the pH differential methods, respectively. Soluble solids were measured by refractometry and titratable acids with NaOH titration. Overall, the phytonutrient contents and antioxidant capacity of the Juneberry fruits were similar to those typically reported for other dark-fleshed small fruits, such as grapes, blackberries and raspberries.

Keywords

Juneberry fruits, phytonutrient content, antioxidant capacity

INTRODUCTION

Juneberry (Amelanchier alnifolia) belongs to the family Rosaceae and is native to Western North America (Figure 1). This hardy shrub was a staple food of
many North American Indians and was used in a wide variety of ways. Berries were eaten fresh, steamed, mashed, or dried to a brick-like consistency to be used in the winter (Hartman 2008). Many wild Juneberry bushes grew near the Missouri River bottom at the Fort Berthold Reservation until they were lost due to the formation of Lake Sakakawea when the Missouri River was dammed. Juneberries are still an important source of nutrition on the Fort Berthold Reservation and have also become a potential crop for the region’s developing wine and small fruit industries. Several cultivars have been developed for the fruit industry and most are well adapted to the cold climates and mildly alkaline soils of the northern Great Plains (St. Pierre et al. 2005).

Juneberries are an excellent source of bioactive components such as anthocyanins, flavonols, procyanidins, and phenolic acids (Ozga et al. 2007; Bakowska-Barczak and Kolodziejczyk 2008). These phenolic compounds have attracted much interest due to their antioxidant properties and perceived health benefits, including antimicrobial, anti-inflammatory, and anticarcinogenic activities. They have also been implicated as having insulin secretion ability and neuroprotective effects (Han et al. 2007). This study was designed to determine the nutriceutical content (total phenolic and total monomeric anthocyanin content, as well as antioxidant capacity) of the fruit as a food source for residents of the Fort Berthold Reservation and to measure the variability in factors that influence eating quality and wine production (soluble solids and titratable acid content), as a step in the development of commercial orchards.

Figure 1. Juneberry plants in flower and fruit.
METHODS

Chemicals and Standards. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from EMD Biosciences, Inc. (San Diego, CA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), and trifluoroacetic acid (TFA) were supplied by Sigma Chemical Co. (St. Louis, MO).

Planting Sites. In 2004, three Juneberry cultivars, Honeywood, Martin, and Smoky, were planted in three unique locations on the Fort Berthold Reservation. Two of the sites (War Coulee- WC (47°42’ N, 101°50’ W) and White Shield –WS (47°38’ N, 101°50’ W)) were irrigated and the third site at Mandaree (47°41’ N, 102°38’ W) was not irrigated. Fruit production at Mandaree was very limited and inconsistent and was therefore not included in this study. At both WC and WS, the dominant soil series was Williams-Bowden (Brockmann et al. 1979). The two sites differed in that WC was planted on a 3% terraced slope and had no protection from the wind, while WS was planted in a nearly level concave swale and was protected from the wind by a tree belt and housing development (Hartman 2008).

Fruit was collected for the first time in 2009 to evaluate the impact of cultivar and location on fruit quality at WC and WS. Three field samples were made for each cultivar, frozen and brought to Brookings for biochemical analyses.

Fruit Extraction. Three replications of frozen Juneberries (100 grams) from each of the 2 field locations (WC and WS) and the 3 cultivars (Martin, Smoky and Honeywood) were pureed in a Waring® blender. Three replicate subsamples of each preparation for each of the tests were placed in 15 ml conical centrifuge tubes and stored at -80°C.

Total Phenolic Measurement. Sample TP contents were measured according to Singleton and Rossi (1965) with slight modifications. To determine levels of TP, we added 1 mL of each extract to Folin-Ciocalteu’s phenol reagent and water 1:1:20 (v/v) and incubated for eight minutes followed by the addition of 10 mL of 7 % (w/v) sodium carbonate. After 2 h, the absorbance of each was measured at 750 nm. Values of TP were estimated by comparing the absorbance of each with those of a standard response curve generated with gallic acid. Results were expressed as µg gallic acid equivalents on a gram fresh weight basis (GAE/gfw).

Total Monomeric Anthocyanins. TMA levels were measured by the pH differential method described by Giusti and Wrolstad (2005). Sample extracts were combined in a 1:20 ratio (v:v) with potassium chloride and with sodium acetate buffers (pH 1.0 and 4.5, respectively) in separate vessels. After an equilibration period (15 min), the raw absorbance of each solution was measured at 520 and 700 nm. A corrected absorbance value was calculated as [(A520–A700) pH 1.0 - (A520–A700) pH 4.5]. The anthocyanin content was calculated using the molar absorptivity (ε) and molecular weights (MW) of cyanidin 3-glucoside (ε = 26,900; MW = 449.2). Results are expressed as µg cyanidin 3-glucoside equivalents (Cy3-GE)/gfw.

Antioxidant Capacity Analysis. The antioxidant capacity of Juneberry extracts and standard compounds was determined using the three antioxidant assays: fer-
ric reducing antioxidant power (FRAP), 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH). In brief, FRAP assay was performed as previously described by Benzie and Strain (1999). The analysis was conducted at 25-30°C under pH 3.6 condition with a blank sample in parallel at 593 nm. The ABTS assay was performed according to the modified method of Ozgen et al. (2006). Levels of reduced ABTS reactants were measured at 734 nm. DPPH assay was performed according to the method of Brand-Williams et al. (1995) and the absorbance was determined at 517 nm. All assay reactions were kept in the dark for 60 min prior to measurement of the absorbance using Beckman Coulter DU-650 spectrophotometer. The results were expressed as micromoles of Trolox equivalents (TE) per gram fresh weight of Juneberry extracts.

**Fruit Quality.** Juneberry purees were assayed for soluble solid (SS) contents by refractometry and for levels of titratable acidity (TA) using the methodology of Perkins-Veazie et al. (1996).

**Statistical Analyses.** Means, standard errors, regression equations, and Pearson’s correlation coefficients were calculated using Microsoft Excel (Microsoft International, Redmond, WA) spreadsheet functions. Analyses of variance and multiple comparisons using the Tukey HSD were performed using StatCrunch® Data Analysis on the Web (http://www.statcrunch.com/).

**RESULTS AND DISCUSSION**

This study is a first examination of Juneberry production in newly established orchards on the Fort Berthold Reservation. Cultivation methods, plant attrition and long-term survival have been previously described for these plants (Hartman 2008). The data presented here represent the first harvest of fruits from these orchards and provide an initial look at fruit quality.

![Figure 2. Total Phenolic Content, in gallic acid equivalents (X ± SE), was determined by the Folin-Ciocalteu method. A) Phenolic content by location, was not significantly different. B) Phenolic content by cultivar were was different, (F = 4.446, P = 0.0.31, df = 17.). HHoney wwood contained higher levels of phenolics that than did Smoky (α = 0.05), Tukey HSD.](image-url)
Total phenolic content, phenolic composition and antioxidant capacity in fruit have been shown to vary in response to cultivar, abiotic stresses such as temperature, water loss and soils, and harvest year (Wang and Lin 2000; Wang and Zheng 2001; Ozgen et al. 2008). This study examined the impact of cultivar and environment on fruit quality and phenolic antioxidants in Juneberries grown on the Fort Berthold Reservation. Juneberry fruit phenolic content (Figure 2) was not significantly different between WC and WS. Cultivars, however, showed a consistent pattern of phenolic content distribution with Honeywood having a significantly higher phenolic concentration compared to that of Smoky, over both locations (\( P = 0.05 \)). Martin was consistently intermediate in phenolic content at the two sites. Location by cultivar interaction was not significant.

Total anthocyanin content (Figure 3) was measured by the pH differential method. Anthocyanin content is reflective of the stage of ripeness as well as the fruit color of a specific cultivar (Ozgen et al. 2008). At the WC site, all three cultivars had significantly higher anthocyanin content than those harvested at WS. The relatively large SE may reflect the difficulty in harvesting uniformly ripe pomes. Juneberry fruit tends to ripen in an uneven manner that makes harvesting labor intensive (St. Pierre 2006). Selection of more uniformity in maturation is a long–term goal of our research.

Regardless of the unevenness of ripening, Honeywood consistently produced the largest amounts of anthocyanins. Over the two locations the Honeywood fruit contained almost twice as much anthocyanin as did the Smoky cultivars and was significantly different \(( P = 0.05 )\) from both the Martin and Smoky fruit. Although the Martin fruit anthocyanin level appears intermediate between the two other cultivars, it was not significantly different from that of Honeywood or Smoky.

That the fruit at the two locations had similar amounts of total phenolics, but showed significant differences in anthocyanin concentrations, which com-
prise the majority of the phenolic compounds in these fruits (Bakowska-Barczak and Kolodziejczyk 2008; Kraft et al. 2008), suggests that the environment at the two locations had an impact on the non-anthocyanin phenolics. This variation in phenolic composition affects the responses of the three different antioxidant measurements (Ozgen et al. 2008) which can be seen in our results.

The three antioxidant assays (Figure 4) utilize different mechanisms to measure the antioxidant capacity of the fruit. Each method provides a different type of data and use of multiple methods has been recommended to provide a complete evaluation of phenolic antioxidants (Ozgen et al. 2006; 2008). ABTS is driven by a direct hydrogen atom transfer from the antioxidant molecules. FRAP measures the ferric reducing antioxidant power of the antioxidant, and DPPH is a radical scavenger. The antioxidant capacity of Honeywood fruit was significantly greater \((P = 0.05)\) than that of Smoky fruit over all locations by both ABTS and FRAP measurements, but there was no difference in antioxidant capacity by location. DPPH analyses indicated a significant difference in antioxidant capacity by location \((P = 0.05)\), but there was no difference between cultivars.

Figure 4. Total Antioxidant Capacity \((\% \pm SE, n = 3)\) was measured using three separate methods, ABTS, FRAP and DPPH assays, and expressed at Trolox equivalents. A) Only DPPH showed differences across location, \((F = 19.977, P = 0.004, df = 17)\). B) ABTS, \((F = 15.012, P = 0.0003, df = 17)\) and FRAP \((F = 10.369, P = 0.0015, df = 17)\), showed significant differences between cultivars with Honeywood having higher antioxidant potential compared to Martin and Smoky \((\alpha = 0.05)\), Tukey HSD) in both tests. Smoky and Martin were also different \((\alpha = 0.05)\), Tukey HSD) in the FRAP assay.
Variation in the relative ranking of the 3 cultivars within a specific assay results from variations in the types of phenolic compounds produced in the fruit. Differences between the DPPH results and those of ABTS and FRAP suggest that the non-anthocyanin phenolic species present in the fruit vary more in response to environment than cultivar, as these types of compounds have previously been implicated in the differences in assay responses (Ozgen et al. 2006).

The quality and flavor of fruits and wines made from them depend upon their acidity and soluble solid (e.g. sugars, organic acids, etc.) content (Kader 1999). A low pH with titratable acidity of 0.6-0.9 % and a Brix of >8 are generally preferred by fresh fruit consumers and wine makers (Gallander 1987; Kader 1999). The analysis of all of the Juneberry fruit grown on the Fort Berthold Reservation demonstrated that at both locations for all three cultivars the acid and Brix were found to be within the recommended parameters.

Titratable acids showed no significant differences by location, but Honeywood fruit contained significantly more acid ($P = 0.05$) than did the Smoky fruit across locations (Figure 5). Fruit at WS had a significantly greater percent soluble solid concentration ($P = 0.05$) than did the fruit from WC. As WS is drier on average than WC, these values may represent a variation in fruit water content (Figure 6).

The consistently high Brix and titratable acid content of Honeywood fruit suggest that it is a better candidate for making wines than is the Smoky cultivar grown in North Dakota. However, all three appear to be of value to winemakers and further study into fruit size and yields needs to be made. As flavor is a personal response, sensory panels will need to be conducted before any final judgments can be made as to the best cultivars to grow for food or wine production. In informal tastings, Smoky was often the favorite choice for eating and overall taste.

Our results show that Juneberry fruit quality was impacted by growing location and cultivar. In general, Honeywood appeared to produce the highest

![Figure 5. Titratable acids (X ± SE) were measured by NaOH titration and calculated as citric acid equivalents. A) There were no significant differences by location. B) The three cultivars showed significant differences in acidity (F = 5.93, $P = 0.013$, df = 17). Honeywood fruit contained significantly more titratable acid than did the Smoky fruit ($\alpha = 0.05$), Tukey HSD.)](image_url)
quality fruit, with regard to phenolic content, anthocyanins, antioxidant capacity, soluble solids and titratable acids. The fruit from the Smoky cultivar tended to be lowest in quality when measured by these parameters and the fruit from the Martin cultivar almost always intermediate between the two. Average size of the pomes, their water content and total yields must still be ascertained before we can complete development of our recommendations. However, all three cultivars have proven to be successful choices on the Reservation and each provides a variation in flavor and sensory appeal. All of the Juneberry fruit quality measurements fell within the range of other health-promoting small fruits such as grapes, strawberries and raspberries and show great potential as a new crop for production at Fort Berthold.

ACKNOWLEDGEMENTS

Funding was provided by grants from the South Dakota Department of Agriculture Specialty Crop Block Grants, the USDA-CREES and the SDSU Agricultural Experiment Station.

LITERATURE CITED


MODEL FOR CLASSIFYING AND MONITORING HACKBERRY (*CELTIS OCCIDENTALIS* L.)–SHRUB ECOLOGICAL TYPE IN SAND HILLS PRAIRIE ECOSYSTEM

Daniel W. Uresk1*, Daryl E. Mergen2, and Jody Javersak3

1USDA-Forest Service
231 East St. Joseph Street
Rapid City, SD 57701
2Colorado State University
Fort Collins, CO 80523
3Mergen Ecological Delineations, Inc.
Colorado Springs, CO 80905
3Sitka, AK 99835
*duresk@fs.fed.us

ABSTRACT

A classification and monitoring system for the hackberry-shrub ecological type was developed based on plant succession in the Sand Hills ecosystem in northern Nebraska and southern South Dakota. Seral stages are quantitatively derived groupings of vegetation composition based on the range of variability within the current ecological type. Multivariate statistical methods determined three key variables that best assign data (sites) to one of four seral stages within the contemporary hackberry-shrub ecological type and represent one of multiple possible stable states. Basal area of hackberry (*Celtis occidentalis* L.), combined canopy cover values of chokecherry (*Prunus virginiana* L.) and wild plum (*Prunus americana* Marsh.), and canopy cover of western snowberry (*Symphoricarpos occidentalis* Hook.) were the key plant variables required for classification and monitoring to determine succession or retrogression. Four seral stages (early to late) were quantitatively identified with an accuracy of 97%. Classification involves a single sample at a site where monitoring trends require multiple seral stage assignment at the same site over time. The assigned seral stages provide resource managers with a quantitative method to evaluate their management objectives by monitoring trend. We list all coefficients required to assign data to seral stage and calculate posterior probabilities, however details of methods for monitoring protocol, seral stage classification, calculation of seral stage probabilities can be viewed at the Forest Service web site http://www.fs.fed.us/rangelands/ecology/ecologicalclassification/index.shtml,

Keywords

Succession, seral stages, woodland, management, state and transition model
INTRODUCTION

Woodlands in the Northern Great Plains represent a very small portion of the area and are often confined to river channels, drainages, swales, or north facing slopes with higher soil moisture. Hackberry (Celtis occidentalis L.) trees represent less than 2-3% (measured by volume) of all tree species found in Nebraska (Meneguzzo et al. 2007). In the Sand Hills, the hackberry ecological type is limited to a small percentage of plant communities found in the Sand Hills prairie ecosystem (Bleed and Flowerday 1990), but are very important for wildlife habitat, biodiversity, livestock cover, and even carbon sequestration (Meneguzzo et al. 2007; Lesica and Cooper 1998; Schmidt 1986; Nudds 1977). Hackberry and the associated shrub species chokecherry (Prunus virginiana L.), wild plum (Prunus americana Marsh.), and snowberry (Symphoricarpos occidentalis Hook.) are all native to Nebraska (Kaul 1990). Hackberry (Celtis) fruit has been found in fossil sediment as early as Eocene (54-38 m.yago) to present and common to abundant during the Miocene (26-7 m.y ago) in northern Nebraska and southern South Dakota (Retallack 1983; Gabel et al. 1998). Hackberry fruits were described as being common to abundant in 78% of the fossil floras (14-19 m.y ago) investigated by Gabel et al. (1998). Hackberry trees are considered a mesic, shade tolerant, late successional tree species (Abrams and Knapp 1986). The hackberry-shrub community is not officially recognized as a terrestrial community in Nebraska, but all community classification systems are subjective, scale based, and possibly poorly studied in a large grassland ecosystem like the Sand Hills (Steinauer and Rolfsmeier 2003). This ecological type is partially contained within two communities in a more recent classification, the snowberry shrubland (early seral stages) and the chokecherry-plum thicket (mid-late seral stages) (Rolfsmeier and Steinauer 2010).

The hackberry-shrub community is one of the few tree-containing communities that are found in upland prairies of the Sand Hills and often found on sides and bottoms of north-facing slopes in areas less than an acre in size (Schmidt 1986) and low slopes, mesic swales, depressions, bottoms of ravines and floodplains (Rolfsmeier and Steinauer 2010). This community type may be currently more common since fire suppression has allowed trees and shrubs to become established in areas predominantly grasslands over the past century (Steinauer and Bragg 1987). Trees and shrubs were observed to increase by 34% in Kansas in the Flint Hills on unburned prairie sites, but soil texture, topography, and distance to seed source were also important (Bragg and Hulbert 1976; Ibanez and Schupp 2002).

Grassland and woodland ecological status undergoes changes over time, following natural and anthropogenic induced disturbances. Sandy soils are erodible and drain quickly and are therefore susceptible to vegetation disturbances like fire, drought, grazing, wind, and wildlife burrows (Loucks et al. 1985; Steuter et al. 1990). Changes to the vegetation condition result in different seral conditions within a community and throughout the landscape (Sousa 1984; Pickett and White 1985). These changes can be quantified using multivariate statistical models of plant succession (MacCracken et al. 1983; Uresk 1990; Benkobi et al. 2007). Plant succession has been used in classification studies for western forests
and rangelands for many years (Sampson 1919; Humphrey 1947; Dyksterhuis 1985; Westoby et al. 1989; Uresk 1990). However, subjective interpretations make it difficult to obtain consistent measurements of vegetation trend. Multivariate quantitative models of plant succession allow resource managers to easily obtain quantitative measurements and relate current condition to management effects at one-time and over long-term on a repeatable basis.

Results of this research provide a multivariate statistical model that can be used by managers to determine seral stage classification within a contemporary tree-shrub ecological type and a method to determine succession and retrogression trends within and among seral stages. In addition, this model can be included in conceptual and working models of multiple stable states (state and transition) to describe vegetation dynamics common where a tree-shrub community can replace or retrogress to a grassland community demonstrating alternative stable states at a single site (Briske et al. 2005; Bestelmeyer et al. 2003). State and transition models are conceptual models that can include vegetation change from fire, climate, management activities that include grazing and succession and also encompass threshold components like structural or functional changes at a single site. Our model consists of an interrelationship from a set of plant species (variables) that best characterizes the ecological type throughout the course of succession within a single stable state. The model also provides managers with quantitative site-specific data and statistically valid sample methodology which have been identified as important for assessment and monitoring (Pyke et al. 2002). The objectives of this study were to (1) develop an ecological classification and monitoring model for the Sand Hills prairie ecosystem hackberry–shrub community and (2) define seral stages.

STUDY AREA

The study was conducted on lands managed by the Nebraska National Forest and emphasized habitat that included woodland vegetation consisting of a hackberry-shrub plant community type in the Sand Hills region that extends into southern South Dakota. The specific study site is located in the north-central part of Nebraska on two areas of the National Forest. The north area is the Samuel R. McKelvie National Forest that encompasses 46,280 hectares (115,700 acres). The south area, the Bessey Ranger District of the Nebraska National Forest, has 36,183 hectares (90,456 acres), with about 24% of the area forested with planted ponderosa pine. The two areas are about 50 miles apart.

Climate is described as semi-arid (Burzlaf 1962). Long-term mean annual precipitation average compiled from three weather stations (Valentine, Halsey, and Nenzel) starting in 1903 was 53 cm (21 in). Annual precipitation for 1988 and 1989 were averaged from the Valentine and Halsey stations (HPRCC 2007). Precipitation is 41 cm (16 inches) in the western Sand Hills to near 61 cm (24 inches) in the southeast part (Farrar 1979). Seventy to 85% percent of the precipitation falls during the growing season (April-Sept.) from short duration intense thunderstorms (Perez et al. 1998). The average monthly temperature
ranges between -45 °C (-48 °F) in the winter to 43 °C (110 °F) in the summer (Farrar 1979).

The Sand Hills flora has been described numerous times over the last century (Pool 1914; Weaver and Albertson 1956; Burzlaff 1962; Stubbdendieck and Tunnell 2008). The descriptions are primarily concerned with grassland communities and production of vegetation in plant communities that represent considerable area. In contrast, the hackberry community occupies a very small area in the grasslands. Although trees are represented as small areas in the upland grassland ecosystem (Jakes and Smith 1982), they often are very important within the ecosystem for specific wildlife species or cover for livestock (Sisson 1970, 1976; Kauffman and Kruger 1984; Hodorff et al. 1988; Bleed 1990; Rumble and Go- beille 1998; Uresk et al. 2009).

METHODS

Data collection and analyses followed Uresk’s (1990) procedures in addition to measuring diameter of all tree species within a 20 m x 40 m macroplot (800 m²). The U.S. Forest Service Permanent Staff, familiar with the project area, selected study sites and collected all data. Sites were selected so that the existing full range of natural variability of the hackberry-shrub woodland type would be collected. All plant species names follow the nomenclature by NRCS Plants database (2010).

Data were collected on 39 macroplots during the summer of 1989. Each macroplot was randomly selected within one of three perceived seral stages (early, mid, late). At each macroplot, two 30 m parallel transects were set 20 m apart. Sampling of canopy cover for individual plant species, plant litter, and bare ground occurring within 0.1 m² (20 cm x 50 cm) microplots following the Daubenmire method (Daubenmire 1959) was completed at 1 m intervals along each transect. Tree diameters were measured with calipers at 4 feet (1.22 m) height in inches within each 800 m² macroplot. All macroplot data (60 microplots) for each site were averaged for individual plant species and for totals of plant cover, litter, and bare soil. Basal area of trees and stem numbers were also averaged for each site. Canopy cover was collected for the most common plant species.

Principal component analysis (Norusis/SPSS Inc. 1992) was used to identify plant variables that contributed various levels to the variation in the data. Additional variables like total grasses, total shrubs, and a combination of species at the genus level were also added to the data set to provide and identify the best variables to include for model selection. The combined basal area and canopy cover percentages of *Prunus* species, the diameter (inches) of all tree species, and combined shrub canopy cover were included in preliminary analyses. Data were then subjected to a non hierarchical cluster analysis, ISODATA, which grouped the 39 sites into 4 distinct cluster groups (seral stages) based on within group similarities and between group differences (Ball and Hall 1967; del Morel 1975; Hall and Khanna 1977).

The greatest possible variation among groups compared to within group variation will result with fewer errors of classification in discriminant analysis when
applying discriminant functions (Afifi and Clark 1990). Therefore, a stepwise discriminant procedure was used to calculate discriminant function coefficients that simultaneously were used to analyze differences among variables for cluster groups (Klecka 1987). Information about numerous independent variables (plant species and basal area) is contained within a single index (SPSS 2003). Fischer classification coefficients (Klecka 1987) were calculated to provide a method to assign (classify) any unclassified case (marcoplot) into a group (seral stage) that it most closely resembled.

RESULTS

Four seral stages (early to late) were defined in the hackberry – shrub ecological type. Seral stages were distinguished from one another by the distribution and abundance of three key plant species that help illustrate the dynamics of these species in the hackberry-shrub ecological type (Figure 1). Four distinct seral stages were identified based on the range of variability of the contemporary vegetation ranging from early to late succession. Results of stepwise discriminant analysis (Norusis/SPSS Inc. 1992) showed significant differences among these seral stages \( (P < 0.05) \). Basal area of hackberry, percent canopy cover of combined \textit{Prunus} species and western snowberry are the best variables to be used in the classification and monitoring model (Table 1).

The late hackberry seral stage represents sites with greatest basal area of hackberry trees and other variables (Table 2). Late seral sites also had the greatest percent of bare soil and the lowest percent cover of litter, total grasses, and total shrubs compared to the other seral stages (Figures 1 and 2). These differences are partially explained by livestock use of the shaded habitat under the mature hackberry trees. Over time shading may also reduce understory development of shrubs, grasses, and forbs. The late-intermediate stage had the greatest canopy cover of the \textit{Prunus} category (chokecherry + plum) and greater cover of shrubs compared to the other stages (Table 2).

This late-intermediate stage was similar in amount of litter and had twice the total grass canopy as the late seral stage. Some of the observed differences in the late and late-intermediate seral stages may be partially influenced by the low number of samples, 3 and 4 respectively, in each of these groups, compared with 21 and 11 samples in the early-intermediate and early seral stages. These two late seral stages had less percent cover of bluegrass (\textit{Poa} spp.), little bluestem (\textit{Schizachyrium scoparium}), and the forb starry false Solomon’s seal (\textit{Smilacina stellata}) compared to the earlier seral stages.

Tree numbers were the same in the late and early intermediate stage compared to the other stages, but diameters were much greater in the late sere. Percent canopy cover of litter was nearly double in the earlier two stages compared to the later two stages and bare ground decreased greatly from the late to late intermediate stage (Table 2, Figure 2).

The early seral stage represents the stage of this type where the transition from a grassland community to an early tree-shrub community has started. This state of the system has an increase of shrub cover, primarily western snowberry. Total
Table 1. Fisher’s classification discriminant function coefficients used for classification of seral stages in Nebraska National Grasslands hackberry-shrub ecological type.

<table>
<thead>
<tr>
<th>Species</th>
<th>Late</th>
<th>Late intermediate</th>
<th>Early intermediate</th>
<th>Early</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal area <em>Celtis occidentalis</em></td>
<td>0.8249947</td>
<td>-0.08342129</td>
<td>0.02235409</td>
<td>0.07811445</td>
</tr>
<tr>
<td>Canopy cover <em>Prunus</em> (P. virginiana + P. americana)</td>
<td>-0.02100902</td>
<td>0.5371982</td>
<td>0.1850731</td>
<td>0.07539636</td>
</tr>
<tr>
<td>Canopy cover <em>Symphoricarpos occidentalis</em></td>
<td>0.1436558</td>
<td>-0.1181865</td>
<td>0.09415513</td>
<td>0.6525852</td>
</tr>
<tr>
<td>Constant</td>
<td>-22.97096</td>
<td>-18.64775</td>
<td>-3.968076</td>
<td>-12.87400</td>
</tr>
</tbody>
</table>

Table 2. Means and standard errors (in parentheses) of most common plant species or species characteristic (% canopy) among four seral stages in Nebraska National Grasslands hackberry-shrub ecological type.

<table>
<thead>
<tr>
<th>Species (% cover) or variable</th>
<th>Late</th>
<th>Late intermediate</th>
<th>Early intermediate</th>
<th>Early</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal area hackberry(ft•a⁻¹) *</td>
<td>51.1(4.8)</td>
<td>0.1(0.1)</td>
<td>3.4(1.3)</td>
<td>6.8(3.3)</td>
</tr>
<tr>
<td>Tree numbers</td>
<td>6.5(2.8)</td>
<td>2.0(2.0)</td>
<td>6.6(2.7)</td>
<td>3.8(2.8)</td>
</tr>
<tr>
<td><em>Prunus</em> species combined</td>
<td>7.3(5.3)</td>
<td>64.3(10.3)</td>
<td>24.1(1.8)</td>
<td>17.2(4.3)</td>
</tr>
<tr>
<td>Wild plum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Prunus americana</em></td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>6.7(2.1)</td>
<td>3.8(1.4)</td>
</tr>
<tr>
<td>Chokecherry</td>
<td>7.3(5.3)</td>
<td>64.3(10.3)</td>
<td>17.0(3.0)</td>
<td>13.3(4.1)</td>
</tr>
<tr>
<td>Snowberry</td>
<td>8.2(3.3)</td>
<td>0.0(0.0)</td>
<td>6.6(1.4)</td>
<td>32.4(2.6)</td>
</tr>
<tr>
<td>Bluegrasses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Poa</em> species</td>
<td>0.0(0.0)</td>
<td>0.8(0.8)</td>
<td>6.9(3.7)</td>
<td>9.2(4.0)</td>
</tr>
<tr>
<td>Little bluestem</td>
<td>0.8(0.8)</td>
<td>1.0(1.0)</td>
<td>4.0(1.7)</td>
<td>3.1(2.4)</td>
</tr>
<tr>
<td><em>Schizachyrium scoparium</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starry false Solomon’s seal</td>
<td>0.1(0.1)</td>
<td>1.7(1.7)</td>
<td>1.3(1.0)</td>
<td>1.4(1.1)</td>
</tr>
<tr>
<td><em>Smilacina stellata</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rose species</td>
<td>0.3(0.2)</td>
<td>1.7(1.2)</td>
<td>2.5(0.8)</td>
<td>3.5(1.4)</td>
</tr>
<tr>
<td>Total Shrubs</td>
<td>21.4(6.2)</td>
<td>77.1(5.4)</td>
<td>56.9(4.1)</td>
<td>71.1(4.1)</td>
</tr>
<tr>
<td>Total Grasses</td>
<td>11.1(2.2)</td>
<td>24.7(14.5)</td>
<td>28.6(5.2)</td>
<td>42.4(3.1)</td>
</tr>
<tr>
<td>Total Forbs</td>
<td>16.8(5.4)</td>
<td>6.5(3.8)</td>
<td>14.1(2.4)</td>
<td>11.7(2.6)</td>
</tr>
<tr>
<td>Litter</td>
<td>30.2(4.4)</td>
<td>34.6(6.5)</td>
<td>66.5(5.8)</td>
<td>67.1(6.3)</td>
</tr>
<tr>
<td>Bare Ground</td>
<td>60.9(6.6)</td>
<td>13(8.1)</td>
<td>6.5(3.8)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

* To convert from ft²•acre⁻¹ to m²•ha⁻¹ multiply basal area by 0.229.
grass canopy decreases from about 40% in the early stage to near 10% in the late stage. The changes in canopy cover, basal area of hackberry, and bare ground and litter can be seen in Figures 1 and 2.

An examination of the magnitude of the canonical discriminant function coefficients (Klecka 1987) indicated the relative importance that 3 key variables had among groups within each function (Table 3). Basal area of hackberry was most important in function 1 and explained 54% of the total variation. Cover of western snowberry was important in function 2, followed by hackberry basal area, and this function explained 40% of the total variation. Function 3 explained 6% of the total variation, and the combined cover of *Prunus* species (chokecherry + wild plum) was the most important variable in the final function (Table 3).

Each key variable is weighted as a separate function that reflected the biotic contribution of each key plant variable in characterizing vegetation dynamics within this ecological type, and the magnitude of the Fisher's classification function coefficients indicated the contribution that each variable had among seral stages (Table 1). Mean values of each variable can be viewed graphically in Figure 1. Fisher’s classification discriminant function coefficients represent the equations that are easily calculated to define seral stages only (Table 1) (see Benkobi and Uresk 1996 for an example of the calculation). Posterior probabilities, calculated from the unstandardized canonical coefficients, group centroids, and Fisher’s classification function coefficients, indicate not only the most likely seral

![Figure 1. Means of 3 key plant species variables through four stages of succession in a hackberry-shrub ecological type. Smoothed lines connect the means of each key variable at each stage and provide an approximate representation of the mixture of key species at each seral stage. To convert from ft^2 acre^-1 to m^2 ha^-1 multiply basal area by 0.229.](image)
stage, but also the location of a sample site within a seral stage. The magnitude of the posterior probabilities (between 0 and 1) calculated for any combination of key variable values is used to determine if a site is at a seral stage center or between two seral stages. The result of the discriminant analysis model presented in this paper (Tables 1 and 3) defines seral stages and provides quantitative information about successional trend within and among seral stages. This model can be used easily to assign sites to seral stages. Excel based spreadsheets can be downloaded from the Forest Service web site (www.fs.fed.us/rangelands/ecology/ecologicalclassification/index.shtml) and used as both data sheet and a tool to provide all required calculations. All coefficients required to assign data to seral stage and calculate posterior probabilities are listed in Tables 1 and 3.

DISCUSSION

The ability to identify seral stages and relate them to prescribed management activities is essential for resource managers to achieve a desired seral stage.
The developed statistical model related to the hackberry-shrub succession, which was based on ecological concepts of plant succession (Clements 1916; Dyksterhuis 1949; Daubenmire 1968), allows the determination of seral stages, thus providing a useful tool for resource managers with field measurements of only a few key variables (Table 1). The current model was developed using data collected from a contemporary ecological type and thus can be used to determine seral stages regardless of hypothetical past or future climax vegetation. It is more realistic to monitor existing ecological types based on key plant species currently present for grazing and wildlife management purposes than attempt to predict hypothetical past or future climax vegetation. Therefore, a range of seral stages (early to late) and dominant plant species collected during sampling were used in a sequence of multivariate statistical analyses to determine the key plant species that best characterizes the seral stages of the hackberry – shrub ecological type.

(Dyksterhuis 1985; Uresk 1990). Monthly precipitation was above average the year before data collection (1988) and much below compared to the long-term average of the region in 1989 during data collection. Average monthly precipitation for the Nebraska locations compared to long-term monthly data for 1988 show above average precipitation (106-380% greater) in all but four months. Monthly precipitation for 1989 represented 12% - 92% below the long-term average for all months except January and February of 1989, which were 35% greater and equal, respectively (HPRCC 2007). This probably had some effect on annuals and herbaceous vegetation, but less measurable impacts on the trees and shrub species selected as key variables for the model.
State and transition models of plant succession have received much attention as a method to describe ecological processes and transitions from multiple possible stable states at a single site (Briske et al. 2005). The hackberry–shrub multivariate statistical model provides a quantitative process and method to assign seral stages at a given site over time within a contemporary ecological type to measure succession. Natural processes (drought, wildfire, wildlife burrows and others) and management activities (livestock grazing, prescribed burns, fire suppression and others) as they impact vegetation at a site can be monitored using the model and defined seral stages. This type of plant community succession model fits well into the state and transition conceptual model.

The greater canopy cover of western snowberry in the early stage of this ecological type may be the results of fire suppression or other environmental change and/or management prescription. Once snowberry or other shrubs become established and begin to increase, then the site condition has crossed or begins to cross a structural threshold and begins to change from grassland site to shrub-woodland site. Depending on site conditions and management, the site progresses toward woodland, remains static as a snowberry shrubland, or reverts back to grassland. The establishment of snowberry or other shrubs alters the succession process by altering the grassland microclimate and enables the growth and establishment of *Prunus* species and hackberry saplings. Snowberry canopy in the early seral stage can triple the amount of aboveground biomass and increase the mass of large roots at a site (Bai et al. 2009). The shrub canopy increases, and high levels of litter in the early seral stages may inhibit or enhance emergence survival of some tree and shrub species (Ibanez and Schupp 2002). The establishment of shrubs and the greater litter cover in the early seral stages can change the chemical and physical condition of the microhabitat (Carson and Peterson 1990; Paez and Marco 2000). These changes may include an increase in mineral and nutrient availability from greater mycorrhizal fungus activity, an increase in available soil moisture and decreases in soil temperature, light levels, evaporation, and evapotranspiration at a site. All of this can affect seedling germination and survival (Facelli and Pickett 1991a, 1991b; Ibanez and Schupp 2002).

The hackberry-shrub type provides a food source for many mammal and bird species, provides nesting cover for many prairie bird species, and provides shade and protective winter cover for wildlife and livestock (Martin et al. 1961; Severson and Boldt 1978; Uresk 1982; Kauffman and Krueger 1984; Lesica and Cooper 1998). The different structural stages of this type may offer various thermal regimes and the ability to intercept and alter snow accumulation. The vertical structure is also important for numerous wildlife species, like deer for bedding, or forage areas (Nudds 1977; Rumble and Gobeille 1995; Rumble and Gobeille 1998) for birds (Hodorff et al. 1988; Bleed 1990; Fritcher et al. 2004; Uresk et al. 2009).

Sharptail grouse are an important ground nesting game bird in the Nebraska Sand Hills ecosystem and prefer dense cover of shrubs, grasses and forbs (Hillman and Jackson 1973; Sisson 1970; Prose 1987; Meints et al. 1992) and depend on grass-dominated habitats intermixed with patches of trees and shrub cover year round (Connelly et al. 1998). Patches of tree and shrub cover provide
sharp-tailed grouse food and protective winter cover (Sisson, 1976; Oedekoven 1985; Meints 1991; Giesen and Connelly 1993). McDonald (1998) in eastern Washington found litter cover and visual obstruction were greater at nest sites and bare ground was less at nest sites compared to random sites. Litter cover at successful nest sites was greater than 80% compared with near 70% observed in the early seral stages of the hackberry-shrub type. Therefore, maintaining different seral stages within the habitat type and providing some hackberry-shrub ecological type throughout the landscape are important for managers in order to provide year-round habitat for multiple species.

This model was specifically developed on National Forest System lands within boundaries of the Nebraska National Forest and limited to the hackberry – shrub ecological type. It is important that the same ecological type be present when collecting data. Verification testing of this model should be completed when using this model beyond National Forest System lands.

ACKNOWLEDGEMENTS

This study was completed with cooperation of the Nebraska National Forest System and Colorado State University, Department of Forest, Rangeland and Watershed Stewardship (28-CR3-752 and 03-JV-1221609-272). D. Mergen was Research Associate working under these agreements with Colorado State University. Special thanks to the late Dr. Dennis Child. Thanks are also extended to Mr. Rudy King, Rocky Mountain Research Station statistician, for his efforts over a twenty-year period to develop a statistical protocol for seral stage model development. Additional thanks to Carin J. Corley for preparation of tables and figures, and Mr. Dave Stricklan for his efforts with project development, data collection, and discussions during model development. Thanks for review are extended to Dr. Chuck Bonham and Mr. Gerry Steinauer.

LITERATURE CITED


Sisson, L.H. 1970. Vegetational and topographic characteristics of sharp-tailed grouse habitat in Nebraska. Project W-38-R-3, Nebraska Game and Parks Commission, Lincoln, NE.

Sisson, L.H. 1976. The sharp-tailed grouse in Nebraska. Nebraska Game and Parks Commission, Lincoln, NE.


ABSTRACT

The objectives of this study were to develop a multivariate statistical model related to plant succession for boxelder ecological type (Acer negundo-Celtis occidentalis-Symphoricarpos occidentalis) to classify plant seral stages for monitoring resource changes based on management or environmental stresses in northwestern Nebraska. The developed boxelder classification and monitoring system was based on plant succession. Multivariate analyses and methods were used to determine key plant variables that best predict seral stages. Key plant variables were boxelder basal area, hackberry basal area, and percent canopy cover of snowberry. Four seral stages were quantitatively defined with a 96% accuracy of seral classification. These seral stages give resource managers four options to evaluate management alternatives and objectives. However, all stages must be present to maintain plant and animal diversity. In addition, plant and animal species diversity, livestock grazing and relationships to seral stages can be related to each of the four seral stages. This tool provides a simple, reliable, repeatable, accurate, and cost effective (time saving) alternative for classification of sites and for monitoring changes between and within seral states.

Keywords
modeling, succession, trees, seral stages, monitoring trends

INTRODUCTION

Little attention has been given to Boxelder (Acer negundo L.) ecological type (Acer negundo-Celtis occidentalis-Symphoricarpos occidentalis) for monitoring and seral stage classification. The sustainability of boxelder has been a concern for land managers. Boxelder is primarily found within deciduous woodlands confined to riparian drainages and upland draws on the Northern Great Plains and occupies less than 1% of the area (Bjugstad 1978). Maeglin and Ohmann (1973) present a review of boxelder which shows that it has a wide range of distribution throughout most of the temperate world. Boxelder is generally more
abundant in areas of greater soil moisture, but can also occupy drier sites (Mac-Cracken et al. 1983; Girard et al. 1989). Boxelder may be common to rare in woodlands and grows in soils ranging from heavy clays to sandy soils.

Over the past few decades, the concepts of seral classification have given resource managers a framework to evaluate the response of plant communities to both current management and natural events. State and transition models have received much attention for describing plant succession. The model that we developed is similar in concept and quantitatively defines discrete categories based on a multivariate statistical analysis of fundamental key variables to define plant community phases within a state and transition model of plant succession (Mac-Cracken et al. 1983; Uresk 1990; Benkobi et al. 2007). The boxelder ecological model defined by ecological plant seral stages reported in this research provides resource managers a statistically accurate quantitative tool for measuring effects of resource management and/or natural events such as drought or disease. This model is based on the interrelationships of key variables that best describe the ecological type throughout the range and variability of plant succession. The objectives of this study were to (1) develop a model for monitoring the boxelder ecological type in northwest Nebraska, (2) define seral stages and (3) provide sampling and monitoring protocols with management implications.

STUDY AREA AND METHODS

This study was conducted on the Nebraska National Forest near Chadron in wooded draws and riparian areas. Trees in this woodland type are boxelder, hackberry (*Celtis occidentalis*), green ash (*Fraxinus pennsylvanica*) and cottonwood (*Populus deltoides*). Common shrubs included snowberry (*Symphoricarpos occidentalis*) and chokecherry (*Prunus virginiana*). Grasses, sedges, and forbs were common in the understory, but data were not collected for herbaceous species.

Data collection for canopy cover followed (Daubenmire 1959) and analyses followed procedures outlined by Uresk (1990). Data were collected on 28 macroplots randomly selected within three perceived seral stages; early, mid-, and late. Each macroplot was 20 m x 40 m with an area of 800 m$^2$. Some sites that were narrow required the use of two 10 m x 40 m sub-plots that were combined as one site for analyses and classification. At the macroplot boundaries, two 30-m transects were established for canopy cover. A single transect was established at the macroplot boundary for each of the two sub-plots (10 m x 40 m) for the narrow sites. Canopy cover for the major shrub species was sampled at 1-m intervals along each 30-m transect following methods by Daubenmire (1959). Diameter at breast height (DBH) of trees greater than 2.54 cm (1 in) in diameter and number of stems were recorded for the 800 m$^2$ macroplots. All macroplot data (28 sites) were averaged for each site for shrub and tree species. A total of 16 variables were collected.

Principal component analysis identified variables that accounted for much of the variation in the data; these variables were selected for further cluster and discriminant analyses. Data were then subjected to a nonhierarchical cluster analysis using ISODATA (for standardized data) which grouped the variables

into seral stages (Ball and Hall 1967; del Moral 1975). Stepwise discriminant analysis (SPSS 2003) identified important variables for seral stage classification and produced a quantitative model that can be used for future classification and monitoring ($P < 0.05$). Misclassification error rates were estimated using cross validation procedures (SAS 1988). We field tested the model by collecting additional data during the second year.

RESULTS

The boxelder ecological type was classified into four distinct seral stages ranging from early to late and was different between and among the groups ($P < 0.001$). This model was best defined by these four seral stages and by three variables: basal area of boxelder, basal area of hackberry, and percent canopy cover of snowberry (Figure 1). Boxelder is dominant in the late seral stage, hackberry in the late intermediate stage, and snowberry dominates in an early intermediate stage. Lesser amounts of all three species describe the early seral stage. Mean basal area of trees, number of tree stems, and canopy cover for snowberry are presented by seral stage in Table 1.

Fisher’s discriminant function coefficients define the seral stages (Table 2). Variables with the greatest coefficient weights by seral stage reflect the biotic potential of each key plant species in predicting dynamics within the ecological system. An example of seral stage assignment is presented in Table 3. Multiplying the mean site values of boxelder and hackberry basal area and percent snow-

![Figure 1. Boxelder ecological type variables by species through 4 seral stages in Northwestern Nebraska (adapted from Uresk et al. 2010).](image-url)
Table 1. Mean basal area (BA = ft²/acre), stems per acre and canopy cover (%) by seral stage based on three variables boxelder, hackberry and snowberry. n = sample size, mean ± standard error.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Late n=9</th>
<th>Late Intermediate n=4</th>
<th>Early Intermediate n=10</th>
<th>Early n=5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boxelder (BA)</td>
<td>58 ± 4</td>
<td>29 ± 15</td>
<td>18 ± 5</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>Hackberry (BA)</td>
<td>8 ± 2</td>
<td>47 ± 6</td>
<td>11 ± 4</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Boxelder (stems/a)</td>
<td>28</td>
<td>7</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Hackberry (stems/a)</td>
<td>2</td>
<td>15</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Snowberry (%)</td>
<td>6 ± 2</td>
<td>8 ± 8</td>
<td>32 ± 3</td>
<td>3 ± 2</td>
</tr>
</tbody>
</table>

1BA * 0.229 = m²/ha
2stems * 2.47 = stems/ha

Table 2. Fisher’s classification coefficients for ecological seral stages for boxelder ecological type in western Nebraska.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Late</th>
<th>Late Intermediate</th>
<th>Early Intermediate</th>
<th>Early</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boxelder (BA)</td>
<td>0.260</td>
<td>0.124</td>
<td>0.130</td>
<td>0.028</td>
</tr>
<tr>
<td>Hackberry (BA)</td>
<td>0.067</td>
<td>0.500</td>
<td>0.124</td>
<td>0.038</td>
</tr>
<tr>
<td>Snowberry (%)</td>
<td>0.168</td>
<td>0.147</td>
<td>0.419</td>
<td>0.048</td>
</tr>
<tr>
<td>Constant</td>
<td>-9.636</td>
<td>-15.351</td>
<td>-10.095</td>
<td>-1.608</td>
</tr>
</tbody>
</table>

1BA = ft²/a * 0.229 = m²/ha, % canopy cover

The ability to identify seral stages and relate them to prescribed management over time by measuring just three plant variables provides resource managers a powerful tool to evaluate and monitor conditions (Uresk 1990; Benkobi et al.)

DISCUSSION

As an alternative method for calculating seral assignment, Uresk et al. (2010) used canonical discriminant function coefficients and mean canonical coefficients (group centroids) for placement of posterior probabilities by seral stage and assignment of seral stage for new data. The seral stage assigned to a set of plant measurements is always associated with the greatest probability value. Details are available online at: http://www.fs.fed.us/rangelands/ecology/ecological-classification/index.shtml.
Knowledge of hypothetical climax vegetation or a reference plant community is not required with an ecological model when managers evaluate ecological types for management options. Selection of the three key plant variables for the model was quantitative and free from subjective information. Thus, for management purposes and monitoring, measuring the three plant variables is accurate and cost effective, providing a quantitative estimate of the system. Additional information and details on seral classification and monitoring may be obtained from Uresk et al. (2010).

State and transition models have received much attention during the past several years as an approach for describing the ecological processes for plants (Briske et al. 2005). The classification model developed for boxelder can be incorporated into state and transition models since the indicator species are diagnostic for differentiating seral stages or community phases. Our boxelder model is based on quantitative data collected throughout the existing range of succession for this vegetation type, and provides discreet categories along a continuum based on fundamental ecological processes. These discreet categories relate to plant community phases (seral stages) and can be incorporated into a state and transition model for this ecological type. Processes of plant succession can be at a steady state, continuous through succession, reversible or defined as at a transition based on data collected for this model (Uresk 1990; Benkobi and Uresk 1996; Benkobi et al. 2007). Management objectives can be defined and results can be monitored within the context of defined seral stages or community phases.

Boxelder ecological type is uncommon on the northern Great Plains, but trees are found throughout the plains, generally within green ash woodlands (Maeglin and Ohmann 1973; MacCracken et al. 1983; Girard et al. 1989). MacCracken et al. (1983) found boxelder stem densities ranging from 260•ha\(^{-1}\) (105•a\(^{-1}\)) to 524•ha\(^{-1}\) (212•a\(^{-1}\)) within green ash woodlands in southeastern Montana. Girard et al. (1989) reported boxelder densities of 58•ha\(^{-1}\) (24•a\(^{-1}\)) in southwestern North Dakota. In the current study, by seral stage, mean stem densities ranged from 5•ha\(^{-1}\) (2•a\(^{-1}\)) to 144•ha\(^{-1}\) (58•a\(^{-1}\)). These lower stem densities in our study suggests that this area is a drier site than the area in southeastern Montana.

These woodlands are very important for wildlife including large and small mammals and non-game birds. In northeastern Montana, where boxelder was abundant, mule deer (Odocoileus hemionus) use was greatest during spring and
summer while white-tailed deer (*O. virginianus*) use was primarily during the winter months (MacCracken and Uresk 1984). MacCracken et al (1985) further reported on rodent abundance within these same woodlands. Relative rodent abundance was greatest for deer mice (*Peromyscus maniculatus*), followed by meadow vole (*Microtus pennsylvanicus*). *Microtus* spp. were significantly correlated with boxelder (*P < 0.001*). Boxelder woodlands are important for nesting and maintaining species diversity for non-game birds on the Northern Great Plains (Walcheck 1970; Rumble and Gobeille 1998; Emmerich and Vohs 1982; Faaness 1984). Bird species richness varied by seral stages in green ash woodlands (with boxelder present) from early to a late seral stage (Rumble and Gobeille, 1998). To maintain plant and animal diversity in a boxelder ecological type, all seral stages must be available and managed (Benkobi and Uresk 1996). Presence of an early seral stage may require additional management for boxelder; trees can disappear as a result of impacts of herbivore use and lack of regeneration (Boldt et al. 1978; Smith and Flake 1983; Uresk and Boldt 1986; Lesica 2009; Uresk et al 2009).

ACKNOWLEDGMENTS

This study was completed with cooperation and support of the Nebraska National Forest System and Colorado State University, Department of Forest, Rangeland and Watershed Stewardship (28-CR3-752 and 03-JV-1221609-272). Special thanks to the late Dr. Dennis Child. Daryl Mergen was research associate, Colorado State University, working under these agreements and located in Colorado Springs, CO. Jody Javersak conducted validation sampling and analyses while employed by Rocky Mountain Research Station. Rudy King, Rocky Mountain Research Station statistician, provided invaluable advice throughout the study. Thanks are also extended to Rick Peterson and the staff of the Nebraska National Forest for help with data collection.

LITERATURE CITED


LANDSCAPE CONTENT IN RELATION TO
BIRD SPECIES OCCURRENCE IN SAGEBRUSH
HABITATS OF NORTH AND SOUTH DAKOTA

Amy R. Lewis* and Kenneth F. Higgins
1Department of Wildlife and Fisheries Sciences
South Dakota State University
Box 2140B, Brookings, SD 57007
(Currently: Department of Biology
Augustana College
2001 S. Summit Ave
Sioux Falls, SD 57197)
2U.S. Geological Survey
South Dakota Cooperative Fish and Wildlife Research Unit
South Dakota State University
Box 2140B, Brookings, SD 57007
* amy.lewis@augie.edu

ABSTRACT

Sagebrush lands in the western Dakotas are the eastern edge of the sagebrush ecosystem, forming a transition zone from the shrubsteppe of Wyoming and Montana to the mixed-grass prairie of the Dakotas. Because this transition zone is different from the two abutting ecosystems, the landscape characteristics are unique and the avian associations consist of species from both grassland and shrubsteppe. We examined percent composition of habitat types within three landscape-scale buffers centered on transects surveyed for avian species. In 2001-2002, avian occurrence and percent habitat composition were determined on 295 sites in North and South Dakota. Percent habitat composition characteristics were summarized from landscape-scale buffers with diameters of 400, 800, and 1600 m centering on bird survey transects. Landscape characteristics were unrelated to bird species, except for the Horned Lark (Eremophila alpestris) and Brewer’s Sparrow (Spizella breweri). Analyses revealed that Horned Lark occurrence was negatively associated with increasing proportions of hayfields, whereas Brewer’s Sparrow occurrence increased with increasing proportions of pasture with sagebrush. Results of this study show that one conservation plan will not work for all species in an area; the needs of each species must be carefully studied. In addition, recommendations for one geographic area or ecosystem should not necessarily be implemented in other areas.

Keywords

avian occurrence, landscape composition, sagebrush, North Dakota, South Dakota
INTRODUCTION

Sagebrush (Artemisia spp.) habitats in the Dakotas occur at the eastern edge of the sagebrush ecosystem in the United States, forming a transition zone from the sagebrush shrubsteppe of Wyoming and Montana to the mixed-grass prairie that dominates most of the Dakotas. This transition area has not been characterized, either vegetatively or in terms of the avian species that occupy this landscape-scale “edge” environment.

Various human activities have significantly altered the native prairie grasslands of the Great Plains (Herkert 1995). Whether due to drought or grazing (Branson 1985), herbicides (SDOU 1991), conversion to agriculture (Higgins et al. 2002), exotic species invasion, road building (Igl and Johnson 1997), or energy development (Doherty et al. 2008; Walker et al. 2007), the fragmentation and degradation of the sagebrush shrubland of the United States has been extensive, with estimates of sagebrush loss ranging from 10% across the west (Baker et al. 1976) to more than 50% in Washington and 70% in Idaho (Welch 2005).

Roughly 60% of the mixed grass prairie in South Dakota, North Dakota, and Montana has been converted to tillage agriculture (Higgins et al. 2002). Similar to sagebrush habitats, landscape-scale changes in the grassland habitats of the western Dakotas are the result of various grazing practices, introduction of exotic grasses and forbs, construction of stock ponds for cattle, and conversion to agriculture (SDOU 1991). All of these changes have the potential to alter the avian assemblages of shrubland and grassland habitats in the western Dakotas.

Grassland bird populations of the Midwest have shown steeper, more consistent population declines than other groups of birds (Herkert 1995), and yet grassland species have received far less attention than forest nesting birds (Igl and Johnson 1997), and shrubland species even less. Information regarding the population status of shrubland birds is scarce (Baker et al. 1976), sometimes being combined with either “grassland” or “early-successional forest” categories (Herkert 1995), where the effects of their population changes have been diluted. Many bird species commonly found in the shrublands of western North and South Dakota are also found often in grasslands, so it may be that shrubland birds are declining in a manner similar to grassland species. Although there are numerous publications examining the community dynamics of shrubland birds in the core of shrubsteppe habitat distribution (e.g. Rotenberry and Wiens 1998; Knick and Rotenberry 1995; and Rotenberry and Wiens 1980), to date, there have been no studies examining the bird communities that inhabit the sagebrush shrublands of North and South Dakota and the effects of the loss of their specific habitats on their populations.

When trying to explain the habitat use of animal species in an area, researchers often use local variables, such as height of vegetation or percent cover of vegetation types. However, fragmentation of the surrounding landscape can affect birds in several ways, including presence/absence of species, density of individuals, and nesting success (Bakker et al. 2002; Winter and Faaborg 1999). In the northern Great Plains, recent studies of bird species have included landscape variables to try to improve our ability to predict habitat use. Smith et al. (2005)
found that fragmentation, in the form of tilled land, had increased in the vicinity of abandoned Greater Sage Grouse (*Centrocercus urophasianus*) leks. Bakker et al. (2002) found that certain grassland obligate species reacted positively to a greater amount of grassland within landscape buffers, and negatively to a greater amount of wooded habitat; others were affected only by local habitat variables. Winter et al. (2006) found bird density was influenced by patch size, landscape, region, and local vegetation, but that the magnitude of the response to these factors differed by species, among regions, and among years.

In regard to sagebrush obligate species, Knick and Rotenberry (1995) found that fragmentation and human disturbance in shrubsteppe habitats significantly affected such obligate species as Sage Sparrow (*Amphispiza belli*), Brewer’s Sparrow (*Spizella breweri*) and Sage Thrasher (*Oreoscoptes montanus*). Brewer’s and Sage Sparrows in Oregon spent more time in large patches, and in patches with large, vigorous sagebrush (Rotenberry and Wiens 1998). Tweit (2000) claimed that the main problem for sage grouse was the fragmentation and reduction of sagebrush shrubland area. In general, mosaics of uneven-aged, native communities within large tracts of sagebrush have been suggested to provide suitable habitat for sagebrush birds (Paige and Ritter 1999). Conservation of shrubland bird species may rely on the conservation not only of nesting sites, but of landscapes that promote movement among populations, facilitating genetic exchange. The objective of this study was to examine the relationships that exist between the occurrence of avian species and the percent composition of major habitat types within landscape-scale buffers in North and South Dakota.

**STUDY AREA**

This study was conducted in Fall River, Butte, and Harding counties of western South Dakota (SD) and Bowman, Slope, and Golden Valley counties of southwestern North Dakota (ND) (Figure 1). This region extends approximately from 43°N to 46° 50’ N latitude, excluding the Black Hills of South Dakota, and from 102° 55’ W to 104° 05’ W longitude. Elevations in the study counties range from 640 m in Golden Valley County to 1223 m in Harding County (Aziz 1989; Johnson 1988; Kalvels 1982; Thompson 1978; Johnson 1976;; Opdahl et al. 1975). Most of the South Dakota sagebrush study area consisted of privately-owned grazing land (although some National Grassland in Fall River County), with inclusions of cropland on the eastern edge. The North Dakota study area consisted of a mixture of privately- and publicly-owned rangeland (mostly grazed) and privately-owned cropland in the southern portion, and predominantly badlands formations in the northern portion. Of the 200 study sites surveyed in SD, 57 were in Butte County, 54 were in Fall River County, and 89 were in Harding County. In ND, 45 of the 95 study sites were in Bowman County, 48 were in Slope County, and two were in southern Golden Valley County. Western South Dakota and southwestern North Dakota commonly are classified as mixed-grass prairie (Brown 1985; Rotenberry and Wiens 1980); however, Johnson and Larson (1999) characterized the area for this study as Wheatgrass-Big Sagebrush Plains containing scattered to fairly dense dwarf
shrubs and dominated by western wheatgrass (*Pascopyrum smithii*), big sagebrush (*Artemisia tridentata Wyomingensis*), Junegrass (*Koeleria macrantha*), Sandberg’s bluegrass (*Poa secunda*), and needleandthread (*Stipa comata*).

**METHODS**

**Bird Surveys**—One bird survey was conducted on each study site during each field season. Use of detection probabilities was deemed unnecessary due to the low, relatively sparse vegetation found on the majority of the study sites. Bird surveys began between 15 minutes before sunrise and 3 hours after sunrise.

*Figure 1. Gray-shaded counties indicate study area in North and South Dakota: from the bottom, the counties are: Fall River (SD), Butte (SD), Harding (SD), Bowman (ND), Slope (ND), and Golden Valley (ND). Dots indicate study sites.*
Surveys were not conducted when winds exceeded 19 kph or during heavy rain events. A fixed-width (50 m) belt transect survey was used to determine presence or absence of species (Wakely 1987). Transects were at least 30 m from the edge of each sagebrush patch. Global positioning system coordinates of each transect starting point and a compass reading of the direction of each transect were noted for future reference (Lewis 2004).

Each survey transect was 100 m long and was walked at a rate of approximately 1.6 kph. All birds detected (seen or heard) within 50 m of either side of the transect centerline were recorded. To avoid duplication of data, we were careful to avoid recording individual birds more than once. Thirty-four bird species were detected on surveys over the course of the study, but only six species were detected on > 10% of the sites: Western Meadowlark (*Sturnella neglecta*) (73%), Grasshopper Sparrow (*Ammodramus savannarum*) (27%), Brewer’s Sparrow (24%), Lark Bunting (*Calamospiza melanocorys*) (23%), Vesper Sparrow (*Poecetes gramineus*) (17%), and Horned Lark (*Eremophila alpestris*) (16%). The data related to these six species are the basis for the analyses reported in this paper.

Quantification of Landscape Variables—The locations of study sites were overlaid on landcover images after which buffered landscape areas were clipped out. The buffered landscape areas consisted of 400-, 800-, and 1600-m diameter circles centered on the midpoint of the bird survey transects. Due to overlap of buffer areas, a number sites had to be eliminated from the analyses, reducing the total number of sites for each analysis; for 400-m buffers n = 226, for 800-m buffers n = 195, for 1600-m buffers n = 168. Using the landcover images to help identify land features and boundaries between cover types, we revisited each site and visually classified the landcover types within the area of the buffers by dominant vegetation or habitat. We calculated percent coverage for the following landcover types: pasture with sagebrush, pasture with sagebrush and greasewood (*Sarcobatus vermiculatus*) (neither shrub noticeably dominant), pasture with greasewood only, pasture without sagebrush or other shrubs, hayfield (ungrazed), tillage agriculture, and other use (including urban development and water). Because they were found in very few buffers (particularly the 400-m buffers), pasture with sagebrush and greasewood and pasture with greasewood only were grouped with pasture with sagebrush for certain analyses, under the more general category pasture with shrubs. Proc CORR (SAS Institute 1999) was used to determine whether the percentages of each landcover type were correlated among the three buffered landscape areas.

Statistical Methods—Analysis of variance (ANOVA) (SAS Institute 1999) was used to evaluate whether percent landscape composition of the buffered areas was associated with the presence or absence of bird species. Levene’s test for homogeneity of variance among group means was used to test for inequality of variances. When Levene’s test revealed variance inequality, we tested the ratio of largest variance to smallest variance. If the ratio was not more than three, the variances were considered homogeneous and the ANOVA F-test was deemed appropriate (Gomez and Gomez 1984). In cases where the ratio was greater than
three, arcsine transformations or Kruskal-Wallis nonparametric tests were used. Results with a $P$-value less than 0.05 were considered to be significant.

**RESULTS**

The percent landscape composition of the habitat types in the 400, 800, and 1600-m buffer areas did not differ depending on the presence or absence of Western Meadowlarks, Lark Buntings, Vesper Sparrows, and Grasshopper Sparrows. For Brewer’s Sparrows, the percent of the buffer that was pasture with shrubs (all shrub types combined) was higher in the 400-m and 800-m buffers where Brewer’s Sparrows were present. In the 400-m buffer, the percent of the buffer made up of pasture with shrubs was 95.2% where Brewer’s Sparrows were detected, and 82.8% where they were absent (arcsine transformation, $F = 7.50, P = 0.0067$). In the 800-m buffer, the percent of pasture with shrubs was 86.3% where Brewer’s Sparrows were present, and 75.6% where they were absent ($F = 4.67, P = 0.0320$). In addition, where Brewer’s Sparrows were present, the percent of the 800-m and 1600-m buffers in other habitat types (i.e., urban, water) (0.2% and 0.04%) was lower than where Brewer’s Sparrows were absent (1.3% and 1.6%) ($\chi^2 = 5.5545, P = 0.0184$ and Kruskal-Wallis $\chi^2 = 5.6419, P = 0.0175$, respectively). Horned Larks had significantly lower percentages of hayfield in the 400-m and 1600-m buffers where they were present (0.0% and 1.1%, respectively) than where they were absent (3.5% and 6.5%, respectively) ($\chi^2 = 4.0327, P = 0.0446$ and Kruskal-Wallis $\chi^2 = 3.8709, P = 0.0491$, respectively).

**DISCUSSION**

We did not find a relationship between the landscape attributes we measured in the vicinity of our study sites and presence or absence of four of the six most abundantly detected bird species on our study sites. Knick and Rotenberry (1995) also found that landscape variables had no influence on the habitat-selection models that they developed for Western Meadowlarks and Horned Larks. Ribic and Sample (2001) reported Grasshopper Sparrows were influenced by the amount of grassland within 400 m of transects in southern Wisconsin. Fletcher and Koford (2003) found that edges between forest and grassland influenced Bobolinks ($Dolichonyx oryzivorus$) more than edges between roads or agricultural fields and grasslands. Perhaps the landscapes of the western Dakotas are more homogeneous in the eyes of the bird species than the habitats analyzed by Ribic and Sample and Fletcher and Koford. For example, the habitat of Ribic and Samples’ Grasshopper Sparrows included far more woodlots and hedgerows than our sites in the western Dakotas, and the edge between grasslands and woodlots may be a more influential edge (a “harder” edge) for the sparrows than the edge between pastures with shrubs and pastures without shrubs. Also, even in the “high density” areas of shrubs and sagebrush in western North and South Dakota, there exists a fairly high proportion of grass, so that, to a grassland bird, the
habitat still may appear to be acceptable grassland (see Lewis 2004 for quantification of local vegetation variables).

Vander Haegen et al. (2000) found a significant relationship between percent shrubsteppe within 5 km of study sites and Sage Sparrows, Sage Thrashers, and Brown-headed Cowbirds (*Molothrus ater*), but not between their landscape variables and Brewer’s Sparrows, Horned Larks, or Grasshopper Sparrows. In contrast to the findings of Vander Haegen et al. (2000), we found an association of habitat composition and Brewer’s Sparrows, the only sagebrush obligate species found in abundance on the study area. Perhaps the location of the western Dakotas, on the edge of the range of sagebrush, in what may be poorer-quality habitat, increases the importance of sagebrush/shrub availability for sagebrush obligates. The scarcity of Sage Sparrows on our sites may be a function of their reliance on large, continuous tracts of sagebrush, as indicated by Vander Haegen et al. (2000). Brewer’s Sparrows, occurring on the edge of the sagebrush ecosystem, possibly a marginal area for sagebrush obligates, may require larger areas of sagebrush than they would in the interior of sagebrush distribution—thus our findings of a strong relationship. So, the quality and the regional context of the habitat may change the relative importance of habitat variables for the same species in different areas. Kaczor et al. (in press) found that Greater Sage Grouse in the Dakotas persisted in areas with much less sagebrush cover than is “preferred” in the core of sagebrush distribution, and utilized the greater grass component of their environment to make up for the lack of sagebrush cover.

Horned Larks in this study were rarely found where hayfields were a significant part of the landscape. This is probably because hayfields tend to have tall grasses much of the year, and Horned Larks in a concurrent study were associated with shorter herbaceous vegetation and low percent cover of grasses (Lewis 2004).

The results of this landscape study and other studies (e.g., Bakker et al. 2002; Winter et al. 2006) indicate that there is no single habitat type that will satisfy the needs of all species or even the same species in all parts of its range. Apparently, there are interactions between species and their habitats that require scientists and land managers to evaluate species’ needs at multiple scales, from local vegetation and landscapes, to regions, and even across continents for long-distance migratory species. Quality of the habitat (in this case sagebrush) may influence the relative importance of local and landscape characteristics; e.g., in places where the sagebrush is more robust, the landscape may be more important than the local characteristics, and vice-versa. Geography may also influence the relative importance of landscape-scale variables in determining species occurrence; thus what is true in one region may not be true for another. In relation to landscape attributes, our findings show that bird species in the western Dakotas behave in similar but not identical ways to birds to the east [e.g., Wisconsin (Ribic and Sample 2001)] and west [e.g., Washington (Vander Haegen et al. 2000)]. This study, by shedding light on landscape relationships on the edge of the sagebrush ecosystem, has perhaps revealed another layer of complexity in the challenge of avian grassland/shrubland conservation. Management strategies to conserve grassland birds will need to take into account the differences between different parts of a species’ range, and will need to be tailored to the species in a given area and their interactions with their local and landscape features. The
sagebrush transition zone in the western Dakotas is a unique area, and the birds living there have their own specific requirements which may or may not be extrapolated successfully from the results reported for the same species occurring in other geographic areas, which, in itself, is reason for future conservation attention to this habitat type.

ACKNOWLEDGMENTS

We would like to thank Drs. A.A. Boe, L.D. Flake, V.C. Kelley, G.E. Larson, K.C. Jensen, K.K. Bakker and Mr. N.A. Kaczor of South Dakota State University, Dr. N.T. Wheelwright of Bowdoin College, Dr. R.H. Yahner of The Pennsylvania State University, and Dr. R.W. Klaver of USGS for earlier reviews of the paper, Mr. P.D. Evenson, Mr. M.J. Fincel, Drs. M.R. Wuellner, M.L. Brown, Z. Wicks, B.D.S. Graeb, and the late Dr. T.A. Wittig for help with the statistical analyses, and R. Murano, R. Peterson, R. Flake, G. McClintock-Ames, T. Parkin, R. Young, B. Zahn, and D. Cunningham for technical help in field data collection. We would also like to thank the SD and ND GAP programs staff for supplying landcover images. Funding was provided by SD Game, Fish, and Parks, Federal Aid to Wildlife Restoration Fund (Project W-107-R, Amend. 14, No. 1012); ND Game and Fish, Federal Aid to Wildlife Restoration Fund (Project W-67-R-40, No. B-V-4); U.S. Forest Service Agreement No. 00-CS-1102; BLM Contract Agreement ESA000013 Task Order 1; and the South Dakota Cooperative Fish and Wildlife Research Unit of the U.S. Geological Survey in cooperation with the U.S. Fish & Wildlife Service, the Wildlife Management Institute, South Dakota State University and the South Dakota Game, Fish, and Parks Department.

LITERATURE CITED


SUGGESTIONS FOR A NEW APPROACH FOR USE IN ASSESSING INDICATED BREEDING PAIR POPULATIONS OF DUCKS DURING SPRING IN NORTH AMERICA

Kenneth F. Higgins*, Kent C. Jensen¹, and Kristel K. Bakker²

¹Wildlife and Fisheries Sciences
South Dakota State University
Brookings, SD 57007-1696
²College of Arts and Sciences
Dakota State University
Madison, SD 57042
*terri.symens@sdstate.edu

ABSTRACT

Waterfowl biologists have been concerned with various elements of duck count methodologies and data treatments for several decades. We present a brief review of past efforts to assess spring duck breeding populations and then we propose a new conceptual approach that should help to improve spring breeding duck population assessments in Canada and the U.S.

Keywords

Duck count methods, spring breeding pairs, wetland attributes

JUSTIFICATION AND CONCEPT DISCUSSION

Alison (1978) provided numerous excerpts of records of waterfowl-related activities including hunting that spanned 21 known civilizations and which occurred periodically in the past 6,000 years of recorded history. Likewise, the archaeological evidence for South Dakota, and very probably for the other waterfowl breeding ground regions, shows that the history of waterfowl presence and duck hunting extends back for thousands of years in North America (Parris and Higgins 2010). Even though the interaction of humans with waterfowl has a long history in North America (Weed and Dearborn 1903), the history of structured attempts to census waterfowl populations is relatively brief, and especially for duck censuses done at larger landscape scales (e.g., physiographic units or regional scales).

The purpose of this paper is not to provide an exhaustive review and synthesis of the duck census accounts and protocols that exist in North American literature, but rather to give a brief description of some past efforts to assess duck
populations and to bring some consideration to a new approach to duck population assessments.

Very likely, most of the earliest attempts to estimate the status of waterfowl populations were simply based on general observations made mostly by untrained people and were categorized in general terms such as “lots more ducks this year than last year” or “there’s so many ducks this year that they darken the skies in all directions” or “there’s too few ducks to hunt this fall”. For sure, many of today’s statisticians would refer to such observations as anecdotal and unreliable, but in contrast how sound were the data on which decisions were made in the establishment of the 1918 Migratory Bird Treaty Act or the “Duck Stamp Act”, both of which are still in effect today and both of which were founded in response to general-observational derived concerns relative to significant declines in duck populations and their habitats.

In 1929, at the 16th American Game Conference, committee chairman Aldo Leopold stated “There is pressing need to know more about the status, not only of the migratory game crop as a whole, but of each constituent species.” “Game yields can be greatly increased, and the costs and risks of management decreased, by more research.” Key words in these statements by Leopold are status, migratory game crop, and game yield for each constituent (duck) species. The above quotes of Leopold were taken from page 6 in Hawkins et al. (1984).

In similar fashion, a report entitled “The 1935 International Wild Duck Census” occurred in relation to the effects of the 1930s drought which was reported in a publication entitled “The Duck Decline in the Northwest: A Report on the Prairie Duck-Breeding Region”. Both reports were published by a Foundation: More Game Birds in America, the progenitor of Ducks Unlimited of today, respectively in 1935 and 1933. The information in the 1933 report was gathered during a trip through the primary prairie duck breeding area of North America during approximately the first half of July in 1933. The information in the 1935 report was gathered during August in 1935 with the cooperation of thousands of individuals and organizations. The 1935 census yielded 42.7 million ducks (page 5) but the 1935 continental population (page 79) was estimated at 65 million ducks (also reported in Hawkins et al. [1984, pg. 17]) which was much below what they believed to be a satisfactory number for the overall welfare of the continental population. In comparison, the 1993 fall flight population of ducks in North America was estimated to be 59 million and the fall-flight index of total ducks has ranged from 55 to 88 million since 1970 (Caithamer et al. 1993). Since 2001 the USFWS has not issued a total fall-flight duck population forecast and the last time they completed a total duck production (broods) survey was 2003.

Besides providing the results of the 1935 wild duck census, the authors (unknown) also provided a fairly detailed set of descriptive guidelines on how to organize and implement large-scale duck counts from the air or via ground or water transportation means in Chapters 3 and 10 in the 1935 report, all of which, in all probability, were the basis for all of the duck census protocols that have been developed since 1935 by various state, federal and private organizations. In general, the period from circa 1930 to 1970 was the time when considerable efforts were made to standardize duck counting methodologies, to define what
duck group elements represented a breeding-pair unit during the nesting season, and how to tabulate the duck count data from a structured waterfowl census (See Dzubin 1969, Hammond 1969 and others in the Saskatoon Wetlands Seminar publication and Cowardin and Blohm 1992). During the same time period that guidelines were being developed toward standardizing duck surveys on small to state-sized census units, guidelines and sampling protocols were being developed for multi-regional scale duck surveys in the U.S. and Canada (see Henny et al. 1972, USFWS 1976, Hawkins et al. 1984).

Technological and computerized analytical advances that evolved from circa 1970 to the present have enabled waterfowl researchers and managers to work with larger data sets from larger geographic areas in a more timely manner to aid their decision processes. Paramount among these advances are the capabilities to capture, file, archive, and electronically transfer and/or manipulate the data sets electronically. Some recent examples incorporating various kinds of digital and remotely-sensed data sets for purposes of waterfowl management are Cowardin et al. (1995) and Reynolds et al. (2006). Waterfowl management agencies, particularly the U.S. Fish and Wildlife Service, have been involved in assessments of duck and wetland conditions in the U.S. and Canada since the 1950s (Martin et al. 1979; Reynolds 1987) primarily for use in establishing annual duck hunting regulations that are published annually.

Annually assessing the status of the continental duck populations, the suitability of their habitats for production of young, and the expected fall flight is not a simple task. It entails hundreds of personnel and the cooperation and coordination of dozens of agencies and organizations. For the most part, the collective information and data summaries from such surveys have been funneled through various information transfer channels in four Flyways for decades, eventually culminating in the regulation setting process relative to the onset of fall duck hunting.

Unfortunately in some respects, most, but not all, of the earlier-established, smaller but intensively-monitored duck study research units, were discontinued circa the 1970s-1980s as duck population and habitat monitoring efforts shifted toward larger-scale monitoring operations that are based on numerous sampling processes enabling rigorous statistical treatment of the resultant data sets. However, even though the same habitats and the same population of ducks are being sampled by more than one affiliate, the summary data results are not always in close agreement. For example, data summaries relative to the population of wetlands (a static sample unit) are in fairly close agreement when compared between the annual monitoring efforts whereas data summaries relative to spring breeding-pair populations of ducks (a highly mobile sample unit) may differ annually by as much as 2-fold (R. Reynolds, pers. comm. in 2009, Bismarck, ND) for the same basic geographic area.

Disparity among duck survey results, even when conducted in the same region or time period, is not a new phenomenon. For example, Cowardin and Blohm (1992) expressed that all systems for the estimation of breeding-population sizes of ducks are subject to errors of biological interpretation such as determining whether an observed pair of ducks represents a resident or a migrant pair and whether observed social groups such as lone males represent breeding pairs.
Likewise, they qualified that data sets such as the National Wetlands Inventory (Wilen 1990), even small ones, may contain errors of omission that when used in data expansion processes, will cause underestimation of breeding-population sizes and/or recruitment rates.

Further confounding duck counts is the fact that all duck species do not settle and begin nesting at the same time. For example, an early count which might be best for mallards (*Anas platyrhynchos*) and pintails (*A. acuta*) may be the worst possible count time for lesser scaup (*Aythya affinis*) and ruddy ducks (*Oxyura jamaicensis*) relative to their late nesting chronologies. This census problem was addressed during a lesser scaup study in South Dakota which identified how the timing of the USFWS breeding pair count overestimated the number of nesting pairs by 11-fold (Naugle et al. 2000:180). Very likely, a large proportion of the scaup that were/are counted in South Dakota were/are recounted again in North Dakota and perhaps a third time in Manitoba or other points northward because the USFWS duck surveys also progress from southern to northern transects as the season progresses. The best estimate of the number of nesting (breeding pairs) scaup is further confounded by the fact that few of the yearling cohort of each year’s scaup population engage in nesting efforts; most do not do so until they are 2-years-olds, and a few perhaps not until their third year (Trauger 1971). Very likely, a similar phenomenon occurs during surveys of other duck species, particularly during springs that are deemed early or late, or during seasons of extremely wet or dry wetland conditions. For example, in abnormally dry springs, breeding pairs are prone to overfly the prairies to wetter conditions farther north in Canada or Alaska (Crissey 1969: 162). In brief, even though data from the USFWS surveys are viewed mainly as index estimates or trend data only, there is opportunity for adjustments in survey protocols and timing which would yield better summary data products (For example see: Diem and Lu 1960, Bennett 1967, Martinson and Kaczynski 1967, Dzubin 1969:224, Sauder et al. 1971, Ringelman and Flake 1980, Rumble and Flake 1982, Blohm 1989, Smith 1995, Austin et al. 2000, Giudice 2001, Pagano and Arnold 2009 A and B).

Another potential bias that accrues in duck count data summaries is related to basin size or fragmentation. Duck pair densities per unit area of habitat (pairs/acre of water) or (pairs/mi^2) have been published for more than 50 years, and in nearly all publications, duck pair density per unit area of wetland decreases as basin size increases (e.g., see graph by Jerome H. Stoudt 1949:147) even though numerically there were more pairs on larger water areas (see Stoudt 1949:147; Table 4). Unfortunately, the importance of this relationship has been over-looked or neglected through the past several decades, and in fact, it may be responsible for many apparent discrepancies among current study results and in past and recent duck population estimates. We believe this is further exacerbated by duck population expansion or modeling exercises based on pair data estimates collected on count data from fragmented segments of individual wetland basins.

Other factors affecting the quality of duck data sets include, but are not limited to, capabilities of duck species identification (Pagano and Arnold 2009A), visibility limitations due to emergent vegetation or environmental conditions (Pagano and Arnold 2009B) for which the actual count data for specific species
are manipulated with index adjustment factors (e.g., in the 1970s each indicated pair of American wigeon (*A. americana*) or green-winged teal (*A. crecca*) was multiplied by a factor of 15x resulting in 2 pairs being indexed as 30 pairs (Higgins et al. 1992: 64). However, throughout the prairie pothole region of the U.S. and Canada, historical reports rarely, if ever, provide any evidence of breeding pair densities of either of these two species greater than 2 pairs per mi², and very often only 1 pair/mi² or less.

**Suggestions Relative to Future Duck Surveys**—As stated previously, our intent is not to criticize any specific study per se, but to point out that there is potential for modifications and/or improvements in operational procedures, protocols and technologies that would or could improve our ability to produce more accurate estimates of the North American duck populations in a timely-manner. These modifications might originate as simply as the adoption of past suggestions that now have more merit of consideration or they might originate from the addition of new data or analytical techniques.

Most biologists would agree that an ideal duck count would be one where the total census area could be covered instantly, similar to a single photo coverage. However, in reality, even when suitable help is available and weather conditions are favorable, most larger study areas take at least one full day of effort for a total census and more often several days or weeks of effort are necessary to complete a total duck and wetlands survey. Ducks are very mobile and often flush to another wetland with most types of minimal disturbance or by random chance due to feeding, nesting or other behavior needs. Therefore, it is all but impossible to prevent multiple counting (roll-up) of some birds at different times during any single census. Averaging two or more counts in the same season helps to minimize roll-up effects. Perhaps at some future date, instant or remotely-sensed photography might be an option, but at present, duck counts are still done while walking, riding or flying.

The following is an unrefined conceptual approach that, in our opinion, has the potential as a better means of estimating the numerical populations of duck breeding populations and/or duckling production on an annual basis, and it also has possible application to other wetland wildlife and plant species.

First, ducks must be treated in similar fashion to any other farm crop, except their fields are wetland basins, and most importantly, the basins must have enough water to be suitable for a duck. John Lynch in a reference memo pertaining to “Waterfowl Crop-Forecasting” (see page 7 in the 1969 Saskatoon Wetlands Seminar publication stated “The best possible stock of potential breeders [ducks] ain’t going to pay off if there ain’t enough water. Our seed won’t germinate if the land ain’t ready.” In the same context, the success of any year is dependent on the number and size of the fields (in our case wetlands) and the number of bushels (ducklings) that each field yields. But unlike plants, ducks are not rooted to one place within one field (wetland) and each wet basin may be occupied or unoccupied by ducks and broods at any specific time for any number of reasons.

Fortunately, determining if a wet basin is occupied or not by ducks is a fairly easy task, and results from several basins are usually expressed as “percent
occupancy” or “the number of wet basins per pair” (see Dzubin 1969:138-160) either of which provide a probability value relative to duck pairs or broods using a sample of wet basins. Furthermore, such wet basin % occupancy values can be partitioned according to wetland basin size, wetland basin water condition, wetland basin classification or the ratio of emergent cover to open water. These data (Table 1) can also be partitioned spatially within landscapes or land use types, physiographic zones, or temporally (e.g., daily, weekly, monthly, seasonally, etc.) by duck species and/or brood size and age classes (Tables 2 and 3 and Figs. 1 and 2).

Thus, because all ducks are wetland obligate species and because wetland basins are a more static unit to sample in time and space, we contend that the development and use of a new approach of assessing duck nesting populations (breeding pairs) and annual brood production based on the assignment of % occupancy values/species/wetland basin attributes all of which are collected in relation to the on-set of nesting and/or the size and age class of broods per wetland, will yield a more robust and less variable duck population data set which should enhance our management capabilities of the North American duck population.

Table 1. An example list of wetland basin attributes.

<table>
<thead>
<tr>
<th>Duck Species</th>
<th>Wetland Class/Type*</th>
<th>Basin Size</th>
<th>H₂O Condition</th>
<th>Emergents</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tiny</td>
<td></td>
<td>Dry (zero)</td>
<td>None</td>
</tr>
<tr>
<td>II</td>
<td>Small</td>
<td></td>
<td>Nearly Dry</td>
<td>None</td>
</tr>
<tr>
<td>III</td>
<td>Medium</td>
<td>¼ Full</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>IV</td>
<td>Large</td>
<td>½ Full</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>V</td>
<td>Extra Large</td>
<td>¾ Full</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Stock Ponds</td>
<td></td>
<td>Full</td>
<td></td>
<td>Choked</td>
</tr>
<tr>
<td>Dugouts</td>
<td></td>
<td>Over Full</td>
<td>Full</td>
<td></td>
</tr>
</tbody>
</table>

*Wetland classification according to Stewart and Kantrud (1971).

Table 2. Summary data example for blue-winged teal (Anas discors) counted in Figs. 1 and 2 assuming a wetland basin population of 2,000 basins for each of the five wetland water condition categories, totaling 10,000 seasonal basins regardless of wet or dry condition.

<table>
<thead>
<tr>
<th>Basin Water Condition Category</th>
<th>Pairs/Basin (% Occupancy)</th>
<th>Pairs/2,000 Basins</th>
<th>Broods/Basin (% Occupancy)</th>
<th>Broods/2,000 Basins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full (N=2,000)</td>
<td>2/1 (200%)</td>
<td>4,000</td>
<td>1/5 (20%)</td>
<td>400</td>
</tr>
<tr>
<td>¾ Full (N=2,000)</td>
<td>1/4 (25%)</td>
<td>500</td>
<td>1/20 (5%)</td>
<td>100</td>
</tr>
<tr>
<td>½ Full (N=2,000)</td>
<td>1/10 (10%)</td>
<td>200</td>
<td>1/100 (1%)</td>
<td>20</td>
</tr>
<tr>
<td>¼ Full (N=2,000)</td>
<td>1/25 (4%)</td>
<td>80</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>Dry (N=2,000)</td>
<td>0 (0%)</td>
<td>0</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>Totals (N=10,000)</td>
<td></td>
<td>4,780</td>
<td></td>
<td>520</td>
</tr>
</tbody>
</table>
**Summary Suggestions**—1. Counts need to include entire basins for ducks regardless of their size; i.e., no partial wetland basin counting to induce bias in population estimates (Stoudt 1949:147).

2. Breeding pair counts need to be conducted more in sync with the chronology of spring migration for all 12 duck species (±); i.e., this means at least two survey periods per year, perhaps three. For example, in 1995, Holland (1997) showed that the earlier FWS survey in South Dakota indicated 17 times more scaup breeding pairs than his later survey (110,900 vs. 6,500) that same spring. If we assume a similar annual error factor for the past 20 years, the total accumulated error for South Dakota would be a little over 2 million scaup. Considering similar error rates for roll-over counting in North Dakota and perhaps the southern tiers of Alberta, Saskatchewan, Manitoba, and the Northwest Territory

<table>
<thead>
<tr>
<th>Basin Water Condition Category</th>
<th>Pairs/Basin (% Occupancy)</th>
<th>Pairs/2,000 Basins</th>
<th>Broods/Basin (% Occupancy)</th>
<th>Broods/2,000 Basins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full (N=2,000)</td>
<td>1/100 (1%)</td>
<td>20</td>
<td>1/2,000 (1%)</td>
<td>1</td>
</tr>
<tr>
<td>¾ Full (N=2,000)</td>
<td>1/200 (.5%)</td>
<td>10</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>½ Full (N=2,000)</td>
<td>0 (0%)</td>
<td>0</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>¼ Full (N=2,000)</td>
<td>0 (0%)</td>
<td>0</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>Dry (N=2,000)</td>
<td>0 (0%)</td>
<td>0</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>Totals (N=10,000)</td>
<td></td>
<td>30</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

**Blue-winged teal / Seasonal Basin**

- Pairs / Basin: 2 / 1
- Broods / Basin: 1 / 5

- Pairs / Basin: 1 / 4
- Broods / Basin: 1 / 20

- Pairs / Basin: 1 / 10
- Broods / Basin: 1 / 100

- Pairs / Basin: 1 / 25
- Broods / Basin: 0 / 0

**Ruddy Duck / Seasonal Basin**

- Pairs / Basin: 1 / 100
- Broods / Basin: 1 / 2000

- Pairs / Basin: 1 / 200
- Broods / Basin: 0 / 1

- Pairs / Basin: 0 / 1
- Broods / Basin: 0 / 3

- Pairs / Basin: 0 / 1
- Broods / Basin: 0 / 1

- Pairs / Basin: 0 / 1
- Broods / Basin: 0 / 1

**Figure 1.** An example of breeding pair and brood ration of occurrence of blue-winged teal on a population of 10,000 seasonal basins, either wet or dry, that ranged from 1/4 - 1/2 acre in size and which had variable degrees of basin inundation at the time of the census.

**Figure 2.** An example of breeding pair and brood ration of occurrence of ruddy ducks on a population of 10,000 seasonal basins, either wet or dry, that ranged from 1/4 - 1/2 acre in size and which had variable degrees of basin inundation at the time of the census.
adds up to an accumulative sum of millions of scaup breeding pairs that do not really exist.

3. Count adjustment factors for some species (e.g., visibility multipliers as used for wigeon, etc., Higgins et al. 1992:64) need to be re-evaluated and perhaps even eliminated, so if we error, it will be directed towards more conservative instead of liberal regulation setting.

4. Consideration should be given to setting hunting regulations for the U.S. one year following the survey year (e.g., the 2007 survey data would be used to set the 2008 season regulations) in a similar manner to that currently being implemented in Canada. Using the delayed approach would remove the urgency to summarize and model data in a rush to meet time schedules for the Flyway Technical and Council meetings, Federal Register, Public Hearings, etc. It would also enable the use of late summer, fall and winter precipitation and wetland conditions relative to the next spring’s pond counts and would also enable use of brood count data (actual yield estimates).

5. Consideration should be given to the use of a new census methodology approach that connects the breeding pair and brood count data to wetland occupancy rates of individual wetland basins and also to some specific basin attributes (e.g., basin size, basin classification, basin emergent vegetation coverage, and especially to the basin water condition: see Table 1 and Figures 1 and 2). For example, Trauger and Stoudt (1979) stated, “Pond occupancy rates indicate that the decline in waterfowl [ducks] is occurring more rapidly than losses of wetland habitat. There is an increasing number of ponds without ducks, suggesting that the available breeding habitat is underutilized.”

6. Better information is needed relative to criteria that best represents a breeding pair of ducks for each species (e.g., see Brashier et al. 2002), and/or the percentage of each species spring population that could be expected to nest each year (e.g., see Trauger 1971).

7. To ensure intergenerational (historic background) connectiveness through time relative to duck counting criteria, methods and protocols, a single-source comprehensive literature synthesis should be compiled and published in a similar fashion to earlier publications such as Flyways (Hawkins et al. 1984), or like reviews by Bowden (1973), Blohm (1989), and Smith (1995). Copies of historically significant works such as the “Saskatoon Wetlands Seminar”, “The 1935 International Duck Census”, etc., should be reproduced to ensure greater availability for future generations of waterfowl management personnel.

8. And lastly, we urge the responsible agencies to give full consideration to the reimplementation of the annual late summer production (brood) surveys and the annual fall-flight forecast for all duck species combined as well as for each individual species separately. Such data summaries would facilitate duck population audits and performance accountability of duck management efforts by various agencies. They would also provide simplified information for use by various waterfowl hunters and other interested parties. And, in retrospect, many older waterfowl biologists, if asked if they had to choose between conducting only a breeding pair survey or a brood survey, would have opted for brood surveys; however, most would have also expressed the need and value of conducting both types of surveys as their first choice.
ACKNOWLEDGEMENTS

We thank Kurt Forman and Rick Warhurst for their reviews and suggestive edits of this manuscript. Terri Symens assisted with manuscript typing and format preparations.

LITERATURE CITED


ABSTRACT

Five historic waterfowl research areas in Canada and the U.S. were revisited in 2008, and duck populations and land use were reassessed on each area. Results from the 2008 field work, when compared to earlier published results, indicated some readily apparent temporal differences relative to wetland conditions, % cropland, and estimates of duck breeding-pair population attributes. General observations indicated several obvious changes in flora and fauna presence or absence.

Keywords

Ducks, land use, wetlands, Canada, U.S.A.

BACKGROUND AND INTRODUCTION

Historically, baseline data from several historic waterfowl study areas in Canada (Smith 1971; Stoudt 1971, 1982) and the United States (U.S.) (Evans and Black 1956; Higgins et al. 1992; McLeod and Higgins 1998) were used to access the general status and productivity of prairie duck populations. Data from these field studies were also used by Central and Mississippi Flyway biologists during late-summer regulation-setting processes. Thirty years have passed since the last waterfowl and habitat condition surveys were conducted on the Canadian parklands study areas (Trauger and Stoudt 1978) at Redvers, Saskatchewan (1952-1977), Lousana, Alberta (1953-1977), and Minnedosa, Manitoba (1952-1955; 1963-1977). In the U.S., waterfowl populations and habitat conditions were assessed on the Waubay Study Area on the Prairie Coteau in northeastern South Dakota during 1950-1953 by Evans and Black (1956) and during 1992-1993 by McLeod and Higgins (1998) and on the Woodworth Study Area on the Missouri Coteau in southcentral North Dakota during 1965-1981 by Higgins et al. (1992).

Since these earlier field studies were last conducted, some species (e.g., lesser scaup and northern pintail: scientific names of ducks are listed in Table 6) have
declined in population status to levels of special concern by natural resource agencies. Technical advances in farm and industrial equipment have also enabled macro-changes in waterfowl habitats and landscapes. Historic maps and field data files as well as recent advances in aerial photography, Geographic Information Systems (GIS), and computer processing techniques also now enable scientists to more easily make temporal comparative assessments of landscape-scale changes in habitat conditions over large geographic regions. Furthermore, several biologists that were previously involved with these earlier studies are still available and are willing to offer advice and background information relative to any future research on these historic study areas.

Repeating these earlier waterfowl studies on the same historic research areas should provide some insights into long-term changes in waterfowl populations and habitat conditions. Thus, the goal of this project was to identify any long-term changes in waterfowl populations and habitats on five historic study areas (three in Canada and two in the U.S.) during the past 60 years, more or less.

In order to achieve this goal, my primary objectives were:

1. to assess the current relative abundance of 12 duck species common to all five study areas,
2. to assess the status and changes of the major habitat types (wetlands and uplands) and agriculture practices, and
3. to compare recent (2008) data with historic data sets from all five study sites.

STUDY AREAS

Maps of historic study areas or the survey routes (Figures 1-5) are provided to enable uniformity during any future assessments. The three Canadian study areas occur along the southern boundary of the aspen parklands zone of southern Canada. The Lousana Study Area is located about 25 miles (40 km) east of Red Deer in southcentral Alberta (Smith 1971) (Figure 1). It consists of four transects forming the sides of a rectangle and containing 3.625 square miles (9.4 km²) (Figure 1). The transects are one-eighth mile (0.2 km) wide and 29 miles (46.7 km)
Figure 2. Redvers Study Area in the Aspen Parklands of Saskatchewan, Canada.

Figure 3. Canvasback Habitat Study Area near Minnedosa, Manitoba.
The Redvers Study Area is located about 10 miles (16 km) east of Carlyle in southeastern Saskatchewan (Stoudt 1971). It is a reverse L-shaped transect, 40 miles (64.4 km) long by one-eighth mile wide, and is 5 square miles (12.9 km²) in size (Figure 2). The transect extends east from Manor for 20 miles (32.2 km) to Redvers and then north 20 miles nearly to Fairlight (Figure 2). The Minnedosa Study Area is located about 20 miles (32.2 km) north of Brandon in southwestern Manitoba. A series of 12 transects crisscross 100-square-miles (259 km²) of waterfowl habitat immediately south of Minnedosa (Figure 3). Between 1963 and 1966, waterfowl data were obtained from a 3-square mile (7.8 km²) block encompassing the former Roseneath Study Area (Evans et al. 1952, Dzubin 1969). Five one-eighth-mile-wide (0.2 km) transects covering 32 linear miles (51.5 km) and totaling 4 square miles (10.4 km²) were used from 1967 through 1977 (Figure 3).

The two United States study areas occur on coteau landscapes; the Missouri Coteau in North Dakota and the Prairie Coteau in South Dakota. The Waubay Study Area is located on the Prairie Coteau physiographic region in Day County in northeastern South Dakota and about 50 miles east of Aberdeen and 13 miles north of Waubay. It includes 11.25 square miles (29 km²) and contains 504 wetlands (Figure 4).

The Woodworth Study Area is located on the Missouri Coteau physiographic region in Stutsman County in southcentral North Dakota about 3 miles (4.8 km) east of Woodworth and about 45 miles (72 km) northwest of Jamestown. It includes 4.75 square miles (12.35 km²) and contains 548 wetlands (Figure 5).
METHODS

For the most part, the historical duck population data are available in published reports. Protocols for collecting data on waterfowl followed that described by Smith (1971), Stoudt (1971), Higgins et al. (1992) and McLeod and Higgins (1998).

Waterfowl counts were made while walking or from pickups with the use of binoculars or spotting scopes. Permission for access was obtained by the Waubay NWR staff for the Waubay Study Area and by the Chase Lake staff for the Woodworth Study Area. Roadside duck counts on the three Canadian Study Areas were done totally from a pickup because time constraints precluded the possibility of obtaining access permission. Pair counts on the five study areas were completed within a 2-day period on each area.

The 2008 duck pair counts were conducted on the Waubay Study Area in South Dakota on May 13 and 14; the Woodworth Study Area in North Dakota on May 15 (first count) and on May 28 – June 2 (second count); the Redvers Study Area in Saskatchewan on May 20–21; the Lousana Study Area in Alberta on May 22; and the Minnedosa/Roseneath Study Area in Manitoba on May 24. Due to lack of access permission in 2008, counts on the Minnedosa/Roseneath...
area were made along transects totaling 9 miles x 1/8 mile wide on both sides of boundary roads, 220 yards on each side of the roads, that bordered the original 3 sections of the Roseneath Study Area, which were also the 3 beat-out sections of Stoudt’s Minnedosa Study Area (Figure 3).

Counts on the Minnedosa Canvasback Study Area (Figure 3) were made on a larger scale (nearly 10 miles x 10 miles) block site that consisted of 12 roadside transects totaling 85 linear road miles x 1/8 mile on each side of each road transect and included 21.25 square miles of habitat. It also encompassed the original Roseneath Study Area (Figure 3). Canvasback pair counts were conducted on May 25 and 26 and canvasback brood counts were conducted on July 6, 7 and 8 with the aid of Dr. Wayne Cowan. We surveyed for canvasback broods on both sides of each road transect from a pickup. Brood ages were recorded as per Gollop and Marshall (1954).

For all five study areas, population estimates of indicated pairs per-square-mile were calculated based on the original land/water acreages and numbers even though some wetland basins had been permanently altered or eliminated due to farming or road construction activities during the past 50-60 years. If a wetland basin had water covering 5% or more of the basin area, it was considered wet and useable by ducks; if the basin had less than 5% of the basin covered by water it was considered dry. The percentage of land use (e.g., tillage cropland, seeded grassland or hayland and native grassland or parkland) was calculated using odometer metrics per length of habitat type along each roadside transect or by notations on field acreage maps for the Woodworth and Waubay study sites.

**RESULTS AND DISCUSSION**

**Wetland Basin Water Conditions**—Compared to historical data, wetland water conditions during 2008 were the driest on record at Redvers, Saskatchewan; were below average at Lousana, Alberta; were about average (estimated) at Minnedosa, Manitoba; were far below average at Woodworth, North Dakota; and were nearly equal to the wettest year on record at Waubay, South Dakota (Table 1). Thus, in 2008, of the five Study Areas, only Waubay had suitable to good upland habitat conditions and excellent wetland water conditions.

**Percent Cropland**—From 1976 to 2008 (Table 2) the proportion of the study area acreage in annually-tilled cropland remained nearly the same on the Redvers, Minnedosa, and Waubay Study Areas, whereas it decreased due to grassland establishment programs on the Woodworth and Lousana Study Areas. At Woodworth all tillage acres were seeded back to some form of grassland nesting cover. In Alberta and on the Lousana Study Area, many landowners have seeded fields of former cropland acres back to hayland or pasture lands in support of greater livestock production.

**Breeding Duck Pair Indices**—The density (#/mi²) and % species composition of breeding pairs of ducks varied within and among the five study areas (Tables 3, 4, 5, 6, 7 and 8). Except for the Woodworth study area, breeding duck popula-
tion density estimates generally were related directly to wetland water conditions (Table 1), i.e., less water equated to fewer breeding pairs per square mile. The anomaly with the inverse relationship of high breeding duck pair densities to poor water conditions at Woodworth (Table 7 and 8) was also evident in the North Dakota Game and Fish Department (NDG&FD) May duck survey/wetland indices for 2008 (Figure 6; data supplied by Mike Johnson, NDG&FD, Bismarck, ND) and also for eight of the 10 years preceding 2008 (Figure 6). Mike and I suspected that this anomaly may have occurred residually due to former duck production resulting from CRP grasslands in the region or drought conditions in prairie Canada or both.

**Minnedosa Roadside Canvasback Transects**—The average density of breeding canvasback pairs/square mile (4.0) in 2008 was the lowest recorded through the years (Table 9), and the average density of broods (0.24/mi$^2$) was also the lowest recorded but similar to the density (0.28) recorded in 1968 (Table 10). For 2008, division of the 5 broods by the 84 canvasback pairs yielded a hen success rate estimate of 6%.

**Percent Species Composition Ranks**—At Redvers, mallards, wigeons, and pintails ranked lower than in past years, whereas gadwalls, shoveler, and green-winged teal ranked higher (Table 5). Other species occurred in similar ranks through time. At Lousana, mallards, blue-winged teal, pintails, green-winged teal, and lesser scaup ranked lower than in past years whereas gadwalls, shoveler, redheads, ruddy ducks and buffleheads ranked higher (Table 6). Other species occurred in similar ranks through time. At Waubay, blue-winged teal, pintails, wigeons, green-winged teal, and wood ducks ranked lower, whereas mallards, canvasbacks, ring-necked ducks, and lesser scaup ranked higher (Table 3). Other species occurred in similar ranks. At the Minnedosa/Roseneath Study Area, mallards, pintails, wigeons, and ruddy ducks ranked lower, whereas gadwalls, shoveler, canvasbacks, lesser scaup and buffleheads ranked higher (Table 4). Other species occurred in similar ranks through time.

At Woodworth (Tables 7 and 8), from the second count data (Table 8), blue-winged teal, pintails, wigeons, green-winged teal, canvasbacks, redheads, and ruddy ducks ranked lower, whereas mallards, gadwalls, and shoveler ranked higher. Other species occurred in similar ranks through time. The NDG&FD has the longest running continuous duck breeding pair data set in North America. It began in 1948 (Table 8; Figure 6) at which time blue-winged teal were the most commonly occurring duck species followed by pintails, and both of these species occurred more than twice as often as did mallards and shoveler. When compared to the Woodworth data sets (Table 8), apparently there has been a major shift in duck species composition ranks through the past 60 years.
General Observations—In 2008 giant Canada geese (*Branta canadensis maxima*) were scattered across the Dakota’s and the Aspen Parklands and prairie regions of Canada. In 2008 there were also more cattails (*Typha*) than bulrushes (*Scirpus*) in semi-permanent wetlands, bigger and better roads with wider rights-of-ways, fewer summer fallow fields, fewer farms and ranches but larger operations and equipment, fewer farms with barns and dairy cows, more coyotes (*Canis latrans*) and raccoons (*Procyon lotor*), the presence of over-water nesting structures, and most of the small towns were much smaller in size.

Across prairie Canada, there were fewer and older stands of aspen/poplar trees, the presence of oil wells near Redvers, a high frequency of ravens (*Corvus corax*), much more canola cropping, and wetlands were beginning to be drained with rolls of plastic tile. Fewer farms with dairy cattle, beef cattle and horses may have, in part, contributed to the increase in cattails and the decrease in bulrushes in many prairie wetland basins.

DISCUSSION AND CONCLUSION

As anticipated, there was considerable variation amongst densities and percent species composition of breeding duck populations on the five historic study areas in Canada and the U.S. in 2008. Much of this variation was attributable to the variation in wetland water conditions that existed in spring 2008. However, when duck population demographics are compared to the data sets from earlier decades, biologists must consider the effects of cropping and land use changes (e.g., the influx of acreages of canola, corn and soybeans), less summer fallow, and changes in predator populations. Recent hunting regulations including some earlier, longer, and special bonus seasons, many of which have been relatively liberal during the past decade, may have had some effects on current duck population structure. Further confounding effects on duck populations are the effects of cultural changes including fewer farm families and small farming operations and the increase in larger, cleaner farming operations and much larger farming equipment (see Higgins et al. 2002), and wider roads with wider road rights-of-ways.

Unfortunately, data collection on wetlands, land use, and duck populations were discontinued or experienced long lapses of inactivity on all five of the historic study areas before the onset of computer and statistical analytical treatments of large complex data sets were commonly available and applied. Perhaps, we would be better able to identify temporal changes in waterfowl populations in relation to habitat changes today if administrators of the major wildlife agencies and research centers had supported a longer-range vision that placed greater value on continuous long-term field-study data sets.

Fortunately, there are several currently on-going short-term studies being supported by Delta Waterfowl, Ducks Unlimited, the U.S. Fish and Wildlife Service, and by several states and provinces. However, recent concerns over potential climate change and land use change effects on waterfowl habitats and populations and some measureable declines in some species such as pintails and lesser scaup, in my opinion, warrant consideration of reevaluation of historic
data sets and/or restoration of the five historic studies discussed in this paper plus several other historic waterfowl research sites, e.g., the Yellowknife NWT scaup research area.

ACKNOWLEDGEMENTS

Thanks to Dr. Wayne Cowan for his assistance with canvasback surveys at the Minnedosa Study Area; to Robert Woodward and several volunteer staff from the Arrowood NWR/Complex for their help with duck surveys on the Woodworth Study Area; and to Scott McLeod and Rick Warhurst of Ducks Unlimited (Bismarck, ND) and to Laura Hubers and Tom Wickstrom and several seasonal technicians at Waubay NWR for assisting with the Waubay Study Area duck and habitat surveys. Thanks also to Rex Johnson for assisting with the overall project and to the U.S. Fish and Wildlife Service and Ducks Unlimited, Inc., for providing indirect funds toward partial assistance for travel expenses incurred during field work. Rex Johnson, David Trauger and Kent Jensen provided earlier reviews of this manuscript all of which helped it considerably. Terri Symens provided manuscript typing and format preparations.

LITERATURE CITED


Table 1. Wetland water conditions and the estimated density of duck pairs per-square-mile on the five duck study areas during 2008.

<table>
<thead>
<tr>
<th>Study Site</th>
<th>Original # of Basins</th>
<th># Wet Basins and (%)</th>
<th># Breeding Pairs/mi²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minnedosa/Roseneath</td>
<td>Unknown</td>
<td>Avg.</td>
<td>59.6</td>
</tr>
<tr>
<td>Redvers</td>
<td>306</td>
<td>70 (17.3)*</td>
<td>14.0</td>
</tr>
<tr>
<td>Lousana</td>
<td>265</td>
<td>115 (43.4)</td>
<td>34.2</td>
</tr>
<tr>
<td>Waubay</td>
<td>504</td>
<td>329 (65.3)</td>
<td>103.4</td>
</tr>
<tr>
<td>Woodworth 1st Count</td>
<td>548</td>
<td>154 (28.1)</td>
<td>97.9</td>
</tr>
<tr>
<td>Woodworth 2nd Count</td>
<td>548</td>
<td>123 (22.4)</td>
<td>171.8</td>
</tr>
</tbody>
</table>

*Percent of the original number of basins that were wet.

Table 2. Proportion of the five duck study areas in annually-tilled cropland in 1964, 1976, and 2008.

<table>
<thead>
<tr>
<th>Study Site</th>
<th>1964</th>
<th>1976</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lousana</td>
<td>29%</td>
<td>60%</td>
<td>46%</td>
</tr>
<tr>
<td>Redvers</td>
<td>60%</td>
<td>67%</td>
<td>67%</td>
</tr>
<tr>
<td>Minnedosa</td>
<td>High</td>
<td>75%</td>
<td>High</td>
</tr>
<tr>
<td>Woodworth</td>
<td>30%</td>
<td>30%</td>
<td>0%</td>
</tr>
<tr>
<td>Waubay</td>
<td>63%</td>
<td>30%*</td>
<td>33.6%</td>
</tr>
</tbody>
</table>

Table 3. Summary of the full duck count at the Waubay Study Area, South Dakota during May 13-14, 2008.

<table>
<thead>
<tr>
<th>Species</th>
<th>2008 Indicated Pairs</th>
<th>% Species Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mallard</td>
<td>399</td>
<td>35.5</td>
</tr>
<tr>
<td>Gadwall</td>
<td>144</td>
<td>12.8</td>
</tr>
<tr>
<td>Blue-winged Teal</td>
<td>353</td>
<td>31.4</td>
</tr>
<tr>
<td>Northern Shoveler</td>
<td>60</td>
<td>5.3</td>
</tr>
<tr>
<td>Northern Pintail</td>
<td>15</td>
<td>1.3</td>
</tr>
<tr>
<td>American Wigeon</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>Green-winged Teal</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Wood Duck</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Canvasback</td>
<td>25</td>
<td>2.2</td>
</tr>
<tr>
<td>Redhead</td>
<td>53</td>
<td>4.7</td>
</tr>
<tr>
<td>Ruddy Duck</td>
<td>32</td>
<td>2.8</td>
</tr>
<tr>
<td>Ring-necked Duck</td>
<td>22</td>
<td>2.0</td>
</tr>
<tr>
<td>Bufflehead</td>
<td>4</td>
<td>0.4</td>
</tr>
<tr>
<td>Lesser Scaup</td>
<td>52</td>
<td>4.6</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>1,163</strong></td>
<td><strong>103.4</strong></td>
</tr>
</tbody>
</table>

Table 4. Summary of the duck count on the Minnedosa/Roseneath Study Area, Manitoba during May 24, 2008.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># Pairs</td>
<td>#/mi&lt;sup&gt;2&lt;/sup&gt;</td>
<td>% Spp. Comp.</td>
</tr>
<tr>
<td>Mallard</td>
<td>28</td>
<td>12.4</td>
<td>20.9</td>
</tr>
<tr>
<td>Gadwall</td>
<td>24</td>
<td>10.7</td>
<td>17.9</td>
</tr>
<tr>
<td>Blue-winged Teal</td>
<td>34</td>
<td>15.1</td>
<td>25.4</td>
</tr>
<tr>
<td>Northern Shoveler</td>
<td>9</td>
<td>4.0</td>
<td>6.7</td>
</tr>
<tr>
<td>Northern Pintail</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>American Wigeon</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Green-winged Teal</td>
<td>4</td>
<td>1.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Canvasback</td>
<td>12</td>
<td>5.3</td>
<td>9.0</td>
</tr>
<tr>
<td>Redhead</td>
<td>7</td>
<td>3.1</td>
<td>5.2</td>
</tr>
<tr>
<td>Lesser Scaup</td>
<td>5</td>
<td>2.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Ruddy Duck</td>
<td>7</td>
<td>3.1</td>
<td>5.2</td>
</tr>
<tr>
<td>Ring-necked Duck</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Bufflehead</td>
<td>4</td>
<td>1.8</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>134</strong></td>
<td><strong>59.6</strong></td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Summary of full duck counts made on May 20-21, 2008 on the Redvers Study Area in Saskatchewan.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mallard</td>
<td>23</td>
<td>4.6</td>
<td>32.9</td>
<td>42.2</td>
</tr>
<tr>
<td>Gadwall</td>
<td>7</td>
<td>1.4</td>
<td>10.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Blue-winged Teal</td>
<td>19</td>
<td>3.8</td>
<td>27.1</td>
<td>23.8</td>
</tr>
<tr>
<td>Northern Shoveler</td>
<td>6</td>
<td>1.2</td>
<td>8.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Northern Pintail</td>
<td>3</td>
<td>0.6</td>
<td>4.3</td>
<td>10.0</td>
</tr>
<tr>
<td>American Wigeon</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Green-winged Teal</td>
<td>5</td>
<td>1.0</td>
<td>7.1</td>
<td>2.9</td>
</tr>
<tr>
<td>Canvasback</td>
<td>2</td>
<td>0.4</td>
<td>2.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Redhead</td>
<td>2</td>
<td>0.4</td>
<td>2.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Lesser Scaup</td>
<td>2</td>
<td>0.4</td>
<td>2.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Ruddy Duck</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Bufflehead</td>
<td>1</td>
<td>0.2</td>
<td>1.4</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>70</strong></td>
<td><strong>14.0</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Summary of full duck counts at the Lousana Study Area in Alberta made on May 22, 2008.

<table>
<thead>
<tr>
<th>Species</th>
<th># Pairs</th>
<th>#/mi²</th>
<th>% Spp. Comp.</th>
<th>14-yr. Avg. % Spp. Comp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mallard (<em>Anas platyrhynchos</em>)</td>
<td>29</td>
<td>8.0</td>
<td>23.4</td>
<td>28.5</td>
</tr>
<tr>
<td>Gadwall (<em>Anas strepera</em>)</td>
<td>11</td>
<td>3.0</td>
<td>8.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Blue-winged Teal (<em>Anas discors</em>)</td>
<td>23</td>
<td>6.3</td>
<td>18.5</td>
<td>22.6</td>
</tr>
<tr>
<td>Northern Shoveler (<em>Anas clypeata</em>)</td>
<td>16</td>
<td>4.4</td>
<td>12.9</td>
<td>2.2</td>
</tr>
<tr>
<td>Northern Pintail (<em>Anas acuta</em>)</td>
<td>2</td>
<td>0.6</td>
<td>1.6</td>
<td>5.1</td>
</tr>
<tr>
<td>American Wigeon (<em>Anas americana</em>)</td>
<td>7</td>
<td>1.9</td>
<td>5.6</td>
<td>6.7</td>
</tr>
<tr>
<td>Green-winged Teal (<em>Anas crecca</em>)</td>
<td>2</td>
<td>0.6</td>
<td>1.6</td>
<td>5.1</td>
</tr>
<tr>
<td>Canvasback (<em>Aythya valisineria</em>)</td>
<td>1</td>
<td>0.3</td>
<td>1.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Redhead (<em>Aythya americana</em>)</td>
<td>15</td>
<td>4.1</td>
<td>12.1</td>
<td>2.8</td>
</tr>
<tr>
<td>Lesser Scaup (<em>Aythya affinis</em>)</td>
<td>8</td>
<td>2.2</td>
<td>6.5</td>
<td>16.1</td>
</tr>
<tr>
<td>Ruddy Duck (<em>Oxyura jamaicensis</em>)</td>
<td>5</td>
<td>1.4</td>
<td>4.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Ring-necked Duck (<em>Aythya collaris</em>)</td>
<td>1</td>
<td>0.3</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Bufflehead (<em>Bucephala albeola</em>)</td>
<td>4</td>
<td>1.1</td>
<td>3.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Cinnamon Teal (<em>Anas cyanoptera</em>)</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Goldeneye (<em>Bucephala clangula</em>)</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.01</td>
</tr>
<tr>
<td>White-winged scoter (<em>Melanitta fusca</em>)</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>124</strong></td>
<td><strong>34.2</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 7. Summary data for the first duck count made at Woodworth, ND, May 15, 2008.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mallard</td>
<td>213</td>
<td>24.2</td>
<td>77</td>
<td>136</td>
<td>28.6</td>
<td>29.2</td>
</tr>
<tr>
<td>Gadwall</td>
<td>111</td>
<td>12.7</td>
<td>35</td>
<td>76</td>
<td>16.0</td>
<td>16.3</td>
</tr>
<tr>
<td>Blue-winged Teal</td>
<td>154</td>
<td>17.6</td>
<td>53</td>
<td>101</td>
<td>21.3</td>
<td>21.7</td>
</tr>
<tr>
<td>Northern Shoveler</td>
<td>64</td>
<td>7.3</td>
<td>8</td>
<td>56</td>
<td>11.8</td>
<td>12.0</td>
</tr>
<tr>
<td>Northern Pintail</td>
<td>31</td>
<td>3.5</td>
<td>0</td>
<td>31</td>
<td>6.5</td>
<td>6.7</td>
</tr>
<tr>
<td>American Wigeon</td>
<td>16</td>
<td>1.8</td>
<td>0</td>
<td>16</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Green-winged Teal</td>
<td>5</td>
<td>1.0</td>
<td>0</td>
<td>5</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Canvasback</td>
<td>6</td>
<td>1.0</td>
<td>0</td>
<td>6</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Redhead</td>
<td>28</td>
<td>3.2</td>
<td>15</td>
<td>13</td>
<td>2.7</td>
<td>2.8</td>
</tr>
<tr>
<td>Lesser Scaup</td>
<td>215</td>
<td>24.5</td>
<td>194</td>
<td>21</td>
<td>4.4</td>
<td>4.5</td>
</tr>
<tr>
<td>Ruddy Duck</td>
<td>25</td>
<td>2.9</td>
<td>24</td>
<td>1</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Ring-necked Duck</td>
<td>9</td>
<td>1.0</td>
<td>6</td>
<td>3</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Bufflehead</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>877</strong></td>
<td><strong>412</strong></td>
<td><strong>465</strong></td>
<td><strong>97.9</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*2008 count minus the non-breeding pairs.

Table 8. Summary data for the second duck count made at Woodworth, ND, May 28-June 2, 2008.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mallard</td>
<td>273</td>
<td>57.5</td>
<td>33.5</td>
<td>10.8</td>
<td>12.9</td>
</tr>
<tr>
<td>Gadwall</td>
<td>121</td>
<td>25.5</td>
<td>14.8</td>
<td>13.8</td>
<td>7.1</td>
</tr>
<tr>
<td>Blue-winged Teal</td>
<td>214</td>
<td>45.1</td>
<td>26.2</td>
<td>45.7</td>
<td>31.1</td>
</tr>
<tr>
<td>Northern Shoveler</td>
<td>74</td>
<td>15.6</td>
<td>9.1</td>
<td>5.9</td>
<td>13.1</td>
</tr>
<tr>
<td>Northern Pintail</td>
<td>31</td>
<td>6.5</td>
<td>3.8</td>
<td>6.3</td>
<td>27.6</td>
</tr>
<tr>
<td>American Wigeon</td>
<td>13</td>
<td>2.7</td>
<td>1.6</td>
<td>2.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Green-winged Teal</td>
<td>1</td>
<td>0.2</td>
<td>0.01</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Canvasback</td>
<td>4</td>
<td>0.8</td>
<td>0.1</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Redhead</td>
<td>20</td>
<td>4.2</td>
<td>2.5</td>
<td>3.7</td>
<td>2.8</td>
</tr>
<tr>
<td>Ruddy Duck</td>
<td>15</td>
<td>3.2</td>
<td>2.0</td>
<td>4.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Ring-necked Duck</td>
<td>5</td>
<td>1.1</td>
<td>0.1</td>
<td>0.02</td>
<td>0.0</td>
</tr>
<tr>
<td>Bufflehead</td>
<td>4</td>
<td>0.8</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Lesser Scaup</td>
<td>41</td>
<td>8.6</td>
<td>5.0</td>
<td>4.7</td>
<td>2.1</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>816</strong></td>
<td><strong>171.8</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Because this 2nd duck count wasn't adjusted for non-breeders according to criteria for indicated breeding pairs, data from the 1st count (Table 7) minus non-breeder are the most representative of the true nesting population.
Table 9. Number of canvasback breeding pairs (density/mi\(^2\)) counted during May 25 and 26, 2008 along 12 roadside transects near Minnedosa, Manitoba totaling 21.25 mi\(^2\).

<table>
<thead>
<tr>
<th>Least (pairs/mi(^2))</th>
<th>Average (pairs/mi(^2))</th>
<th>Most (pairs/mi(^2))</th>
<th>2008 (pairs/mi(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>110 (5.2)</td>
<td>152 (7.2)</td>
<td>210 (9.9)</td>
<td>84 (4.0)</td>
</tr>
</tbody>
</table>

Table 10. Number of canvasback broods (density/mi\(^2\)) counted during July 6, 7 and 8, 2008 along 12 roadside transects totaling 21.25 mi\(^2\) near Minnedosa, Manitoba.

<table>
<thead>
<tr>
<th>Least (broods/mi(^2))</th>
<th>Average (broods/mi(^2))</th>
<th>Most (broods/mi(^2))</th>
<th>2008 (broods/mi(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (0.28)</td>
<td>36.5 (1.72)</td>
<td>91 (4.28)</td>
<td>5* (0.24)</td>
</tr>
</tbody>
</table>

*All 5 were IIc or class III brood age and 5 broods ÷ 84 pairs = 6% hen success.
WATERFOWL IN THE PREHISTORY OF SOUTH DAKOTA

David C. Parris* and Kenneth F. Higgins2
1New Jersey State Museum
P.O. Box 530
Trenton, NJ 08625-0530
2Wildlife and Fisheries
South Dakota State University
Brookings, SD 57007
*david.parris@sos.state.nj.us

ABSTRACT

The paleontological and archaeological records of waterfowl have provided extensive evidence of a sixty million year prehistory of the Order Anseriformes. The distinctive shapes of the skulls and many of the limb bones have enabled recognition of this group in many paleofaunas of the Cenozoic Era, especially in North America. Abundant and useful, waterfowl have had a long association with human populations as well, especially in South Dakota.

We compiled an extensive bibliography and examined a number of actual specimens in order to provide this review of waterfowl prehistory, focused on South Dakota. The earliest waterfowl, related to the Anseranatidae (Magpie Geese), are fossils from the earliest Paleocene. They are found in sediments laid down shortly after the extinction event that ended the reign of the dinosaurs. Later fossils attributed to waterfowl have been reported from Eocene sediments in Wyoming and from Oligocene, Miocene, and Pliocene rocks in South Dakota. One bone of a duck was recovered from the Late Pleistocene Lange-Ferguson Site (Shannon County), a mammoth kill attributed to early humans.

Archaeological sites of the Missouri Basin dam salvage projects produced waterfowl bones, demonstrating that the Arikara and other cultures made use of at least thirteen species of swans, geese, and ducks. Other sites in South Dakota, notably the Black Hills, have yielded isolated records of various species, notably of goose and teal species. Explorer accounts and ethnographic descriptions supplemented these records, confirming the venerable cultural associations of waterfowl in this region.

Keywords
Anseriformes, paleontology, archaeology, South Dakota
INTRODUCTION

Ducks, geese, and swans have a place in human cultures that is exceeded by no other group of birds. Their abundance, usefulness, and ease of domestication are part of recorded history. The Order Anseriformes, to which most living species belong, has long served human needs for food (flesh and eggs), sport, and even aesthetics (as with swans in parks). They are included within some of our most cherished traditions. (Dickens’ enlightened Ebenezer Scrooge quickly ordered a Christmas goose for the Cratchit family.)

The full story of the waterfowl begins far earlier, of course, and greatly exceeds that of humanity. The duck family was recognizable as such at a time when the ancestors of humans were small furry tree-dwellers. It is a fascinating story, much of it well-documented in the paleontology of North America, including what is now South Dakota. From the time of the earliest known records about sixty million years old, these ancestral ducks were prominent among bird life and diverged to become the varied and abundant group known to nearly everyone today.

The archaeological record demonstrates that waterfowl species were important to human cultures throughout prehistory as well. Nowhere is this more evident than in South Dakota, where the record of ducks and duck hunting extends back for thousands of years. It is likely that some of the twenty-first century practices of sport hunters (such as camouflaging and decoying) were also used by the earliest residents of the Missouri River Basin and the Black Hills. Research elsewhere in North America (and throughout the world) has shown that the interactions of human cultures with waterfowl have astonishing complexity over long periods of time (Alison 1978, McAndrews and Turton 2007), confirming the importance of research on the subject.

The Fossil Record of the Waterfowl—The earliest birds thus far discovered are from the rocks of the Jurassic System in the Old World. The famous (but very rare) Archaeopteryx is still considered to be the earliest known creature recognizable as a bird, although it resembles its reptilian ancestors in many ways. The few specimens all come from the lithographic limestones of Germany. These birds lived within what is known as the Mesozoic Era, or time of “Middle Life”, also remembered as the Age of the Dinosaurs, the probable ancestors of birds. At the end of the Mesozoic Era, about sixty million years ago, the dinosaurs abruptly became extinct, along with vast numbers of other animal species. Precisely coinciding with that cataclysmic event, the fossil record has yielded evidence of the earliest ancestral ducks.

The few paleontologists who specialize on fossil birds deserve a few words of tribute, for they face a daunting task. Because most birds are flying creatures, their bones tend to be very light and thin, easily destroyed and rarely preserved as fossils. Only a few of the more durable bones of a bird skeleton are likely to ever be found, and whole bones are particularly rare. Fossil mammals are often represented by their teeth, but only a few of the earliest birds have any teeth at all. Some dinosaurs are known from nearly complete skeletons, but many fossil bird species are known from only one end of a single bone specimen!
In the coastal plain of New Jersey, in remarkable sediments called greensands, bird fossils come from the earliest Paleocene Age, immediately after the time of dinosaur extinction. The several dozen known specimens are among the most precious collections of fossil birds in the world. Two of the bones, given the name of *Anatalavis rex*, represent the earliest known waterfowl (Olson and Parris 1987). Each of the bones is a humerus, a wing bone that has a very distinctive shape in members of the Order Anseriformes, to which waterfowl belong. These specimens are a modest early record of what was to become one of the most important groups of birds. *Anatalavis* also is known from later (Eocene Age) sediments in Europe (Olson 1999), and the better specimens found there have confirmed that it belongs to the primitive waterfowl Family Anseranatidae, which has but one living species, the Australian Magpie Goose (*Anseranas semipalmata*).

Within the next few million years, waterfowl developed the very distinctive physical features by which we now recognize them, notably the broad bill and tongue. Their association with aquatic environments, where their remains are likely to be preserved in muddy sediments, has provided a somewhat better fossil record than is true of most birds. Substantial numbers of fossils are found in several sites, some of which are in Wyoming, where an evolutionary branch family, the Presbyornithidae, is represented by great numbers of specimens.

*South Dakota Duck Fossils*—The Cenozoic Era, or “era of modern life”, is well represented in South Dakota, notably by the geological formations of the Big Badlands region. It is there that waterfowl (Family Anatidae) fossils have been found which have close relationships to the species of the present. Some fossil eggs, questionably attributed to ducks, have been reported from the Big Badlands rocks of Oligocene Age, perhaps 40 million years old (Adolphson 1973).

In the Miocene Age that followed (about 30 million years ago), South Dakota duck fossils are definitely known. The Batesland Formation in Bennett County includes the Flint Hill North Local Fauna, which has yielded many fossil bird specimens. In early descriptions of the Flint Hill North Local Fauna (Miller and Compton 1939, Miller 1944), it was noted that bird fossils were uncommon in most fossil sites, but that in this paleofauna they were well represented.

Among the various bird species from Flint Hill North are three ducks. A rather large (swan-sized) supposed Diving Duck was described from the deposit, represented by two bones. It was given the name *Paranyroca magna*. A Whistling Duck, *Dendrochema robusta*, also was described as a new genus and species. Several bones were referred to it, an unusual situation for fossil birds, many of which are based on unique specimens. A new species of Teal, *Querquedula integra*, was also described, with several referred specimens. The ducks from Flint Hill North were among those birds cited as evidence for a very aquatic environment (large river or lake) of the fossil deposit, although woodland bird species were also found (Miller 1944).

One fossil Teal from South Dakota has been reported from sediments of Late Miocene Age (Brodkorb 1964a). Typical of fossil birds, the type (originally described) specimen is but one end of a long bone, a humerus. Named *Nettion greeni*, it too comes from Bennett County, in the Ash Hollow Formation, and
is probably about ten million years old. A fossil goose, referred to the genus *Branta*, was reported from somewhat later (Pliocene) sediments by Compton (1935).

Although few in number, these records of ducks from the Cenozoic are sufficient to demonstrate a substantial presence and variety of ducks in what was to become South Dakota during the past 60 million years, up to the time of the Pleistocene, known as the “Ice Age”. Toward the end of the Pleistocene, the archaeological (human cultural) record begins, and one of South Dakota's most venerable sites is from that time. The Lange/Ferguson Site, designated as 39-SH-33, is a mammoth kill site of Paleo-Indian (Clovis) hunters. The site is at least 10,000 years old. Although best known for its association of humans with an extinct elephant, it also produced a single bone of a duck, which at least indicated that an aquatic environment was nearby at that time (Martin 1987).

**The Archaeological Record of Ducks in South Dakota**—The last one million years, from the “Ice Age” to the present day, have given South Dakota a wonderful heritage of waterfowl. With the arrival of human populations from Asia across the Bering Sea, the stage was set for an archaeological (rather than paleontological) record. As they always have, waterfowl became a part of human culture and resources (Zimmerman 1985). Our knowledge of this record, which we call zooarchaeology, is rapidly increasing. There are several reasons why this is so.

The records of Pleistocene and prehistoric waterfowl were meticulously summarized by conscientious scientists during the middle of the twentieth century (Brodkorb 1964b). There was little mention of South Dakota in such summaries, but that did not mean that no studies had taken place. In fact, South Dakota had been the site of some of the most comprehensive archaeological studies ever attempted, when the Missouri River dams were constructed. Among the considerations in planning the projects was the possibility that some wildlife would benefit from the water impoundments (Aitken 1949). These ambitious engineering projects also inspired government-sponsored excavations so that cultural resource losses would be mitigated. Scientists, engineers, and planners of that period deserve much credit for preserving so much of our heritage, but the analysis and publication of these excavations has taken many decades (Thiessen 1994). This was especially true for zooarchaeology, but much has been revealed during the last thirty years (Parmalee 1977a,b), and further analyses with the latest methods are continuing (Tiffany 2007). With new studies continuing, South Dakota's archaeological heritage is becoming even better understood.

The zooarchaeological record of the Black Hills has been recently summarized (Sundstrom 1996) and contains a few records of human usage of waterfowl. For example, two well-documented records of geese in Fall River County sites are cited, but a fair number of Black Hills sites are listed with bird remains that were not determined to species. There has been a strong emphasis on large mammals in Black Hills area sites however, and birds may not seem to be significant resources in such situations. However, detailed excavations in the Black Hills almost always produce significant archaeological records of bird remains, including waterfowl (Martin et al. 1993).
In contrast, the archaeological surveys of the Missouri River basin have produced many bird specimens, and these were studied by one of the nation's most noted zooarchaeologists, Paul Parmalee (1977a,b). His meticulous research revealed that Missouri River sites had yielded specimens of 13 species of waterfowl from 51 different sites, ranging over a period from A.D. 900 to A.D. 1780, most notably representing the Arikara cultures. Swans, geese, and ducks were all represented, and additional information from early ethnographic studies was correlated with the species recorded and the bone elements that had been identified. The study was so thorough that a number of specimens with healed injuries or diseased malformations were also described, despite the difficulty of identifying them to species. Parmalee had accepted the task of research on nearly all bird bones from nearly all sites in the River Basin Surveys, over 3000 specimens in all. It is not surprising that his work was not completed and published until several decades had elapsed since the excavations.

During recent years greater attention has been given to bird bones from archaeological sites, and an accurate faunal list and analysis is expected as part of all site reports. The renewed work at the Bloom Site in Hanson County (Haug et al. 1994) was typical of such investigations. Animal bones were identified and published with the rest of the site report and included the remains of Blue-winged Teal.

When sites from earlier excavations are reexamined, the results often differ considerably from the initial analyses, an indication of how much the science of zooarchaeology has advanced. The Swanson Site (Tiffany 2007) is typical of these. The original report of the site (Hurt 1951) included a faunal list with four waterfowl species, Blue-winged Teal, Green-winged Teal, Canada Goose, and Snow Goose. However, the faunal material was not so fully curated as were other specimens from the site, and the extant collection includes but a few species of any vertebrates. Waterfowl represented in the extant collection include only a Snow Goose maxilla (upper jaw portion) and a duck pelvic fragment unidentifiable to species (Tiffany 2007). If the curation had followed the standards of the present day, all specimens would have been saved and given catalogue numbers, for the faunal material is actually artifactual evidence.

The primary evidence for cultural use of birds is the presence of identifiable bones in an excavated site. Of course, other cultural evidence is welcome, but not always present, especially in the case of waterfowl. Although there is anecdotal evidence of decoying waterfowl throughout human history, evidence of prehistoric use of decoys is extremely rare. The few known examples may explain the reasons for this; decoys found in a prehistoric site near Lovelock, Nevada, were made of tule reeds (Scirpus acutus) and feathers, materials that would be preserved only in the most extraordinary environmental conditions (Byrd 2009). There were no decoys of the more durable materials now used.

Although this review is intended primarily to document prehistoric records of waterfowl, we also note the importance of records in historical sites. These may give evidence of reliance upon hunted fauna rather than domestic animals, a very important distinction in interpretation of sites. Exemplary among such records are the species listed by Bozell (1996) for historic features in a site in Lead (numbered 39 LA 3001). Possible Canada Goose and Teal remains were
identified, but conservatively interpreted as possibly wild or semi-domestic, thus worthy of further investigation. Another historic site that produced anseriform remains was at Fort Randall in Gregory County (numbered 39 GR 15), reported by Lees (1991).

**Records of Waterfowl Species in South Dakota Prehistory**—The more than 40 North American waterfowl species (Order Anseriformes) may range widely and possibly occur as accidentals in most of the North American continent (Wylie and Furlong 1972). While only a limited number of species would be expected in South Dakota archaeological site records, an essentially complete list is presented here. Paleoentologists and zooarchaeologists generally classify them into tribes, of which eight are to be expected in North America.

**Swans (Tribe Cygnini)**

The Trumpeter Swan (*Cygnus buccinator*), although now considered to be accidental in the Mississippi Flyway and rare in the Central Flyway, was recorded from Missouri River sites by Parmalee (1977a,b). There were few specimens, but some included recognizable butcher marks and other certain indications of the importance of swans in prehistoric cultures. The spectacular size and plumage of swans has had appeal in many world civilizations, exceeding their obvious value as food. Parmalee (1977a) quotes several early ethnographic accounts of Plains cultures that incorporated decorative and ritualistic aspects of swans.

The Tundra Swan (*Cygnus columbianus*) is rare in the mid-continent and has not been recorded from South Dakota archaeological sites. The Mute Swan (*Cygnus olor*) is an introduced species with no archaeological records from South Dakota.

**Geese (Tribe Anserini)**

Of the best-known genus of geese (*Chen*), the five major American forms are the White-fronted Goose (*C. albifrons*), Snow Goose and Blue Goose (*C. caerulescens*), Ross' Goose (*C. rossi*), and Emperor Goose (*C. canagius*). They are difficult to distinguish from bones alone, and Parmalee (1977a) noted only *Chen caerulescens* (the two most common color morphs or subspecies) from Missouri River prehistoric sites, while noting that other forms might be represented. More than one hundred bones of these geese were identified, a strong indication of their cultural importance, especially for food.

The Canada Goose (*Branta canadensis*) was recorded by Parmalee (1977a), who identified more than one hundred bones of this species that had been found in Missouri River sites. Although the resident and migratory patterns of this very common species have changed considerably in modern times, it was apparently always common in what is now South Dakota. The Canada Goose was also reported from a site in the Black Hills, 39-FA-23, by Wheeler (1995), and repeated in the summary of Sundstrom (1996). Published records of this species include the Swanson Site (Tiffany 2007), Crow Creek Site (Kivett and Jensen 1976) and Lead Site (Bozell 1996). The other two species of *Branta*, the Brant (*B. bernicla*).
and Barnacle Goose (*B. leucopsis*), have coastal preferences and would not be expected in mid-continent sites. As noted previously, a fossil of *Branta* from Pliocene sediments was reported by Compton (1935).

In some respects, the various species of geese may have been the most important waterfowl to the prehistoric people of what is now South Dakota. Geese are generally large birds and migrate in sizable groups, often being quite gregarious and relatively approachable. Would they have been easy to hunt? The significant number of records from archaeological sites seems to suggest that they were. Also, the ethnographic notes of early explorers seem to confirm the importance of geese. Prince Maximilian (Thomas and Ronnefeldt 1976) noted in the expedition journal for the Sojourn at Fort Clark (now Bismarck, North Dakota) that:

“The women at Mih-Tutta-Hang-Kush celebrated the spring corn feast, of which Mr. Bodmer made a sketch. This feast is always observed on the return of the wild geese, which are the messengers of the old woman who never dies. The Indians had already killed some of these birds.” (Entry for April 2 1834)

Whistling Ducks (Tribe Dendrocygnini)

The Fulvous Whistling Duck (*Dendrocygna bicolor*) and Black-bellied Whistling Duck (*D. autumnalis*) are rare in the Mississippi and Central Flyways and have no records in South Dakota archaeological sites. However, as previously noted, a fossil Whistling Duck (*Dendrochon robusta*) was described from the Miocene of South Dakota by Miller (1944).

Pond and Puddle Ducks (Tribe Anatini)

The Mallard (*Anas platyrhynchos*) is the most common of ducks and was identified in a number of Missouri River sites by Parmalee (1977a), who considered it to be indistinguishable from the Black Duck (*Anas rubripes*) on the basis of bones alone. Needless to say, the two subspecies variants, the Mexican Duck (*Anas platyrhynchos diazi*) and the Florida Duck (*Anas platyrhynchos fulvigula*) would not be distinguishable either, and neither would be expected in the northern plains region. Mallards are representative of the larger species of puddle ducks (*Anas*), and are the most expected in any site.

Green-winged Teal (*Anas crecca carolinensis*) is perhaps the most typical of the smaller species of Anas, and thus recognizable on size alone. Fifteen bones of this species were identified by Parmalee (1977a) from Missouri River sites. A number of additional specimens were identified as possibly teal species, including some possible bones of Blue-winged Teal (*Anas discors*), a slightly larger species. The Blue-winged Teal was positively identified in the assemblage from the Bloom Site (Haug et al. 1994). As previously noted, there are also much older records of fossil teal species from what is now South Dakota. The Shoveler (*Anas clypeata*) has a distinctly large bill and can sometimes be distinguished from other small puddle ducks, if the most diagnostic bones are found. Two specimens were noted by Parmalee (1977a) in collections from early sites along the Missouri River. Other species of *Anas*, such as the Gadwall (*A. strepera*), Wigeons
(A. penelope and A. americana), and Northern Pintail (A. acuta) have not been recorded from South Dakota archaeological sites, although most of them are well known in the region.

Diving Ducks (Tribe Aythyini)

The Redhead (Aythya americana) is fairly distinctive among diving ducks in size and bone anatomy, and was found in bone collections from a few sites along the Missouri River collections, cited by Parmalee (1977a). Larger and smaller species of Aythya, such as the Canvasback (A. valisneria), Ring-Necked Duck (A. collaris), and Lesser Scaup (A. affinis), were possibly also present in the collections, but identified with less certainty.

Sea Ducks (Tribe Mergini)

Although this rather large subfamily includes mostly ducks of coastal and marine environments, a few species are found regularly in the mid-continent. The Hooded Merganser (Lophodytes cucullatus) is distinctive in size and skull shape. This small merganser was recorded from a number of Missouri River sites by Parmalee (1977a), although it is considered to be an uncommon species there at present. The other mergansers, the Common Merganser (Mergus merganser americanus) and Red-Breasted Merganser (Mergus serrator) have not been found in South Dakota sites.

Ducks of the Genus Bucephala include the American Goldeneye (B. clangula) and the Bufflehead (B. albeola). Both are considered common in the mid-continent, but have no archaeological record from South Dakota as yet. The Barrow’s Goldeneye (B. islandica) has no record either and would not be expected, as it is rare there.

The Eiders (Genus Somateria), Scoters (Genus Melanitta), Harlequin Duck (Histrionicus histrionicus), and Oldsquaw (Clangula hyemalis) are not known from South Dakota archaeological sites. All are predominantly coastal or marine.

Stiff-tailed Ducks (Tribe Oxyurini)

This tribe includes the Ruddy Duck (Oxyura jamaicensis) and Masked Duck (O. dominica). Neither has any archaeological record in South Dakota, although the Ruddy Duck is common there, and the bones of these two species are quite distinctive and easily identified.

SUMMARY

South Dakota has substantial records of the paleontology (fossil prehistory) of waterfowl and also the archaeology (human cultural aspects) of waterfowl hunting and usage. The extensive research from excavations now and in the future will surely add greatly to this record, and may well contribute further perspec-
tives that will assist in conservation and management of this culturally important group of birds.

ACKNOWLEDGEMENTS

We are grateful to George Grigonis for his assistance in information technology and media presentations. James E. Martin, Kent C. Jensen, and Margaret M. F. O’Reilly reviewed the manuscript and made important suggestions for improvements.

LITERATURE CITED


Olson, S.L. 1999. The anseriform relationships of Anatalavis Olson and Parris (Anseranatidae), with a new species from the Lower Eocene London Clay. Smithsonian Contributions to Paleobiology 89: 231-244.


A curious occurrence of gnawed rock fragments was discovered on the Kenneth Brown Ranch southeast of Rapid City, along Spring Creek in Pennington County, South Dakota. The fragments are concretions derived from the Pierre Shale Group (Late Cretaceous) and commonly contain invertebrate fossils, principally inoceramid pelecypods. The concretionary fragments contain paired gnaw marks, similar to those made by rodents, and were found lying adjacent to holes in a prairie dog town. The area is inhabited by *Cynomys ludovicianus*, and the paired grooves matched those of paired incisors of the black-tailed prairie dog. Chemical analysis of the fossiliferous concretions indicates high concentrations of calcium, suggesting the rodents were chewing the rocks in order to derive these salts. Another possibility for the occurrence is that the concretionary fragments were gnawed as part of the burrowing process and pushed to the surface. However, non-burrowing rodents have been observed to gnaw calcium-bearing rocks and bones. Maintenance of incisor length and health may also be involved in the gnawing habit.

Keywords

Black-tailed prairie dog, fossiliferous concretions, gnawing

INTRODUCTION

The second author found fragments of concretionary material, which exhibit paired grooves (Figure 1), scattered around prairie dog holes on his ranch along Spring Creek in Pennington County, South Dakota. Mr. Brown brought the rock fragments to the Museum of Geology where they were identified; subsequent field investigations produced additional specimens from the prairie dog town. The prairie dog town had been developed on Quaternary alluvium that overlies the Pierre Shale Group of Late Cretaceous Age (Martin et al. 2007). The alluvium is part of the terrace fill from Spring Creek, which flows easterly from the Black Hills. Although the Cretaceous shale was not well exposed at the...
Figure 1. Rodent-gnawed concretionary clasts. A) Fossil inoceramid clams found near prairie dog holes. The middle specimen (SDSM 84601) exhibits paired grooves. B) Grooves on ventral side of SDSM 84601; arrows indicate paired grooves, and the middle arrow points to the grooves illustrated in the SEM photograph, Fig. 1G. C-E) SDSM 84602, concretionary clast that exhibits extensive gnawing and fossil clam. F) SDSM 84603 broken concretionary clast with incisor grooves. G) SEM photograph of paired grooves on SDSM 84601. H) Cynomys ludovicianus cranium with incisors placed on paired grooves of SDSM 84601. All scales in centimeters except Fig. 1G.
prairie dog town, regional stratigraphic investigation indicates that the lithostratigraphic unit subjacent to the alluvium appears to lie stratigraphically above the Mitten Black Shale Formation (Martin et al. 2007), probably a Red Bird Silty equivalent (Gries and Martin 1985), but the precise formational entity could not be proven. The prairie dog town was inhabited by *Cynomys ludovicianus*, the black-tailed prairie dog, a common species east of the Black Hills in western South Dakota (Turner 1974).

Abbreviations include: N = number of measurements; OR = observed range; SDSM = Museum of Geology, South Dakota School of Mines and Technology; SEM = Scanning Electron Microscope; X = mean.

**ANALYSIS**

Both fossiliferous and unfossiliferous concretionary materials were found with paired grooves. Most concretions include Cretaceous fossil invertebrates, and some consist of steinkerns (Figure 1A) and indicate original derivation from the Late Cretaceous Pierre Shale Group. The rock specimens are principally in the 5-6 cm range, but some can be up to 10 cm (Figure 1C). Most concretionary fragments are subrounded, but whether the rounding is the original concretionary shape or the result of fluvial reworking cannot be positively determined. Some angular specimens (Fig. 1F) indicate relatively recent breakage, but the breakage occurred before the paired grooves were incised. This suggests, but is not conclusive proof, that reworking had occurred, and these concretionary materials may have been derived by the rodents from the Quaternary alluvium rather than from the underlying Pierre Shale Group.

Invertebrate fossils are common among the concretionary specimens with paired grooves; some specimens represent steinkerns or actual fossil material (Fig. 1A). Original shell material is preserved on some, but only one specimen (Fig. 1C) exhibited any intersection of the original shell material by the paired grooves. Most fossils (e.g. SDSM 84601) are relatively poorly preserved, but all appear to be pelecypods, and most appear to be smaller inoceramids of the *Inoceramus perplexus* morphotype (Kauffman 1967), which are relatively common in the Pierre Shale Group (Fox 2007); one specimen (SDSM 84602, Fig. 1C) appears to represent the anomid pelecypod, *Anomia*.

Scanning electron microscopy revealed the grooved structure (Fig. 1G), as well as providing a quantitative analysis of the concretionary material. Figure 2 indicates that the concretions are calcium-aluminum silicate. The high concentration of calcium was expected, given the associated fossil remains, although the matrix, not the shell material, was sampled for this analysis.
The paired grooves occur principally around the edges of the concretionary clasts, although some occur on the larger faces (Figures 1B,E). One specimen exhibits very long grooves (Figure 1E), although most pairs are short (Figures 1B,D). Paired width measurements of the three specimens indicate a similar width of the grooves among various concretionary clasts (SDSM 84602, N = 15, X = 4.3 mm, OR = 4.0-4.7 mm; SDSM 84601, N = 6, X = 4.2 mm, OR = 3.9-4.6 mm; SDSM 84603, N = 6, X = 4.2, OR = 3.9-4.7 mm). The observed ranges are small, and the variation can be explained by some grooves being incised deeper or by individual variation, although the latter is less likely
because most grooves on each concretionary clast were probably the result of a single individual. When Recent crania with incisors were placed in the grooves, they matched (Figure 1H). Moreover, when width measurements were taken of the incisor tips of *Cynomys ludovicianus* (N = 16, X = 4.2, OR = 3.8-5.1), the means were found to be very similar to those incised into the concretionary clasts, indicating that the incised grooves were made by the black-tailed prairie dog. This conclusion was expected given the source of the specimens from around the openings of prairie dog burrows.

Prairie dogs are principally herbivores, although they may include some invertebrates (arthropods) in their diet (Uresk 1984). The black-tailed prairie dogs normally ingest grasses and forbs, with lesser amounts of shrubs and seeds; selective feeding is practiced (Summers and Linder 1978; Uresk 1984); however, inorganics are not commonly found as consumables. Nevertheless, rodents characteristically gnaw bones and antlers. The reason for the gnawing behavior may have multiple purposes. Not only could dietary supplement be causative, but because prairie dogs are burrowing, the grooves may be the result of the burrowing process. However, one might not expect great number of grooves widely distributed over the concretions if the grooves were a by-product of the digging process. Moreover, other non-burrowing rodents (tree squirrels) have been observed by the first author gnawing concrete fragments in Louisiana. Obviously, these fragments were not the result of burrowing, and calcium is a major component of concrete. Another possibility to explain the grooves is for maintenance of length and health of the incisors. Because rodent incisors are ever-growing, if malocclusion occurs, the result can be detrimental or even result in the demise of the individual as documented by Martin (1983).

**SUMMARY**

Concretionary clasts with paired grooves (Figure 1) were found in the debris around the burrow openings made by black-tailed prairie dogs. Many of the clasts contained fossil bivalves characteristic of the Late Cretaceous Pierre Shale Group, and this contribution represents the first report of such an occurrence. The concretions may have been reworked by fluvial processes into Quaternary alluvium. Composition of the clasts is calcium-aluminum silicate. The paired grooves were expected to have been made by the incisors of black-tailed prairie dog (*Cynomys ludovicianus*), and incisor width measurements supported this hypothesis. The high calcium content of the concretions (Fig. 2) is likely the reason that the prairie dogs gnawed these concretionary clasts. Paired rodent gnawing marks to gain salt content are common on Recent bones and antlers. Alternatively, the marks may only be the result of gnawing when rocks were dislodged during the burrowing process. However, the first author has observed non-burrowing rodents (tree squirrels) in Louisiana gnawing concrete fragments, which obviously have high calcium content. The gnawing habit may also be involved in maintaining incisor length and health.
ACKNOWLEDGEMENTS

We thank Dr. Edward Duke, SD School of Mines and Technology, who aided in SEM and chemical analyses. Dr. Darrin Pagnac, Museum of Geology, and David Parris, New Jersey State Museum, provided critical reviews of the manuscript for which we are appreciative.

LITERATURE CITED


CULTURE OF ADVANCED-SIZED LARGEMOUTH BASS FOR STOCKING INTO SOUTH DAKOTA IMPOUNDMENTS

Matthew J. Ward1*, Michael L. Brown2 and Isak J. Csargo2
1South Dakota Department of Game, Fish and Parks
Blue Dog Lake State Fish Hatchery
44437 139A St., Waubay, SD 57273
2South Dakota State University
Department of Wildlife and Fisheries Sciences
Northern Plains Biostress Lab 138, Box 2140B
Brookings, SD 57007
*matthew.ward@state.sd.us

ABSTRACT

We investigated a combination of culture techniques for producing advance-sized largemouth bass (Micropterus salmoides) that may yield increased stocking contributions to South Dakota impoundments. During the initial extensive phase (May – July), 8,800 age-0 largemouth bass at a size of 350 bass/kg were produced and brought into the hatchery. Inside (August – April), acceptance of dry diets was high with overall survival of 93%. Specific growth rate (% body weight / d) was correlated significantly with water temperature (r = 0.82, P < 0.01). A significant regression equation predicted that water temperatures of 18 ºC during the intensive culture period would have led to growth rates of 1.3% body weight/d. All bass were freeze branded and 99.5% of the brands were readily identifiable at seven and 60 d following branding. In the final extensive phase, a natural prey was provided and survival was 87%. Overall, a total of 7,100 age-1 largemouth bass at a size of 73 bass/kg were produced for stocking into South Dakota waters. Important aspects contributing to growth performance included initiating the intensive phase while Blue Dog Lake water was 25 ºC and using a heat pump (Westinghouse Templifier) to increase and sustain water temperatures during the winter months. The combination of extensive and intensive techniques was effective in producing an advance-sized largemouth bass product for stocking.

Keywords
largemouth bass, stocking, small impoundments, water temperature, growth

INTRODUCTION

Largemouth bass (Micropterus salmoides) have been stocked into numerous small impoundments across the United States (e.g., Dillard and Novinger 1975;
Modde 1980). Although largemouth bass (hereafter referred to as bass) were likely not native to South Dakota waters (Bailey and Allum 1962), the species has been widely stocked into small water bodies throughout the state with variable degrees of success. Where populations have become established, bass have become a popular sport fish among South Dakota anglers. Stone’s (1996) angler survey indicated that bass were a highly preferred sportfish, ranking fourth statewide.

In South Dakota, fingerlings are produced and reared in ponds (i.e., extensively) then stocked in early to mid-September at a rate of 250 per ha, ranging in size from 374 to 198 bass/kg (61 to 74 mm total length). Previous research has documented that the stocking contribution of these fingerlings has been highly variable. Low returns of stocked bass have been attributed to size at stocking, likely due to increased predation and reduced energy reserves that may affect overwinter survival. Size-related survival has been documented in other predatory fishes. Longer smallmouth bass (*Micropterus dolomieu*) had increased survival in an overwinter experiment (Oliver et al. 1979). Increased size at stocking improved survival of channel catfish (*Ictalurus puncatus*) (Storck and Newman 1988), walleye (*Sander vitreus*) (Brooks et al. 2002), and muskellunge (*Esox masquinongy*) (Szendrey and Wahl 1996). A recent study by Diana and Wahl (2009) investigated four sizes of pellet-reared bass (55, 100, 150 and 200-mm total length) stocked into four Illinois reservoirs, but found no difference in long-term survival among size groups. Although high levels of initial predation were observed in the 55 to 150 mm sizes, low long-term survival rates of all sizes were attributed to starvation or an inability to compete with the existing population caused by difficulties adjusting to live prey. In general, these studies infer that stocked largemouth bass should be of sufficient size and habituated to actively avoid predators, be fully converted to live prey (actively search, capture and consume live fish) to offset competition effects, and stocked sufficiently early in the growing season to allow an acclimation period and building of energy stores prior to winter. The latter may be particularly important given the seasonally harsh climate and dynamic precipitation/drought cycles characteristic of South Dakota.

A variety of techniques for culturing bass exist. Some rely solely on pond rearing techniques (Broughton et al. 2008), whereas others have successfully spawned broodstock in raceways (Mayes et al. 1993) and feed trained fry intensively (Willis and Flickinger 1981). Studies in Kentucky and Illinois reported using a combination of pond and raceway methods (Coyle et al. 2009; Diana and Wahl 2009). Out of season spawning of walleye (Malison et al. 1998) and yellow perch (*Perca flavescens*) (Kolkovski and Dabrowski 1998) has been accomplished. This provides potential to produce a larger fish by a particular time compared to the size of fish produced at normal spawning times. Out of season spawning of northern bass is not well documented, and implementing that process may be logistically difficult for multi-species hatcheries. Initially using pond procedures and transitioning to raceway techniques during the winter months may be an effective strategy to raise advance-sized bass for spring stocking in South Dakota waters.
Intensively cultured fish are typically fed a pelleted diet (Twibell et al. 2009, Clayton et al. 2009, Skudlarek et al. 2007) and the feed training success on these diets will ultimately affect survival and growth. Feed training success of fishes can be influenced by turbidity (Bristow et al. 1996), fish size (Kubitza and Lovshin 1997), and possibly rearing density. Largemouth bass acceptance of artificial diets has been reported to be lower for dry pellets (17.2%) compared to moist pellets (92.3%) (Lovshin and Rushing 1989). Feed training and growth rate of intensively cultured bass are also known to be influenced by water temperature (Coyle et al. 2009).

The objective of this study was to investigate a combination of extensive (i.e., pond) and intensive (i.e., raceway) culture techniques to produce advance-sized bass for stocking into South Dakota waters.

**METHODS**

**Phase 1: Initial Extensive Culture**—Bass broodstock (18:12, male:female ratio) were introduced into a 0.4-ha pond on 21 May 2009 and allowed to spawn. On 11 July, fry were harvested from the spawning ponds and 300 were weighed to estimate the number of bass/kg. Total kilograms harvested were determined by weighing standardized nets filled with bass to the nearest 0.04 kg (Intercomp Model CS200 scale, Medina, MN). Total kilograms harvested were then multiplied by the number of bass/kg to estimate the number harvested. Fry were then stocked into a 0.8-ha “grow-out” pond that was fertilized every ten days with chopped alfalfa hay at a rate 142 kg/ha. On 7 August, the 0.8-ha pond was harvested and total kilograms of bass, bass/kg, and number of bass were determined as described for harvest from the spawning pond. The number of bass harvested was divided by the number stocked and expressed as a percentage to represent survival.

**Phase 2: Intensive Culture**—Water Source and Temperature. A combination of filtered Blue Dog Lake water and unfiltered well water was used to intensively culture bass from 7 August 2008 through 5 May 2009. Filtered lake water was used from 7 August through 10 October until water temperatures dropped below 10.0 °C. Water temperature was recorded daily at 1500 h using a YSI 55 meter (Yellow Springs, OH). On 10 October, the water source was changed to unfiltered well water which maintained a constant 9.4 °C. From 5 February to 26 March 2009, a Westinghouse Templifier heat pump (Staunton, VA) was used to gradually increase the temperature of the well water to a constant 18.7 °C. Water temperature returned to 9.4 °C on 26 March and remained there through April. An average water temperature was calculated for each interval that corresponded to the bass growth estimates. Also, an average water temperature was calculated for each month based on the daily temperature values recorded at 1500 h. Flow rates were maintained at 113 L/m in each raceway.

**Procedures**—On 7 August, all juvenile bass were brought into the hatchery for feed training. Initial rearing densities from 7 August through 29 October were 2.2 kg/m³ (805 bass/m³) for two raceways and 3.1 kg/m³ (1,085 bass/m³) for four
raceways. Tank covers were used on each raceway and covered 2/3 of the area with a partial opening in the middle to allow pelleted food to be dispensed from the Loudon II Feeders (Gaston, VA). At first, BioVita Fry (2.5-mm diameter pellet, Bio-Oregon, Longview, WA) was fed daily at half hour intervals starting at 0800 h and continuing until 1500 h; this resulted in a total of 14 feedings daily. On 29 October, all juvenile bass were divided equally among four raceways to better accommodate the hatchery production schedule. Each raceway received 13.9 kg of bass resulting in a rearing density of 2.8 kg/m$^3$ (415 bass/m$^3$). Tank covers were not used after 29 October. On 16 September, the starter diet was gradually switched to the Richloam diet (3.5-mm diameter pellets, Richloam State Fish Hatchery, Webster, FL), a specialty largemouth bass diet. Starting on 23 September, the Richloam diet was fed for the remainder of the intensive culture period at hourly intervals from 0800 h to 1500 h. Fish were fed daily when filtered lake water and heated well water were used (7 August through 9 October, 5 February through 23 March); however, food was administered on a continuous cycle of 5 days feed, 2 days fast when ambient well water was used (10 October through 4 February, 24 March through 5 May). Tanks were cleaned daily and a weekly 0.25% NaCl bath was administered as a prophylactic disease treatment.

Daily feeding rations were recorded and an average ration was calculated for each month. Rations were adjusted to maintain a specific feeding rate for desired growth. Bass total weight (kg) was determined periodically by randomly collecting a minimum of 100 fish and calculating the number of bass per kg for each raceway. The estimated number of bass in each raceway was divided by the bass per kilogram estimate to determine the total weight of bass within each raceway. Bass per kilogram estimates were converted to grams per bass and specific growth rate was calculated as follows: 

$$\left(\log_{e} W_f - \log_{e} W_i \right) / t \times 100,$$

where $W_f = $ final weight (g), $W_i = $ initial weight (g), and $t = $ time in days. Throughout the intensive culture period, the number of mortalities (mostly due to starvation) was recorded daily and a mean percent survival was calculated for each month. Mortality due to cannibalism was determined as follows: $N_i - N_f - N_m$, where $N_i = $ initial number of bass, $N_f = $ final number of bass, and $N_m = $ cumulative number of deceased bass removed.

On 30 March and 1 April, juvenile bass were freeze branded on their right side by methods described by LaJeone and Bergerhouse (1991). Mark identification was quantified seven days following freeze branding by determining the percentage of fish that had recognizable marks in a sample of 100 fish per tank. The amount of time required to freeze brand all bass was recorded.

**Phase 3: Final Extensive Culture**—On 5 May, all bass were transferred from raceways to a drainable, 0.2-ha pond. This rearing pond was stocked bimonthly with 89,000 fathead minnows to habituate bass to a common natural prey source. On 2 June, the bass were harvested, four sample counts were completed and average size (bass/kg) was estimated. Percent survival and growth were recorded following harvest on 2 June. Freeze brand recognition from a sample of 100 bass was also determined during pond harvest.
Statistical Analysis—Simple correlation and linear regression analyses were conducted using SYSTAT software (Wilkinson 1990). Specific growth rate (%BW/d) was used as the dependent variable, and water temperature was used to explain the variation in bass specific growth rate. Statistical significance was set \textit{a priori} at alpha = 0.05.

RESULTS

Phase 1—On 11 July, 45,375 fry at a size of 7,960 bass/kg were harvested from the spawning pond. This result yielded an estimate of 3,780 age-0 bass per female. The 0.8-ha grow out pond was stocked the same day with 14,520 (36,300 bass/ha) of those fish. On 7 August, a total of 8,800 fish at a size of 350 bass/kg, or 2.9 g/bass were harvested from the grow-out pond. Overall survival in the grow-out pond was 61%.

Phase 2—Overall survival during the intensive phase was 93%. An estimated 340 fish were lost due to cannibalism and an additional 300 were removed from raceways due to mortality factors such as starvation or disease. With cannibalistic mortality removed, average monthly survival was greater than 98% for every month during intensive culture (Table 1).

Daily water temperatures ranged from 9.4 to 25 ºC. The highest temperature was recorded on 7 August when intensive culture began. Average monthly water temperature ranged from 22.6 ºC in August to 9.4 ºC in November, December, January, and April (Table 1). Average feeding ration (i.e., percent body weight/d) was highest during the month of August (4.4%) and lowest during the months of April and January (Table 1).

<table>
<thead>
<tr>
<th>Month</th>
<th>Water temperature</th>
<th>Percent survival</th>
<th>Daily ration</th>
</tr>
</thead>
<tbody>
<tr>
<td>August</td>
<td>22.6 (0.45)</td>
<td>99.7 (0.05)</td>
<td>4.4 (0.003)</td>
</tr>
<tr>
<td>September</td>
<td>16.1 (0.47)</td>
<td>99.6 (0.04)</td>
<td>3.3 (0.002)</td>
</tr>
<tr>
<td>October</td>
<td>10.6 (0.36)</td>
<td>98.6 (0.3)</td>
<td>1.9 (0.002)</td>
</tr>
<tr>
<td>November</td>
<td>9.4 (n/a)</td>
<td>99.6 (0.17)</td>
<td>1.6 (0.002)</td>
</tr>
<tr>
<td>December</td>
<td>9.4 (n/a)</td>
<td>99.8 (0.07)</td>
<td>1.7 (0.002)</td>
</tr>
<tr>
<td>January</td>
<td>9.4 (n/a)</td>
<td>99.8 (0.02)</td>
<td>0.9 (0.002)</td>
</tr>
<tr>
<td>February</td>
<td>15.1 (0.61)</td>
<td>99.8 (0.04)</td>
<td>1.4 (0.001)</td>
</tr>
<tr>
<td>March</td>
<td>18.2 (0.64)</td>
<td>99.8 (0.04)</td>
<td>1.7 (0.001)</td>
</tr>
<tr>
<td>April</td>
<td>9.4 (n/a)</td>
<td>99.9 (0.04)</td>
<td>1.0 (0.001)</td>
</tr>
</tbody>
</table>

Table 1. Means for water temperature (ºC), percent survival, and daily ration (percent body weight fed) for each month juvenile largemouth bass were intensively cultured at Blue Dog Lake State Fish Hatchery during 2008–2009. BioVita fry starter was provided from 7 August 2008 to 16 September 2008, followed by Richloam feed through the duration. Standard errors are in parentheses. Sample sizes for mean water temperature and daily food ration are based on the number of days in each month, and sample sizes for mean percent survival was based on the number of raceways used that month.
Average bass per kg estimates showed an immediate decrease during August and then remained consistent through January. During February and March a decrease in average bass/kg was observed again (Figure 1). Specific growth rates (% BW/d) were strongly correlated with water temperature ($r = 0.82, P < 0.01$; Figure 2). Average water temperature was significant and explained 66% of the variation in bass specific growth rate ($R^2 = 0.66, P < 0.01$).

From November through January when water temperature was maintained at 9.4 °C, bass size remained unchanged (Figure 1). If water temperature could have been maintained at 18 °C, growth rates would have been 1.3% body weight per day according to the significant linear regression model:

$$\text{Largemouth bass specific growth (BW/d)} = -1.34 + 0.144(\text{water temp °C}).$$

All juvenile bass were successfully freeze branded at a rate of approximately 210 fish per hour per person. Average mark recognition was above 99% seven days following branding. Mortality due to freeze branding was less than 1%. Recognition of freeze brands was greater than 99% two months after branding.

**Phase 3**—Bass survival was 87% and size increased from 84 to 73 bass/kg during the transition to feeding on fathead minnows in a pond. This resulted

![Figure 1. Mean largemouth bass/kg estimates with standard error bars during intensive culture at Blue Dog Lake State Fish Hatchery from August 2008 through April 2009.](image-url)
in approximately 7,100 age-1 bass at an average size of 73 bass/kg available for stocking into South Dakota waters.

**DISCUSSION**

The initial extensive phase was effective at producing age-0 bass that readily accepted the dry diets, BioVita Fry Starter and Richloam, as indicated by the overall survival of 93% during phase 2. Lovshin and Rushing (1989) reported feed training success of only 13% when largemouth bass were provided a dry diet. Skudlarek et al. (2007) found that pellets soaked in oil or water improved bass training success compared to dry pellets. The initial size of a largemouth bass in this study was 2.9 g, whereas 1.2-g bass were used in Lovshin and Rushing (1989) and 2.2 g bass were used in Skudlarek et al. (2007). Kubitza and Lovshin (1997) documented that as largemouth bass weight increased, their feed training success also increased. The larger fingerlings in this evaluation likely enhanced the feed training success on the dry diets.

Daily rations varied throughout the intensive culture period due to water temperature. Initially, 5% body weight was fed to improve feed training success. As intensive culture progressed, daily rations decreased to as low as 0.9%. These deviations occurred because of inconsistent water temperatures and attempts to
reduce the amount of uneaten food. In other studies, feeding rations of bass have been reported to be 10 to 15% initial body weight/d when water temperatures were 25 ºC (Lovshin and Rushing 1989, Skudlarek et al. 2007). Increasing rations may result in improved growth when water temperatures are high (e.g., > 20 ºC), but consistent monitoring of water quality variables may be necessary to avoid deleterious conditions (e.g., ammonia spikes) due to increased decomposition of uneaten high protein feed and/or increased excretion. Visual observations provided evidence that reduction in feeding ration did not affect growth during this evaluation. Little to no feeding activity and excessive amounts of uneaten food were observed during periods of low water temperature (i.e., 9.4 ºC) indicating that low water temperature, not reduction in ration, was most likely responsible for no growth during the use of unheated well water.

Water temperature was significant in explaining 66% of the variation in specific growth rate. The remaining variation may be attributed to temperifier shutdowns resulting in drastic water temperature decreases (9 ºC in 15 minutes), turbid water episodes when using lake water, or differences in rearing density from 7 August to 29 October (805 and 1,085 bass/m³) compared to 29 October to 5 May (415 bass/m³). From November through January when water temperature was maintained at 9.4 ºC, bass size remained unchanged. In a study that compared largemouth bass feed training and growth at three temperatures, bass reared at 28 ºC experienced the highest feed training success and highest growth rates (Coyle et al. 2009). In that study, the intermediate temperature group (24 ºC) also performed better than largemouth bass at 20 ºC. Water temperature was the most important factor affecting bass growth in this evaluation. The water temperatures used during intensive culture were below optimal for bass feed training and growth.

Increasing water temperature results in a greater hatchery operating expense. In addition, the size of largemouth bass necessary to produce increased stocking contribution is unknown. Future stocking evaluations will provide necessary data on the size of bass required to increase stocking contribution and reveal if heating water for a longer interval is required. Field studies that determine the optimal size of bass for stocking South Dakota waters should allow for a hatchery cost:benefit analysis to provide information on feasibility of bass production efforts in the state.

Freeze brands have been readily identifiable up to 60 d following branding indicating that methods described by LaJeone and Bergerhouse (1991) are effective on largemouth bass. LaJeone and Bergerhouse (1991) described recognizable brands remaining on walleyes after 40 months. Freeze brands have been readily identifiable up to 60 d following branding indicating that methods described by LaJeone and Bergerhouse (1991) are effective on largemouth bass. LaJeone and Bergerhouse (1991) described recognizable brands remaining on walleyes after 40 months. Future stocking evaluations of the age-1 bass will provide information on mark duration, but at this point, there is no reason to suspect mark deterioration will jeopardize future stocking contribution studies.

Presumably, the majority of bass successfully switched to feeding on fathead minnows during phase 3 since survival was 87% and size increased from 84 to 73 bass/kg. Weather conditions and subsequent pond water temperatures likely are important factors regulating the habituation back to a natural prey. In most years, pond temperatures during May will not exceed 15.5 ºC, which is below optimal for bass growth. Another factor likely regulating survival and growth
during this phase involves providing an appropriate sized prey fish. At the start of phase 3, bass were approximately 94 mm total length. Lawrence (1958) estimated that a 130-mm bass could swallow a prey fish up to 38% of its length. Assuming a constant relationship, a 94 mm bass would then be able to swallow a 36-mm prey fish. No length measurements were made on the fathead minnows that were provided during phase 3 due to manpower shortages. Obtaining appropriate sized prey fish is a factor to consider for possibly improving bass survival and growth during phase 3.

We conclude that the combination of extensive and intensive culture techniques were successful at producing an advance-sized bass product available for spring stockings into South Dakota impoundments. An important aspect involved the initiation of phase 2 when lake water temperatures were high (25 °C). Prolonging phase 1 until lake temperatures become lower (e.g., late August) may result in increased mortality and reduced growth due to reduced acceptance of a dry diet. Additionally, 9.4 °C well water (ambient temperature) was adequate to maintain bass body weight over a 123-d interval. However, increased water temperature is necessary if largemouth bass are expected to grow during the winter months. The size necessary to provide increased stocking contribution in South Dakota impoundments is still unknown. If a larger product is ultimately desired, well water will have to be heated for longer intervals. The fish raised in this evaluation will be used to provide information regarding bass size and stocking contribution in South Dakota waters.

ACKNOWLEDGMENTS

Special thanks to Jerry Broughton, Randy Smidt, Ryan Rasmus, and Eugene Holm as without their effort and guidance this project would not have occurred. Partial funding for this project was through Federal Aid in Sport Fish Restoration, administered through the South Dakota Game, Fish and Parks project no. F-15-R, study 1512.

LITERATURE CITED


OFF-SEASON SPAWNING BEHAVIORS IN NORTHERN LARGEMOUTH BASS

Daniel E. Spengler and Michael L. Brown*
Department of Wildlife and Fisheries Sciences
South Dakota State University
Brookings, SD 57007
*michael.brown@sdstate.edu

ABSTRACT

Northern largemouth bass *Micropterus salmoides salmoides* were subjected to a compressed photothermal schedule to induce off-season spawning. The natural reproductive cycle, previously quantified for a small impoundment largemouth bass population in southeastern South Dakota, was shortened from 360 to 135 d in three phases. Periods of fall, winter, and spring were compressed by 32% (44 to 30 d), 73% (167 to 45 d), and 12% (68 to 60 d), respectively. Brood pairs (*n* = 9) were held in sixteen 757 L circular tanks and maintained on commercial feed and fathead minnows *Pimephales promelas*. During the spring compression, spawning mats were deployed in each tank and brood pairs began to exhibit courtship behaviors (i.e., nipping, nudging, parallel or inverted swimming behavior) when water temperatures reached 16.0 °C and photoperiod was 15.0 h daylight/d. Courtship behavior increased in complexity when temperatures exceeded 16.0 °C and remained constant when temperatures exceeded 20 °C. Spawning did not occur at 20 °C so brood pairs were simultaneously injected with human chorionic gonadotropin (HCG) at 4,000 IU/kg body weight. Courtship behavior increased following HCG injections. Despite increased courtship activity following HCG treatment, voluntary gamete release did not occur and courtship activity declined. Failure to induce an early spawn may be related to several stress-related factors including bacterial infections, reduced body condition, and elevated water temperatures, all of which are known to negatively influence fish reproduction. Despite no spawning, the presence of courtship behaviors during the expected spawning period suggests the photothermal schedule developed for this study, with minor modification, may be useful in future spawning trials.

Keywords
Largemouth Bass, Spawning, Reproductive Behavior, Stress

INTRODUCTION

In South Dakota, northern largemouth bass (*Micropterus salmoides salmoides*) provide recreational angling opportunities and, as a predator, function in the
regulation of panfish abundance and size structure in small impoundment communities (Guy and Willis 1990; Guy and Willis 1991; Willis and Neumann 1994). However, natural recruitment of largemouth bass in many South Dakota small impoundments is often problematic (Kolander 1992; Lucchesi et al. 2004). Thus, populations are periodically augmented with age-0 fingerlings (~75 mm TL) during late-summer.

Fingerling production occurs at Blue Dog State Hatchery (BDSH, Waubay, South Dakota) using traditional (extensive) pond culture methods. Survival of these fingerlings when stocked into established fish communities is generally low, likely due to a combination of size related mortality, losses due to environmental extremes, and insufficient overwintering resources (Peeters 1978; Beck 1986; Kolander 1992; Wahl 1995; Diana and Wahl 2009). Due to these factors, fisheries managers in South Dakota have generally been concerned with the size of the stocking product currently produced at BDSH (Broughton et al. 2005) and attempts by BDSH to overwinter age-0 fingerlings to produce advanced-sized stocking products for spring stockings have been met with limited success. Additionally, some fisheries managers in South Dakota have stressed the need to develop a hatchery protocol capable of producing larger stocking products to potentially enhance stock contributions (Lucchesi et al. 2004).

Off-season spawning (OSS) may provide hatcheries the opportunity to produce larger, age-0 stocking products by extending the growing season. Off-season spawning procedures generally induce early or regulated spawning by subjecting brood fishes to synchronous water temperature and photoperiod compressions (e.g. Ciereszko et al. 1997; Kelly and Kohler 1996). Development of a photothermal schedule to regulate spawning requires a thorough examination of a species reproductive cycle (Matsuyama et al. 1988; Berlinsky et al. 1995), especially the key stages of gonadal maturation (Banner and Hyatt 1975). After the reproductive cycle has been characterized, the cycle can be shifted, extended, or compressed to influence gametogenesis and regulate the timing of reproduction (Kolkovski and Dabrowski 1998; Kelly and Kohler 1996).

Off-season spawning studies have been conducted on several species of fishes (Kelly and Kohler 1996). Important freshwater species for which OSS has been attempted include channel catfish Ictalurus punctatus (Kelly and Kohler 1996), yellow perch Perca flavescens (Ciereszko et al. 1997; Kolkovski and Dabrowski 1998), walleyes Sander vitreus (Malison et al. 1998), bluegill Lepomis macrochirus (Banner and Hyatt 1975) and other sunfish L. species (Mischke and Morris 1997), smallmouth bass M. dolomieui (Cantin 1987), and largemouth bass (Brauhn et al 1972; Carlson and Hale 1972; Carlson 1973). Producing fishes outside of their typical spawning season can be highly beneficial for both public hatcheries and private aquaculture organizations (Malison 1998) because compressed or extended spawning periods can be used to collect multiple spawns from different fishes (Mayes et al. 1993). By shifting spawning periods to earlier dates, hatcheries may also be able to produce larger stocking products than normally possible (Malison 1998). In addition, extended spawning cycles may increase the seasonal availability of certain species (Banner and Hyatt 1975; Kelly and Kohler 1996) and provide the opportunity for staggered production schedules.
When the gonads reach the final stage of maturation (Banner and Hyatt 1975), fishes are allowed to spawn naturally or can be induced to spawn via hormone injection. Kelly and Kohler (1996) indicated that 50% of their channel catfish successfully spawned off-season without hormonal control. The remaining females which failed to spawn naturally often spawned readily after hormone treatment with human chorionic gonadotropin (HCG) or mammalian luteinizing hormone-releasing hormone analog (LHRH-A). Other hormones, such as carp pituitary extract, have been effective in promoting OSS in bluegill (Banner and Hyatt 1975).

Human chorionic gonadotropin, which induces spawns by directly interacting with the gonads, has effectively induced spawning in largemouth bass (Mayes et al. 1993). Relative to other teleosts, largemouth bass generally require higher doses of HCG to successfully induce spawning (Stevens 1970; Wilbur and Langford 1975; Mayes 1991; Mayes et al. 1993). Stevens (1970) reported that a single injection of 4,000 IU/kg body weight stimulated effective ovulation. Additional studies (e.g., Mayes et al. 1993) found that simultaneously injecting males and females with 4,000 IU/kg body weight of HCG was more successful than using LHRH-A to induce spawning. Additionally, Mayes et al. (1993) found longer acclimation periods (1-2 weeks) improved spawning performance by shortening latency periods.

To date, few studies have attempted to develop OSS schedules for northern largemouth bass (e.g., Carlson and Hale 1972; Carlson 1973) and further information regarding OSS techniques for largemouth bass is necessary (Williamson et al. 1993). Therefore, our goal was to develop an OSS protocol for northern largemouth bass using a compressed photothermal schedule formulated from natural water temperature and photoperiod cycles observed in southeastern South Dakota. A successful OSS protocol for northern largemouth bass may provide opportunities to produce larger, age-0 products capable of enhancing stock contributions in sport-fish applications, in addition to providing staggered production schedules for private producers in South Dakota and other Midwestern states.

METHODS

Husbandry—Sixty-five known age-1 largemouth bass (previously stocked as fingerlings from BDSH) were captured from three small private impoundments near Brookings, South Dakota using a combination of angling, C-phase pulsed DC electrofishing, and seining during fall 2007. After an initial temperature acclimation period, all fish were transferred to a closed recirculating system at South Dakota State University (SDSU) comprised of 16 757 L circular tanks connected in parallel to a biofiltration system. All tanks were insulated with foil-back bubble insulation and equipped with covers constructed of 50% wood and 50% plastic mesh to minimize disturbances. Mean flow rate for each tank was 2.2 L per min and diffused air was supplied by an oil-less compressor. Tank temperatures were controlled with a thermostat-controlled heat pump (Aquanetics
Systems, San Diego, California) and photoperiod was controlled with automatic timers. Light intensity at the surface of each tank was 21.3 ± 1.0 lux.

Initially, largemouth bass were held in two tanks and allowed to acclimate (20 °C and 12 h light: 12 h dark) to the recirculating system for three days following capture. After acclimation, each tank received daily *ab libitum* rations of live fathead minnows for a period of three weeks. Fish were then transitioned to consume frozen fatheads (*n* = 10 d) and gradually weaned (~ 20 d) to accept 4.5 mm floating salmonid pellets (45% crude protein, 16% crude lipid; Nelson and Sons, Inc., Murray, Utah). After a successful transition to artificial feed, each fish was tagged with a passive integrated transponder tag, separated by size among four tanks, and allowed to grow to maturity. During the domestication period (fall 2007 to fall 2008), fish were fed to satiation daily and periodically measured and weighed to monitor condition. All fish remained on artificial feed until diets were supplemented with live fathead minnows (2 to 3 weeks) prior to water temperature and photoperiod manipulation, which commenced during the fall of 2008.

In addition to feedings, periodic water quality assessments were conducted to maintain a quality environment. Total concentrations of ionized ammonia (NH$_4^+$, Nessler method, Hach # 8038), nitrite (NO$_2^-$, Ferrous Sulfate method, Hach # 8153), nitrate (NO$_3^-$, Cadmium Reduction method, Hach # 8039), and chlorine (Cl$^-$, DPD method, Hach # 8167) in mg/L were analyzed with a DR/2000 spectrophotometer (Hach Company, Loveland, Colorado). Dissolved oxygen (DO) and water temperature for individual tanks were monitored using a YSI model 55 DO/temperature meter (YSI Incorporated, Yellow Springs, Ohio). Additional water losses to the system, either through evaporation or siphoning, were compensated with dechlorinated municipal water.

**Development and application of a compressed photothermal schedule**—Following the feed training and domestication period, brood fish were subjected to a synchronous water temperature and photoperiod compression formulated from natural water temperature and photoperiod cycle data. The natural cycle (previously recorded in a 1.7-ha impoundment containing a self-sustaining population of largemouth bass in Brookings County, South Dakota, Figure 1a) was shortened from 360 to 135 d in three phases, where periods of fall, winter, and spring were compressed by 32% (44 to 30 d), 73% (167 to 45 d), and 12% (68 to 60 d), respectively (Figure 1b). The fall compression was initiated on 14 November 2008 when water temperature and photoperiod were simultaneously reduced from 20.7 °C and 14 h daylight per day to 10 °C and 11 h daylight by 17 December (rate: 0.36 °C/d, 0.1 h daylight/d). During the winter compression, brood fish were sexed externally (Parker 1971) and segregated. Water temperature during this phase remained stable at 10 °C from 17 December to 30 January while photoperiod continued the compression trajectory (11 to 13.5 h daylight/d, rate: 0.06 h daylight/d). Unlike the water temperature typically experienced by Midwestern largemouth bass in winter (i.e., extensive periods near 4 °C), water temperature during the winter compression was reduced only to 10 °C to allow brood fish to continue feeding and maintain adequate condition prior to entering the spring compression phase. Spring compression
began on 31 January when water temperature and photoperiod were simultaneously increased from 10 to 20 °C and 13.5 to 15.5 h daylight/d by 19 March (rate: 0.17 °C/d, 0.033 h daylight/d). Water temperature and photoperiod at this juncture (representing summer conditions) were held constant until termination of the experiment. Thermal control during these compressions was achieved using the thermostat system previously described. Photoperiod adjustments were standardized across tanks.

During the spring compression phase, a single male and female (males = 296.6 ± 6.0 mm TL, females = 310.4 ± 4.8 mm TL) were paired together in each tank (n = 9 male: female pairs) on 22 February when water temperature reached 15.5 °C. Pairing procedures utilized the external method for sexing largemouth bass described in Parker (1971). After the pairs had acclimated, spawning mats were deployed into each tank when water temperatures reached 16.8 °C. Each mat was constructed with 39.37 x 39.37-cm squares of grey poultry Astroturf (Prod. No. CR004, Solutia Inc., St. Louis, MO) attached to 38.1 x 38.1-cm frames of 1.27-cm diameter PVC pipe. Mats were weighted by filling the PVC frame with sand and water and by placing two stones on opposite corners of the mat. After deployment, spawning mats were surreptitiously checked twice daily for the presence of eggs at 0800 to 0900 and 1500 to 1600 hours using a flashlight.

As water temperature and photoperiod approached spawning conditions, male and female reproductive condition was monitored twice (14 and 19 March). Males were lightly stripped to monitor milt production. Females were not stripped and reproductive condition assessments were restricted to visual interpretation (urogenital pore protrusion and color, in addition to the distended appearance of the abdomen). During these evaluations, fish were anesthetized with the appropriate MS-222 solution, given the water temperature. When brood pairs began to exhibit consistent courtship behavior (~ 19 d after water temperatures exceeded 16 °C), each pair was immersed in a 290 ppm solution of MS-222. When anesthetized, each fish received a 4000 IU/kg body weight intramuscular injection of HCG (Intervet Inc., Millsboro, Delaware) with 3-mL sterilized BD syringes equipped with 21 gauge needles. Hormone injection occurred on 24 March when water temperatures had stabilized at 20 °C for 3 d. After injections, brood pairs were returned to their tanks and left undisturbed. Daily egg checks resumed the following morning. The experiment was terminated on 13 April, 26 d after water temperatures had reached 20 °C.

Reproductive behavior—When water temperature and photoperiod approached pre-spawn conditions, brood pairs were observed randomly for a period of five minutes each day to monitor temporal advancement in spawning courtship behavior. To observe spawning behavior, observers would sit motionless next to each tank. Tank lids were lifted no greater than 6.35 cm, allowing the observer to remain inconspicuous during observational periods. Courtship behavior recorded included nipping, nudging, parallel, inverted, or lateral swimming, circling the spawning mat, closeness or separation between the brood pair, and sexual dimorphism reflected by differences in color.
Figure 1. a) Water temperature and photoperiod profiles (Aug 2007 to Aug 2008) retrieved from data loggers submerged in a 1.7-ha impoundment located in southeastern South Dakota. b) Compressed water temperature and photoperiod schedule formulated from natural water temperature and photoperiod data retrieved from a 1.7-ha impoundment in southeastern South Dakota. Photothermal compressions were separated into three phases (fall, winter, and spring). Vertically oriented grey bars depict separation among compression phases.
RESULTS

Brood pairs began to exhibit courtship behaviors during the spring compression when water temperature reached 16.0 °C and photoperiod was 15.0 h daylight/d. Courtship behaviors gradually increased in complexity as water temperature surpassed 16 °C and remained consistent when water temperatures stabilized around 20 °C. After brood pairs were injected with HCG, courtship behavior increased, particularly the nipping, nudging, inverted and lateral swimming behaviors. At this time, male and female fish could be distinguished from one another based on melanophore activation (i.e., dark male versus light female). Most of these behaviors either occurred over the spawning mats or in close proximity to the mats. Despite increased courtship behavior among brood pairs following HCG treatment, voluntary gamete release did not occur. Additionally, males did not guard spawning mats at any point while exhibiting courtship behavior. Approximately 6 d after HCG injections, courtship behaviors among most brood pairs began to decrease. Fifteen d after initial HCG injections, a second HCG injection was administered to two brood pairs which had continued some courtship behavior; however, this second injection failed to increase courtship behavior. Additionally, milt production among brood males, although present, was negligible during courtship behavior. Body condition also appeared relatively low for some females during courtship behavior. Termination occurred 26 d after water temperatures had reached 20 °C.

Brood fish frequently nipped and nudged (mechanical damages) by spawning partners often exhibited columnaris *Flexibacter columnaris* infections during this study. Despite multiple 1-h immersion treatments in 20 ppm oxytetracycline-hydrochloride solutions, columnaris infections generally progressed until the fish had difficulty maintaining equilibrium. As females became more gravid during courtship activity, columnaris infections were nearly inevitable, causing body condition to rapidly decline. In some cases, columnaris infections led to mortality, as fish were constantly subject to simultaneous stresses of courtship, tank confinement, and elevated water temperature.

After the experiment was terminated, three brood fish were randomly selected for necropsy by the Animal Disease Research & Diagnostic Laboratory (ARDL) at SDSU. Diagnostic findings determined that these wild-caught fish had visceral infections of the parasitic nematode *Contracaecum spiculigerma*. Additionally, the liver, kidney, and spleen tissues possessed a large number of space-occupying metacercaria cysts typical of white grub *Posthodiplostomum minimum* infections (Figure 2).

DISCUSSION

Off-season spawning has been documented in a number of fresh-water fishes (e.g., walleye, Malison et al. 1998, *Lepomis* spp., Mischke and Morris 1997), including northern largemouth bass (Carlson and Hale 1972; Carlson 1973). However, unlike previous studies with largemouth bass, successful spawning (i.e., gamete release and fertilization) did not occur in this study, despite the use of
water temperature and photoperiod manipulation (Carlson and Hale 1972; Carlson 1973) and HCG treatment (e.g., Mayes et al. 1993).

Because our photothermal compression design was essentially a compressed version of the natural water temperature and photoperiod cycles typical of this region, it is unlikely that our compression was the deciding factor which prevented spawning. Further, largemouth bass have been induced to spawn using more abnormal photothermal schedules (e.g., 4.5 to 20 °C in 7 days, Carlson 1973). Spawning in these studies generally occurred when water temperatures stabilized at or above 20 °C (Carlson and Hale 1972; Carlson 1973). In this study, we observed advancements in courtship behavior during the predicted spawning period typical for largemouth bass observed under natural conditions (Breder and Rosen 1966; Scott and Crossman 1973), particularly when water temperatures approached 20 °C. The presence of these courtship behaviors suggest that the photothermal schedule developed and applied in this study can effectively progress spawning behavior in this species. These courtship behaviors have also been reported in other largemouth bass OSS studies where spawning was successful (Carlson and Hale 1972; Carlson 1973).

Figure 2. Photomicrographs depicting space-occupying cysts typical of trematode or metacercaria infections. Images (a) and (b) depict hepatic and spleen tissues, respectively. Image (c) depicts metacercariae encysted in spleen tissue. Images provided by Regg Neiger (SDSU, ARDL).
Stress-mediated factors may have played a role in spawning success, particularly during the spring phase of the reproductive compression (late stage of gonadal development). During this period, columnaris infections (under optimal water quality conditions) appeared on the caudal fin on many brood fish, which may be associated with courtship-mediated physical injury (i.e., lesions formed from excessive nipping and nudging behavior) at elevated water temperatures. Although infected fish were treated, these fish were continuously subject to additional nipping behavior, and therefore, continuous stress by the other brood fish when returned to their experimental tanks. Columnaris infections are commonly observed in cultured fishes (Plumb 1999), and infection susceptibility has been shown to increase due to physical injury (Hussain and Summerfelt 1991).

Stress can play an influential role on fish reproduction (Schreck 2009), as fishes may shift focus to survival, rather than other important life functions, such as reproduction (Schreck et al. 2001). Although information regarding the influence of stress on largemouth bass reproduction is limited, periods of acute stress during the later stages of gonadal development have been suggested to adversely influence reproduction in largemouth bass (Ostrand et al. 2004).

Under confinement, stress has been shown to significantly influence reproductive hormones and gamete production in fishes (Campbell et al. 1992; Coward et al. 1998; Consten et al. 2002), particularly chronic stressors (i.e., tank confinement), which elevate corticosteroid production in animals trying to maintain homeostasis (Coward et al. 1998; Consten et al. 2002). During periods of continuous or chronic stress, elevated cortisol levels in fishes have been shown to suppress many of the key sex steroid hormones which regulate gamete production (Foo and Lam 1993; Coward et al. 1998; Consten et al. 2002), and suppression of sex steroid hormones such as testosterone and estradiol—17β (E2) have been suggested to inhibit reproduction in some fishes by reducing E2-stimulated vitellogenic processes in females (e.g., Tilapia zillii, Coward et al. 1998). Although we did not directly verify sex steroid suppression in this study, we postulate that the presence of stressors prior to and during the expected spawning time may have been sufficient enough to adversely influence endogenous sex steroid hormone production. If corticosteroid-induced sex steroid hormone suppression did occur in this study, it could potentially explain the limited sperm production exhibited by males, as well as the minimal gonadal development observed in females. If similar stress-related circumstances arise in future OSS applications, blood samples could be collected from a few female brood fish when E2 levels are suspected to be elevated (spring compression). Blood samples from these fish could be subjected to E2 radioimmunoassay analysis and the results could then be compared to the natural E2 cycle to determine if sex steroid hormone suppression is occurring.

Another stress-related factor potentially influencing reproduction during this study is parasitism. Parasite infections, which can be energetically demanding, can influence fish reproduction (Barber et al. 2000; Schreck 2009). Necropsy and histopathological findings (R. D. Neiger, SDSU-ARDL) showed that the brood fish used in this study possessed extensive internal parasites. Visceral infections of C. spiculigerma, in addition to high numbers of metacercariae cysts in the liver, spleen, and kidney tissues may have affected energy dynamics. The
potential use of energy by parasites may add to the stressors already encountered by the brood fish (i.e., tank confinement, consistent courtship behaviors, and elevated water temperature), and this parasitic demand may partially explain the susceptibility to columnaris infections and reduced body condition during the predicted spawning period.

Other factors, in addition to stress and parasitism, may have influenced reproduction. Brood fish condition may be an important factor to consider, as feeding activity appeared to be negatively influenced by the winter compression when water temperatures remained static at 10 ºC. During this period, brood fish feeding was minimal and body condition began to decline. Although feeding did increase during the spring compression, feeding activity never rebounded to pre-winter compression levels, and remained relatively low throughout courtship activity. Food limitations have been found to influence reproduction in some fishes by reducing fecundity (Billard et al. 1981), and reduced nutrition can also influence gamete quality in fishes (Schreck et al. 2001). Therefore, to address feeding concerns in future trials, we recommend increasing the water temperature during the winter compression from 10 to 12—13 ºC to maintain higher feeding activity.

Experimental design should also be considered, because brood fish were selectively paired with another individual of the opposite sex, preventing natural mate selection. In a previous OSS investigation with largemouth bass (Carlson and Hale 1972), male-to-female brood ratios of 3:2 and 4:3 successfully produced early spawns, suggesting that mate selectivity may be important. Mate selectivity based on life-history characteristics (i.e., male size) is generally considered an important regulatory mechanism of fish reproduction (Wiegmann and Baylis 1995), and therefore, may be important to largemouth bass reproduction as well. However, information regarding largemouth bass mate selectivity based on life-history characteristics is not available in the literature at this time, particularly in artificial settings.

Conclusions—Many of the factors listed in this discussion, independently or collectively, may have contributed to the lack of reproduction in this study. In some experimental situations, individual stressors (e.g., tank confinement) alone can be sufficient enough to inhibit reproduction in some fishes within controlled environments. Because other potential factors known to influence spawning in largemouth bass were accounted for in this study (e.g., acclimation time and benefits of HCG application, Mayes et. al. 1993), we postulate that the brood fish in this study failed to spawn due to collective stressors associated with parasitism, reduced condition, and an increased susceptibility to columnaris infections due to open wounds caused by courtship behavior (i.e., constant nipping of the caudal peduncle) at elevated water temperatures.

Recommendations—Although spawning did not occur, the progression of brood fish courtship behavior observed during the projected spawning period suggests that the photothermal schedule developed for this study may be appropriate in future spawning trials. However, several factors should be considered prior to implementing future experimentation. Because stress can influence
fish reproduction, a list of potential stressors that could adversely influence largemouth bass reproduction should be compiled and evaluated prior to experimentation. We also recommend that a representative sample of brood fish be submitted for diagnostic evaluation prior to experimentation to determine the presence of parasites. If parasites are documented in the brood fish, the necessary treatment procedures to eliminate these parasites should be initiated, or a new brood source should be selected for study. Brood fish condition should also be considered, as foraging activity was negatively influenced by the static 10 ºC water temperature during the winter compression. In future applications, we recommend elevating the static water temperature of 10 ºC during the winter compression to 12-13 ºC to keep feeding activity acceptable and maintain brood fish condition.

Prolonged periods of elevated water temperature during the predicted spawning period should also be considered in future studies, as elevated temperatures increase the likelihood of epizootics in laboratory experiments. During this study, peak columnaris infections occurred when water temperatures exceeded 18 ºC. Therefore, reducing the number of days where water temperatures exceed 18 ºC may help to reduce columnaris outbreaks in future applications, however, water temperatures exceeding 20 ºC may still be required to successfully induce OSS in this species (Carlson and Hale 1972; Carlson 1973).

ACKNOWLEDGMENTS

We thank all the professors and biologists who provided assistance or insight on this project: Charles R. Berry, Jr., Jeffrey A. Clapper, Tanya D. Graham, Regg D. Neiger, Matt J. Ward, Brian G. Blackwell, Carl J. Kittle, and Juan Martinez. Several graduate students and technicians also provided assistance: Travis Schaeffer, Will French, Matt Hennen, Will Schreck, Mike Greiner, Tom Baccula, Michael Weber, Brent Martens, Seth Lundgren, and Chris Dekker. Special thanks to Dr. William Gibbons, who allowed us to collect study fish from his property. To Solutia Incorporated, thank you for providing discounted materials for our research. We also thank Matt Ward and Mike Barnes of the South Dakota Game, Fish and Parks, who provided technical reviews of this manuscript.

Funding for this project was through Federal Aid in Sport Fish Restoration, administered through the South Dakota Game, Fish and Parks project no. F-15-R, study 1512. Additional funding was provided by the South Dakota State University Ag Experiment Station.

LITERATURE CITED


Fitting Circles for Phase Space Analysis

Michael Janes, Edward Corwin*, and Antonette Logar
Department of Mathematics and Computer Science
South Dakota School of Mines and Technology
Rapid City, SD 5770
edward.corwin@sdsmt.edu

Abstract

Friction stir welding is a relatively new joining technology. Work has been done that correlates feedback forces to weld quality, but finding a value that captures the information in the feedback forces has proved difficult. This paper extends previous work by quantifying the shape of feedback force trajectories in phase space by fitting a circle to the spindle frequency and computing deviation from this ideal trajectory. Experimental results are given showing the effectiveness of the algorithm in terms of both evaluating weld quality and computational efficiency.

Keywords
friction stir welding, phase space, nondestructive evaluation

Introduction

The fundamental goal of this research was to develop a method for quantifying the extent to which a set of points approximates an ellipse in phase space. While there are known algorithms for generating an elliptical curve fit, they are complicated and too slow for some real-time applications. The most obvious choice, a least-squares quadratic fit, also has the drawback of potentially generating a hyperbola instead of an ellipse. Algorithms are also known for fitting circles to data using the property that the perpendicular bisectors of two chords of a circle intersect at the center. Once the center is known, a least-squares fit can be done to find the radius. This technique is not computationally prohibitive but is only applicable to fitting data to circles.

This research defines an iterative process in which the circular fit algorithm is applied to an ellipse to find a major axis. The data are then rescaled and the process is repeated until the error in the circular fit is below some pre-defined threshold. Signals captured during friction stir welding can be viewed and analyzed as trajectories or curves in phase space. The spindle frequency, the speed at which the welding pin tool rotates, defines the frequency which should dominate in the captured feedback. A single frequency will appear elliptical in phase space, but the presence of other frequencies alters that shape. Previous research focused on visual inspection of the trajectories has shown a correlation between the shape of the phase space plot and weld quality (Boldsaikhan et al. 2007). The algorithm
developed in this research quantifies that approach by providing a measurement of the extent to which a curve deviates from the ideal. Friction stir welding provides the spindle frequency as a starting point for the algorithm. However, Fourier analysis can be used to define initial conditions which will allow wider application of this technique to other types of signals.

METHODS

If a given set of data is periodic with an ideal frequency, then one could analyze this data set by applying a Fourier transform. However, in some circumstances the Fourier transform can be difficult to quantify. Observing a phase space plot of the data along side the ideal frequency can provide a clear indication of how well the data match the ideal frequency.

Notice in Figure 1 that the divergence from the ideal frequency in the phase space on the right is caused by secondary frequencies in the data, and thus by simply looking at the phase space one can obtain an accurate representation of how well the data match the ideal frequency. This research uses a circle fitting algorithm to quantify these phase spaces plots and to provide a numeric representation of how well the data fit the ideal frequency.

Since the ideal frequency is a single frequency, it can be modeled by a single sine wave: $F = \beta \sin(\alpha x) + \delta$, with the derivative: $F' = \alpha \beta \cos(\alpha x)$. Graphing $F$ vs. $F'$ will produce an ellipse with major and minor axes parallel to the coordinate axes. If this ellipse can be found, a sum of squared distances from the data to this ellipse can be computed and used as a numeric representation of how well the data fit the ideal frequency. A problem arises when trying to find an ellipse-fitting algorithm that can perform in real time. To mitigate the computational

The intensity of fitting the data to an ellipse, we scaled the phase space data to fit the ideal frequency with a circle.

Since the ellipse has a major axis that is parallel to the X-axis or the Y-axis depending on $\alpha$, it can be scaled easily to be nearly circular. If $\alpha$ is known, then scaling is the trivial process of multiplying $F'$ by $1/\alpha$, but in certain circumstances $\alpha$ may be unknown and hard to find, in which case the iterative algorithm described here can be used to scale the ideal frequency.

The first step in the scaling algorithm is to fit the ellipse with a circle. The major axis of the ellipse can now be found by noting the points outside the circle are closer to the major axis than the points inside the circle. Therefore, if the points outside the circle are given a positive error, and the points inside the circle are given a negative error, then summing the individual components of the distance between the ideal frequency and the circle will produce one positive sum and one negative sum. The positive sum will correspond to the major axis. The major axis can now be scaled, and the process repeated until the ideal frequency becomes circular to some predetermined threshold.

Once the ideal frequency is scaled to be nearly circular, it can be fit with a circle and a sum of squared distances between the data points and the circle can be computed. Since the phase space is scaled, the sum will have to be divided by the radius squared in order to keep the error value independent of the scale of the phase space.

Friction stir welding (FSW) is an innovative technique for joining metals and was developed and patented by The Welding Institute of Cambridge, England in 1991. The process of friction stir welding begins by plunging a rotating pin tool in between two work pieces (Figure 2). The rotation of the pin tool produces two important side effects. First, it generates enough heat to plasticize the work pieces, and second, it stirs the two work pieces together.

While welding, the operator must define three important parameters: the pin tool’s revolutions per minute, its forward speed, and the downward force exerted on the pin tool. An improper selection of these parameters may result in a weld with defects (the phase space analysis discussed in this paper is being used to detect these defects).

The ISTIR-10 located in the AMP Center at SDSM&T is

![Figure 2. Drawing of a typical FSW pin tool.](image-url)
capable of recording a variety of feedback forces, three of which were found to be useful in analyzing a weld: the X, Y, and Z feedback forces. The X force measures the resistance to the forward motion of the pin tool, the Z force measures the downward pressure exerted on the pin tool, and the Y force measures the amount of sideways movement made during the weld (Figure 2).

Previous work has shown that the Y force acting on the pin tool should be periodic with a frequency that matches the pin tool itself. The Y force, as shown in Figure 3, results from the pin tool being acted upon by two competing shear forces: the leading-side shear force and the trailing-side shear force. The two competing shear forces act in opposite directions. The leading-side shear force tends to decrease the Y force, while the trailing-side shear force tends to increase the Y force. Material flow originates at the leading side of the pin tool. During this stage, the leading-side shear force increases and the Y force is decreased. When the material flow reaches the trailing side of the pin tool, the trailing-side shear force increases and the Y force is increased (Boldsaikhan et al. 2010). Therefore, given a regular material flow, the frequency of the Y force will match the frequency of the pin tool. However, if the material flow is irregular, then secondary frequencies will appear in the Y force, and defects may appear in the weld. By comparing the phase space of the Y force against the phase space of the spindle frequency (rotational speed of the pin tool), one can compute an error value that can be used to determine weld quality.

RESULTS

A software application was developed to use the method discussed in this paper to analyze the data produced from friction stir welding. The application takes an FSW data file as input and produces a screen similar to Figure 4. The application provides four graphs: the raw Y force data (top), the raw Y force data currently being analyzed (middle), a Fourier transform of the analyzed data (bottom left), and a phase space of the analyzed data (bottom right).

The material used for the welds discussed here was 0.250 inch thick 7075 Aluminum plates. The data in Figure 4 are from a transition weld, which is a weld that starts off with parameters known to make a good weld (400 RPM, 14 IPM), and half way through the weld the parameters were changed (150 RPM, 18 IPM) in order to produce a bad weld. By examining the raw Y force data, we are able to break the
weld into three sections: warm up section during which process forces have not stabilized sufficiently for analysis, good section, and bad section (Figure 5). The application then analyzes the entire weld, recording the error values (differences between the Y-force phase space and the spindle-frequency phase space), and produces a plot of the error value vs. weld location (Figure 6). Note that the data acquisition rate was 102.4 Hz which is certainly high enough to capture the spindle frequency which was less than 7 Hz.

The transition from good weld to bad weld can be seen clearly in Figure 6. Note that the high peak in the middle is due to the window (The section of the data being analyzed is shown in the red box in the top graph of Figure 4.) containing data from both the good weld region and the bad weld region. More importantly, when the window is entirely in the bad section of the weld, the error value has an average of 1.5, and when the window is in the good section of the weld, the error value remains nearly constant at about 0.5.

Figure 4. Screen shot of the FSW application.
DISCUSSION

If the feedback from a process should be periodic with a single ideal frequency, then the algorithm outlined in this document can be used to analyze the phase space of the data and produce a quantitative analysis of how well the data fit the ideal frequency. The algorithm relies on the fact that the phase space of a single frequency produces an ellipse with a major axis parallel to the $x$-axis or the $y$-axis, and because of this, the ellipse can be scaled easily to be nearly circular. The phase space of the single ideal frequency can then be fit with a circle. Once the ideal frequency is fit with a circle, summing the squared distances between the circle and the data provides a quantitative analysis of how well the data fit the
ideal frequency. This algorithm is fast enough to allow real-time evaluation of weld quality which makes future extension to process control possible.

This approach has been successfully implemented in an application to predict weld defect in welds produced by a friction stir welder. Although the ideal frequency is always known for the friction stir welding data, a Fourier transform can be used to find a dominant frequency if the ideal frequency is unknown.

LITERATURE CITED


ABSTRACT

This work presents results of research on constructing resistive loads using direct-write (AKA: printed electronics) technologies for use in two passive microwave devices— a resistive and a Wilkinson divider. The primary goal of this research is to extend the range of devices that can be constructed by direct-write fabrication. A secondary goal was to demonstrate the ability to “tailor” the electrical conductivity of the deposited resistive ink. This was accomplished by mixing a resistive ink with a more conductive ink. The resistive loads and devices were constructed on rigid microwave substrates using an nScrypt 600-3Dn-HP direct-write system to deposit commercial inks. The copper traces were fabricated using a milling machine. The first device built was a three-port, equal-split, resistive divider. It requires three, physically small, resistive loads with values equal to one-third of the characteristic impedance of the transmission lines connected to the ports. The next device built was a three-port, equal-split Wilkinson divider. The Wilkinson divider requires a physically small resistive load between ports 2 and 3 with a value equal to twice the characteristic impedance of the transmission lines connected to the ports. For both devices, experimental measurements were made using vector network analyzers and numerical simulation models were run. The measured results and simulations agreed well with the results predicted by theory, demonstrating success.

Keywords

Direct-write, printed electronics, resistive loading

INTRODUCTION

This work presents results of research on constructing resistive loads for use in two passive microwave devices, resistive and Wilkinson power dividers, using direct-write technologies (AKA: printed electronics). This is a continuation of prior work (Montoya 2008 and Montoya 2009) where antennas and resistive traces were built using direct-write fabrication techniques. Further, it comprises part of Mr. Downs’ graduate research (Downs 2010) at the South Dakota School
of Mines and Technology (SDSM&T). The intent of this research is to extend the range of microwave/RF devices that can be constructed by direct-write fabrication beyond those consisting only of conductive materials. In addition, the ability to “tailor” the electrical conductivity of deposited resistive inks to a desired value is demonstrated by mixing a resistive ink with a more conductive ink. The term ‘resistive load’ is meant to imply that the resistive loading is implemented with dimensions small enough for it to behave as a point resistance (i.e., a discrete resistor). The power dividers were constructed on rigid microwave substrates. Since the research focus is on the resistive materials, the conductive portions of the devices were made using the copper foil on the microwave substrates. These devices can be modeled both analytically and numerically which allows the performance of the devices with resistive loads fabricated by direct-write to be evaluated.

First, a three-port, equal-split, resistive power divider was selected for fabrication and testing. The schematic for this well known device (Pozar 2005, pp. 317-318) is shown in Figure 1 where the parameter $Z_0$ refers to the characteristic impedance of the transmission lines feeding into the device. This device is considered to be wideband and is limited in bandwidth (range of frequencies) by the size of the “point” resistances and interconnections between the resistances. They need to be a small fraction of a wavelength in length at the desired frequencies of operation for the device to perform as expected by theory.

The electrical performance of an ideal three-port, equal-split, resistive power divider is described by its scattering matrix or $S$-parameters matrix

$$S = \begin{bmatrix} S_{11} & S_{12} & S_{13} \\ S_{21} & S_{22} & S_{23} \\ S_{31} & S_{32} & S_{33} \end{bmatrix} = \begin{bmatrix} 0 & 1 & 1 \\ 0.5 & 1 & 0 \\ 1 & 1 & 0 \end{bmatrix}.$$
The individual S-parameters are defined by the ratio of normalized voltage waves either entering or leaving each port of the device (see Pozar 2005) when all the ports are terminated into matched loads ($Z_0$). Note that the “input” S-parameters ($S_{11}$, $S_{22}$, and $S_{33}$) have values of zero. This indicates that the ports are matched, i.e., no reflections. The other S-parameters with non-zero values are the “through” S-parameters which indicate how much of a voltage wave incident on one port exits another port. For this device, all of the “through” S-parameters have values of 0.5. This tells us that the resistive power divider is reciprocal/symmetrical, i.e., the device behaves the same regardless of which port is used as the input. Further, the ports are not isolated, e.g., power reflected from or entering port 2 will arrive at both ports 1 and 3. Moreover, the magnitude of the “through” S-parameters being 0.5 tells us that this device is not lossless because power is proportional to the square of S-parameters. In this case, given a total input power $P_1$ entering port 1, half of the power ($0.5P_1$) is absorbed in the resistive components, while the other half of the input power is split between the output ports 2 and 3 (i.e., $P_2 = P_3 = 0.25P_1$) and exits the device when those ports are terminated into matched loads.

The other passive microwave device selected for investigation is an equal-split, three-port, Wilkinson power divider (Pozar 2005, pp. 318-323). The schematic for this device is shown in Figure 2. This device is considered to be narrowband. It is limited in bandwidth primarily by the fact that it incorporates two (curved) transmission lines of characteristic impedance $\sqrt{2}Z_0$ that are a quarter of a wavelength ($\lambda/4$) in length. An additional limiting factor for the bandwidth is the size of the $2Z_0$ “point” resistance.

![Figure 2. Schematic for ideal three-port, equal-split, Wilkinson power divider.](image-url)
The electrical performance of an ideal equal-split, three-port, Wilkinson power divider is described by its scattering matrix

\[
S = \begin{bmatrix}
S_{11} & S_{12} & S_{13} \\
S_{21} & S_{22} & S_{23} \\
S_{31} & S_{32} & S_{33}
\end{bmatrix} = \frac{j\sqrt{2}}{2} \begin{bmatrix}
0 & 1 & 1 \\
1 & 0 & 0 \\
1 & 0 & 0
\end{bmatrix}.
\]

Again, the “input” S-parameters (\(S_{11}, S_{22}, \text{ and } S_{33}\)) have values of zero which indicates that the ports are matched. Note that the remaining S-parameters do not all the same value. Therefore, this device is not reciprocal/symmetrical, i.e., it has a definite input port and two output ports. \(S_{13}, S_{21}, S_{32}, \text{ and } S_{31}\) are the “through” S-parameters and characterize voltage and power flow between the input (port 1) and outputs (ports 2 and 3). \(S_{23}\) and \(S_{32}\) are the “isolation” S-parameters and characterize voltage and power flow between the outputs (ports 2 and 3).

Since the “through” S-parameters have a magnitude of \(\sqrt{2}/2\), it can be inferred that the Wilkinson divider is “lossless” when the output ports are matched. For example, given a total input power \(P_i\) entering port 1, half of the power will leave each of the output ports (i.e., \(P_2 = P_3 = 0.5P_i\)). Under these conditions, no power is absorbed by the 2\(Z_0\) “point” resistance. However, the 2\(Z_0\) “point” resistance does serve a purpose by enabling this device to provide isolation between the output ports as indicated by the “isolation” S-parameters having values of zero. This means that a signal entering or reflected back into one of the output ports will not go to the other output port (no crosstalk).

**METHODS**

The resistive and Wilkinson power dividers were constructed using equipment located at SDSM&T. The devices were fabricated on Rogers Corporation (http://www.rogerscorp.com) RO4003C™ rigid microwave substrates with a thickness of 0.813 mm, 1/2 oz copper cladding (18 µm) on both sides, and an electrical relative permittivity (dielectric constant) of 3.38. The conductive portions of the devices were made using a milling machine, T-TECH 5000 Quick Circuit System (see Montoya 2009), to cut the outlines of the conductive portions of the devices in the copper cladding. After milling, we removed (peeled) the excess copper foil from the microwave substrates on the milled side. The copper foil on the other (back) side was left intact to serve as a ground plane.

The resistive traces for the devices were printed or deposited on the substrates using an nScrypt 600-3Dn-HP (http://www.nscrypt.biz/) material deposition or printing system (see Montoya 2009). The nScrypt system uses a direct contact microdispensing or syringe process to deposit liquid materials onto a substrate (similar to a fountain-pen or caulk gun). The system uses air pressure to push material through a nozzle or precision syringe. After printing, we heat cured the resistive traces in an Omegalux LMF-3550 oven for 45 minutes at 145 °C. The oven temperature was increased from room temperature to the curing temperature at a ramp rate of 20 °C/min.
Prior to the construction of actual devices, the electrical and mechanical properties of several commercial materials/inks were evaluated (Downs 2010). The electrical property evaluated was electrical conductivity $\sigma$ which is a key parameter for designing resistances. The electrical conductivity of traces was evaluated for pure as well as various mixes of materials. For mechanical properties, we tested the inks’ adhesion to substrates and resistance to wear or abrasion resistance. The first test method was the “fingernail” test where a cured trace was repeatedly scratched by hand and observed for damage and/or removed material. The second test consisted of “flex” testing where traces were deposited and cured on flexible polyimide substrates. Then the substrate was repeatedly flexed perpendicular and parallel to the trace to see if the trace delaminated and/or cracked. Only inks with good electrical and mechanical properties were considered for the devices.

For the equal-split, three-port, resistive power divider (see Figure 1), a characteristic impedance $Z_0$ of 50 $\Omega$ was selected. This is a standard impedance for RF and microwave test equipment. Therefore, the value for each point resistance $Z_0/3$ is 16.67 $\Omega$. On the Rogers substrate, the design called for the copper microstrip traces to have a width 1.85 mm at 2 GHz to achieve this characteristic impedance.

For the initial design, the resistive traces were assumed to be 130 $\mu$m thick, 1.85 mm wide and 1 mm long. These dimensions were selected based on experience with the resistive materials, nScrypt, and the limitations of the milling machine. From the equation

$$\sigma = \frac{l}{Rwt}$$

where $l$ is the length of the resistance, $w$ is the width of the resistance, $t$ is the thickness of the resistance, and $R$ is the desired resistance, the desired electrical conductivity $\sigma$ is approximately 250 S/m. Based on this conductivity, a commercial carbon-based polymer overprint ink was selected- Goldstone 3100, made by the Methode Development Company™ (http://www.methodedevelopment.com/). This ink has an electrical conductivity near the desired value. If necessary, the thickness of the resistive traces can be trimmed after deposition to achieve the design resistance. The initial design layout is shown in Figure 3 where $l_{gap} = 1$ mm and $w_{port} = w_{gap} = 1.85$ mm. The length of the copper microstrip transmission lines ($l_{port} = 5.0$ mm) feeding the three ports is arbitrary. Note that a copper “island” has been inserted between the three resistances to allow for accurate fabrication and measurement.

We completed the construction of the equal-split, three-port, resistive power divider by soldering 50 $\Omega$ end-launch SMA connectors to the ends of each transmission line. A photograph of the three-port, equal-split, resistive power divider is shown in Figure 4. Due to machine errors, the final dimensions of the device after fabrication were slightly different than those of the original design, e.g., $w_{port} = 1.69$ mm, $l_{port1} = 3.85$ mm, $l_{port2} = 3.15$ mm, and $l_{port3} = 3.88$ mm. The dimensions and parameters of the fabricated resistive traces are given in Table 1. A multimeter with 4-point probes was used to measure the DC resistances of the resistive traces.
Table 1. Dimensions and parameters of the resistive traces after fabrication

<table>
<thead>
<tr>
<th>Resistive Gap</th>
<th>Measured Length $l_{gap}$ (mm)</th>
<th>Measured Width $w_{gap}$ (mm)</th>
<th>Measured Thickness (µm)</th>
<th>Measured Resistance (Ω)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.367</td>
<td>1.78</td>
<td>241</td>
<td>16.11</td>
</tr>
<tr>
<td>2</td>
<td>1.335</td>
<td>1.69</td>
<td>207</td>
<td>15.93</td>
</tr>
<tr>
<td>3</td>
<td>1.475</td>
<td>1.62</td>
<td>200</td>
<td>16.29</td>
</tr>
</tbody>
</table>

For the equal-split, three-port, Wilkinson power divider (see Figure 2), a characteristic impedance $Z_0$ of 50 Ω and a center frequency of 2.4 GHz were selected. On the Rogers substrate, the design again calls for the input copper microstrip traces to have widths of 1.85 mm. The quarter-wavelength copper microstrip traces with characteristic impedances $\sqrt{2}Z_0$ have widths of 1.05 mm. The value for the ideal point resistance $2Z_0$ is 100 Ω. Numerical simulations, using Advanced Design System (ADS) Momentum™, resulted in the design shown in Figure 5. Here, the overall
resistive trace is 3.25 mm long, spans a 1.4 mm gap, 1.85 mm wide, and 50 µm thick and requires an electrical conductivity of about 150 S/m. To achieve this electrical conductivity, we selected a mixture of CI-2020 resistive ink and CI-2002 conductive ink from Engineered Conductive Materials (both carbon-based polymers). When the device was constructed, the desired conductivity (149 S/m) was achieved with a mixture of 47% CI-2002 to 53% CI-2020.

We completed the construction of the equal-split, three-port, Wilkinson power divider by soldering 50 Ω end-launch SMA connectors to the ends of each transmission line. Due to machine errors, the final dimensions/parameters of the device after fabrication were slightly different than those of the original design as shown in Figure 6. The isolation performance of the device was optimized while connected to a vector network analyzer by manually trimming the thickness of the resistive trace. A multimeter with 4-point probes was used to measure the 142 Ω DC resistance of the resistive trace. A photograph of the three-port, equal-split, Wilkinson power divider is shown in Figure 7.

RESULTS

To demonstrate that the devices perform as expected, we made a full set of two-port S-parameter measurements for each device up to a frequency of 6 GHz. We used an Agilent 8753ES vector network analyzer (VNA) to perform these measurements (see Montoya 2009). We calibrated the VNA using a Thru-Reflect-Line (TRL) fixture to remove the effects of the SMA connectors and initial 1.5 cm of the copper microstrip traces.

For an ideal equal-split, three-port, resistive power divider, the input S-parameters will have magnitudes of 0 (-∞ dB) while the through S-parameters will have magnitudes of 0.5 (-6.02 dB). Ideally, these values are independent
of frequency. The measured magnitudes of the $S$-parameters up to 6 GHz are shown in Figure 8.

For an ideal equal-split, three-port, Wilkinson power divider, the input and isolation $S$-parameters will have magnitudes of 0 (-∞ dB) while the through $S$-parameters will have magnitudes of $2/\sqrt{2}$ (-3.01 dB) at the design/center frequency. The measured magnitudes of the $S$-parameters up to 6 GHz are shown in Figure 9.

**DISCUSSION**

Actual physical devices have many limitations which affect performance. One important limitation is the manufacturing tolerances on the dimensions of the various components making up the devices. Also, these devices are limited in bandwidth by the length of the resistive traces and the interconnections between the resistances, i.e., the performance of the devices changes when these lengths become a significant fraction of a wavelength at higher frequencies instead of being “points.” Another set of limitations arise from the use of actual physical materials. For example, the resistive materials can have manufacturing
imperfections such as air bubbles. Also, the microwave substrates are not lossless, but experience dielectric and conductive losses. Part of the design and construction process is to manage these various limitations to achieve a usable device or product.

For the equal-split, three-port, resistive power divider, the input $S$-parameters, shown in Figure 8a, are below -20 dB up to about 5 GHz and below -15 dB across the entire measured frequency range. For input $S$-parameters, a magnitude of less than -20 dB is considered a good match since it means that less than 1% of the incident power at a port is being reflected. The through $S$-parameters,
shown in Figure 8b, start at the ideal value of -6.02 dB and stay within ±0.1 dB up to about 3 GHz and within ±0.6 dB through the entire measured frequency range. Therefore, the measured performance of the device compared well to the theoretical ideal resistive power divider over a large frequency range, up to the limitations imposed by the length of the resistive traces and the interconnections between the resistances. In addition, both sets of measurements agreed well with numerical simulations of the device made with ADS Momentum.

For the equal-split, three-port, Wilkinson power divider, the input and isolation S-parameters, shown in Figure 9a, are all below -20 dB from about 2.2 to 2.7 GHz. The input S-parameter at the input port (S11), where the power

should be largest, has the very low value of about -38 dB at 2.4 GHz. Figure 9b shows the measured through S-parameters to be approximately -3.05 dB from 2.1 to 2.8 GHz. This is only a few hundredths of a decibel lower than the ideal value of -3.01 dB, indicating that the device has minimal power losses. These frequency ranges include the design/center frequency of 2.4 GHz. Again, the measured performance of the device compared well to the theoretical ideal Wilkinson power divider.

In summary, this paper presents results demonstrating the successful use of resistive loads fabricated using direct-write techniques on rigid microwave substrates in the construction of two passive microwave devices, specifically, resistive and Wilkinson power dividers. The devices performed well from an electrical standpoint, indicating that the resistive properties of the inks are stable with respect to frequency up to the limits of the tests (6 GHz). Also, this work demonstrated that inks can be mixed to achieve a desired or “tailored” electrical conductivity. This allows the dimensions of resistive traces, in terms of wavelengths, to be kept small, i.e., in the range where they perform like point resistors. In addition, the inks used in these devices performed well mechanically, demonstrating good adhesion and wear/abrasion resistance during testing.

ACKNOWLEDGEMENTS

This material is based upon work supported in part by the National Science Foundation/EPSCoR Grant No. 0554609 and by the State of South Dakota. The authors wish to thank Dr. Decker and Mr. Randle of the SDSM&T Technology Development Laboratory and Dr. Whites of the LAEC for their assistance with test equipment.

LITERATURE CITED

INTEGRATED PEST MANAGEMENT IN YARDS AND GARDENS: COMPARING SOUTH DAKOTA AND REGIONAL MASTER GARDENERS

Rhoda L. Burrows
Horticulture, Forestry, Landscape and Parks Department
South Dakota State University – West River Agricultural Center
1905 Plaza Blvd.
Rapid City, SD 57702
rhoda.burrows@sdstate.edu

ABSTRACT

Extension Master Gardeners (MGs) undergo intensive horticultural training in preparation for volunteer service assisting with extension programs. A significant portion of volunteer time is directed towards addressing homeowners’ pest and disease problem questions, so MG knowledge of Integrated Pest Management (IPM) practices to manage these problems in a sustainable and environmentally responsible manner is critical. Masters Gardeners in the north central United States were surveyed to determine their knowledge and use of IPM in a home yard and garden context. The 14-question online survey was developed by Consumer Horticulture extension personnel in the region, and was completed by 3852 MG volunteers in ten states in Spring 2009. The average response rate was 16% of all MGs across the states; in South Dakota, 156 volunteers completed the survey (39% of active S.D. MGs.) Responses of South Dakota MGs were similar to those of the region, although they were significantly more confident that they had enough knowledge to make recommendations regarding insects (38% compared with 31% regionally), diseases (32%, 21% regionally) and weed identification (53%, 42% regionally). They were also significantly more likely to use cultural IPM methods and chemical controls on vegetables, but were less likely to use some of these same practices with ornamentals, compared to regional MGs. All MGs indicated a need for more training in disease and insect identification, and organic and biological controls. These results will be used to identify areas for further training for the volunteers.

Keywords
IPM, pesticide use, pest control, homeowner

INTRODUCTION

Consumers and homeowners are a vast population using pesticides that can have significant environmental impacts. Over 45,000 tons (40,820 t) of herbicides are applied on lawns and garden per year in the United States, with
3 to 10 lbs applied per acre (3 to 11 kg ha\(^{-1}\)). This is more than agricultural use (EPA 2004). Yet, when surveyed, 89% of households felt that it was important to maintain residential, commercial and municipal lawns and landscapes “in an environmentally friendly way” (Butterfield 2008). However, only 14% of those consumers indicated that they were “very knowledgeable” about how to accomplish that environmentally friendly maintenance.

Integrated Pest Management (IPM) is a “science-based, decision-making process that identifies and reduces risks from both pests and pest management strategies.” It seeks to “use pest biology, environmental information, and available technology to prevent unacceptable levels of pest damage by the most economical means, while posing the least possible risk to people, property, resources, and the environment” (NC IPM Center 2010). The use of IPM relies heavily on monitoring and identifying pests, setting action thresholds (i.e., how much damage can be tolerated), and considering a range of prevention and control mechanisms, including cultural, mechanical, and biological controls. When chemicals are employed, they are carefully targeted. While IPM was developed primarily for agricultural producers, the same principles can be applied in the home yard and garden, but until recently, little effort has been made to educate homeowners about IPM principles.

Extension MGs are intensively trained (from 36 to over 60 hours, depending on the state) in the science of horticulture, in order to help educate the public on lawn and garden issues, including recommendations for pest control. If IPM practices are to be extended to the public through MG volunteers, it is critical that these volunteers have sufficient knowledge of IPM principles in order to be confident in recommending those practices to the public. Although a number of studies of the MG program have been undertaken (e.g., Meyer and Hancheck 1997, Swakhamer and Kiernan 2005), until now, none have directly examined MG volunteers’ knowledge and use of IPM principles. Therefore MG coordinators in the north central U.S. created an on-line survey to assess the knowledge and use of IPM by the MG volunteers in their states.

**METHODS**

Master Gardener volunteers in eleven states (Table 1) in north central U.S. were invited to complete a 14-question on-line survey assessing their familiarity with and use of IPM principles in their own yards and gardens, and in making recommendations to others. The survey was available for a six-week period on SurveyMonkey (Portland, OR). The volunteers’ knowledge of IPM was not assessed directly; rather questions asked them to assess their own level of knowledge (and use) of specific practices such as pest identification, cultural controls, use of chemicals, and other IPM components. These were evaluated for each of five kinds of plants: fruits, vegetables, flowers, shrubs and trees, and lawns. Demographic data in the survey included the respondents’ years as MG volunteers, state and county, and whether they had completed advanced training. Chi-square analysis (\(\alpha = 0.05\)) was used to compare responses of South Dakota MGs with those of the region.
RESULTS

Survey Response rate—The survey was completed by 3,842 MGs, or about 17% of the region’s active MG volunteers, with response rates per state varying from 5% to 39% (Table 1); South Dakota had the highest response rate.

Reasons for gardening—“Being outdoors” and “Enjoying nature” were the answers most frequently selected when regional MGs were asked for their primary

Table 1. North Central Master Gardener (MG) IPM Survey Response Rate.

<table>
<thead>
<tr>
<th>State</th>
<th># Responses</th>
<th>% of state’s active MGs</th>
<th>Active MGs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illinois</td>
<td>623</td>
<td>17%</td>
<td>3600</td>
</tr>
<tr>
<td>Indiana</td>
<td>189</td>
<td>5%</td>
<td>3600</td>
</tr>
<tr>
<td>Kansas</td>
<td>178</td>
<td>20%</td>
<td>900</td>
</tr>
<tr>
<td>Michigan</td>
<td>987</td>
<td>21%</td>
<td>4600</td>
</tr>
<tr>
<td>Minnesota</td>
<td>228</td>
<td>10%</td>
<td>2200</td>
</tr>
<tr>
<td>Missouri</td>
<td>465</td>
<td>19%</td>
<td>2500</td>
</tr>
<tr>
<td>Nebraska</td>
<td>104</td>
<td>18%</td>
<td>587</td>
</tr>
<tr>
<td>North Dakota</td>
<td>34</td>
<td>6%</td>
<td>600</td>
</tr>
<tr>
<td>Ohio</td>
<td>342</td>
<td>18%</td>
<td>1860</td>
</tr>
<tr>
<td>South Dakota</td>
<td>155</td>
<td>39%</td>
<td>400</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>522</td>
<td>26%</td>
<td>2019</td>
</tr>
<tr>
<td>TOTAL</td>
<td>3842</td>
<td>17%</td>
<td>22916</td>
</tr>
</tbody>
</table>

Figure 1. Primary reasons Master Gardeners list for gardening or yard care (multiple responses allowed). Asterisks indicate significant differences between the proportion of SD and regional MGs listing a response (P < 0.05).
reasons for gardening or yard care (Figure 1), followed closely by “Having an attractive landscape.” South Dakota MG’s were significantly more likely than other MGs in the region to list “Having freshly grown produce,” “Knowing where my produce comes from,” and “Saving money on food” as one of their primary reasons for gardening (Figure 1); and 92% of the South Dakota MGs grew vegetables, compared to 81% regionally (data not shown).

**Integrated Pest Management practices used in their own yards & gardens—Monitoring.** With the exception of lawns, the majority of MGs (64% to 94%, depending on the plant type) routinely inspect their plants (Figure 2). South

Figure 2. Master Gardener reported use of selected IPM practices for various plant types. Asterisks indicate significant ($P < 0.05$) difference between percent of SD and of regional MGs reporting use of a particular practice.
Figure 3. Proportion of Master Gardeners who reported using cultural IPM practices on various plant types. Asterisks indicate significant ($P < 0.05$) difference between percent of SD and of regional MGs reporting use of a particular practice on each plan.
Consider all control options:
- Fruits
- Vegetables
- Flowers
- Trees & Shrubs
- Lawns

Read & follow labels:
- Fruits
- Vegetables
- Flowers
- Trees & Shrubs
- Lawns

Do not use chemical pesticides:
- Fruits
- Vegetables
- Flowers
- Trees & Shrubs
- Lawns

Figure 4. Proportion of Master Gardeners who reported using various IPM control practices on different plant types. Asterisks indicate significant (P < 0.05) difference between SD and regional responses.

Figure 5. Primary concerns when choosing a pest management practice. Asterisks indicate significant (P < 0.05) difference between SD and regional responses.
Dakota MGs were significantly more likely than their peers to inspect their vegetables, and less likely to inspect their flowers, and correspondingly, less likely to identify flower pest problems before treatment. Most MGs reported that they “accurately identify pest or disease before taking action,” with SD MG percentages ranging from a low of 44% (lawns) to 80% (vegetables). As a group, MGs are poor record-keepers, with fewer than 20% (Figure 2) keeping records of “pests and management practices I have used in my yard or garden.”

Prevention. Use of resistant cultivars by MGs regionally was highest for vegetables (over 65%) and flowers (over 60%), and lowest for lawns (under 20%). South Dakota MGs lagged significantly behind their peers in use of resistant flower cultivars; fewer than half of SD MGs selected plant cultivars (other than vegetables) for their pest resistance (Figure 2).

Controls. The use of cultural methods for disease and insect control are reported more often for vegetables and flowers than for other types of plants (Figure 3). Plantings to attract beneficial insects were one of the least implemented IPM practices, with fewer than 20% of these being used for fruits or trees and shrubs. South Dakota MGs were more likely to use various cultural IPM practices on vegetables than were their regional counterparts, but lagged in their use of some of the same practices on trees and shrubs (Figure 3).

Fewer SD MGs indicated that they “do not use chemical pesticides” compared to regional MGs (Figure 4); however, they also were significantly more likely to indicate that they “consider all control options” (vegetables), and that they read and follow the labels in applying pesticides (fruit and vegetables). Effectiveness and “Health and safety to family and pets” were primary concerns when selecting pest management practices for all MGs (Figure 5). Environmental impact was also important for most MGs, although significantly fewer SD MGs listed it as a primary concern than did their regional counterparts.

Level of confidence in using various IPM aspects in gardening activities—South Dakota MGs’ reported level of confidence with IPM practices such as monitoring pests, and using cultural and biological controls and pesticides, was similar in most responses to those of the region. Although SD MGs were significantly more confident than their counterparts that they had enough knowledge in insect, disease, and weed identification to “make recommendations to other gardeners,” fewer than 40% felt qualified to identify diseases and insects for others (Figure 6). MGs were most knowledgeable in using adapted plants and cultural controls (over 60% indicated they could make recommendations), and least confident in their knowledge of biological controls, conventional and organic pesticides (fewer than 30% were confident that they could make recommendations to others).

Need for more training: Congruent with their confidence levels (Figure 6), MGs were least interested in further training on cultural controls and the use of adapted plants (Figure 7). South Dakota MGs were significantly less interested in weed identification training than their counterparts, consistent with their greater level of confidence in this area. Over half of MGs indicated a need for more training on organic and biological controls, as well as further training on insect and disease identification.
Figure 6. MG IPM confidence levels. Bars indicate % of MGs confident enough to use IPM at home (Home Use), or to make recommendations to others (SD, Region). There were no significant differences between SD and regional MGs at the Home Use level; asterisks indicate significant (P < 0.05) difference at the recommendation level.

Figure 7. IPM areas in which Master Gardeners indicate a need for additional training in order to be confident enough to make recommendation to other gardeners. Asterisk indicates significant (P < 0.05) difference between SD and regional MG response.
DISCUSSION

The higher survey response rate of South Dakota MGs (39% SD vs. 17% regionally) may be in part due to South Dakota volunteers’ strong sense of personal engagement engendered in part by the SD model of intensive face-to-face training with topic specialists, as well as our smaller number of volunteers overall. The intensive training may also be in part responsible for our volunteer’s somewhat higher level of confidence in their knowledge in several areas.

Given the restricted availability of quality fresh produce in many isolated rural areas across the state, it is not surprising that South Dakota Master Gardeners are more likely to garden for food production, compared to their counterparts in other states. A person’s reasons for gardening may impact his or her decisions on pest and disease control. Their interest in food production may explain why SD MGs tend to have a higher reported use of various IPM methods with vegetables in particular, compared to their regional counterparts.

A core practice of IPM is routine monitoring for pests and diseases, and a high percent of MGs reported routine inspections of all but their lawns. However, a survey of attendees at a Pennsylvania flower show reported even higher numbers of scouting their landscape (82%), identifying pests prior to treating them (71%), and keeping records of pests and treatments (21% always kept records and another 51% occasionally did so) (Sellmer et al. 2003). Based on their responses to various horticultural questions, 69% of the Pennsylvania survey participants were classified by cluster analysis as “horticulturally savvy,” which suggests a similar population to those who participate in master gardener programs.

Use of plant cultivars that are resistant to pests and diseases is integral to IPM. Although use of resistance is advocated throughout the MG training, MG reported use of resistant cultivars is relatively poor (Figure 2) in general, especially since resistant cultivars are available for most plant types. Effort should be made during training to identify barriers to use of resistant plants and to help MGs to be able to select those cultivars when purchasing seed or plants.

Although the vast majority of MGs ranked their knowledge of various IPM practices strong enough to use at their own homes (Figure 6), their use of these same practices often lagged (Figures 2, 3). The survey did not ask them to rate the importance of using the various practices; presumably they use the ones they feel are most important. The purpose of the Master Gardener program is, however, to train volunteers to extend their knowledge to the general public, and fewer than 30% of the volunteers felt they had enough knowledge to train the public on using organic pesticides, biological controls, or conventional pesticides (Figure 6). Consistent with this lack of knowledge regarding biological controls and organic pesticides was MGs’ desire for further training with these practices and materials (Figure 7).

South Dakota MGs are similar in most respects to those of the region, although slightly more confident in their identification skills, and more versed in using pesticides correctly. They also tend to apply IPM practices more frequently to vegetables than their counterparts in the region. The survey indicates areas to target in additional training, including use of biological controls and organic
pesticides (an area of evolving knowledge in general), insect identification and monitoring methods, and use of resistant cultivars.

LITERATURE CITED


Abstracts of
Senior Research Papers
presented at
The 95th Annual Meeting
of the
South Dakota Academy of Science
A PRELIMINARY STUDY ON ESSENTIALITY OF DIHYDROXYACETONE KINASE IN METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

Jordan Foos¹, Chun Wu¹ and Adhar Manna²*

¹Division of Natural Sciences
Mount Marty College
Yankton, SD 57078
²Sanford Medical School,
University of South Dakota
Vermillion, SD 57978
*Adhar.Manna@usd.edu

ABSTRACT

Methicillin resistant Staphylococcus aureus (MRSA) is the leading cause of nosocomial infections in the United States and is resistant to multiple antibiotics. Our previous in silico analysis (Baye, N., K. Velk and C. Wu. 2009. Genomics approach for the identification of drug targets in Methicillin/Multiple-Resistant Staphylococcus aureus (MRSA). Proceedings of the South Dakota Academy of Science 88:202.) identified 126 and 132 potential drug targets in MRSA 252 and MRSA Mu50 strains respectively that are essential to pathogen survival and absence in the human genome. In this research, we examined the preliminary work of using an allelic replacement approach (Manna A. C., and A. L. Cheung. 2006. Expression of SarX, a negative regulator of agr and exoprotein synthesis, is activated by MgrA in Staphylococcus aureus. J. Bacteriology 188:4288-4299.) to validate the essentiality of one target, MRSA dihydroxyacetone kinase (DAK). A DNA fragment containing 1 kb upstream and 1 kb downstream region of the dak gene was cloned into a plasmid pCR 2.1 TOPO-TA easy vector system. An erythromycin sensitive gene was inserted into the recombinant pCR 2.1 plasmid to silence the dak gene. Our future work includes a subclone of the resulting silent gene into a pCL52.2, a temperature sensitive E. coli–Staphylococcus aureus shuttle vector, transformation of recombinant pCL52.2 into Staphylococcus aureus RN4220 strain then into clinical strains and induction of homologous recombination in Staphylococcus aureus chromosomes.
ENZYMATIC IDENTIFICATION OF THE PHOSPHORYL GROUP DONOR IN A HYPOTHETIC PROTEIN, MRSA DIHYDROXYACETONE KINASE

Kelly Schilling and Chun Wu*
Division of Natural Sciences
Mount Marty College
Yankton, SD 57078
*cwu@mtmc.edu

ABSTRACT

Methicillin/multiple-resistant Staphylococcus aureus (MRSA) are bacteria that cause human infections and are resistant to commonly used antibiotics. An in silico approach (Baye, N., K. Velk and C. Wu. 2009. Genomics approach for the identification of drug targets in Methicillin/Multiple-Resistant Staphylococcus aureus (MRSA). Proceedings of the South Dakota Academy of Science 88:202.) identified MRSA dihydroxyacetone kinase (DAK) as one of the potential drug targets, which was further cloned and expressed in E. coli (Baye, N., K. Velk and C. Wu. 2009. Cloning of a gene encoding dihydroxyacetone kinase from methicillin/vancomycin-resistant Staphylococcus aureus Mu50. Proceedings of the South Dakota Academic of Science 88:203.) Herein, we report the purification and initial characterization of MRSA DAK. Molecular weight and extinction coefficient were measured by ESI-TOF MS and UV-Vis spectrometry respectively. MALDI-TOF/MS analysis revealed a very low degree of structural similarity with other proteins. Kinetic assay demonstrated that MRSA DAK is not an ATP dependent dihydroxyacetone kinase. Future work includes a kinetic assay to determine if a bacterial phosphotransferase system (PTS) is the phosphoryl group donor and a test of phosphoglycolohydroxamate as a potential MRSA DAK inhibitor.
INNATE IMMUNE RESPONSES OF PORCINE INTESTINAL EPITHELIAL CELLS TO BACTERIA-ASSOCIATED MOLECULAR PATTERNS

C. Sreenivasan and R. S. Kaushik*
Dept of Biology and Microbiology and Veterinary Science
South Dakota State University
Brookings, SD 57007
*radhey.kaushik@sdstate.edu

ABSTRACT

The goal of this study was to define the changes in the expression of genes involved in intestinal innate immunity to bacteria-associated molecular patterns. We stimulated porcine intestinal epithelial IPEC-J2 cells for 3 and 24 hrs with lipopolysaccharide (LPS), peptidoglycan (PGN), flagellin (FLA), CpG oligonucleotides and GpC oligonucleotide as negative control for CpG. Cells with media alone were used as negative control. Cell lysates were processed for total RNA extraction which was used to prepare cDNA using reverse transcriptase reagents. The gene expressions of various TLRs, antimicrobial peptides, cytokines, and chemokines were quantified using real time RT-PCR relative to cyclophilin. The expression of TLRs and NODs did not change upon stimulation at 3 hrs. TLR-2 expression was significantly upregulated by LPS at 24 hrs. PGN significantly decreased TLR-6 but increased NOD-2 expressions; CpG decreased TLR-2, 5 and 6 expressions; and GpC decreased TLR-2, 5, -6 and -7 expressions at 24 hrs. LPS did not induce any change in cytokine and chemokine expression at 3 and 24 hrs. PGN upregulated expression of IL-12p40, IL-15 and beta-defensin-2 at 3 hrs and downregulated IL-15 and osteopontin at 24 hrs. FLA significantly upregulated IL-8, IL-15, MCP-1, beta-defensin 1 and 2 expressions at 3hrs and downregulated IL-6 at 24 hrs. CpG induced an increase in IL-15, beta-defensin 1 and 2 at 3 hrs and decreased IL-8 at 24 hrs. GpC induced a significant increase in IL-18, beta-defensin 1 and 2 at 3 hrs and decreased IL-6, IL-7, IL-8, IL-12p35, beta-defensin 1 and 2 at 24 hrs.
DIFFERENTIAL EXPRESSION OF 
CANDIDA ALBICANS GENES IN 
RESPONSE TO SILVER COMPOUNDS

Polly A. Hall, Pankaj Mehrotra and Cynthia Anderson*
CCBR/WestCore
Black Hills State University
Spearfish, South Dakota 57799
*Cynthia.Anderson@bhsu.edu

ABSTRACT

Biofilms are complex networks of microorganisms, including bacteria of several genera and opportunistic fungi, which invade acute and chronic wound beds. Such invasion makes treatment of wounds extremely difficult because some components of the biofilm are resistant to antimicrobial treatment. However, advancements in the medical field have yielded new products for the treatment of wounds, including the use of silver. Although there has been much research on the effects of silver on bacterial pathogens, little is known about the level of sensitivity and resistance of dermatophytic fungi to silver. In this study, we designed and optimized primer pairs for use in real-time amplification studies of genes we hypothesize to be influenced by silver exposure in Candida albicans. C. albicans was grown in the presence of various levels of silver nitrate up to and including the minimum inhibitory concentration (MIC). Differential expression of the proposed target genes were measured by real time PCR for each treatment level. Patterns of altered expression were present for multiple genes. Specifically, expressions of metallothionein and glutathione synthetase were downregulated significantly. Many genes had similar expression in which regulation was comparable for each treatment level except for the middle level, which had amplified regulation. Increased knowledge of the effect of silver on gene expression in fungi will provide insight into the mechanism(s) underlying silver resistance in fungi.
EXPRESSION OF TWO *NITROSONOMAS EUROPAEA* PROTEINS, HYDROXYLAMINE OXIDOREDUCTASE AND NE0961, IN *ESCHERICHIA COLI* BL21 (DE3)

Pankaj Mehrotra and David Bergmann*

Integrative Genomics Program
Department of Science
Black Hills State University
Spearfish, SD 57799
*dave.bergmann@bhsu.edu*

ABSTRACT

Hydroxylamine oxidoreductase (HAO) is a homotrimer of 63 kDa subunits, each with 7 c-hemes and an active site heme (heme P460) with a unique heme-tyrosine crosslink. HAO has been characterized from the autotrophic ammonia oxidizing bacterium *Nitrosomonas europaea*; the gene encoding HAO (*hao*) is present in other bacteria, always in a cluster with a gene (“ORF2”) for a putative membrane protein (NE0961 in *N. europaea*). We report the heterologous expression of the genes for HAO and NE0961 of *N. europaea*, cloned separately and together, on a T7lac dual promoter plasmid vector, pETDUET-1, in the host *Escherichia coli* BL21 (De3). A second plasmid, pEC86, overexpressed the *E. coli* Ccm A-H gene products for c-cytochrome processing. SDS-PAGE of *E. coli* proteins, followed by in-gel tryptic digestion and mass spectrometry, confirmed expression of both HAO and NE0961 in the membrane fraction of the host. However, expression of HAO was much higher than that of NE0961. When *hao* was expressed alone, HAO was found entirely in the membrane fraction and had partial attachment of c-hemes, but no periplasmic holocytochrome was formed. When *hao* and ORF2 were expressed together, a large amount of membrane-bound HAO with partial c-heme attachment was formed, as well as a very small amount of high molecular mass, periplasmic cyctochrome. Due to the low expression of NE0961 relative to HAO with the pET-DUET-1 expression system, the role of NE0961 in HAO processing remains unclear.
REAL-TIME T-P KNOT ALGORITHM FOR BASELINE WANDER NOISE REMOVAL AND ECG-DERIVED RESPIRATION (EDR) SIGNAL ESTIMATION FROM THE ELECTROCARDIOGRAM (ECG) FOR RESPIRATORY RATE

S. P. Arunachalam* and L. F. Brown
College of Engineering
Department of Electrical and Computer Science Engineering
South Dakota State University
Brookings, SD 57007
*shivaram.poigai@sdstate.edu

ABSTRACT

Numerous techniques have been reported for deriving a respiration waveform from a patient’s electrocardiogram (ECG). Such respiratory waveforms are called ECG-Derived Respiration (EDR) waveforms. In this paper we report a new real-time T-P knot algorithm to obtain the EDR signal that can yield the respiratory rate. This algorithm utilizes a real-time baseline wander removal technique which is based on the repetitive backward subtraction of the estimated baseline from the ECG signal. The estimated baseline is interpolated from the ECG signal at midpoints between each detected R-wave. As each segment of the estimated baseline signal is subtracted from the ECG, a “flattened” ECG signal is produced for which the amplitude of each R-wave is analyzed. The respiration signal is estimated from the amplitude modulation of R-waves caused by breathing. The algorithm depends only on the ECG morphology, interpolation and subtraction and is functional in real-time. Testing of the algorithm was conducted in a pseudo real-time environment using MATLAB™, and test results are presented for simultaneously recorded ECG and respiration recordings from the PhysioNet/PhysioBank Fantasia database. Test data from a patient were chosen with particularly large baseline wander components to ensure the reliability of the algorithm under adverse ECG recording conditions. The algorithm yielded respiration rates of 4.4 breaths/min for Fantasia patient record f2y10 and 13.3 breaths/min for Fantasia patient record f2y06. These were in good agreement with the respiration rates of the simultaneously recorded respiration data provided in the Fantasia database, thus confirming the efficacy of the algorithm.
A NEW PALEONTOLOGICAL FACILITY FOR EDUCATION, RESEARCH, AND REPOSITION AT THE MUSEUM OF GEOLOGY, SOUTH DAKOTA SCHOOL OF MINES AND TECHNOLOGY, RAPID CITY, SOUTH DAKOTA

James E. Martin*
Museum of Geology
South Dakota School of Mines and Technology
Rapid City, SD 57701
*James.Martin@sdsmt.edu

ABSTRACT

For over 20 years, personnel at the Museum of Geology, SD School of Mines and Technology, have pursued the dream of a new paleontological building to house systematic collections and provide a center for research and education. Currently, only 9000 ft² house the laboratories and storage facilities for approximately 500,000 fossil, mineral, and rock specimens, as well as their associated contextual and archival data. The fossil library at the Museum of Geology contains systematically arranged geological and paleontological specimens derived from federal and state holdings, as well as those from private holdings. Nonrenewable resources from every federal land-managing agency repose in the systematic collections. State-owned specimens from the South Dakota Office of School and Public Lands and the South Dakota Game, Fish, and Parks also are preserved within the systematic collections. Now, the Museum is becoming an important repository for specimens derived from required surveys and mitigation resulting from the newly passed Paleontological Resources Preservation Act. Therefore, the repository provides an important service in curation and preservation of public nonrenewable resources for education, research, and future technologies.

Multiple benefits of the facility to the citizens of South Dakota revolve around education, research, and public service. The School of Mines houses the largest paleontology program in the country based upon student enrollment and boasts the only Master's degree in Paleontology with a mandatory field emphasis. Therefore, many students are attracted to the program because of its uniqueness and location in the midst of the great western fossil beds. The repository functions as a library of fossil resources and is an integral portion of the undergraduate and graduate programs in paleontology. The current collections, even though they are not entirely accessible, are world renowned, and numerous researchers from around the world utilize the collections to document the history of life on this planet. The collection library forms the basis for comparison of fossils, particularly those brought by the public for identification. The collection also is the basis for numerous educational programs from K-12 outreach to television
programs, such as those on public television, National Geographic, and Discovery channels.

As the result of funding from the State of South Dakota and with the generosity of private donors, a new 33,000 ft² building has been erected on the School of Mines campus. The new repository includes a geological-paleontological library, archival/curation center, special specimen storage, examination/study rooms, and dedicated storage for vertebrate, invertebrate, and plant fossils, Recent vertebrate skeletons, and minerals from the world over. Supporting laboratories include those for preparation of fossil specimens, exhibits, replication, chemistry, and conservation. Therefore, the new repository serves the undergraduate and graduate programs at the university, provides a research center to undertake paleontological and geological studies, and allows conservation and preservation of present and future specimens that represent our natural history heritage.
THE EARLIEST RECORD OF PROBOSCIDEA IN SOUTH DAKOTA FROM THE MIDDLE MIocene (BARSTOVIAN) FORT RANDALL FORMATION

D. C. Pagnac*
Museum of Geology
South Dakota School of Mines and Technology
Rapid City, SD 57701
*darrin.pagnac@sdsmt.edu

ABSTRACT

The earliest appearance of proboscideans for the Great Plains is calibrated between 14.2 and 14.0 Ma. Proboscideans (gomphotheres, mastodons, and elephants) were common constituents of Great Plains paleofaunas by the late Barstovian (15.0-12.5 Ma). Late Barstovian proboscidean remains are most commonly found in the Valentine Formation of Nebraska, but have also been reported from the Madison Valley beds of Montana, paleofaunas from central Wyoming, and from correlative assemblages from the Pawnee Creek Formation in northeastern Colorado. Previously, the earliest occurrences of Proboscidea from South Dakota were from the Wolf Creek and Mission assemblages of early Clarendonian age. I report the earliest record of proboscideans in South Dakota from the Fort Randall Formation.

The Fort Randall Formation consists of several hundred feet of fluvial channel sands and overbank muds intermittently exposed in southern South Dakota. The Fort Randall Formation unconformably overlies the Cretaceous shales of the Pierre Group, and is overlain by the Miocene Valentine Formation. The highly resistant Bijou Quartzite commonly caps exposures of the Fort Randall Formation serving as a useful marker bed. The Fort Randall Formation contains a diverse and well-documented late Barstovian fossil mammal assemblage.

SDSM 78602, a large fragment of tooth enamel from a gomphotheriid molar, was recovered from the McConnell/Trenholm Site (V2006-1), approximately 1.5 miles from the Fort Randall Fm. type section. The fragment contains part of the characteristic “trefoil” molar cusp (diagnostic of the family) and lateral portion of the tooth crown. While the specimen is not of exceptional quality, its recovery from the Fort Randall Formation extends the record of Proboscidea in South Dakota to the late Barstovian.
SURVEY OF THEROPOD  
(DINOSAURIA, SAURISCHIA) DENTITION:  
MORPHOLOGY AND FUNCTION

Jason J. Testin* and Ryan T. Tucker  
Department of Geology and Geologic Engineering  
South Dakota School of Mines and Technology  
Rapid City, SD 57701  
*jason.testin@mines.sdsmt.edu

ABSTRACT

Dental morphology and its significance to theropod (Dinosauria: Saurischia) taxonomy are poorly understood. Dentition morphology is closely linked to feeding; changes in dental morphology could provide a basis for adaptations linked to utilization of new feeding niches. Due to this relationship, it is possible to rely on preservation of dentition in the fossil record to aid in the understanding of theropod evolution. Isolated theropod teeth are fairly widespread in dinosaur-bearing deposits, unfortunately a significant majority of these isolated teeth are shed during hunting or scavenging and lack any root structures. In several previous studies shed teeth have been used to extend pre-existing paleobiographical ranges. A study based solely on isolated dentition must be viewed critically, and to date there are little quantitative data to assign isolated crowns to distinctive theropod taxa. In this study detailed measurements were taken from Allosaurus along with Tyrannosaurus, Carcharodontosaurus, Spinosaurus, Ceratosaurus, Torvosaurus, and Acrocanthosaurus. These measurements and counts were complied into a database of morphological standards that can be used to identify shed teeth found in rocks of the Late Jurassic age Morrison Formation. Our goal for this project is to discuss in detail the dentition of a single theropod, Allosaurus fragilis, and create a database of dentition values from A. fragilis and other large theropods. The emphasis will be placed on in-situ dentition, and the use of morphologic standards in the taxonomic identification of isolated teeth.
THE TYRANT KING OF MUD BUTTE:
NOTES ON A TYRANNOSAURUS REX SPECIMEN
FROM BUTTE COUNTY, SOUTH DAKOTA

M. M. Pinsdorf
South Dakota School of Mines and Technology
Museum of Geology
Rapid City, SD 57701
michelle.pinsdorf@mines.sdsmt.edu

ABSTRACT

SDSM 12047 is an approximately 40% complete skeleton of a Tyrannosaurus rex collected by the South Dakota School of Mines & Technology's Museum of Geology in the early 1980s. This study represents the first osteological description of this specimen, reinforcing its diagnosis and detailing unique and useful information in comparison to other tyrannosaurid specimens. Unique features of SDSM 12047 include pronounced rugosity of the posterior cranium, potential pathology of the left maxilla, and unusual tooth counts in the dentaries. SDSM 12047 preserves features absent or poorly preserved in other T. rex specimens, including the "intercoronoid"/"supradentary", and a nearly complete series of cervical vertebrae. Cranial characteristics were scored and compared to those of other T. rex specimens to establish an adult age for SDSM 12047. Establishing skeletal characteristics and an ontogenetic stage for this individual adds data to further our understanding of this popular yet poorly represented taxon.
PALEOZOIC FISH FROM THE PERMIAN MINNEKAHTA LIMESTONE OF THE BLACK HILLS

N. Dierks* and D. Pagnac
South Dakota School of Mines and Technology
Rapid City, SD 57701
*Nick.Dierks@gmail.com

ABSTRACT

The Permian Minnekahta Limestone was deposited in an ancient, shallow marine environment. While both invertebrate and vertebrate fossils are typically rare in the Minnekahta, nearly 50 specimens of fossil fish have been accumulated and subsequently housed in the collections of the Museum of Geology, South Dakota School of Mines and Technology. To date, only one of these fish has been described. Our purpose is to identify the fossil fish that have been collected, and to compare this faunal assemblage with those of correlative depositional environments or deposits from the same time period. Currently, identified specimens include the genera Acrolepis and Platysomus, as well as material that may belong to acanthodian fishes and ctenacanthid sharks. In addition to the fossil fish, numerous specimens of the shrimp-like Mamayocaris have been found in association with fish remains. The provenance of this genus has been attributed previously and erroneously to the unfossiliferous Opechee Shale, which underlies the Minnekahta Limestone.
EXPERIMENTAL ADIPOCERE FORMATION: IMPLICATIONS FOR CONCRETION FORMATION ON VERTEBRATE FOSSILS

Randolph J. Moses*
Department of Geology and Geologic Engineering
South Dakota School of Mines and Technology
Rapid City, SD  57701
*moses.wyogeo@gmail.com

ABSTRACT

Adipocere, or grave wax (adipo=fat, cere= wax) is a special decomposition product composed primarily of fatty acids and their alkali salts (soap). Fatty acids result from the bacterial enzymatic hydrolysis of body fats. Ammonia produced by decay of tissues reacts with fatty acids to produce ammonium salts of fatty acids. Further reactions between ammonium salts and alkali metals (Na, K, Ca, Mg) originating from body fluids and pore waters of the depositional environment produce alkali salts of fatty acids (soap). Over time, as Ca replaces Na and K, reactions with bicarbonate can eventually form a calcium carbonate concretion.

Optimal conditions for adipocere formation include warm, moist, mildly alkaline, anaerobic situations. However, adipocere can form over a rather wide range of conditions. The basic requirement for adipocere formation is the presence of anaerobic bacteria. Adipocere formation is generally associated with burial of complete corpses in which ample adipose tissue is available. No indications that adipocere can form on defleshed remains have been presented in the literature.

The result herein reveals that adipocere can indeed form on defleshed bones under the right conditions. This indicates that even residual adipose tissue and other lipids found in defleshed bones are sufficient to produce adipocere growth on the surface of bone. These adipocere growths on bone are of similar form and appearance to concretions that can be observed on many fossils. Although a direct correlation between these two phenomena cannot be drawn based on these results, it certainly may be envisioned. Further research and understanding of this correlation may provide a wealth of information about the environment of deposition and other taphonomic factors.
AN ECOLOGICAL COMPARISON OF THE GREEN RIVER AND FLORISSANT INSECTS

L. J. Clarke
South Dakota School of Mines and Technology
Rapid City, SD 57701
laura.clarke@mines.sdsmt.edu

ABSTRACT

The Green River and Florissant formations are separated by a span of 16 million years. The Green River Formation consists of basin deposits from a series of three lakes that existed around 50 million years ago in the Middle Eocene Laramide from western Wyoming, western Colorado, and northern Utah. The deposits at Florissant are from a lake in the vicinity of a volcano about 34 million years ago in the Late Eocene Priabonian. Based on past analysis of flora, the Green River Formation had been described as the more tropical of the two locations. The Florissant Fossil Beds appear to have been at a higher elevation, with flora of a more temperate climate. However, the insect fauna from the two locations appears to reflect ecologies opposite to those interpreted through the flora.

Around 300 species have been identified from the Green River Formation, while around 1,500 species have been identified at Florissant. Research in the Green River insects has been somewhat neglected since 1890, partially due to a research bias towards increasing interest in the Florissant Beds insects. A study of Green River insects in the collections at the South Dakota School of Mines and Technology is establishing groundwork for an overdue review. The work could provide new information for insights on insect diversification and paleoecology through the progression of the Eocene Epoch.
SAMPLE ANALYSIS OF *ARCHAEOOTHERIUM* (ARTIODACTYLA: ENTELODONTIDAE) FROM THE CONATA PICNIC GROUND “BIG PIG DIG”, BADLANDS NATIONAL PARK, SOUTH DAKOTA

Matthew T. Miller*
Museum of Geology
South Dakota School of Mines and Technology
501 East Saint Joseph Street
Rapid City, SD 57701
*matthew.miller@mines.sdsmt.edu

ABSTRACT

Cranial variation in members of *Archaeotherium* (Mammalia, Entelodontidae) has caused wide discrepancies in the diagnoses of many included species. Since 1993, thousands of associated elements of *Archaeotherium* have been excavated from the lower Scenic Member of the Brule Formation (Orellan North American Land Mammal Age) at the Conata Picnic Ground “Big Pig Dig” site in Badlands National Park, South Dakota. Better understanding of the variation in *Archaeotherium* from the Conata Picnic Ground revealed new insights into the taxonomy of the genus. The specimens of *Archaeotherium* represent a sample population and were analyzed utilizing principle component analysis and statistical analyses, and given ontogenetic stages based on tooth wear. Morphologic features previously thought to be taxonomically significant (shape and size of the suborbital process, and mandibular tubercles) are discussed and refuted to be of any taxonomic importance for *Archaeotherium*. Rugosity above the orbits and on the frontals is thought to be characteristic of “male” *Archaeotherium* and is absent in “female” specimens. Crania from the “Big Pig Dig” have been referred to *Archaeotherium mortoni* and *Archaeotherium wanlessi*. This analysis indicates the first instance of multiple species of Entelodontidae from a single locality.
A NEW STRATIGRAPHIC OCCURRENCE OF THE LEPTAUCHENIINE OREODONT *SESPIA* IN THE UPPER BRULE FORMATION OF SOUTH DAKOTA

E. Welsh
Department of Geology and Geological Sciences
South Dakota School of Mines and Technology
Rapid City, SD 57701
edtwelch@hotmail.com

ABSTRACT

Fossil collecting in the White River Group has been done for over 150 years, yielding limited new information on the fossil fauna. A new find out of an old discovery, however, provided an opportunity to re-examine specimens that had been overlooked. The Museum of Geology at SDSM&T has a skull of *Leptauchenia (Sespia) nitida* that had been on display since the 1932. What is interesting about this particular specimen is the recorded stratigraphic location which expands the genus *Sespia* from the Sharps Formation to the Upper Brule Formation in South Dakota. This stratigraphic unit is known to contain fauna from the Whitneyan North American Land Mammal Age, but not *Sespia*.

Measurements taken under the convention of previous researchers and morphological characters of the skull were used to validate the integrity of the initial identification. Resulting data were compared to previously identified species and type specimens, which were used as taxonomic anchors. This study shows that this specimen was correctly identified as *Seipsia nitida*. The lithology of the remaining matrix on the specimen was utilized to verify its geologic origin based on the recorded locality. The matrix secured to the skull is siltstone comparable to the Whitney member of the Brule Formation, as seen in Nebraska, which is close to the approximate physical location of the site where the specimen was collected. This new information from a skull collected about eighty years ago is providing new insights on the Oreodont subfamily Leptaucheniiinae.
HOW FABERGE EGGS ARE MADE IN THE BADLANDS: UNIQUE PRESERVATION OBSERVED IN FOSSIL EGGSHELS

E. Welsh* and H. Minkler
Department of Geology and Geological Sciences
South Dakota School of Mines and Technology
Rapid City, SD 57701
*edtwelch@hotmail.com

ABSTRACT

Eggs and eggshell remains are rare in the amniote fossil record, but have been known to occur in the White River Group in Nebraska and South Dakota. Identification of such material is difficult because no specimens have been found with associated skeletal material. Only one previous attempt to identify eggs from the White River Group has been made, relying entirely on egg shape. However, because eggshell microstructure is formed under the physiological processes of an animal which produces calcite microcrystals, these microcrystals have been demonstrated to provide the most useful characters in aiding analyses, correlations and identifications.

The Highway 44 locality in South Dakota, Chadron Formation, has produced fossil eggshells that display unique mineral replacement. Eggshell fragments from this locality were analyzed using SEM and EDS microscopy revealing nearly complete mineral replacement of eggshell calcite with silica. Silica replacement in these specimens likely resulted due to high volcanic ash content typical in the White River Group. Fossil eggshells are already composed of a relatively stable mineral (calcite) and rarely undergo diagenetic processes as seen in the unique preservation discussed herein. This study demonstrates a previously undocumented preservation in fossil eggshell material from the White River Group and the difficulty of studying fossil eggshell material from the White River Group for taxonomic comparison and identification. It also refutes previous taxonomic correlations.
THE FINAL CLOSING OF THE BIG PIG DIG: AN UNUSUAL DILEMMA ENCOUNTERED AT THE UNIQUE SITE IN BADLANDS NATIONAL PARK

W. A. Thompson*1 and M. W. Weiler2
1South Dakota School of Mines and Technology
Rapid City S.D. 57701
2University of North Dakota
Grand Forks, North Dakota 58202
*wayne.thompson@mines.sdsmt.edu

ABSTRACT

The Big Pig Dig at Badlands National Park was closed in August of 2008 after nearly 15 seasons of field work which yielded over 15,000 individual elements and over 15 taxa characteristic of the White River Chronofauna. The site represents a significant and unique fossil locality within an already unequaled region for fossil occurrences. While the practice of careful documentation was generally followed, a number of problems began to arise during the final closing of the site. The origin, backsite and Easting stakes required for constructing the mapping grid were to be removed and replaced with stainless steel monument markers encased in a sleeve of concrete in order to remediate the site to as natural a state as possible. This required driving the original stakes farther into the ground, while simultaneously maintaining the integrity of the original markers. This was done with the aid of a total station mapping system. A further problem arose during excavation of the original origin. An Archaeotherium cranium was discovered directly beneath the origin stake. The exposure of the skull created a unique ethical dilemma, and the choice was made to remove the skull while maintaining the integrity of the origin. This was accomplished by setting a grid around the origin and using the total station to ensure the origin remained in place. The cranium was then excavated and the original origin marker set back into place.
RETROACTIVELY GEO-REFERENCING HERBARIUM SPECIMENS OF \textit{PHRAGMITES AUSTRALIS}

K. I. Miller and C. A. Johnston*
Department of Biology and Microbiology
South Dakota State University
Brookings, SD 57007
*Carol.Johnston@sdstate.edu

ABSTRACT

\textit{Phragmites australis} (giant reed grass) is a wetland plant that is native to South Dakota, but non-native genotypes of \textit{P. australis} are invasive. The non-native \textit{P. australis} was recently declared a noxious weed in Butte and Meade Counties in western South Dakota, but the extent and origin of \textit{P. australis} in the state is unknown. As a first step to increasing understanding of historical versus present \textit{P. australis} occurrence in South Dakota, we mapped the state distribution of \textit{P. australis} based on collected specimens from herbaria at South Dakota State and Black Hills State Universities. We tallied specimens by decade of collection, and developed a GIS protocol to improve the georeferencing of specimens. There were 89 South Dakota \textit{P. australis} specimens, sampled as early as 1893. Eighty-five specimens could be georeferenced. Of these specimens, 60\% were located west of the Missouri River, and 40\% in the glaciated terrain east of the Missouri River, primarily in the Prairie Coteau ecoregion. \textit{P. australis} usually occurred in wetlands or on lakeshores, but \textit{P. australis} in unglaciated western South Dakota mostly occurred on dams, creeks, and stock ponds. There was no evidence of a recent increase in \textit{P. australis} occurrence: 38 of the specimens were collected during the 1980s, 26 in the 1990s and 4 in the 2000s. \textit{P. australis} was found west of the Missouri River as early as 1914. Future research should revisit areas where \textit{P. australis} was sampled to determine whether stands are of native or non-native origin.
DIAGNOSIS AND APPRAISAL OF BACTERIAL LEAF STREAK DISEASE SEVERITY IN WHEAT


Plant Science Department
South Dakota State University
Brookings, SD 57007
*lawrence.osborne@sdstate.edu

ABSTRACT

* Xanthomonas campestris pv. translucens causes bacterial leaf streak (BLS) and black chaff in wheat (Triticum aestivum). Proper diagnosis and severity assessment are important for disease management and effective germplasm screening. Conventional diagnostic techniques are often inefficient and inaccurate. Severity assessment is difficult as there are no standardized methods in regular use for bacterial diseases of small grains. Here we examined application of the Biolog micro-plate system for pathogen identification and a double digit (00-99) disease severity rating scale. Bacteria were isolated from symptomatic wheat leaf tissue and identified by using the Biolog system. The isolates were inoculated onto wheat seedlings to test their pathogenicity, then re-isolated following ‘Koch’s postulates’ for validation. Field experiments were conducted in Brookings and Codington counties in SD in 2009. Forty-five spring wheat lines were spray-inoculated using isolate XtSD-017. After heading, disease severity was assessed four times at 7-day intervals using a modification of Saari and Prescott’s (00-99) scale. Area under disease progress curve (AUDPC) was estimated from the scores. Results showed that the Biolog system was efficient and economical in identifying bacterial isolates. AUDPC scores ranged from 639 to 1449 and analysis revealed differential reactions among wheat lines to pathogen challenge, indicating the utility of the double-digit scale as a good means of severity assessment. Of the 45 genotypes, most were susceptible, however, one genotype (SD4205) appeared resistant and two were moderately resistant. The findings of the study have implications on diagnostics and disease screening for bacterial diseases of wheat.
ESTABLISHMENT OF AN EFFICIENT CALLUS INDUCTION AND REGENERATION PROTOCOL FROM IMMATURE EMBRYOS OF WHEAT

Manali Shirke, Kim Buehner, Ansuman Roy, Suman Rohila and Jai S. Rohila*
Department of Biology and Microbiology
South Dakota State University
Brookings, SD 57007
*jai.rohila@sdstate.edu

ABSTRACT

Wheat is one of the most important cereal crops of South Dakota and the world. According to an FAO estimate we need to double our food production by 2040, but severe yield losses in wheat because of biotic and abiotic stresses are a major threat to achieving this goal. The major stress is drought. Recent development of powerful “omics” technologies is enabling us to identify many genes of interest for which comprehensive functional analyses are highly desirable in wheat. However, the production of lines which ectopically express recombinant genes, or those in which endogenous genes are knocked down via stable transformation, remains a major bottleneck for the association between genetics and gene function in wheat. A facile, genotype-independent regeneration protocol is the first key step to pursue genetic improvement of wheat. The purpose of this study was to develop and standardize a protocol to induce a high frequency of callus induction and a good plantlet regeneration system for wheat breeding programs with the final objective of applying genetic engineering to improve its yield in stress environments. Twenty-eight genotypes of winter and spring wheat were examined for potential use in wheat tissue culture studies. Tissue cultures were successfully initiated from immature embryos on a modified MS medium. The induced calli were then subjected to regeneration with varying concentrations of plant growth regulators. Very good genotype-independent growth was seen in two media with IAA/Zeatin and IAA/BAP. This improved protocol can be successfully used in future wheat genetic engineering programs.
GENETIC CHARACTERIZATION OF TWO CHROMOSOMAL SEGMENTS ASSOCIATED WITH SEED DORMANCY AND PLANT HEIGHT IN RICE (ORYZA SATIVA L.)

Heng Ye, Chase R. Mowry and Xing-You Gu*
Plant Science Department
South Dakota State University
Brookings, SD 57007
*Xingyou.gu@sdstate.edu

ABSTRACT

Domestication of cereal crops tended to reduce seed dormancy, while modern breeding practices have favored semi-dwarf plant height. However, little is known about the evolutionary relationship between seed dormancy and plant height traits. In this research, we introduced two chromosomal segments from a line of weedy rice into the same genetic background of a cultivated rice line by recurrent backcrossing to evaluate their effects on seed dormancy and plant height under controlled conditions. These two segments were mapped onto the long arms of chromosomes (chr) 1 and 7, respectively. Each of the two segments was delimited to a genomic region of several centiMorgans in genetic distance, and associated with both seed dormancy and plant height. The chr 1 segment contains dormancy- enhancing/-reducing (D1/d1) and height-reducing/-enhancing (h1/H1) alleles from the cultivar/weedy parent (or D1h1/d1H1); whereas, the chr 7 segment contains dormancy-reducing/-enhancing (d7/D7) and height-enhancing/-reducing (H7/h7) alleles from the cultivar/weed parent (or d7H7/D7h7). We detected significant epistatic effects of the two segments on both seed dormancy and plant height. The effects of the d1 and h1 alleles, but not the d7 and h7 alleles, could be rescued by application of gibberellic acid (GA3), suggesting both the d1 and h1 mutant may arises from the same gene on the GA biosynthetic pathway. The observed tight linkage or pleiotropic effects demonstrated that seed dormancy and plant height are co-evolved traits and to some extent that semidwarf breeding also enhanced seed dormancy. The mutations present at the h1 and h7 loci in cultivated and weedy rice, respectively, provide a genetic mechanism for weeds to mimic their accompanying cultivars for the plant height trait. The h7 allele isolated from the weedy line could be a new source of semidwarf gene for rice breeding.
DETERMINATION OF THE GENETIC BASIS OF PSEUDOFLOWER FORMATION IN *BOECHERA HOLBOELLI*

Riston H. Haugen*, David H. Siemens, and Cynthia Anderson

Integrative Genomics Program
Black Hills State University
Spearfish, SD 57799
*riston.haugen@yellowjackets.bhsu.edu

ABSTRACT

Some rust fungi in the genus *Puccinia* cause a radical transformation of the morphology and physiology of their crucifer host plants, inducing the formation of a pseudoflower. The pseudoflower differs drastically from that of the true flowers of the host and mimics the appearance of a co-occurring species. Pseudoflowers are covered in fungal spermatia, and their similarity to co-occurring flowers fools insect pollinators into visiting the fungus-infested plants, thereby facilitating sexual reproduction by the fungus. To determine the genetic basis of pseudoflower formation, we compared transcript profiles of wild *Puccinia*-infected and uninfected *Boechera holboellii* plants by microarray analysis. Because whole-genome microarrays do not exist for *B. holboellii*, *Arabidopsis* oligonucleotide arrays were used. A majority of differentially expressed genes were repressed in infected samples. A number of genes involved in photosynthesis, flowering, and cell signaling were repressed along with many genes known to be involved in response to pathogen infection including MAPKs and WRKY transcription factors. Among the genes induced in infected samples were numerous cytochrome p450s and genes involved in flowering and auxin metabolism. A few disease resistance genes were induced in infected samples, but a majority were repressed. Preliminary analyses indicate a complex regulatory response in infected *Boechera* plants in which plant growth, flowering, and defense pathways are all possibly being manipulated by *Puccinia*. This experiment represents a novel investigation of the genetic basis of pseudoflower formation and regulation of pseudoflower physiology and should help to elucidate the plant genes involved in these processes.
FINE-MAPPING A GENOMIC REGION
CONTAINING A SEED DORMANCY QTL,
A SEMI-DWARF GENE, AND THE
VIVIPAROUS-1 LOCUS ON CHROMOSOME 1
OF RICE (ORYZA SATIVA L.)

Matt Josephson¹, Heng Ye² and Xingyou Gu¹,²*
¹Department of Biology
²Plant Science Department
South Dakota State University
Brookings, SD 57007
*Xingyou.gu@sdstate.edu

ABSTRACT

Seed dormancy, a major domestication-related trait, is a complex trait controlled by multiple genes currently identified as quantitative trait loci (QTL) in cereal crops. Our previous research mapped the qSD1-2 seed dormancy locus onto the long arm of chromosome 1 in rice and isolated the QTL as a single Mendelian factor to clone and characterize the QTL underlying gene. In this research, we fine mapped the qSD1-2-containing region of about 10 cM, on which there are two known genes: the semi-dwarf gene sd1 controlling plant height and the OSVP1 locus predicted based on its similarity to the Maize viviparous-1 mutant. To determine the physical relation among the qSD1-2, sd1, and OSVP1 loci, we genotyped a population of 3300 plants segregating only for the QTL region with five relatively evenly distributed markers and obtained 32 recombinants for different marker intervals. Progeny testing or marker-germination correlation (r) analysis for progeny lines derived from selected recombinants revealed that both the qSD1-2 and sd1 loci are located within a genomic interval of < 100 Kb, and qSD1-2 or sd1 is about 1.5 Mb from the OSVP1 locus. The OSVP1 locus had no effect on seed dormancy (r < 0.09) in the progeny lines segregating only for the dissected OSVP1-containing sub-region. The narrowed qSD1-2/sd1 sub-region contributed 57% (r = 0.75) to the phenotypic variance in germination percentage and 83% (r = 0.91) to the total phenotypic variances in plant height. A physiological experiment revealed that isogenic lines for effects of the mutant qSD1-2/sd1 alleles could be rescued by application of gibberellic acid, suggesting that the qSD1-2 dormancy QTL could be identical to the sd1 gene.
ANTI-MICROBIAL PROPERTIES
OF *MONARDA FISTULOSA*

C. Geffre*, J. Jacobs, J. Dixson, M. Gabel, D. Bergmann, and J. DeCory
College of Arts and Science
Black Hills State University
Spearfish, SD 57799
Cody.geffre@yellowjackets.bhsu.edu

ABSTRACT

Microbial antibiotic resistance is becoming a major health concern. One case in particular is methicillin-resistant *Staphylococcus aureus* (MRSA). Research aimed at identifying new molecules with antibiotic properties is critical to addressing this ever growing health care need. *Monarda fistulosa* (Bee Balm) was a plant that many American Indian tribes used to treat a variety of conditions such as cuts, colds and fever. This project’s goal was to isolate and identify biologically active molecules present in this species and to screen them for antimicrobial activity. A large sample of *Monarda fistulosa* was collected and dried. The plant material was homogenated and put through a “sequential gradient partition extraction procedure.” This procedure fractionates the plant material based on polarity. The fractions were then screened for antimicrobial activity. Once the active fractions were identified, they were further purified using a variety of chromatographic techniques and rescreened for activity. Once a purified active compound was isolated, its structure was determined using NMR, IR and mass spectroscopy. We have begun structure determination on a purified fraction that has shown inhibition against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* and believe it to be a cyclic aliphatic molecule similar in structure to cholestanol. We have found that medicinal plants used by the American Indians to treat diseases do show antibacterial properties. These compounds may prove to be valuable leads for treatment against diseases we face today.
ANTIMICROBIAL ACTIVITY OF NATIVE SOUTH DAKOTA PLANT EXTRACTS ON *ESCHERICHIA COLI*

Gitanjali NandaKafle and Neil Reese*
Dept. of Biology & Microbiology
South Dakota State University
Brookings, SD 57006
*neil.reese@sdstate.edu

ABSTRACT

Certain *Escherichia coli* (*E. coli*) are the primary cause of lethal diarrhea in weaned pigs. United States swine producers face huge losses due to bacterial infections. Our study was aimed mainly at investigating the antimicrobial effect of native South Dakota plants on *E. coli* for use as a source of new natural antibiotics. Some native plants have the potential to provide new classes of antibiotics with low cytotoxicity that may allow regional swine producers to protect young pigs from lethal diarrhea. We conducted our experiments by using twelve plants previously screened in our lab that had antimicrobial effects on *E. coli*. We treated *E. coli* with plant extracts from both dried as well as fresh plants. Our results indicate that fresh plant extracts were much more effective than those from dried plants. For our experiment, plant materials were extracted with methanol and dried by using a rotary evaporator and then resolubilized with 70% ethanol. Growth of *E. coli* was assayed using standard disk susceptibility tests and micro-dilution protocols. After determining Minimal Inhibitory Concentration (MIC), we plated cultures on blood agar Petri plates to determine whether the extracts were bactericidal or bacteriostatic. All experiments were conducted in triplicate. Out of the 12 plants collected in SD, only four, *Clematis ligusticifolia*, *Monarda fistulosa*, *Centurea stoebe* and *Rhus aromatica* showed strong enough antimicrobial effects in *E. coli* cultures to warrant further study.
THE ANTIBIOSIS OF LEAVES OF SPECIFIC PLANTS ON SELECTED BACTERIA

R. V. Jensen and D. Hazelwood*
College of Arts and Sciences
Dakota State University
Madison, SD 57042
*donna.hazewood@dsu.edu

ABSTRACT

This study examined the antibiosis of leaves of different ages on the growth of selected bacteria. Leaves were collected from trees located in Madison and in Beresford, SD. The tree species collected included buckthorn (*Rhamnus cathartica*), box elder (*Acer negundo*), hackberry (*Celtis occidentalis*), silver maple (*Acer saccharinum*), emerald queen maple (*Acer platanoides* cv Emerald Queen Norway maple), cottonwood (*Populus deltoides*) and American elm (*Ulmus americana*). Leaves were also collected from burning bush (*Euonymus alata*) on the Dakota State University Campus. Preliminary results showed that *Micrococcus roseus*, *Bacillus subtilis*, *E. coli*, and *Pseudomonas fluorescens* were not inhibited on all agar augmented with leaves of all tree species collected in mid-summer. Growth appeared to be stimulated by the formation of large bubbles for some combinations for *P. fluorescens* and *M. roseus*. In contrast, *Arthrobacter globiformis* grew only on agar augmented with hackberry and silver maple.
NITROGEN TRANSPORT AND ITS REGULATION IN THE ARBUSCULAR MYCORRHIZAL SYMBIOSIS

H. Bücking*, E. W. Gachomo¹, S. Choudhari², and Y. Beesetty²
¹Department of Biology and Microbiology
South Dakota State University
Brookings, SD 57007
²Biology Department
Rutgers University
Camden, NJ 08102
*heike.bucking@sdstate.edu

ABSTRACT

Nitrogen (N) is the nutrient required by plants in the highest amounts and often limits plant growth. More than 65% of all known plant species are associated with arbuscular mycorrhizal (AM) fungi. AM fungi can contribute significantly to the N nutrition of their host plant, but the transport pathway and regulation are poorly understood. We examined N transport, activities of enzymes and expression of genes involved in the fungal N metabolism under different supply conditions to better understand how N transport in the symbiosis is regulated. When N was supplied to the extraradical mycelium (ERM) of the AM fungus in the form of NO₃⁻ or NH₄⁺, the activity and expression of urease and arginase were stimulated in mycorrhizal roots. This indicates that the N assimilated by the ERM was translocated in the form of arginine to the intraradical mycelium where it was broken down by arginase and urease to provide inorganic N for transport across the mycorrhizal interface. Our observation that stimulation occurred earlier with NH₄⁺ than with NO₃⁻ confirms previous findings that ERM prefers the more energy efficient NH₄⁺. The mycorrhizal host plant is obviously able to trigger N uptake and transport in the AM symbiosis by its carbohydrate supply. When carbon was supplied, effects on fungal gene expression and on urease and arginase activities were observed. A new model will demonstrate how N is translocated and how the flux is regulated in the AM symbiosis with the host plant.
EFFECTS OF MYCORRHIZAE ON GROWTH OF CORN

Suzanne Swett and Donna Hazelwood*
College of Arts and Sciences
Dakota State University
Madison, SD 57042
*donna.hazewood@dsu.edu

ABSTRACT

We examined the effects of amending germinating corn seeds with mycorrhizae with and without fertilizer, Osmocote® 14/14/14 or Fafard® 20/20/20. Mycorrhizae were obtained from Fungi Perfecti, Olympia, WA 98507. Corn seeds were grown in vermiculite in the green house under constant illumination. Preliminary results show that corn roots were infected with mycorrhizae and corn growth was greatest for treatment with both Osmocote® fertilizer and mycorrhizae. Corn roots plus mycorrhizae alone appeared to have the greatest biomass. To clear the roots, we placed separate treatments in a solution of 10% (wt/vol) KOH and autoclaved them for 15 minutes. Roots were stained with lactophenol cotton blue to detect the presence of vesicular arbuscular mycorrhizae in mycorrhizae treatments. Under microscopic inspection, untreated mycorrhizae roots did not stain and were clear in appearance. We will continue qualitative evaluation of mycorrhizae with and without fertilizer and under simulated drought conditions.
IDENTIFICATION AND CHARACTERIZATION OF ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES ASSOCIATED WITH PRAIRIE CORDGRASS

E. Liepold, C. Fellbaum, J. L. Gonzalez-Hernandez, and H. Bücking*
Department of Biology and Microbiology
South Dakota State University
Brookings, SD USA 57007
*heike.bucking@sdstate.edu

ABSTRACT

Prairie cordgrass (Spartina pectinata) is one of the perennial grasses that is considered to be the future of the bioenergy industry due to low required inputs, high energy production, and low greenhouse emissions compared to annual crop plants. The primary goal of this study is to identify and to characterize natural communities of arbuscular mycorrhizal (AM) fungi that are associated with prairie cordgrass. AM fungi improve the nutrient supply of their host plant and increase the abiotic and biotic stress resistance and could play a significant role as “biofertilizers” and “bioprotectors” in sustainable agriculture of bioenergy crops. Prairie cordgrass samples were collected from different sites in Brookings County, South Dakota, and were examined for their mycorrhizal colonization. The AM fungal communities were characterized by denaturing gradient gel electrophoresis after DNA extraction and amplification through a nested polymerase chain reaction approach with primers targeting the fungal 18S ribosomal gene. All samples were colonized with indigenous AM fungal communities, and the colonization rate in most samples was higher than 60%. The intracellular structures of the fungus within the mycorrhizal roots were primarily of the Paris-type and were characterized by the formation of scarcely branched intracellular hyphal coils, intercellular and intracellular vesicles, and only few intercellular hyphae.
SALT TOLERANCE VARIABILITY IN SOUTH DAKOTA CORDGRASS (SPARTINA PECTINATA BOSC EX LINK) SELECTIONS

Alma Meza, Seth Schmoll and R. Neil Reese*
Biology & Microbiology Department
South Dakota State University
Brookings, SD 57007
*neil.reese@sdstate.edu

ABSTRACT

Prairie cord grass (Spartina pectinata) has been seen as a potential new biomass crop for its ability to grow in varying environmental conditions, including marginal lands containing elevated salt concentrations. Clones of 12 cordgrass selections were analyzed for tolerance to increasing salt concentrations in a hydroponic system, with baseline values being measured before the addition of NaCl. Salt concentrations were increased (0.1 M) on a weekly basis over 5 weeks. Growth of roots and shoots was visually monitored, and plant health was determined using SPAD meter readings. After 6 weeks, plants were harvested, dried, weighed, digested and Na, K and proline levels in the tissues were determined. Noticeable differences were found between the twelve selections including root and shoot development, areas and amount of salt accumulation, and ability to cope with increasing levels of salt concentration. The response of the selections varied, with some selections showing strong inhibition to increased salt and others showing increased growth. The 12 cordgrass selections demonstrated significant variation in salt tolerance, with some selections showing increased plant health (SPAD) values with increasing salt exposures. Correlations of proline concentrations and shoot and root Na⁺ and K⁺ concentrations with plant health values indicated that tissue concentrations of these solutes varied widely with increased salt tolerance among the 12 selections.
EFFECT OF NODULATION ON DROUGHT RESPONSE IN ALFALFA

Peizhi Yang1, Michael Peel2, Bing Li1,4, Bart Weimer3, Dong Chen3, Tianming Hu1, and Yajun Wu4*

1Department of Forage Grass Science
Northwest Agriculture & Forest University
Yangling, Shaanxi 712100
2Forage Range Research Laboratory
USDA-ARS
Logan, UT 84322
3Center for Integrated BioSystem
Utah State University
Logan, UT 84322
4Department of Biology and Microbiology
South Dakota State University
Brookings, SD 57007
*Yajun.wu@sdstate.edu

ABSTRACT

To understand the interaction between nitrogen fixation and drought response in alfalfa (Medicago sativa L.), we examined dehydration response in non-nodulated and nodulated alfalfa plants. Alfalfa plants cultured in a greenhouse in sandy soil, with or without Rhizobia inoculation, were subjected to dehydration stress. We found that plants with nodules showed delayed wilting in a time-course experiment and were able to maintain higher relative water content compared to the plants without nodules. To provide molecular understanding of the interaction between nodulation and drought response in alfalfa, we are studying the expression of a set of drought-responsive genes in non-nodulated and nodulated alfalfa plants under drought conditions.
VEGETATION CHARACTERIZATION WITHIN SELECT PRAIRIE DOG COLONIES IN SOUTHWESTERN SOUTH DAKOTA

E. D. Boyda*, J. Butler#, and L. Xu†

1Department of Biology and Microbiology
South Dakota State University
Brookings, SD 57007
2USDA Forest Service
The Rocky Mountain Research Station
Rapid City, SD 57702
*Eric.Boyda@sdstate.edu

ABSTRACT

On the landscape level, prairie dog colonies contribute substantially to the biotic diversity of grassland ecosystems. Efforts to control prairie dogs have generated considerable concern over a number of listed and candidate species as described by the U.S. Endangered Species Act, including the black-footed ferret. Despite the importance of prairie dog colonies to grassland ecosystems, the vegetation is often described simply as early successional without addressing the considerable inter- and intra-site variation that exists. The objective of this study is to develop site-specific vegetation models that may be used to evaluate ecological thresholds for prairie dog management. Prairie dog colonies in southwestern South Dakota were evaluated for intra- and inter-variability in total biomass, bare ground cover, and biomass and cover of select species and plant functional groups. Sampling occurred on the Buffalo Gap National Grassland and was stratified into interior, edge, and immediate off-colony areas in clayey and loamy ecological sites. Three colonies from each ecological site were sampled for a total of six colonies with four 100 m transects placed within each sampling area with five 0.25 m² plots at 20 m intervals. Preliminary results indicated total biomass was significantly different ($P < 0.05$) among interior, edge, and off colony areas for clayey and loamy sites. Loamy sites had higher total biomass and litter than clayey sites across all sampling areas. Meanwhile, the amount of bare ground in loamy sites was significantly lower on both edge and off-colony areas when compared to clayey sites.
MORPHOLOGICAL CHARACTERISTICS
OF 11 ALFALFA POPULATIONS

J. Schmuck¹, L. Xu*, A. Boe², C. G. Misar¹,
R. N. Gates³, and P. S. Johnson¹

¹Department of Biology and Microbiology
²Department of Plant Science
³Department of Animal and Range Sciences
South Dakota State University
Brookings, SD 57007

*Lan.Xu@sdstate.edu

ABSTRACT

Alfalfa (*Medicago sativa* L.) is a major component of feed for dairy and beef cattle and one of the most productive forage species in North America. Although alfalfa has been planted on millions of acres and more than 100 varieties have been developed over the past 100 years in North America since its introduction, historically, alfalfa persistence under grazing in semiarid rangeland has generally been poor. However, recently, it was discovered that naturally-selected populations of predominantly yellow-flowered alfalfa have been proven to be adapted to rangelands of western South Dakota and adjacent areas. A study was initiated in May 2006 to evaluate persistence and vigor of 11 alfalfa population (conventional-hay type, predominately falcata, pure falcata and pasture type) by transplanting seedlings into native and tame grasslands in South Dakota. The objective of this experiment is to investigate morphological characteristics of 11 alfalfa populations in tame grasslands. The experiment was a randomized complete block design with 3 replications of 5 plants in 1.2 m long single-row plots. For each population, aboveground biomass of all plants was harvested and ten stems were randomly selected on July 25, 2008. For each stem, the morphological characteristics measured included: a) length & basal diameter, b) number of nodes, branches, pods, c) ratios of leaf to stem, branch to stem, reproductive to vegetative biomass. Pure falcata population Don had the shortest and thinnest stem, and the lowest leaf to stem ratio, while naturally-selected predominately falcata population Wind River had the longest, thickest, heaviest and most branched stem compared to the other populations.
EFFECTS OF PRESCRIBED BURNING ON SPECIES DIVERSITY AND BIOMASS IN SMOOTH BROME INFESTED RANGELAND OF THE NORTHERN GREAT PLAINS

B. Arlt¹, C. Grewing², and L. Xu*¹

¹Department of Plant Science
²Department of Animal and Range Sciences
³Department of Biology and Microbiology
South Dakota State University
Brookings, SD 57007
*Lan.Xu@sdstate.edu

ABSTRACT

Invasion and persistence of smooth brome (Bromus inermis) are severely reducing the acreage of remnant tracts of tallgrass prairie and altering the species composition and production of prairie communities. Prescribed burning in the spring is recognized as an effective management tool to restore native tallgrass prairie. The objective of this study is to investigate the effects of prescribed spring burning on species diversity and biomass between burned and unburned sites. The study was conducted at the South Dakota State University Oak Lake Field Station in eastern South Dakota. Two study sites having similar soil type were selected. One site was burned consecutively every year in mid-May for the past two decades. The other adjacent site, dominated by smooth brome, had not been burned during the same time period. Vegetation sampling was conducted in early September of 2005 and 2006. Six 30-m transects were placed 20 m apart on each site along environmental gradients. Cover by species was recorded in ten 0.25 m² quadrats which were located at 3-m intervals along each transect. From each transect, 3 quadrats were randomly selected and harvested for aboveground biomass of native species and introduced species. Prescribed spring burning significantly decreased total biomass and introduced species biomass but increased native species biomass. Total species and native species richness was higher on burned sites than unburned sites. Biomass production was higher in 2005 compared to 2006 for both burned and unburned sites in all categories except for introduced species biomass which showed no difference between burned sites for both years.
EVALUATION OF TECHNIQUES USED TO ESTABLISH YELLOW-FLOWERED ALFALFA (MEDICAGO SATIVA SUBSP. FALCATA) IN CRESTED WHEATGRASS (AGROPYRON CRISTATUM) STANDS

C. G. Misar¹*, L. Xu¹, R. N. Gates², A. A. Boe³, and P. S. Johnson²
¹Department of Biology and Microbiology
South Dakota State University
Brookings, SD 57007
²Department of Animal and Range Sciences
South Dakota State University,
Rapid City, SD 57702
³Department of Plant Science
South Dakota State University
Brookings, SD 57007
*christopher.misar@sdstate.edu

ABSTRACT

Monocultures of crested wheatgrass (Agropyron cristatum) are a major component of grassland vegetation in many areas of the Northern Great Plains. Crested wheatgrass plays a critical role in livestock production within this region. However, its competitiveness limits the presence of companion plant species, resulting in poor seasonal forage availability and a reduction in ecological goods and services. Interseeding yellow-flowered alfalfa (Medicago sativa subsp. falcata), which is adapted to this semiarid region, into crested wheatgrass monocultures would improve the plant community. Our objective was to evaluate yellow-flowered alfalfa establishment following no-till interseeding into crested wheatgrass using a variety of techniques. A study was initiated at Newcastle, WY, Fruitdale, SD, Buffalo, SD, and Hettinger, ND, in August 2008 evaluating three main factors: seeding date (late summer vs. spring), seeding rate (0.56, 1.12, 3.36, 5.60, and 7.84 kg Pure Live Seed ha⁻¹), and chemical sod suppression (1 L clethodim ha⁻¹ vs. untreated control). Establishment success was determined from alfalfa seedling frequency, seedling height, and below canopy photosynthetically active radiation (PAR). Results revealed that seedling frequency generally increased as seeding rate increased, but this was location dependent. Mean frequency for the highest seeding rate ranged from only 14% at Fruitdale to 94% at Hettinger. At all locations, below canopy PAR was significantly higher (P < 0.05) where sod was chemically suppressed. Moisture conditions and competitiveness of the grass were critical to alfalfa seedling establishment. Conclusions regarding establishment success would be premature as dynamic changes in seedling death and recruitment will continue to occur.
2-D PROFILING OF TOTAL PROTEOME CHANGES IN CEREALS AND OIL CROPS UNDER ABIOTIC STRESSES

Ansuman Roy and Jai S. Rohila*
Department of Biology and Microbiology
South Dakota State University
Brookings, SD 57007
*jai.rohila@sdstate.edu

ABSTRACT

Separation and visualization of proteins by two-dimensional polyacrylamide gel electrophoresis (2D–PAGE) followed by identification and characterization by mass spectrometry (MS) is a common method of choice in proteomic analysis. As a prelude to using functional proteomics towards understanding the effect of abiotic stresses on the developmental processes in various crop plants, we examined the total protein expression pattern of rice, wheat and soybean seedlings. After isolating total protein from plant tissues, we ran 2D-PAGE to check the resolution and reproducibility of the method. The applicability of the method was demonstrated using rice, wheat and soybean tissues. In addition to these preliminary proteomic data, several proteins, which are differentially expressed and expected to be stress regulatory or cellular signaling components, will be identified. After this, a partial proteome map will be constructed for several crop plant species under different stress conditions. By analyzing the roles of the identified proteins that may be involved with several biochemical and metabolic pathways, we expect that this will allow us to develop a drought and heat resistant crop plant.
ABSTRACT

The soybean aphid, *Aphis glycines*, is an important insect pest of soybean, a major crop in South Dakota. First discovered in the U.S. in 2000, it was accidentally introduced to the U.S. from Asia and spread quickly throughout the Midwest. Pesticides are currently the primary control method for this pest, but biological control, the use of beneficial species to control pests, is a promising management approach. The soybean aphid is seldom a problem in its native Asia largely because a group of natural enemies, beneficial predatory insects, are specialized to prey on it there. Since 2007 South Dakota has been part of a multi-state project to introduce a new specialist natural enemy from Asia to the U.S. for biological control of the soybean aphid. University and USDA entomologists identified the parasitoid *Binodoxys communis* as a promising species for release. (Parasitoids have a parasitic lifestyle on the host insect.) Here, we report on the second year of a multi-year study whose first year was described in the Proceedings of the South Dakota Academy of Science previously. In the summer of 2009 we released *B. communis* in 12 locations in 10 counties in Eastern South Dakota ranging from Campbell to Union Counties, with the long-term goal of establishing permanent populations of this parasitoid for soybean aphid biological control. We added small numbers of parasitoids to release sites in soybeans protected by field cages, and allowed parasitoids to reproduce and increase. After two weeks we removed the protective field cages and allowed the parasitoids to colonize the surrounding fields. At the end of the growing season we found next-generation parasitoids in eight out of 12 locations.
THE SWITCHGRASS MOTH,
BLASTOBASIS REPARTELLA

P. J. Johnson¹*, A. Boe¹ and D. Adamski²

¹Insect Research Collection
Department of Plant Science
South Dakota State University
Brookings, SD 57007

²Department of Entomology
National Museum of Natural History
Smithsonian Institution
Washington, D.C., 20560

*paul.johnson@sdstate.edu

ABSTRACT

National interest for native plants as feedstock for cellulosic biomass in ethanol production leads to questions about insect-plant associations. Investigations on the insect biota associated with switchgrass, Panicum virgatum, resulted in discovery of the moth, Blastobasis repartella (Lepidoptera: Coleophoridae: Blastobasinae), and provided new geographic and host records. This obscure moth is rare and difficult to study in native prairie remnants, but migrates to research farm plots of switchgrass and then occurs abundantly. The moth or its larva was found at four sites in South Dakota, and is now also known from seven other states. At the Aurora Research Farm, Brookings Co., SD, adults of B. repartella were monitored with emergence cages and immature stages found in plant parts. Adults are nocturnal from mid-July through mid-August, with females ovipositing under the basal prophylls of mature tillers. Larvae occur singly and bore into the proaxis and basal internodes of single tillers. Pupation occurs inside the proaxis or internode and near to an exit portal. One parasitoid species was found, the wasp Bassus difficilis (Hymenoptera: Braconidae). The best indicator of larval presence is death of a tiller prior to midstage growth and an exit portal with frass. In a two-year investigation in a 3-4 year old stand of mixed switchgrass cultivars we found per plant larval incidence typically varying up to 15%. At a 1% incidence rate and at an average of 750 tillers/m², there may be an estimated 75,000 larvae per hectare. In one instance at a separate site, tiller death rates reached near 40% on one plant.
EFFECTS OF INITIAL EGG EXTRUSION TECHNIQUES AND FERTILIZATION IN OVARIAN FLUID DURING LANDLOCKED FALL CHINOOK SALMON SPAWNING

Matt Wipf1, Dan J. Durben1*, and Michael E. Barnes2
1Black Hills State University
Spearfish, SD 57799
2McNenny State Fish Hatchery
Spearfish, SD 57783
*dan.durben@bhsu.edu

ABSTRACT

Fall Chinook salmon, Oncorhynchus tshawytscha, from Lake Oahe, South Dakota, is a freshwater, landlocked population, with a potamodromous migration pattern. No natural reproduction occurs; the population is maintained entirely through hatchery production. Feral fish are collected during the fall spawning run and artificially spawned, with egg survival dramatically reduced from anadromous Chinook salmon in their native range. In an attempt to improve landlocked fall Chinook salmon egg survival, we compared the initial extrusion of eggs into two different types of containers and two different egg fertilization (sperm activation) protocols. Because the standard air extrusion technique may shock the unfertilized eggs and cause premature micropyle closure, we evaluated the hypothesis that reducing such shock will improve egg survival. Due to potentially negative effects of the high bacterial loads that are typically found in Lake Oahe salmon ovarian fluid, we also investigated adding or removing ovarian fluid from the spawning medium. A total of four treatments was used during the spawning of thirteen females: egg extrusion into a suspended net and fertilization either with or without ovarian fluid, and egg extrusion into a hard plastic pan with subsequent fertilization with or without ovarian fluid. There was no significant difference in survival to the eyed stage from eggs either spawned into the net or directly into the pan. However, the presence of ovarian fluid during sperm activation and fertilization had a small but significantly negative effect on egg survival, decreasing mean eyed egg survival from 24.9% to 22.3%. Overall, eyed egg survival was improved in 10 of the 13 spawns fertilized without ovarian fluid. To maximize embryo survival, removal of ovarian fluid prior to fertilization of fall landlocked Chinook salmon eggs is recommended.
THE EFFECT OF MALE LANDLOCKED FALL CHINOOK SALMON ON EMBRYO SURVIVAL

Matt Wipf, Dan J. Durben*, and Michael E. Barnes
1Black Hills State University
Spearfish, SD 57799
2McNenny State Fish Hatchery
Spearfish, SD 57783
*dan.durben@bhsu.edu

ABSTRACT

Landlocked fall Chinook salmon, Oncorhynchus tshawytscha, from Lake Oahe, South Dakota, are a semelparous fish and therefore have only one chance to propagate filial generations. This strain of Chinook salmon is maintained entirely by hatchery propagation and historically has exhibited relatively poor embryo survival during artificial propagation. To investigate the potential effect of male landlocked fall Chinook salmon on embryo survival, we used milt (sperm) from 16 individual males to fertilize the eggs from 16 randomly chosen female salmon and showed that sperm from individual male salmon had no significant effect on embryo survival. However, embryo survival was affected by interaction between gametes from discrete individual males and individual females. We recommend that in order to maximize embryo survival and minimize potentially negative genetic effects in this population of landlocked fall Chinook salmon, females should be spawned with pooled milt.
ABSTRACT

We examined the feeding habits of game fish in Mille Lacs Lake, MN, from spring to late summer 2009. To examine seasonal patterns, we divided my collections into three seasons: Spring, Early to Mid Summer, and Late Summer. To examine diurnal patterns, we collected data on the feeding habits of walleye (Sander vitreum) and yellow perch (Perca flavescens) as follows: 8 am-12 pm, 1 pm-5 pm, and 6-10 pm. A 45-50 foot boat was taken out during the three time periods throughout the season. On each trip, 10-20 people fished for the target species. The data suggest that for walleye and yellow perch, there is a correlation between water temperature and maximum fish catch, as well as water temperature and water depth which produced maximum fish catch as the season progressed. Also, patterns of maximum fish catch were evident throughout the season as the 6-10 pm time slot proved to be the most productive. Despite great fluctuations in water temperature due to a cool summer, an overall pattern of maximum fish catch in correlation with water temperature and depth was still evident.
COMPARATIVE ANATOMY AND EVOLUTION OF THE SHOVELNOSE CATFISH GROUP
SORUBIM (BLEEKER) (PIMELODIDAE)

Uriel Angel Buitrago-Suarez
Department of Biology
Mount Marty College
Yankton, SD.
Uriel.buitrago@mtmc.edu

ABSTRACT

The genus Sorubim is a monophyletic assemblage of species that inhabit the major South American river drainages. It includes five recognized species. They reach large sizes, 45 cm – 900 cm, a condition that makes these catfishes an important source of food to humans. Few research papers have considered the taxonomy of the group. The present report is the first intent to establish the evolutionary relationships of these species. A broad and comparative anatomic analysis was therefore conducted including the following closely related taxa: Sorubinichthys, Hemisorubim, Zungaro (= Paulicea) and Pseudoplatystoma. The phylogenetic reconstructions of Sorubim and related groups are based on morphological characters recovered from this comparative anatomy study. Morphological comparisons were based on cleared and stained, and dried skeletons. Characters used to reconstruct the evolutionary history of the species of Sorubim and related genera were numbered, named, described and their transformations coded. Several trees were recovered and the most parsimonious tree was selected.
QUEEN NUMBER AND POPULATION STRUCTURE IN THE ANT *CAMPONOTUS MODOC*

John H. Duvall-Jisha* and Garth M. Spellman  
Black Hills State University  
Spearfish, SD 57799  
*John.DuvallJisha@yellowjackets.bhsu.edu

ABSTRACT

Ants (Formicidae) are unique in many aspects, not the least of which is the ubiquity of eusociality within the entire family. Eusociality has been studied intensely, but the existence of polygynous (multiple queen) colonies is a thorn in the side of kin-selection theories that account for the benefit of altruistic behavior in these organisms. The purpose of this study is to determine the prevalence of polygyny in the carpenter ant *Camponotus modoc* across its range and the relationship of polygyny to biotic stressors such as population density and isolation. *Camponotus modoc* is prevalent west of the Rocky Mountains, but ranges as far east as the Black Hills of South Dakota and Wyoming. Because the Black Hills are isolated by large stretches of unsuitable habitat from the main range west of the Rocky Mountains, we might expect to see a higher prevalence of polygyny due to competitive stress or bottleneck events in this area. Six microsatellite loci designed for *Camponotus femoratus* have been successfully amplified and further loci are being investigated. We intend to collect and genotype samples of twenty individuals per mound from the Black Hills and across the western range of *C. modoc* and quantify population density in the sample areas via mound counting. In order to determine polygyny we will use genotype data and the software RELATEDNESS to calculate within mound relatedness and determine if there is a correlation between polygyny and inter/intra-specific competition as measured by population density.
MONITORING OF HYMENOPTERA DISTRIBUTION AND ABUNDANCE GETS A BUZZ

Jake A. Sathe¹, Jordan D. Werner¹, James L. Hansen¹, Lynn C. Geuke¹, Sam Droege², and L. Brian Patrick¹*  
¹Department of Biological Sciences  
Dakota Wesleyan University  
Mitchell, SD 57301  
²Patuxent Wildlife Research Center  
United States Geological Survey  
Beltsville, MD 20705  
*brpatric@dwu.edu

ABSTRACT

The distribution and abundance of bees, wasps and hornets (hereafter “hymenopterans”) in the U.S. is not well monitored. This information is important because hymenopterans are influenced by habitat and climate change, which affects their diversity, distribution, and abundance. We conducted a pilot study using bottle traps to determine the best way to capture hymenopterans for monitoring purposes. We tested our design in four different habitats: a grassland, an orchard, a wetland, and a woodland. We also tested two bottle sizes, 0.591L and 2L clear plastic bottles, and each bottle containing one of four attractants: Blue Moon beer with apple slices, Heineken beer, orange juice, or sugar water. We replicated each bottle size and attractant combination four times in each habitat for a total of 32 bottles per habitat and 128 bottles total. We predicted that the 2 L bottle would collect the most diverse group of hymenopterans and that there would be an edge effect for our rectangular sampling grid. A 2-sample t-test was done to determine whether there was a bottle size effect and/or an edge effect, with the number of species as a variable, and the number of specimens caught as a variable. Both tests showed that there was neither a significant bottle size effect nor edge effect. Based on this information, we conclude that bottle size and edge effect do not have an effect on the number species caught because the attractant is the ultimate factor.
CATCHING A BUZZ: STUDY OF HYMENOPTERA ATTRACTANTS FOR A MONITORIAL PROTOCOL

Lynn C. Geuke¹, James L. Hansen¹, Jake A. Sathe¹, Jordan D. Werner¹, Sam Droege², and L. Brian Patrick¹*

¹Department of Biological Sciences
Dakota Wesleyan University
Mitchell, South Dakota 57301
²Patuxent Wildlife Research Center
United States Geological Survey
Beltsville, MD 20705
*brpatric@dwu.edu

ABSTRACT

Currently, there is not a standardized monitoring protocol for North American bees, wasps, and hornets (hereafter “hymenopterans”). Monitoring hymenopterans is important because we can study the abundance and distribution and whether climate changes affect these groups of species. Based on studies done in Europe, we designed a study to test which of four attractants would catch the highest species richness and diversity of hymenopterans in South Dakota: Heineken beer (control), Blue Moon beer with apple slices, orange juice, and sugar water. We hypothesized that Blue Moon with apple slices would attract and catch the most diverse group of hymenopterans because it is a wheat beer with fruit that would ferment. We also tested bottle sizes (2L and 0.591L) using clear plastic bottles. We tested our design in 4 habitats (a wetland, a grassland, an orchard, and a woodland), and measured the total number of hymenopterans captured, species richness (SR), and Shannon’s Index for diversity. Heineken beer had the highest species richness (SR = 11), while Blue Moon beer and orange juice were equally effective with SR = 8 species. In the orchard and the grassland, orange juice caught the most diverse group of hymenopterans. In the wetland, Heineken beer was the most effective attractant and in the woodland Heineken beer and Blue Moon beer tied. Heineken caught the most diverse groups of hymenopterans according to Shannon’s Index. Orange juice had the second highest diversity of groups according to Shannon’s Index.
THE SOUTH DAKOTA SPIDER SURVEY (SDSS): INVENTORYING THE DISTRIBUTION AND DIVERSITY OF KEY PREDATORS

L. B. Patrick
Department of Biological Sciences
Dakota Wesleyan University
Mitchell, SD 57301
brpatric@dwu.edu

ABSTRACT

Spiders (Araneae) are a diverse group of predators that can significantly impact community and ecosystem dynamics in a wide variety of habitats. Spiders can affect nutrient cycling in grasslands and agroecosystems, and can be used as biological control agents for integrated pest management of crops. Yet, despite the ecological and potentially economic importance of spiders, the state fauna is woefully understudied. Only Peterson’s 1939 statewide study; a smaller study from a single location near Brookings; and a handful of additional state records exist in sporadic publications, but the taxonomy is often outdated. To date, only approximately 130 species are documented in publications. Based on the fauna of other states of similar size and with comparable habitat variability, a conservative estimate of the total number of spider species found in South Dakota would be 650, and potentially as many as 850. A comprehensive survey of the state spider fauna is needed, so I have established the South Dakota Spider Survey (SDSS) to collect, document, and catalog the diversity and distribution of these ubiquitous predators. Modeled after other state spider surveys, the SDSS will generate a general list of species, complete state distribution maps for all species, as well as the development of an atlas of the spiders of South Dakota. For each species the atlas will include high quality images of the dorsal and ventral general habitus, male and female reproductive structures needed for correct identification, and any other unique or important diagnostic characters.
GLACIAL ISOLATION AND POSTGLACIAL COLONIZATION ENHANCE GENETIC DIVERSITY IN A NEOTROPICAL MIGRANT PASSERINE

G. M. Spellman*, K. J. Burns‡, S. E. Cameron§, J. Hudon¶, and J. Klicka®

1Black Hills State Univ., Spearfish, SD, USA
2San Diego State Univ., San Diego, CA, USA
3Harvard Univ., Cambridge, MA, USA
4Royal Alberta Museum, Edmonton, AB, Canada
5Barrick Museum, Univ. of Nevada, Las Vegas, NV, USA

*garth.spellman@bhsu.edu

ABSTRACT

Phylogeographic study of North American vertebrate taxa has revealed that many species were isolated in Pleistocene glacial refugia and only recently expanded to occupy their current distributions. The consequence of these rapid postglacial expansions has been a reduction of genetic diversity throughout the majority of a species’ range. We analyzed genetic variation (mtDNA sequences, N = 344; and nuclear DNA sequences, 6 loci, N = 39) and used paleoecological niche modeling to infer the phylogeographic history of the Western Tanager (Piranga ludovician). Phylogenetic analysis (mtDNA = 180 haplotypes) revealed two well-supported monophyletic lineages (Clades A and B). Populations from the southeastern and eastern Rocky Mountains possessed only A haplotypes, and all other populations contained a mixture of A and B haplotypes. Paleo-niche modeling indicated the species was likely isolated in two glacial refugia. Analysis of genetic variation at multiple loci revealed that genetic diversity in the species was more than double the amount found in most North American passerines. Glacial isolation, postglacial expansion, and lack of reproductive isolation have led to the hyper genetic diversity observed in this species.
SPECIATION IN THE WHITE-BREASTED NUTHATCH (*Sitta carolinensis*): A MULTILOCUS STUDY OF DIVERSIFICATION IN NORTH AMERICAN PINE AND OAK WOODLANDS

V.W. Walstrom*, G. M. Spellman, and J. Klicka  
Department of Biology  
Integrative Genomics Program  
Black Hills State University  
Spearfish, SD 57799  
*veryl.walstrom@yellowjackets.busu.edu

ABSTRACT

Pine and oak woodlands are common North American floral communities with distinct regional species composition. The origination of these distinct communities was the once continentally distributed Tertiary forest. The orogeny of the late Miocene and Pliocene and Quaternary glacial cycles fragmented the Tertiary forest into separate regional communities. The White-breasted Nuthatch (*Sitta carolinensis*) is a common avian resident of these highly disjunct woodlands. One prior study has presented evidence for four distinct and well-supported clades that evolved in situ in these refugia. This study used one mitochondrial gene (ND2) to elucidate the phylogeography of this species. I am adding to these data the use of five known nuclear loci and fifteen anonymous nuclear loci. Multilocus data are pertinent when determining the accuracy of gene coalescence and divergence times of populations. The stochastic variance related to the creation of gene trees can be reduced only by increasing the number of loci sampled as opposed to the number of samples of the same gene. Analysis using the Genealogical Sorting Index, principle component analysis, and Structure agree with the former mtDNA study. It is very likely that when looking at differences in morphology, song, mtDNA and nuclear DNA, we have four distinct species of the White-breasted Nuthatch.
A MULTILOCUS STUDY OF PHYLOGEOGRAPHY IN THE BUSHTIT (PSALTRIPARUS MINIMUS)

Laura Kramer* and Garth Spellman
CCBR/Westcore Laboratories
Black Hills State University
Spearfish, SD 57799
*Laura.kramer@yellowjackets.bhsu.edu

ABSTRACT

The Bushtit (Aves, Passeriformes, Aegithalidae: Psaltriparus minimus) is a resident of the pine and oak woodlands in North America. Historically, the Bushtit has been classified into three distinct subspecies groups: the minimus group, residing in the Pacific coastal regions, the plumbeus group, residing in the interior portion of the Mexico-USA border near New Mexico and Arizona, and the melanotis group, residing south of the Mexico-USA border and into Guatemala. Although these groups are geographically isolated throughout most their range, there exist zones of secondary contact between contiguous populations in California, Texas and northern Mexico. As a result of these contact zones the three distinct subspecies groups have been maintained as a single morphologically diverse species. Using a multi-locus phylogeographic approach, we are reconstructing the evolutionary history of the Bushtit to explore the evidence for genetic distinction among the three subspecies groups. Eighteen anonymous nuclear loci have been sequenced. Genetic diversity and neutrality statistics (e.g., haplotype diversity, nucleotide diversity (π), 95% confidence interval of π, Tajima’s D, and Fu’s F) within populations were estimated using the program DNASP v4.0. These 18 loci will be used in further statistical analysis to infer gene coalescence and divergence times of populations. Preliminary results in Structure v2.2 have shown divergence between the subpopulations but further analysis still needs to be completed. We plan to further evaluate this in the program Isolation with Migration Analysis (IMA) to gain a more accurate interpretation of models of speciation in regards to divergence times and effective population sizes.
CRYPTIC SPECIATION IN A WIDESPREAD NORTH AMERICAN SONGBIRD (CERTHIA AMERICANA)

Joseph D. Manthey\textsuperscript{1,2,*}, John Klicka\textsuperscript{3}, and Garth M. Spellman\textsuperscript{1,2}
\textsuperscript{1}Center for the Conservation of Biological Resources
\textsuperscript{2}Western South Dakota DNA Core Facility
Department of Biology
Black Hills State University
Spearfish, SD
\textsuperscript{3}Marjorie Barrick Museum of Natural History
School of Life Sciences
University of Nevada
Las Vegas, NV 89154
*Joseph.manthey@yellowjackets.bhsu.edu

ABSTRACT

Although considered one taxonomic unit, the Brown Creeper (\textit{Certhia americana}) is variable throughout its range; it exhibits clinal coloration and size variation with a marked difference between northern and southern populations, resulting in up to 13 recognized subspecies. Mitochondrial DNA data (\(n = 344\)) suggest geographical structuring of regional Brown Creeper populations with the largest divergence between northern and Mexican populations. However, individual genes may have different histories due to coalescent stochasticity and may not accurately represent the true species tree. To better explore the history of speciation in the Brown Creeper, we sequenced 22 additional nuclear loci for 54 individuals from seven populations representing the regional mountain ranges across the Brown Creeper’s distribution. All analyses of the multilocus data (Genealogical Sorting Index (GSI), Structure, principal coordinate analysis, genetic distance) reveal considerable geographic structure in \textit{C. americana}. All of the populations sampled show significant structuring of gene genealogies as measured by GSI. Structure identified five populations, with a complete lack of admixture between northern and southern populations and minimal admixture between populations in the North and South. We tested models of isolation and migration using the program IMa, which show that migration is not significantly affecting the structure of \textit{C. americana} populations, rather the populations have been isolated for several hundred thousand years and the majority of shared variation is due to incomplete lineage sorting. The concordance in the geographic structuring of morphological, mtDNA and nDNA variation indicates northern and southern populations of the Brown Creeper should be recognized as separate species.
MALARIAL PARASITE INFECTION AND MHC VARIABILITY IN THE WHITE WINGED JUNCO

Jess Moser¹,², Pankaj Mehrotra¹,², Daniel Terveen³, Christy Bergeon-Burns⁴, Ellen Ketterson⁴ and Garth Spellman*¹,²
¹Center for the Conservation of Biological Resources
Black Hills State University
Spearfish SD 57799
²Biology Department
Black Hills State University
Spearfish SD 57799
³Science Department,
University of Sioux Falls
Sioux Falls SD 57105
⁴Biology Department
Indiana University
Bloomington, IN 47405
*Garth.Spellman@bhsu.edu

ABSTRACT

Avian malarial parasites play an important role in the study of human malaria, being a stimulus for the development of medical parasitology. Malarial parasites’ use in epidemiological and ecological investigations has led to many new insights into the evolution of host-parasite-vector interactions. These parasites are one of the most well known and well studied groups of parasitic protists. With the introduction of molecular screening techniques, the diversity of avian malaria parasites has increased from what was previously thought. In this study, we examined the impacts of seasonality, sex, body condition, and genetic variation at immune related major histocompatibility class I alleles on malarial infection rates in the white-winged junco (Junco hyemalis aikeni), a subspecies of the dark-eyed junco endemic to the Black Hills. We found that the diversity of malarial parasite lineages in this species changes. Malarial infection rates in males were significantly higher than in females. There was also a significant association with malaria infection and body condition as measured by mass, indicating infection adversely impacts health. To further elucidate the impacts of the malarial parasites, we are now examining loci of the major histocompatibility complex (MHC) in the Black Hills junco. Previous studies have shown that parasite and pathogen diversity promotes diversifying selection in MHC loci. Thus, individuals with higher heterozygosity at MHC loci should have lower infection rates, possibly indicating increased immune function.
USE OF AN EGG HATCH ASSAY FOR EVALUATING THE ANTHELMINTIC ACTIVITY OF EXTRACTS FROM PLANTS NATIVE TO THE NORTHERN GREAT PLAINS AGAINST THE SHEEP NEMATODE, *HAEMONCHUS CONTORTUS*

J. Acharya¹, M.B. Hildreth¹,² and R.N. Reese¹*  
¹Department of Biology & Microbiology  
²Department of Veterinary Sciences  
South Dakota State University  
Brookings, SD 57007  
*neil.reese@sdstate.edu

**ABSTRACT**

*Haemonchus contortus* is an economically important gastrointestinal parasite of small ruminants. Control of this parasite relies mainly on treatment of infected hosts with available anthelmintic drugs, but increasing levels of resistance to these drugs has created the need for alternative treatment compounds. Several studies have shown that herbal preparations from certain plants have some anthelmintic activity, and these plants are being used in small herds to treat *Haemonchus* infections. Unfortunately, little information is known about the anthelmintic efficacy of local plants from the Northern Great Plains. A few *in vitro* assays have been developed for screening plant extracts for anthelmintic activity using eggs and juveniles of *H. contortus*, but no assay has received universal acceptance. In this study, we evaluated an egg hatching assay involving *H. contortus* for measuring anthelmintic activity in extracts from 3 plants native to South Dakota. For this assay, eggs harvested from sheep feces were incubated with plant extracts at 5 concentrations (i.e. 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.2 mg/ml, and 3.1 mg/ml prepared in 0.5% dimethyl sulphoxide). After incubating for 48 hours, we added a drop of Lugol’s iodine solution, and counted the number of hatched juveniles and unhatched eggs. Methanolic extracts from 3 indigenous plants (i.e. *Agastache foeniculum*, *Pedicularis* spp. and *Monarda fistulosa*) were tested. All the extracts inhibited egg hatching in a concentration dependent manner, but at 50 mg/ml, *M. fistulosa* showed complete inhibition of egg hatching, whereas, *Pedicularis* and *A. foeniculum* inhibited hatching by 86.1% and 73.2% respectively.
EFFECTS OF PERIMETER TREATMENTS WITH A RESIDUAL INSECTICIDE AROUND A SOFTBALL COMPLEX IN EASTERN SOUTH DAKOTA ON THE TRAPPING RATES OF CULEX TARSALIS

B. W. Riss¹, K. A. Kastelic¹, A. S. Bastian¹, M. J. Wittry¹, J. A. Wilson², and M. B. Hildreth¹,3*

¹Department of Biology and Microbiology
²Department of Plant Science and Cooperative Extension Service
³Department of Veterinary Sciences
South Dakota State University
Brookings, SD 57007
*michael.hildreth@sdstate.edu

ABSTRACT

Culex tarsalis continues as the primary vector for West Nile virus (WNv) throughout western U.S.A., and this region continues to have high human incidence rates for WNv. Outside community events held during the late evening likely increase Cx. tarsalis transmission of WNv. Insecticide formulations exist which remain active on vegetation for several days, and when sprayed on tall vegetation around the parameter of an area, may help reduce Cx. tarsalis numbers within community event locations. During the summer of 2008, a pilot study was conducted to determine if benefits from residual treatments (Insectrin-X) could be detected within a treated softball complex in Brookings, SD using four CO₂-baited CDC light traps inside the treated ball-complex area, and three control sites outside the treated area. Results from this pilot study were inconclusive. During 2009, the study was expanded to include 14 sites for 53 days; 5 of the sites were inside the softball complex (treatment sites), and 9 sites were just outside the complex (control sites). Tempo Ultra SC was sprayed around the parameter of the complex on July 16 and August 8. During the 53 days, control and treatment sites had a high correlation coefficient (i.e. 0.80), suggesting that sites outside the treatment area served as appropriate controls for the treated sites. Five days before and after the July 16 treatment period, Cx. tarsalis numbers increased significantly in the control sites ($P < 0.005$), and increased slightly in the treated sites ($P < 0.05$). Treated sites were not significantly different from the control sites before treatment, but treated sites had fewer Cx. tarsalis mosquitoes after treatment ($P < 0.05$). The Cx. tarsalis population had dropped low enough in August that any treatment effects were not measurable.
MARGINAL BENZIMIDAZOLE RESISTANCE IN TRICHOSTRONGYLES FROM A SOUTH DAKOTA SHEEP HERD PREVIOUSLY FOUND TO BE RESISTANT TO THE AVERMECTINS

B. R. Boswell¹, J. D. Bierle², D. D. Grosz¹,
L. D. Holler¹, S. Holler¹, and M. B. Hildreth¹,²*

¹Department of Veterinary Sciences
²Department of Biology & Microbiology
South Dakota State University
Brookings, SD 57007
*michael.hildreth@sdstate.edu

ABSTRACT

*Haemonchus contortus* is the most prevalent and detrimental species of trichostrongyles in sheep and goats. Producers normally use anthelmintic treatments to reduce production losses from these nematodes. Unfortunately, continual use of the benzimidazole and avermectin classes of anthelmintics has led to the development of drug resistance to these compounds in certain flocks of sheep and herds of goats. Recently, a flock of sheep from eastern South Dakota was found to be infected exclusively with *H. contortus*, and a large proportion these worms were resistant to the avermectins. Benzimidazole resistance was also suspected in this same flock. The purpose of the present project was to use a fecal egg count reduction test (FECRT) to determine if the *H. contortus* worms from this flock were also resistant to albendazole (a benzimidazole). During the fall of 2009, fecal samples were collected from 35 spring-born pastured lambs, and fecal egg counts (FEC) were determined using the Wisconsin sugar floatation technique (specific gravity 1.26). Lambs were then administered at least the recommended dosage of albendazole (Valbazen®, Pfizer Animal Health) orally, and fecal samples were collected 12 days later so that FECs could again be determined. The pre-treatment FECs averaged 1,092.3 eggs per gram (EPG) with a standard deviation (SD) of 906.3 EPG. After albendazole treatment, the FEC was reduced by 90.3% (mean FEC of 105.9, SD of 171.7). Reductions of less than 95% are considered to be evidence for resistance, but in this flock, this resistance would be marginal for the benzimidazoles. Attempts were made to measure resistance with a commercial larval development bioassay (Drenchrite, Microbial Screening Technologies, New South Wales), but results were inconclusive.
ABSENCE OF *HAEMONCHUS CONTORTUS* AND OTHER ABOMASAL NEMATODES IN EASTERN SOUTH DAKOTA WHITETAIL DEER DURING THE 2010 WINTER SEASON

A. A. Eljaki¹, J. A. Knutsen², and M. B. Hildreth¹,²*

¹Department of Biology and Microbiology
²Department of Veterinary Sciences
South Dakota State University
Brookings, SD 57007
*michael.hildreth@sdstate.edu

ABSTRACT

*Haemonchus contortus* is the most economically important abomasal helminth in sheep and goats, but it also parasitizes wild ruminants. Drug resistance in this species is a growing problem throughout much of the world, including South Dakota. Recent studies from Sweden have shown that, unlike most other trichostrongyles, the free-living J₃ stage of *H. contortus* survives poorly in the soil during cold winter conditions. This weakness of the J₃ stage is being used to control *Haemonchus* populations in domestic flocks and herds, but, unfortunately, the pre-adult parasitic juvenile stage (J₄) can survive winters encysted in a hypobiotic state within the abomasal wall of various ruminants. During the next spring, these juveniles molt to the adult stage, and then re-contaminate pastures with new *Haemonchus* eggs. Therefore, various wild ruminants in North America could serve as reservoir hosts for overwintering *H. contortus*. Because whitetail deer are one of the most common wild ruminants in eastern South Dakota, the purpose of this present study is to determine if the deer are commonly infected with *H. contortus* J₄ juveniles during the winter months. For this study, 35 white-tail deer were collected from various locations in eastern South Dakota during January and February of 2010. The abomasums were removed from each animal and digested with an acidified pepsin solution to free the hypobiotic J₄ juveniles from the submucosal tissues. The abomasum contents and digested abomasal tissue were then carefully observed with a stereomicroscope. Nematode adults and juveniles were not found within any of the samples from the 35 deer, suggesting that whitetail deer do not serve as significant reservoir host for *H. contortus*. 


EFFECTS OF FECAL FLOATATION SOLUTIONS ON THE BINDING OF PEANUT AGGLUTININ LECTIN TO THE SURFACE OF HAEMONCHUS CONTORTUS EGGS

D. D. Grosz\textsuperscript{1*}, K. Pike\textsuperscript{2} and M. B. Hildreth\textsuperscript{1,2*}
\textsuperscript{1}Department of Veterinary Sciences
\textsuperscript{2}Department of Biology & Microbiology
South Dakota State University
Brookings, SD 57007
\*michael.hildreth@sdstate.edu

ABSTRACT

\textit{Haemonchus contortus} is a trichostrongle nematode that commonly parasitizes the abomasums of sheep and goats, and frequently causes damaging effects to production parameters in these animals, occasionally killing individuals within the group. Appropriate management of this problem requires proper diagnosis of heavily infected individuals, and diagnosis is based upon finding \textit{Haemonchus} eggs in fecal samples from the suspect animals. Unfortunately, it is not possible to morphologically differentiate \textit{Haemonchus} eggs from other less pathogenic trichostrongyles also common to sheep and goats. Studies have shown that peanut agglutinin (PNA) lectin binds only to \textit{Haemonchus} eggs, thus enabling specific identification of \textit{Haemonchus} infections. To isolate eggs for diagnosis, fecal samples are added to a floatation solution, and fecal material is centrifuged to the bottom of each tube while the eggs are allowed to float to the surface. For the currently accepted protocol, eggs are then sucked from the surface, washed with water, incubated in fluorescently-labeled PNA, washed again and observed with an epifluorescence microscope. The purpose the present study is to determine if it is possible to add PNA to the eggs prior to floatation, and thereby, eliminate the time-consuming wash steps. In addition to sucrose (solution already being used), NaCl, NaNO\textsubscript{3} and ZnSO\textsubscript{4} were also tested as floatation solutions in order to determine their effect on the PNA staining. Staining intensity was compared with an Olympus AX70 epifluorescence microscope. In comparison to the accepted protocol, staining before floatation decreased PNA binding to some of the eggs, thus increasing staining variability. This also appeared to increase background staining of the fecal debris. This problem consistently occurred for all of the floatation solutions.
INVESTIGATION OF THE POTENTIAL ENERGY SURFACE FOR THE CCO + N_2 REACTION

Maximilian Rude and Justin Meyer*
Department of Chemistry
South Dakota School of Mines and Technology
Rapid City, SD 57701
*Justin.Meyer@sdsmt.edu

ABSTRACT

The potential energy surface of the CCO + N_2 reaction was investigated using theoretical methods at the CBS-QB3 level of theory. Three different product channels were found for the reaction. The enthalpies of reaction for these three product channels were used to estimate a value for \( \Delta H^\circ \) (CCO) of 372 \( \pm \) 5 kJ/mol. Results of this investigation help to answer questions about prompt NO_x formation via the CCO radical.
DEVELOPMENT OF SILVER NANOPARTICLE BASED CONDUCTIVE INKS FOR PRINTED SOLAR CELL APPLICATIONS

Ravi Shankar*, Keith W. Whites¹ and Jon J. Kellar²

¹Nanoscience and Nanoengineering Program
²Department of Materials and Metallurgical Engineering
South Dakota School of Mines and Technology
Rapid City, SD 57701
*ravi.shankar@mines.sdsmt.edu

ABSTRACT

In printed electronics, various printing technologies for metallic patterning require stable and well defined rheological behavior of dispersions of metallic nanoparticles. This work is directed towards the application of silver nanoparticles for the development of conductive nano-inks for printed solar cells. Successful nano-ink formulation involves a mixture of conductive nanoparticles and rheological additives. Various deposition methods (M3D, nScrypt, ink-jet) can be employed for the ink deposition. Lastly, metallization of deposited ink can be done using the thermal sintering process. We have developed both aqueous and organic solvent based nano inks by using silver nanoparticles with different capping agents. For aqueous ink, PVP capped silver nanoparticles have been synthesized using the polyol process. A number of syntheses have been tried for organic solvent based ink using wet chemical reduction reactions. Successful organic solvent based inks have been developed using dodecylamine capped silver nanoparticles. Ink formulations with varying shapes and sizes of particles have also been tried in order to study the variation in conductivity and ease of deposition. Conductivity in the range of 50-60% of bulk silver has been achieved with a single pass using aqueous ink with the M3D deposition technique at low temperature thermal curing. However, this aqueous ink has its limitations for ink-jet printing. With organic solvent based ink about 40% conductivity of bulk silver has been achieved with 5 passes. Also, inks in organic solvent are particularly interesting for ink-jet printing of solar cell collector networks.
EXAMINATION OF THE RULE OF MIXTURES FOR TWO POLYMERIC SYSTEMS: GLASS TRANSITION TEMPERATURE ($T_g$) AND SPECIFIC REFRACTIVE INDEX INCREMENT (DN/DC)

Shady Awwad*, Tsvetanka Filipova and David A. Boyles
Department of Chemistry
South Dakota School of Mines and Technology
Rapid City, SD 57701
*shady.awwad@mines.sdsmt.edu

ABSTRACT

Can differential scanning calorimetry (DSC) and refractive index increment (dn/dc) distinguish between two solutions having identical numbers of monomers, one solution being a solution of a copolymer, and the other solution being identical in monomer concentration but being a mixture of two separate homopolymers? Solutions of copolymers and novel miscible polycarbonates (which show a single glass transition temperature constituting proof of miscibility) have been investigated by DSC and Optilab DSP refractometer to ascertain how the glass transition temperature ($T_g$) and specific refractive index increment (dn/dc) data obtained from the copolymeric solutions are related to data from homopolymeric mixtures having identical stoichiometric compositions of the copolymers. In particular, we compared homopolymers BPA-PC ($M_w 26,410$ g/mol, PDI = 1.526) and triBPA-PC ($M_w 39,090$ g/mol, PDI = 3.162), their copolymers, and corresponding homopolymeric solutions having identical mole fractions. Additional comparisons were made by proton NMR ($^1$H NMR). DSC thermograms of tri-BPA-PC: BPA-PC blends showed a composition dependent glass transition ($T_g$s). A method for determining the specific refractive index increment will be described as well as the results of the mixtures/copolymer DSC investigations.
INFLUENCE OF CARBONATE UNITS ON THE CHEMISTRY OF BISPHENOL A POLYCARBONATE: SYNTHESIS OF POLY(ETHERCARBONATE)S CONTAINING PRECISELY CONTROLLED MONOMERS OF VARIABLE LENGTH

Mohammad S. Alomar,* and David A. Boyles,
1Nanoscience and Nanoengineering Program
2Department of Chemistry
South Dakota School of Mines & Technology
Rapid City, SD 57701
*Mohammad.Al-Omar@mines.sdsmt.edu

ABSTRACT

Although bisphenol A polycarbonate (BPA-PC) has many property advantages such as heat resistance, clarity, and ductility, the sensitivity of the carbonate units to hydrolysis and photo-initiated rearrangements leads to degradation of mechanical properties with time. While many commercial polyethers exist which do not have these problems, their synthesis is restricted to nucleophilic aromatic substitution of a limited category of starting materials, which latter is not possible with bisphenol A. Although some investigators have sought previously to synthesize an all-bisphenol A polyether, its synthesis has continued to elude any viable synthetic methodology. It was therefore of interest to design a series of polycarbonates which were precisely tailored to have differing carbonate-ether ratios to circumvent these difficulties as well as to produce materials having greater ether-imparted chain backbone flexibility for dielectric studies. Several attempts to synthesize the requisite monomers failed until a recently-published method for diaryl ether synthesis was found. Previously used for the production of smaller pharmaceutical molecules only, this liganded copper-catalytic method was successfully used for the synthesis of the envisioned poly(ether-carbonate)s. The molecular structure of the newly synthesized monomers and their corresponding polymers was confirmed by proton nuclear magnetic resonance spectroscopy. Molecular weight and glass transition temperatures of the polymers were determined by laser light scattering and differential scanning calorimetry, respectively. The polymers possessed remarkably high glass transition temperatures in spite of the high ether-to-carbonate ratio in the backbone chain. Application and preliminary results of the synthetic methodology to the homopolyether of bisphenol A are also described.
DESIGN AND SYNTHESIS OF A HIGH POLARITY MONOMER FOR ELABORATION INTO A FIELD RESPONSIVE MATERIAL FOR WOUND FILM CAPACITORS

Hammad Younes* and David A. Boyles
Department of Chemistry
South Dakota School of Mines and Technology
Rapid City, SD, 57701
*hammad.younes@mines.sdsmt.edu

ABSTRACT

Pulse power capacitors are under intense consideration by the U.S. Navy as a power source for all-electric missiles. Polycarbonate has been used for decades in wound film capacitors owing to its excellent ability to form consistent, well-behaved films of moderate dielectric permittivity and good breakdown strength. Newer materials with fast relaxation times are required to meet energy density specifications for military requirements such as in rail guns. Urea-related molecules have high dipoles which make them respond to externally applied electric fields. We have designed a molecule containing a key imidazolidinone unit for subsequent polymerization and dielectric film testing. Several synthetic routes were considered for synthesis of this material and will be described. The successful synthesis ultimately employed a recently-published variation on the well-known Goldberg reaction as modified by Buchwald. Obstacles initially encountered included deprotection which led to a modification in the choice of protective group. Molecular characterization data by proton nuclear resonance spectroscopy confirmed the expected structure.
SPECTROSCOPIC STUDIES OF IODINE-DOPED POLYCARBONATES

M. R. Barth*, D. A. Boyles, and T. S. Filipova,
Department of Chemistry
South Dakota School of Mines and Technology
Rapid City, SD 57701
*mackenzie.barth@mines.sdsmt.edu

ABSTRACT

The behavior of iodine in polycarbonates as thin films or in solution was investigated by spectroscopic methods. It is well known that the color of a solution containing iodine is dependent on the solvent. It is assumed that the difference in color is due to charge-transfer interactions. Solutions of 3, 5, and 25% (w/w) iodine in commercial polycarbonate (BPA-PC) and nitrile polycarbonate (CN-PC) in dichloromethane were studied. Films of iodine-doped BPA-PC and CN-PC exhibited a difference in color, which could be attributed to a difference in the formation of the iodine charge transfer complex in the polymer films. Unlike BPA-PC, CN-PC contains a dipole from the nitrile functional group, which may cause increased charge-transfer interactions. UV-Vis absorption spectra of the investigated polymer films showed different wavelengths of maximum absorption in the visible range. Infrared spectroscopy of iodine-doped PC films in comparison with pure PC films showed a shift in the band at 1774 cm$^{-1}$, which was assigned to the vibrational mode of the carbonyl group in the polymer chain. This shift may be due to an electrostatic interaction between iodine and the carbonyl group, which could result in the formation of a charge transfer complex.
EFFECTS OF STRUCTURE ON SORPTION OF SELECTED TETRACHLOROBIPHENYL CONGENERS BY NATURAL SOLUBLE ORGANIC MATTER

N. K. Kadukuntla\textsuperscript{1} and H.V. Mott\textsuperscript{2*}
\textsuperscript{1}ACCENTURE Consultants
India
\textsuperscript{2}Department of Civil and Environmental Engineering
South Dakota School of Mines
Rapid City, SD 57701
\textsuperscript{*}henry.mott@sdsmt.edu

ABSTRACT

Observations of data collected from the literature regarding the sorption from aqueous solution of hydrophobic, synthetic organic contaminants (SOCs) by soluble humic substances (characterized as dissolved organic carbon, DOC) suggest behaviors that cannot be reconciled solely by the hydrophobicity of the solute (as characterized by the octanol/water partition coefficient, $K_{ow}$). Various researchers have postulated that sorption by DOC of SOCs having three-dimensional molecular structures (e.g., polychlorinated biphenyls) might be less intense for a given degree of base hydrophobicity than that of planar SOCs (e.g., polynuclear aromatic hydrocarbons). A significant problem associated with comparisons of sorption of various classes of SOCs by DOC arises from necessarily employing differing experimental methodologies in measuring the magnitude of the SOC-DOC distribution coefficient ($K_{DOC}$). In order that values of $K_{DOC}$ for SOCs of varying structure could be measured using a common methodology – Apparent Aqueous Solubility Enhancement (ASE) – three tetrachlorobiphenyl congeners were selected for study based on their differing ortho, meta, and para substitutions of chlorine for hydrogen on the base biphenyl molecule. Two sources of natural soluble humic-like material (aqueous extracts from Canadian peat moss and fresh silver maple, \textit{Acer saccharinum}, leaves) were prepared, sorption experiments were conducted using the ASE methods, and results were analyzed and interpreted based on the degree to which rotation about the single carbon-carbon bond of the biphenyl molecule might impart hindrance to the physical association of the particular congener within the structure of the natural organic material. Experimental results indicated that the chlorine positions indeed affected the capacity for sorption.
SYNTHESIS AND CHARACTERIZATION OF MESOPOROUS MANGANESE-DOPED FERRITE FOR POTENTIAL APPLICATION IN HIGH TEMPERATURE THERMOCHEMICAL HYDROGEN PRODUCTION

Michael Opoku, Rajesh Shende, and Jan A. Puszynski*
Chemical & Biological Engineering Department
South Dakota School of Mines & Technology
Rapid City, SD 57701
* Jan.Puszynski@sdsmt.edu

ABSTRACT

Mesoporous manganese-doped ferrite (MnFe$_2$O$_4$) material was synthesized and investigated as a potential redox material for thermochemical production of hydrogen. The basic idea is that the doped ferrites can split water molecules by extracting oxygen atoms and reversibly incorporating them into their (doped ferrites) lattices. MnFe$_2$O$_4$ was produced from manganese acetate, iron citrate and sucrose precursors via a novel microwave-assisted synthesis method which resulted in the formation of a pure crystalline material. Average crystallite size of MnFe$_2$O$_4$ was 15 nm as determined by x-ray powder diffraction (XRD) peak broadening technique. Transmission electron microscopy and scanning electron microscopy images of the synthesized material revealed that MnFe$_2$O$_4$ is mesoporous and the average crystallite size matches well with the size obtained by XRD. Average pore diameter of 4.5 nm and specific surface area of 64.2 m$^2$/g were measured by the BET surface area technique. Redox experiments conducted by differential scanning calorimeter and thermogravimetric analyzer (TGA) indicated that synthesized MnFe$_2$O$_4$ material can take up oxygen into its (MnFe$_2$O$_4$) lattice structure up to 3.4 wt%. The results have also shown that this material can be fully regenerated by raising the temperature from 800 °C to 1000 °C under argon or nitrogen atmosphere. Based on the TGA analyses of oxidation and reduction in air and argon, respectively, we estimated that 47.7 STPml/g-solid of H$_2$ might be generated in each cycle. Details of synthesis and characterization of this promising doped ferrite material will be presented. In addition, experimental results of hydrogen generation by high temperature water splitting on MnFe$_2$O$_4$ in a laboratory scale reactor will be presented.
SOL-GEL DERIVED SN-FERRITE FOR H₂ GENERATION FROM WATER-SPLITTING REACTION

Department of Chemical & Biological Engineering
South Dakota School of Mines & Technology
Rapid City, SD 57701
*rajesh.shende@sdsmt.edu

ABSTRACT

Ferrites such as \((A,B)_xFe_{3+y}O_4\) (doped spinel) or \((A,B)_xFe_{1+x}O\) (doped wustite) or their mixtures, where A and B are bivalent metal cations, were reported to be the most active materials for hydrogen generation using thermochemical water-splitting reactions. Although these ferrites have been synthesized by solid-state mixing, oxidation of aqueous metal hydroxide suspension, self-propagation high temperature synthesis and aerosol spray pyrolysis methods, the use of sol-gel derived ferrites for thermochemical water-splitting reactions is very limited. The sol-gel synthesis method provides better control over stoichiometry and powder characteristics. The powders produced by the sol-gel synthesis method usually have higher specific surface area, which may provide a higher number of active sites for water-splitting reactions. In this investigation, Sn-ferrite and Sn doped Ni-ferrite were synthesized using a sol-gel technique wherein Ni, Sn and Fe salts were dissolved in ethanol by sonication with the gel formation being accomplished by adding propylene oxide. The gels were aged for 48 h, dried at 100 °C for 1 h, and finally calcined at 900 °C in an air environment. The calcined powder was characterized using powder X-ray diffraction, Brauner-Emmett-Teller surface area analysis and scanning electron microscopy. After the powder was placed in a packed bed reactor, the water-splitting reaction was carried out at 700 °C. After each water-splitting reaction, oxidized ferrite was regenerated at 900 °C for 2 h in an N₂ environment. Synthesis method, characterization, and transient hydrogen profiles obtained at various experimental conditions will be presented in detail.
RESISTANCE TO AIRFLOW OF POROUS MEMBRANES USED IN PHOTOBIOREACTORS

Gary A. Anderson*, Anil Kommareddy**, Zhengrong Gu¹ and Joanne Puetz Anderson¹
¹Agricultural and Biosystems Engineering
²Geographical Information Systems Center of Excellence
South Dakota State University
Brookings, SD 57007
*gary.anderson@sdstate.edu

ABSTRACT

Mixing and injection of carbon dioxide in a photobioreactor are essential for high density algal biomass production. One method of mixing and injecting carbon dioxide is to force carbon dioxide rich air through a porous/perforated membrane at the bottom of the photobioreactor. Our objective was to determine the resistance to airflow of four membranes for various airflow rates and water depths above the membrane. The membranes were made of sintered plastic beads with a tortuous flow path (porous) and orifice diameters of 15-45, 20, and 35µm and respective membrane thicknesses of 1.588, 0.794, and 0.794 mm. One plastic sheet membrane (perforated) was tested with an orifice diameter of 45µm. Results show that for water depths greater than 75 mm the resistance to airflow is primarily due to water depth and air flow rate significantly affects resistance at lower depths. The membranes had virtually no resistance to airflow (less than 24 Pa) until water was added to the test apparatus, in which case pressure increased abruptly due to the orifices being filled with water (bubble formation). Air flow resistances ranged from 3.4 Pa (no water and an air velocity of 0.93 m/s) to 3800 Pa (0.23 m of water and an air velocity of 6.5 m/s) over all test conditions. We noted that the area of the membrane emitting bubbles increased with airflow rate as well as bubble size. The tortuous path through the membrane gives rise to variation in flow path resistance, and the deflected shape of the pressurized membrane changed water depth across the membrane leading to changes in airflow area of the membrane.
USING THE METHODS OF SCIENCE TO DETERMINE HOW TO TEACH SCIENCE

Andy Johnson*
Center for the Advancement of Math and Science Education (CAMSE)
Black Hills State University
Spearfish, SD 57799
*andy.johnson@bhsu.edu

ABSTRACT

Science distinguishes itself from other fields of knowledge by requiring, among other distinctions, that scientists match their ideas to their observations and data. Scientific reasoning needs not stop at the classroom door. Recent research in science education and particularly in physics education has applied this scientific method by connecting particular classroom approaches to the realities of learning outcomes. A clear picture has emerged of the effectiveness of various approaches to science teaching. Substantial evidence shows that certain inquiry-based approaches result in highly significant improvements in learning, particularly when compared to the traditional lecture format. The implication is that if we are to take a scientific approach to teaching, we need to adjust our thinking to match the data on learning. I will summarize research findings on what works, what doesn’t, and why.
BRINGING AUTHENTIC RESEARCH INTO THE GENERAL CHEMISTRY LABORATORY CURRICULUM: NORTHERN PLAINS UNDERGRADUATE RESEARCH CENTER

Mary Berry¹, Miles Koppang¹, Joe Vitt¹, Krisma DeWitt²*, Gary Earl³, Duane Weisshaar³, Paul Weber⁴, and Carl Fictorie⁵

¹Department of Chemistry
University of South Dakota
Vermillion, SD 57069
²Department of Natural Sciences
Mount Marty College
Yankton, SD 57078
³Department of Chemistry
Augustana College,
Sioux Falls, SD 57197
⁴Chemistry Department
Briar Cliff College
Sioux City, IA 51104
⁵Chemistry Department
Dordt College
Sioux Center, IA 51250
*kdewitt@mtmc.edu

ABSTRACT

The NSF-sponsored Northern Plains Undergraduate Research Center (NPURC) involves a partnership of eight institutions consisting of public universities, private and tribal colleges. The center’s overall goal is increasing access to and opportunities for research for undergraduate students. To reach the greatest number of students, NPURC has implemented a series of research activities designed for first year students. These activities include authentic research experiences in the general chemistry laboratory, weeklong research workshops and intensive summer research experiences. The University of South Dakota, Mount Marty College and Augustana College implemented research experiences centered around a novel group of tetraalkylammonium salts. These ionic liquids have been the subject of intense research efforts throughout the world in the past decade for their possible use as renewable solvents for chemical reactions. In addition, water analysis in conjunction with the Missouri River Institute has been conducted in the general chemistry laboratory at the University of South Dakota as an authentic research experience. The mechanics of implementing the effort and its assessment will be presented in the panel discussion on NSF funded URCs.
ABSTRACT

The University of South Dakota’s College of Arts and Sciences and School of Education established a teacher development partnership with Avon and the 21st Century Rural Schools Consortium (a group of participating high-needs schools in Region 3 and beyond) to provide two week-long short courses in summer 2009. The first course entitled “The Horse before the Cart: Science Problems Driving Mathematical Understanding” provided depth of knowledge enhancement in mathematics by directing that science experimentation illustrate the need for mathematical modeling and understanding of the scientific phenomenon. The second course entitled “One Room Science Schoolhouse: Big Kids teach Little Kids” was designed to partner a high school science teacher and a science teacher at the upper elementary grade level in order to provide innovative “hands on” science experiences to elementary students (little kids). These activities were delivered by high school students (big kids), guided by their teachers during the 2009/2010 academic year. Each short course provided the opportunity for participating teachers to earn two credits of graduate science content coursework through The University of South Dakota. Various aspects of the implementation of these short course activities into the classroom will be presented by two high school teacher participants.
THE SOUTH DAKOTA SCHOOL OF MINES AND TECHNOLOGY’S SCIENCE EDUCATION OUTREACH PROGRAM: EXPOSING YOUNG, INQUISITIVE MINDS TO THE WONDER OF SCIENCE

Rachel Brown*, Natalie Toth, and Sally Shelton
Museum of Geology and Department of Geology and Geological Engineering
South Dakota School of Mines and Technology
Rapid City, SD 57701
*Rachel.brown@mines.sdsmt.edu

ABSTRACT

The Museum of Geology at the South Dakota School of Mines and Technology (SDSMT) is known for its invaluable paleontological and mineralogical resources. Thousands of visitors pass through the Museum’s exhibits every year and give support to the Museum of Geology not only to ensure success of the museum, but also to bring the new Paleontology Research Center to life. Now is the time for the Museum of Geology’s students and staff to give back to the public and reach out to schools, children’s programs, and organizations in the Black Hills Region. SDSMT’s Science Education Outreach Program works to expose students to the disciplines of science that are not part of the traditional core curriculum at their grade level. The Museum of Geology, along with various student organizations, such as the Paleontology Club, Tech Geologic Association (TGA), and the Society of Economic Geology (SEG), allows undergraduate and graduate students the opportunity to create and execute demonstrations for K-12 students touching on the concepts of paleontology/fossils, rocks and minerals, plants and animals in the Black Hills, and hydrology/groundwater. The presentations are unique in that they can be formatted to fit any grade level, in any location, allowing teachers the option to incorporate the various themes into their own curriculum, while still meeting educational standards of South Dakota. When a local school or organization requests an outreach subject, SDSMT will provide teaching kits, as well as an outreach instructor. These kits include books and manuals, pertinent information, hands-on activities, and even suggestions about how to incorporate the presented topic into other subjects aside from science.
BRIDGING THE GAP: EXPANDING SCIENCE CLASSROOM EXPERIENCES USING NEW AND WEB-BASED TECHNOLOGIES

Cathy Ezrailson
Department of Education
University of South Dakota
Vermillion, SD
Cathy.ezrailson@usd.edu

ABSTRACT

The complexion of science classrooms today and tomorrow require that teachers continue to acquire the skills needed to incorporate new and emerging technologies. There are a host of web-based teaching tools, easily learned, free to teachers and immediately available that could enhance and augment science learning. Technology takes many forms in today’s high schools – from smart board, to data acquisition devices to web-based lessons and resources. Using web tech tools such as Google Docs to organize, design, access and assess lessons seamlessly is integral to teaching in the 21st century classroom. This paper illustrates use of and examples of these tools along with suggestions for applications.
MODELING PHYSICS: A RESEARCH-BASED METHOD THAT SUPPORTS LEARNING

Rose Emanuel* and Roberta Traxinger

1Lead-Deadwood High School
Lead, SD 57754-1817
2Douglas High School
Box Elder, SD
*Rose.Emanuel@k12.sd.us

ABSTRACT

The Modeling Physics pedagogy, developed by researchers at Arizona State University and elsewhere, applies proven strategies in a coherent framework to promote learning. Modeling has earned a distinction as one of the most effective materials for teaching physics. Modeling materials are also available in other topics including physical science, chemistry, mathematics. In a Modeling classroom, students are highly involved in the scientific process as they uncover key physics ideas themselves. With Modeling, students come to understand physics much more deeply than with typical methods. And it’s fun! We will present glimpses into how Modeling works in the classroom and how students learn what it means to do science.
FROM THEORY TO HYPOTHESIS TO TEST: APPLYING SCIENCE TO TEACHING BIOLOGY

Robert Tatina  
Biology Department  
Dakota Wesleyan University  
Mitchell, SD 57301  
rotatina@dwu.edu

ABSTRACT

Producing a scientifically literate society has been a goal of science education since the 1980’s. However, numerous authors have shown that such a goal has not been effectively implemented in the pre-college curriculum. Data collected from surveys of the American public have shown that society in general still accepts non-scientific explanations for natural phenomena and that pre-college teachers generally possess an inadequate knowledge of the methods of science. During that same time period scientists and educational researchers have been exhorting science teachers and curriculum designers to teach science like science is done. Students need to learn the tools that will help them judge 1) whether an explanation for some natural phenomenon is indeed scientific and 2) whether a piece of research was done correctly.

For explanations of natural phenomena, a scientifically literate public should be able to recognize the characteristics of a scientific theory and hypothesis. They should realize that theory is more than mere opinion. For experiments they should be able to identify suitable controls and replicates and be able to ask whether the experiment fits the hypothesis. I describe a teaching technique that I used in the lecture and lab portions of an introductory biology class that I taught to majors and non-majors at Dakota Wesleyan University. The technique starts with a theory from which is developed a hypothesis, which is then tested.

In lab: I introduce a theory, explain its nuances and then suggest a hypothesis and show the students how to test it (the theory and test are given in their lab books, but not the hypothesis). The students then carry out the test, after which we discuss the outcome in terms of our hypothesis, accepting or rejecting it. Then I charge them to come up with a hypothesis of their own based on the theory and the test they have completed. In the following week they must have written their hypothesis and described their test and expected outcome. They also must have a copy of one piece of scientific literature that relates to their hypothesis. They set up their experiment, run it, gather data, analyze it and then as a group write a formal paper. The paper is submitted for review, sent back for corrections and then resubmitted for a grade. The papers are graded on style, and experimental design.

In lecture: I propose a theory, give an example from the literature of the test and ask for an expected outcome. Sometimes I will ask groups to select a hypothesis to test, and/or suggest the test and the expected outcome.
INSTRUCTIONS FOR AUTHORS

Editorial Policy: The South Dakota Academy of Science is a forum for the promotion of scientific teaching, research, and service in the state of South Dakota. The South Dakota Academy of Science meets each spring for the purpose of annual business, awards, and the interchange of ideas, information, and results from scientists and students of science. The minutes of the annual meeting and the abstracts of presentations are published annually in the Proceedings of the South Dakota Academy of Science. In many cases, authors choose to submit a full manuscript of their presentation, which is published in lieu of the abstract. Deadlines for submission of full manuscripts are July 15 each year; to submit manuscripts, please adhere to the Submission Procedures listed below. Authors have the option of purchasing additional reprints; information on page and reprint charges will accompany the page proofs sent to the corresponding author. The following guidelines provide instructions for authors submitting manuscripts to the Proceedings. Be aware that these guidelines may vary from year to year, so please try to format your manuscript so that it meets the conventions described below.

Authorship Policy: The following is the policy on authorship of abstracts and manuscripts to be published in the Proceedings:

1) All papers published in the Proceedings of the South Dakota Academy of Science will include all authors that were shown on originally submitted abstracts or manuscripts.

2) Any request to change authors after a submission has been made to the Proceedings must be accompanied by a brief letter of explanation sent to the editor that includes the original signature of all authors (including authors removed from or added to the authorship list).

3) The deadline for requesting changes in authorship is 30 August of the year of submission.

Manuscript Submission Procedure: Manuscripts submitted for publication in Proceedings must be accompanied by two technical reviews. Authors are required to solicit reviewers that have expertise in the topic area of the manuscript. Co-authors of manuscripts are not considered reviewers. Each reviewer must provide their name, title, address, and phone number along with a brief review of the manuscript; to facilitate this process, the reviewers should use the Peer Review Form that can be downloaded from the Academy's website (http://acadsci.sdstate.org). In turn, Authors must provide a brief [written] reconciliation letter of how reviewer comments were addressed along with a final, revised copy of the manuscript. The submission package must include the following: 1) two technical reviews (Peer Review Forms), 2) written reconciliation from authors, and 3) revised manuscript. Failure to adhere to the submission procedure will result in manuscripts being returned to authors. Submission of abstracts for publication in the Proceedings do not need to be peer-reviewed.

Abstracts or manuscripts may be submitted either electronically or by mail. Please do not submit abstracts or manuscripts both electronically and by mail. Include a cover letter with the address and telephone number of the corresponding author.

Electronic submission:
Electronic submissions should be forwarded to Robert Tatina at rotatina@dwu.edu. Manuscripts should be saved as MS Word (Please use Word 97-2003) files that include tables and/or figures. Reviewer comments and the author(s) reconciliation statement must be submitted as separate files (MS Word) along with the manuscript. Manuscript illustration files that have the extension .BMP, .GIF, .EPS, .JPG, .TIF, or .PCX are acceptable. IMPORTANT: Begin all file names with the corresponding author’s last name (e.g., Chippins_ms.doc, Chippins_review1.doc, Chippins_review2.doc, Chippins_reconcil.doc).

Mail submission:
Manuscripts may be submitted by mail by including one hardcopy and one electronic copy (3.5” disk or CD) of each manuscript. Hardcopies of reviewer’s comments and reconciliation statement must also be included. Each diskette or CD should be identified with authors’ names and by the format used (e.g., WordPerfect 6.0). Submit all information in MS Word. Illustration files that have the extension .BMP, .GIF, .EPS, .JPG, .TIF, or .PCX are acceptable. Begin all file names with the corresponding author’s last name (e.g., Chippins_ms.doc).

Submission of Manuscripts. Mail manuscript submissions to:

Robert Tatina, Proceedings Editor
704 South Montana
Mitchell, SD 57301

Phone: 605-996-7670;
E-mail: rotatina@dwu.edu.

Membership. The author or one co-author of each abstract or paper must be a current member of the South Dakota Academy of Science.


Format Conventions: All manuscripts and abstracts should be double-spaced throughout including tables and the literature cited section. All pages should be numbered serially in the upper right-hand corner. Use a 1-inch margin all around but do not justify the right margin or hyphenate words on the right margin (i.e., use word wrap). Avoid footnotes in both articles and abstracts. Use metric measurements unless English units are clearly more appropriate, in which case metric equivalents must appear in parentheses. Give scientific names for all species the first time the species’ common name is used as follows: Culver’s root (Veronicastrum virginicum). Write out numbers under 10, except in a series with numbers >10 or with measurements or percentages. Statistical terms and other measures should conform with the Council of Science Editors Style Manual.
Manuscript Components:

TITLE PAGE
All manuscripts should include a title page that includes the author name(s), affiliation(s), and current mailing address(es). Here are the components and style of the title page:

Microsoft Word
Times New Roman, Font Size = 12
Margin: Left = 0, Right = 6, Paragraph Indent = 4
Title: All Caps, Bold, Centered
Authors: See example:
Alfred Jones*, William Smith† and Lawrence Porter‡
†Biology Department
Cognition University
South Chicago, IN 44230
‡Chemistry Department
Intuition College
Newell, SD 57330
*alfred.jones@sdstate.edu

ABSTRACT
Include a brief but informative abstract. The abstract should be a single paragraph of 200-300 words that concisely summarizes the results and conclusions of the study. The abstract should be followed by a short list of keywords (< 5). For authors submitting only an abstract, you may include a few literature citations, but please use sparingly.

INTRODUCTION
The introduction should include sufficient detail to establish the purpose and importance of the work being summarized. It should include pertinent literature related to the study question and end with a statement of the objectives/hypotheses being examined.

METHODS
The methods should include sufficient information for the reader to evaluate the data or repeat the study. It is better to be inclusive (i.e., overly explicit) when describing the methods used, but keep in mind that clarity and conciseness are important.

RESULTS
Results follow the methods section and should include a concise summary of your findings. Be careful not to ‘discuss’ implications of results; reserve these points for the Discussion section. Results that are presented in tables or figures should not be exhaustively discussed in the text. When statistics are presented they should be explicit (e.g. $t = 34.07, df = 48, P = 0.003$).

DISCUSSION
The common trait among good scientific papers is a well-written discussion section. The discussion section is where you synthesize what has been learned from the study and provide direction or generalities for future studies. The discussion section should not be merely a summary of relevant literature linked to restatements of your results. Rather, it should be an informative essay that advances what is known about the subject area. Be careful, however, not to extend the discussion beyond what can be supported by the data reported in the results.

LITERATURE CITED. All references cited in the text should be listed at the end of the manuscript under the Literature Cited heading. References should be in alphabetical order by the last name of the author(s). If different works by the same author(s) are referenced, references should be in chronological order. Authors cited in the text of abstracts or papers should be indicated in parentheses by last name and year of publication (e.g., Wade 1967; Bremer 1977). When a citation has more than two authors, the last name of the first author followed by et al. should be used (e.g., Seabloom et al. 1978). Where two or more papers by the same author(s) have appeared in one year, the style should be (Stewart 1967a, 1967b).

Examples of Literature Cited

Tables, Figures, Illustrations and Photographs. Tables should be double spaced throughout, without vertical lines, and with page numbers in the upper right-hand corner. If tables are more than one page in length, put "Table x. continued" on all subsequent pages. Each table should begin with a Table number (e.g., Table 1, Table 2, etc.) followed by a descriptive caption which is separated from the table headings and data by a horizontal line. Tables must be referenced in the text.

Figures should be done with black ink on firm quality paper or if computer generated, they should be of camera-ready quality (laser printer) with sharp lines suitable for reproduction. Photographs should be 5x7-inch, sharp, black and white glossy prints. When appropriate, a legend of symbols, etc., scale and a cardinal direction (e.g. North) should be indicated on figures of maps. On the back side, identify each figure or photograph lightly in pencil with the figure’s number, author(s) name(s), and an indication of the top of the figure. Figure captions should be numbered (e.g., Figure x.) and typed on a separate sheet of paper at the end of the manuscript. Letters, symbols, and legends should be large and clear enough on all figures and photographs to be legible after copy reduction. Figures must be referenced in the text.