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MINUTES OF THE EIGHTY-FOURTH ANNUAL MEETING OF THE SOUTH DAKOTA ACADEMY OF SCIENCE

The eighty-fourth Annual Meeting of the South Dakota Academy of Science was held Friday, April 16, 1999, at the Habeger Science Center on the Dakota State University campus in Madison, South Dakota.

The Executive Committee and Arrangements Co-Chairs met on Wednesday, April 14, at 8:00 p.m., for the final check of the agenda and arrangements for the annual meeting on Friday.

Registration on April 16 was facilitated by Ken Higgins, Terri Symens and Pam Schut and several Dakota State University students. Bill Soeffing was absent due to a death in his family.

ANNUAL BUSINESS MEETING

The Annual Business meeting of the 84th Academy was called to order by President Royce Engstrom following the noon lunch. President Engstrom extended the Academy's sincere gratitude to Dakota State University's President, Gerald Tunheim, for hosting the Academy; to Philip Sandberg, Dean of Natural Sciences, for use of his staff in hosting the Academy at the Habeger Science Center, and to co-chairs Donna Hazelwood and Dale Droge for local arrangements for the Annual Meeting.

The 1998 Academy Proceedings Editor Ken Higgins gave a status report on the past and current Proceedings. Emil Knapp was still working on the 1996 and 1997 Proceedings. The 1998 Proceedings were made available at the Annual Meeting. Ken extended thanks to several who were largely responsible for helping him get the 1998 Proceedings completed on schedule, including, but not limited to: Terri Symens, Mary Brashier, Tom Holmlund, Emil Knapp, Dennis Lundgren and several SDSU graduate students. Ken offered to stay on as Proceedings Editor in 1999 if there were no major concerns.

President Engstrom reported that the 2000 Annual meeting of the SD Academy will be held jointly with the North Dakota and Minnesota Academy's at a joint tri-state meeting on April 28-29, 2000, on the Moorhead State University campus, Moorhead, MN.

Lenore Koczon provided a summary of the Jr. Academy as follows:

Twenty-five papers were submitted for the Jr. Academy for 1999 of which 21 were presented and judged. Two papers were recognized as the most outstanding: one by Heather May Anderson, the other by Elaine Kub, both of which were 10th grade students from Ipswich High School. Their advisor was Mr. Bill Mower. Abstracts of their winning presentations appear in this proceedings.

President Engstrom presented awards to the recipients of the Fellow Award (K. Higgins 1998, C. Estee 1999) and to the Outstanding High School Teachers of the Year as follows:

Plaques were awarded to Dr. Kenneth F. Higgins, Assistant Leader-S.D. Cooperative Fish and Wildlife Research Unit, South Dakota State University, who was recognized in 1998 as the First Fellow of the South Dakota Academy of Science and to Dr. Charles R. Estee, Professor of Chemistry at the University of South Dakota, who was recognized in 1999 as the Second Fellow of the South Dakota Academy of Science.

Three High School Teacher Awards were presented in 1999. David Strom of the Hartford-West Central High School was presented with an Honorary Lifetime Achievement in Physical Sciences Award. Angie Heil of Yankton High School was honored as Biology Teacher of the Year and Carolyn Burns of Watertown High School was honored as Physical Science Teacher of the Year.

RESOLUTION COMMITTEE

The South Dakota Academy of Science thanks Dr. Gerald Tunheim, President of Dakota State University, for making campus facilities available and for hosting the 1999 annual meeting of the Academy.

The Academy is grateful to the local planning committee, Drs. Donna Hazelwood and Dale Droge, for their efforts in providing excellent accommodations and arrangements for the 1999 meeting.

Thanks to President-Elect Neil Reese for his illuminating address, "Herbalism in the Modern World."

We also wish to thank Dr. Bruce Hannon for his post-banquet address "Dynamic Modeling."

Our appreciation and congratulations are extended to President Royce Engstrom for a successful year of leadership and for his personal role in stimulating reactivation of the Junior Academy of Science.

We thank Academy Secretary-Treasurer Bill Soeffing for his dedication to our organization, and we extend our sincere sympathies to Bill and his family in the very recent passing of his mother.

Special thanks are extended to Emil Knapp for his many years of service as Editor of the Proceedings. And thank you to Ken Higgins who has ably assumed the role of Editor.

The Academy congratulates Angie Heil of Yankton High School who is honored as the Biology Teacher of the Year. We also extend our congratulations to Carolyn Burns of Watertown High School, recipient of the Physical Science Teacher of the Year award.

The Academy extends its best wishes to David Strom (and wife Betty) as he retires from many years of science teaching at Hartford-West Central High School.

Congratulations to Ken Higgins of South Dakota State University, who in 1998 was recognized as the first Fellow of the South Dakota Academy of Science. Congratulations also to Charles Estee of the University of South Dakota who is this year distinguished as the 2nd Fellow of the South Dakota Academy of Science.

Reported by: *Gary Larson*
Nels Troelstrup

AUDIT COMMITTEE

No Report

MEMBERSHIP COMMITTEE

No Report

NOMINATIONS COMMITTEE

A slate of candidates and results of the election were presented. The Executive Committee Officers for the 1999-2000 South Dakota Academy of Science are as follows:

1999-2000 Executive Committee

PRESIDENT	R. Neil Reese, SDSU Biology, 688-4568 Neil_Reese@sdstate.edu
PRESIDENT-ELECT	Lenore Koczon, NSU Chemistry, 626-2633 Koczon@wolf.northern.edu
FIRST VICE-PRESIDENT	Charles Lamb, BHSU Biology, 642-6026 Clamb@mystic.bhsu.edu
SECOND VICE-PRESIDENT	Steve McDowell, SDSM&T, Chemistry, 394-1229 Smcdowel@msmailgw.sdsmt.edu, FAX: 394-1232
SECRETARY-TREASURER	Bill Soeffing, USF Natural Sciences, 331-6759 Bill.Soeffing@thecoo.edu, FAX 331-6615
PROCEEDINGS EDITOR	Kenneth F. Higgins, SDSU Wildlife, 688-4779 Terri_Symens@sdstate.edu, FAX 688-4515
FIRST PAST-PRESIDENT	Royce Engstrom, USD Chemistry, 677-5370 or 6396 Rcengstr@usd.edu
SECOND PAST-PRESIDENT	Sharon Clay, SDSU Plant Science, 688-4757 Sharon_Clay@sdstate.edu

MEMBERS-AT-LARGE (3-year terms)

Second Year	Gary Larson, SDSU Biology, 688-4552 Gary_Larson@sdstate.edu
Second Year	Donna Hazelwood, DSU Natural Sciences,256-5187 Hazelwood@pluto.dsu.edu
First Year	Dan Heglund, SDSM&T, Chemistry, 394-2421 Dheglund@msmailgw.sdsmt.edu, FAX: 394-1232
First Year	Bob Tatina, Dakota Wesleyan, Biology, 995-2712 Rotatina@dwu.edu

TREASURER'S REPORT

Statement of Receipts, Disbursements and
Changes in Cash Balances for Fiscal Year 1998

Cash Balance on 1 January 1998	
Certificate of Deposit	$ 5500.00
Savings Account	$ 9.15
Checking Account	$ 6908.69
TOTAL BEGINNING CASH	$ 12417.84
Receipts	
Membership Dues (Life-33 / Regular-99+2 / Associate-47	$ 2255.00
Warrant Replacement	$ 14.00
Reprint Charges	$ 25.00
Annual Meeting Registration	$ 580.00
Luncheon and Banquet Tickets	$ 1024.00
Other Income	$ 40.50
Interest on Investments	$ 250.94
TOTAL RECEIPTS	$ 4189.44
Disbursements	
Office Supplies	$ 64.69
Annual Meeting	
Dining	$ 1290.59
Supplies	$ 46.72
Facilities Rental	$ 150.00
Overpayment Returns	$ 20.00
TOTAL DISBURSEMENTS	$ 1572.00
Cash Balance on 31 December 1998	
Certificate of Deposit	$ 5750.94
Savings Account	$ 9.15
Checking Account	$ 9275.19
TOTAL ENDING CASH	$ 15035.28

NEW BUSINESS

President Royce Engstrom graciously passed the office of President and the gavel over to President-Elect Neil Reese and wished him the best for his 1999-2000 presidency. President Reese again addressed the Academy of the need for good membership help to make the Tri-State Academy Meeting in 2000 a success. He also gave the SD Academy's WEB address as follows:

http://www.sdstate.edu/wdas/http/sdas/index.htm

At 2:00 p.m., President Reese declared the Annual Business Meeting of the South Dakota Academy adjourned until the Tri-state Meeting on April 28-29 in Moorhead, Minnesota.

These minutes were collectively submitted for Bill Soeffing, by inputs from Donna Hazelwood, Ken Higgins, and Gary Larson.

1998-99 EXECUTIVE COMMITTEE

PRESIDENT	Royce Engstrom, University of South Dakota
PRESIDENT-ELECT	R. Neil Reese, South Dakota State University
FIRST VICE-PRESIDENT	Lenore Koczon, Northern State University
SECOND VICE-PRESIDENT	Charles Lamb, Black Hills State University
SECRETARY-TREASURER	Bill Soeffing, University of Sioux Falls
PROCEEDINGS EDITOR	Kenneth F. Higgins, South Dakota State University
FIRST PAST-PRESIDENT	Sharon Clay, South Dakota State University
SECOND PAST-PRESIDENT	John Thomas, University of South Dakota
MEMBERS-AT-LARGE	Audrey Gabel, Black Hills State University
	Gary Larson, South Dakota State University
	Donna Hazelwood, Dakota State University
	Tim Sorenson, Augustana College

PRESIDENTIAL ADDRESS

Herbalism in the Modern World

Address to the South Dakota Academy of Science
Dakota State University
April 16, 1999

Presented by R. Neil Reese
South Dakota State University

INTRODUCTION

As the 19th century came to an end, modern orthodox medicine displaced traditional folk medicine in the United States. The advent of modern medicine, with its focus on the scientific method in elucidation of the causes of diseases and their treatments, has led to longer life-spans and generally better health in most of the industrialized world. It has also made medicine more remote from the general public. Furthermore, with the ever increasing use of technology, medicine has been perceived to have become the exclusive domain of corporate America.

During the past few decades, alternative medicinal practices including the use of natural herbal remedies, has begun to regain greater acceptance on our society. To many scientists, the desire to return to a "more natural" approach to health maintenance seems to be a repudiation of the progress made in medicine and the scientific methods responsible for the relative good health that we can expect as members of our affluent society. For many people, however, this shift is not so much a denial of the benefits of modern science, but a recognition that the benefits of modern medicine come at an extremely high financial cost. There is also a feeling that the dismissal of natural remedies by orthodox medicine and pharmaceutical companies may be based more on financial considerations than pure science.

As scientists, we might think that the public's doubts about the integrity of corporate medicine and their questioning of the motives of members of the scientific community are unjustified. However, when you examine the use of herbal medicines in the rest of the world (herbal extracts are prescribed throughout Europe and much of the developing world) and the fact that up to 75 % of prescription drugs in the U.S. are based upon natural plant products or directly derived from secondary plant products (Chevallier, 1996), these concerns may appear more reasonable.

My own interest in herbs and the commercial potential of native plants came about through a very circuitous path. Early in my career I was a student of plant systematics and was always fascinated by the ancient herbals, from both the historical and aesthetic perspectives of these great tomes. (Although access to the original work is almost impossible, high quality reproductions of

many of these texts are readily available. - e.g., Cruz, 1940, Dioscorodies, 1959, Gerard, 1931) Upon my arrival at South Dakota State University, I became involved in the ongoing research with *Echinacea* (Purple Coneflower) and have spent several years examining its basic botany, biochemistry and economic potential. This work and my more recent studies into the economic potential and unrealized medicinal value of secondary products of a broad array of native plant species has contributed to my interest in the resurgence of herbal medicine in general. Although there exists a great wealth of knowledge of plants on a world-wide basis, I will limit my discussion here to the history of herbs and herbals in western culture and personal experiences with modern herbalism, with which I am significantly more familiar.

THE PAST

The use of herbal remedies was a fundamental practice in all primitive societies and in Western culture provided the foundations of both botany and medicine. Careful observation and empirical data allowed early medical practitioners to utilize the cornucopia of biologically active secondary plant products found in nature. Although this knowledge was probably passed down orally for millennia, our first glimpse into this collective knowledge is an herbal by Theophrastis, considered the father of Botany, published around 300 B.C. (Chevallier, 1996). This text contained detailed descriptions of plants and their medicinal uses, but lacked illustrations.

During the first century A.D., Pedacius Dioscorides, a Greek army surgeon, produced one of the most important herbals in the history of western medicine. The text, translated into Latin as *De Materia Medica*, detailed more than 600 plant species and provided descriptions of their preparation for various maladies. Dioscorides' text was used virtually unaltered for 16 centuries, but many of the later versions or Codices were prepared with illustrations that did not accurately reflect his text. This practice at times had profound effects on the value of the empirically derived medical treatments. Although a multitude of different herbals were produced during the ensuing centuries, *De Materia Medica* provided the foundation for much of Western medicine during this period.

Galen, in the second century A.D., greatly affected medicine and the use of herbal remedies through the development of the Doctrine of Naturals (Chevallier, 1996). Based on Hippocrates humoral ideas (Good health was viewed as the result of the balance of four bodily humors or fluids: blood, black bile, yellow bile and phlegm.) and Pythagorean theory, all things, including plants, were determined to have natural characteristics (qualities such as dry, moist, hot cold etc.) that allowed their use to be determined in the light of "pure theory". Thus, the value of empirical observation and experience was diminished. This approach and subsequent theoretical considerations (i.e. Doctrine of Signatures where the appearance of a plant or plant part [God's signature] determined its proper use.) greatly reduced the value and efficacy of much of western medicine. This approach to medicine not only led to a diminished understanding of the power of herbal medicines, but contributed to the success of charlatans who preyed upon the desperately ill.

With the advent of modern orthodox medicine and the formation of medical associations such as the AMA in the late 19th and early 20th century, the ability to prescribe herbs became further removed from botanists, naturalists, and traditional practitioners of folk medicine. In an initial attempt to reduce the many abuses that unscrupulous hucksters were inflicting on the general public, practitioners of orthodox medicine moved to restrict the use of herbal medicine to licensed physicians. Later the use of herbs was greatly marginalized as the medical associations and pharmaceutical companies took control of medical practices in North America and, to a lesser extent, in Europe. Production of synthetic and patentable products hid the use of herbs in the laboratory where valuable biochemicals could be extracted, synthesized and packaged through registered pharmacists, under the control of licensed physicians.

THE PRESENT

During the past 30 years there has be a renewed interest in the use of herbal medicines in western cultures and in the United States in particular. In China the barefoot doctor program of the 60's led to increased use of herbs and other folk medicines and to a general recognition of their potential benefits, especially in regions where orthodox medicine was unavailable. Adoption of a similar strategy by the World Health Organization in several developing nations and the recognition of the continued use of herbs in orthodox medicine in Europe further stimulated interest in use of traditional medicines and a holistic approach to health in the U.S., especially among members of the baby boomer generation.

Since the 1960's, a growing segment of the American population rejects orthodox medicine or at least feels that a holistic approach, in conjunction with orthodox medicine, offers an affordable and practical alternative to the corporate medicine practiced in the US. This movement has created a $12 billion herbal market in this country this year, with an estimated growth potential of >10% each year in the foreseeable future.

The initial restrictions on the sale of herbs as medicines during most of this century and the dismissal of their value by orthodox medicine, which continued to prescribe pharmaceuticals comprised of >75% plant products, created considerable skepticism among the populace. This skepticism was documented recently in an NPR / Kaiser Family Foundation / Kennedy School of Government Survey on Americans and Dietary Supplements where 69% of respondents stated that dietary supplements, other than vitamins and minerals, are important to their personal health. More tellingly, when asked " If a government agency said that the dietary supplement you use most often is ineffective, do you think you would stop using it, or you would you continue using it because it works for you?" (Based on those who use supplements regularly/sometimes; n=412) 72% said they would continue using it.

In 1994, this vocal segment of society successfully lobbied the US Congress to remove the restrictions on the sale of herbs by creating the Dietary Supplement Law. This law virtually removed the FDA's ability to oversee the sale and marketing of herbs. Unfortunately, it not only allowed sincere and educated

practitioners of herbal medicine to ply their trade, but opened the door for charlatans to advertise and sell products without any knowledge of their traditional use, efficacy, toxicity, or requirement for quality control. Changes in the Dietary Supplement Law have also induced many large companies to enter into and exploit the herb market by removing the need for any substantive research or evaluation of their products. In fact, almost any product may be sold as a dietary supplement with little or no oversight as long as the manufacturer does not make any overt health claims. This aspect of the law creates quite a paradox in that most of the compounds are sold based upon their supposed medicinal qualities, but are officially advertised as dietary aids that have no medicinal value. Manufactures rely on word of mouth advertising and the numerous Internet and popular press sites that tout their efficacy. If herbal medicines are ever going to be fully utilized by the majority of the US population and fulfill their theoretical potential, efforts to ensure quality control and efficacy are essential.

In an ongoing study in my laboratory, we have examined the variation in quality and content of several commercially available *Echinacea angustifolia* preparations. Using ethanol extractions and HPLC separation of more than 100 *Echinacea* plants collected from throughout the Midwest as a base line, we found that the capsules and tinctures tested contained < 25% (some <5%) of the ethanol extractable components that would be expected if the products contained the advertised amounts of properly dried root materials (the traditionally used portion of *E. angustifolia*). Furthermore, individual capsules within a lot number and capsules from different lot numbers showed significant variation in ethanol-extractable compounds. Although these are only preliminary findings, they suggest that there is little chance of discovering the potential of herbal medicines with the present lack of regulation.

The demonstration of efficacy, another major weakness of the current system of utilization of herbal medicines, is an even more daunting problem. There is a paucity of data as to the proper dosage and proper method of administration for most herbs. These data are essential before we can measure efficacy. The method of administration and proper dosage of an herb must initially be based upon traditional usage. For herbs used in European or Asian traditions these data are readily available. However, many of the currently "hot" herbs are native to the Americas, where much of the knowledge of the traditional use of these plants has not been recorded or made available to the population in general. Although we know of many plants used by Native Americans, relatively little of the specific methods of preparation and use are available. Their knowledge has been ignored or dismissed, due in part to the nature of the interactions between the early European settlers and the Native peoples. In addition, American Indians hold much of this information sacred - not to be discussed except when specifically directed by their deities. (This is an area that may be addressed in the future through efforts by Native American Land Grant Colleges, as they document the large body of knowledge that is part of the Native American oral tradition.)

Even when there is a basis for their use, efficacy of herbal medicines is the most difficult of the issue to address. Even with the current mandate to explore

alternative medicine, NIH-funded studies of herbal preparations are unlikely, except where specific claims of disease cures and clinical trials can be made. Because many herbal preparations are thought to act in an holistic fashion resulting from synergistic effects of their chemical constituents, and act to improve overall heath and prevent diseases, studies of efficacy require large populations studied over years to determine whether use of an herbal preparation significantly decreases the occurrence of disease. These types of studies demand participation by large pharmaceutical companies or other private sources. However, because plant extracts and other plant preparations are not easily patented, pharmaceutical companies will not be interested.

At present, the prospects for regulation of this market and the elucidation of the true potential of many of these herbal medicines is bleak. Although there is an effort by the FDA to create over-the-counter regulation of herbal medicines using the European model, where traditional use and preparation of the products is the basis for sale, the economic potential of the market is likely to stifle any real efforts toward meaningful evaluation of herbs. The potential of a virtually unregulated, multi-billion dollar market has attracted many of the worlds largest pharmaceutical companies (e.g. Bayer, Centrum) and many other new herb marketers. The probability that these companies and the myriad of other entrepreneurs entering the market will press for, or allow, stronger regulation of marketing and demand costly research that might limit sales is negligible.

Concurrent with the entry of many of these large companies, has been an effort to lower the standards for defining products "organic". Most traditional herbalists and practitioners of holistic medicine insist on all natural products in their treatment regimes. Changes in these regulations would allow large-scale production of plants that could make the claim of being organic without meeting the current rigid standards. For large scale production, this would allow more mechanized and less labor-intensive means of producing these high-value products.

THE FUTURE

It takes little foresight to know that for the near future, there will be an increased use of plants in both orthodox and herbal medicine in the USA. Plants and plant products still make up the majority of pharmaceuticals used in this country, and there is an active search of the tropics for new plants to fill the needs of the pharmaceutical industry. The need for new antibiotics and compounds to treat a host of human and animal diseases has stimulated interest in the herbal traditions of Europe and Asia. There is even beginning to be a recognition by the pharmaceutical industry that the indigenous peoples of the Americas have a vast body of knowledge of native plants and their uses. The knowledge of these peoples was for the large part ignored by the European settlers, but now there is a developing appreciation of the possibility that there may be undiscovered valuable plants growing in our own backyards. Additionally, as discussed above, the market for herbal medicines as replacements for or supplement to orthodox medicine will continue to grow, because they

are seen as cost effective and believed by many to be as effective as standard pharmaceuticals over the long run.

There can be little doubt as the to potential benefit of the vast number of still-to-be-discovered secondary plant products and their medicinal potential. This potential will continue to drive the marketing and use of herbal preparations. Perhaps even more important for the herbal drug market is the change in public opinion of the value of herbal remedies and tonics. Natural herb preparations are more commonly seen as inexpensive alternatives to costly pharmaceuticals and the putative preventive-medicinal value of many of the herbal products has great appeal to many as life expectancies increase and the population becomes more health-conscious.

As was true in the past and is currently common place, the herbal market will be driven by a wide range of people and interests. There are a large number of traditional herbalist (e.g. Christopher Hobbs) and members of the established scientific community (e.g. Varro Tyler - former Dean of Pharmacy at Purdue University, and David Duke - USDA) who believe in and promote the use of herbs based on an empirical and scientifically-grounded approach. There are also many well-meaning, but poorly informed practitioners that will prescribe herbal remedies based upon quasi-religious beliefs and/or scientifically unfounded theories (e.g. the Doctrine of Signatures). In addition, the industry will continue to be plagued by charlatans and unscrupulous entrepreneurs whose only motivation is profit.

This mix of players, the lack of support by a large portion of the medical research infrastructure, and almost no real funding for clinical evaluation of herbal medicines makes the probability of great strides in the use of herbal medicines, except as the basis for production of standard pharmaceuticals, seem very limited. In this way, for at least the near future, we seem destined to repeat the past, with much of the true potential and limitations of herbal therapies remaining in the realm of anecdotal data and statements of belief. Although the market will expand, lack of data will prevent meaningful evaluation of the products.

Although an immediate upswing in our understanding of efficacy of herbal products is not likely, the future of herbal medicinal research is not without some glimmer of hope. The public's general interest in their use, and the entry of the big pharmaceutical companies into the market, with the accompanying participation of local pharmacists through whom the products are marketed, has led to the recognition and prescription of these products by many General and Family Practitioners (e.g., Dr. Gott's syndicated column in many of the region's newspapers and the SD Public Radio monthly medical call in program).

The recognition by the general public that there is a need for regulation and study also has the potential to eventually force a closer examination and generate the funding support for the needed research. In the NPR Poll discussed above, a majority of the same people who said they would continue to use a dietary supplement even if a government agency said if it was ineffective, recognized that the there is a need for more governmental regulation to ensure the purity, ensure truthfulness in the industry's claims and to protect

them from harm. If these desires are conveyed to Congress, especially in light of the ever increasing numbers of people using herbal products, there may be a change in the overall picture. In fact, this process has already started with the development of an NIH funded program for alternative medicine. Congress pushed NIH to fund research in alternative medicine when they passed the Dietary Supplement Law, however, little money provided for research has actually gone toward clinical trials or even examination of traditional uses of plants, instead the money was used to fund development of research centers. Perhaps in the future, this will change in response to the concerns of society at large, and at least for the most widely used herbs, a true understanding of their potential can be reached.

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ACKNOWLEDGMENT

Special thanks to Lorna Reese and Dr. Nels Granholm for help with editing this manuscript.

Complete Senior Research Papers

presented at

The 84th Annual Meeting

of the

South Dakota Academy of Science

ISOLATION, CLONING, AND SEQUENCING OF A PORCINE MELANOCORTIN-1 RECEPTOR LIKE GENE

Michael Halverson, Carl Westby, and Nels Granholm
Department of Biology/Microbiology
and
Donald Marshall
Department of Animal Science
South Dakota State University
Brookings, South Dakota 57007

ABSTRACT

Initially we started out to isolate, clone, and sequence the porcine Melanocortin-1 Receptor (MC1R) in swine (*Sus scrofa*). Using human MC1R primers we used PCR to amplify the swine DNA to isolate the MC1R gene. However, we have isolated what may be a melanocortin-1 receptor-like gene in pigs but not the actual MC1R. BLAST searches indicate that our data sequence is statistically similar to the human melanocortin-1 receptor. However other computer analysis indicate that our sequence is roughly 40% similar to various melanocortin-1 receptors of related species. We attempted to isolate 18 base pairs on the 3`end of this gene. Initial analysis of the 3` sequence indicates that we have not isolated the same gene sequence.

Keywords

Melanocortin-1 Receptor, Agouti protein, Pig, Alpha-Melanocyte Stimulating Hormone (a-MSH).

INTRODUCTION

The Melanocortin Receptor-1 (MC1R) in humans and other mammals is coupled to a G-protein complex that is partially responsible for pigmentation in mammals At least two different gene products competitively bind to this receptor. One of the gene products is alpha-Melanocyte Stimulating Hormone (a-MSH), and it causes normal pigmentation pattern of the mammal when it binds to MC1R. The other gene product is the agouti protein. Agouti protein as well as other melanocortins act through G-protein receptors to modify metabolism in a wide variety of cells.(Cone et al. 1996). Agouti protein, a competitive inhibitor of a-MSH, acts on Melanocortin receptors one and four (MC1R and MC4R, Lu et al., 1994.)

There are five distinct melanocortin receptors (MC1R-MC5R) which exhibit wide tissue distribution in mammals. The lethal yellow allele (A^y) causes an

aberrant overproduction of agouti protein in virtually all tissues. A^y may promote the lethal yellow syndrome (i.e. obesity, yellow coat, diabetes, infertility, compromised immunity, and greater body size) by virtue of its competitive inhibition of the melanocortins. The agouti gene is thought to be highly conserved in mammals (Searle, 1968; Silvers, 1979). Both murine (Bultman et al., 1992; Miller et al., 1993) and human (Kwon et al., 1994; Wilson et al., 1995) agouti genes have been isolated, cloned, and sequenced (Wang et al. 1998).

Alpha-Melanocyte Stimulating Hormone on the other hand does not cause the lethal yellow syndrome problems when it binds to the melanocortin-1 receptor. In this situation the mammal exhibits normal pigmentation, body weight, fat/protein body composition, normal metabolism, and fertility. The purpose of this study is to identify and characterize genes that encode one or more members of the melanocortin receptor family. We have cloned and identified a sequence which may be a melanocortin-1 receptor like gene.

METHODS

Because of their fundamental role in metabolism, we want to characterize the melanocortin-1 receptor (MC1R) in pigs. Since agouti protein and a-MSH ligands competitively bind to MC1R, we were interested in isolating, cloning, and sequencing the MC1R. However, about six months into our research we discovered that Lief Andersson's group in Sweden had isolated, cloned, and sequenced the porcine MC1R from swine. At the same time though through Polymerase Chain Reaction (PCR) we were consistently amplifying an 800 base pair fragment that we believed to be the porcine MC1R. We cloned this 800 base pair DNA into competent *E. coli* cells via the pCRII vector. The transformant E. coli cells were grown in LB-Kanamycin medium and the plasmid DNA was isolated from the cells using the *Power prep minikit* which is used to isolate plasmid DNA from chromosomal DNA and other cellular debris. A restriction enzyme digest was performed using (EcoR1) to determine that the 800 base pair insert was actually in the vector. Analysis of the restriction enzyme digestion by agarose gel electrophoresis indicated that the insert was in fact present. Once we verified presence of the insert DNA in the cells, we then isolated the plasmid DNA that contained the insert DNA and sent it to Iowa State Sequencing Facility. In order to sequence the DNA, they employed a technique called Primer Walking to complete the sequencing. Our next step was to analyze the data from the sequencing facility (Fig. 1). In order to determine what gene or genes we had isolated we employed three computer programs 1) Gene Jockey, 2) Gene Inspector, and 3) BLAST (Basic Local Alignment Tool).

RESULTS

Gene Jockey Analysis

Gene Jockey is used to align two different sequences and determine the percent similarity between the two. Since the PCR primers that we designed were from the human melanocortin-1 receptor it seemed logical to align our

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          10          20          30          40          50
           |           |           |           |           |
ATGAGTCCCT GCCCTGAGAC TGTGGTGTG TGAGGGTCCT GGCATGAGTC
          60          70          80          90         100
           |           |           |           |           |
CTGCTTGGCA AGAGACTTGG AGACGGGGGC GGGGAGCAAG CCCCCCTGGG
         110         120         130         140         150
           |           |           |           |           |
CTAGAACTGT ACGTCACCAC TGATAGCCAT GACTTCAGCC CACCCCACCC
         160         170         180         190         200
           |           |           |           |           |
ACCCCCCACA TGCTGTGGGA TGGAGGTCCC TGTCACCAGG CTGCCCCCAC
         210         220         230         240         250
           |           |           |           |           |
CTGGCTGCTG GAGCCCCTTG AGGAACAGAC CCTTGTCTCA GGCTCAGCAT
         260         270         280         290         300
           |           |           |           |           |
CAGTCTCGGC TGGCCGGTTG GACACTCAGC CACATGGCCC TGGGAGCCGG
         310         320         330
           |           |           |
AGCTGATGAC GCGCGGTGGC CAGAGAGAGA TGCTGA
```

Figure 1: Nucleotide Sequence of Porcine MC1R-like Candidate Gene

data with the human MC1R. In fact we aligned our data with human, bovine, goat, dog, fox, and horse melanocortin-1 receptor sequences. This analysis demonstrated that our sequence was roughly 40% similar with each of the different melanocortin-1 receptors.

Gene Inspector Analysis

Gene Inspector is an alternate program that can be used to align different sequences, determine percent GC content, and derive the amino acid translation from the nucleotides (Fig. 2). This program also yielded results showing our sequence is about 40% similar to the other melanocortin receptors. An interesting aspect of this program was, however, that it was able to determine the possible open reading frame (ORF) in which the isolated gene is read. Our sequence is read in ORF 2.

BLAST Search Analysis

Basic Local Alignment Search Tool is used to compare our sequence data to every entry submitted to and contained in GenBank. This search tool gave encouraging results. It displayed a statistically significant match with the Human Melanocortin-1 Receptor DNA and mRNA. However, these results also conflict with Gene Jockey and Gene Inspector. Both of those programs indi-

```
          10         20         30         40         50
          |          |          |          |          |
MSPCPETVVC EGPGMSPAWQ ETWRRGRGAS PPGLELYVTT DSHDFSPPHP
          60         70         80         90         100
          |          |          |          |          |
PPTCCGMEVP VTRLPPPGCW SPLRNRPLSQ AQHQSRLAGW TLSHMALGAG
          110
          |
ADDAVARERC
```

Figure 2. Deduced Amino Acid Sequence of Porcine MC1R-like Candidate Gene

cated that our nucleotide and amino acid sequence did not match up with the other Melanocortin-1 Receptors any better than 40% similarity. The BLAST search gave a 4e-05 "hit", with any hit smaller than 0.1 being considered significant (Cheesbrough, 1999).

While we believe we have isolated an MC1R-like candidate gene we are still are not sure regarding our ability to define it as an MC1R-like sequence. Therefore we have isolated and sequenced a small segment of the MC1R gene that our initial PCR primers did not cover. This small piece was 18 base pairs at the 3` end of the gene. When we designed these primers, we overlapped our original sequence with roughly 450 base pairs. The purpose of this was two-fold. First, it is not possible to amplify an eighteen base pair segment through PCR because of its size. Second, this overlap would tell us if we had isolated the original gene. This DNA was amplified
through PCR, the DNA was cloned via the pCRII vector into *E. coli* and sequenced at Iowa State as described above.

CONCLUSION

Upon preliminary examination of the most recent results it does not appear that the original and the latter genes are identical. We believe we may have a melanocortin like gene because repeated BLAST searches have indicated the high statistical similarity between the human MC1R and both of our data sets. The computer software used for gene analysis has given some conflicting results that we are still trying to sort through. The BLAST search indicates that both sets of our data are similar to the human MC1R which is what one would expect since our PCR primers were designed from the human MC1R. However, when we line up the sequences with Gene Jockey or Gene Inspector, the percent similarities are only about 40%. Therefore we are still in the midst of determining what our data actually means.

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DESIGN AND CONSTRUCTION OF A PORTABLE WIND TUNNEL FOR SOIL EROSION RESEARCH

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ABSTRACT

A portable wind tunnel has been designed and constructed by senior geological engineering students enrolled in the capstone course 'Geological Engineering Design II' at South Dakota School of Mines and Technology, Rapid City, SD. The project involved research, design, analysis and implementation phases under the constraints of a budget. The experience offered students a first-hand view of an actual design process where critical design decisions were required at various stages of the project. The result was a research quality field wind tunnel that will be used to research wind erosion of soil and sediment from numerous landuse categories across western South Dakota.

The tunnel has an overall length of 8.5 m with a working section 4.5 m long, 0.5 m high and 0.5 m wide. Power was supplied using a 6.5 kW generator driving a 0.62 m axial fan. An in-line power inverter allowed a variable current to be supplied to the fan thereby controlling wind speed. A diffuser was used at the downstream edge of the working section to recover pressure losses. Aerodynamic stability was achieved using a 2:1 expansion of the flow immediately downstream of the fan and passing it through a perforated plate and a plastic honeycomb at low velocity. A subsequent 4:1 constriction increased velocity to the working section where it passed over the natural surface. The tunnel was constructed of composite aluminum-honeycomb fiberglass panels that were cut to size and fiberglassed together yielding a total weight of less than 50 kg. The tunnel consisted of 6 sections that were each 1.5 m long which were connected in the field using an external frame and latch system. The tunnel was transported and moved in the field using a 1.5 x 3.0m trailer and a 1.0m crane.

INTRODUCTION

Wind erosion of soil is a world-wide concern and has been addressed by numerous studies, e.g., Stetler & Saxton (1996, 1995), Stetler et al. (1994), Nickling & Gillies (1993) and Schütz (1980). These studies have focused primarily on degradation of arable lands and reduction in air quality. On the Great Plains, severe drought conditions during the 1930s led to soil erosion on large tracts of arable lands affecting food production and quality of life nationwide. These climatic conditions also stimulated the development of wind erosion research and control practices. In recent years, potential health-related concerns

associated with wind erosion of soil, specifically emissions of particulate matter (fine dust), has become a driving force behind soil erosion studies (Schwartz et al., 1996). The 1990 Clean Air Act (US-Environmental Protection Agency (EPA), 1990) established National Ambient Air Quality Standards (NAAQS) based on acceptable levels of respirable dust defined as particulate matter less than a specified aerodynamic diameter (referred to as PM_x, where the subscript denotes particle size in micrometers). Revisions to NAAQS in 1997 (Federal Register, 1997) specified both primary and secondary standards for $PM_{2.5}$ and PM_{10} that are based on annual arithmetic mean and average daily concentrations, respectively. Primary and secondary annual standards for $PM_{2.5}$ and PM_{10} are mean annual arithmetic concentrations ≤ 15 μgm^{-3} and ≤ 50 μgm^{-3}, respectively. Primary and secondary 24-hour daily standards are based on a 98th percentile 24-hour concentration ≤ 65 μgm^{-3} and a 99th percentile 24-hour concentration ≤ 150 μgm^{-3}, respectively.

The potential for wind erosion of soil is highest in arid and semiarid climates. Western South Dakota has a climate characterized by average annual precipitation of 40 to 60 cm, annual average evaporation of 75 to 90 cm (Linsley & Franzini, 1979) and average annual temperatures of ~16 °C. Using the scheme of Wilson (1968), this climate can be classified as semiarid. Dominant surface processes in such a climate include running water, mass movement (landslides, etc.) and wind action that produce pediment, fan and deflation surfaces, debris slopes and badlands. Fortunately, South Dakota's climate generally provides sufficient precipitation to form stable vegetated land surfaces that are unaffected by wind processes. However, once a stable surface has been disturbed, wind erosion processes can potentially mobilize substantial amounts of sediment until such time as a cover becomes reestablished. Agricultural and rangeland management practices as well as various types of construction and commercial activities can exacerbate the wind erosion process by mechanically altering the surface and stripping vegetation. As such, predicting where and when any surface may be affected by wind action is problematic. In addition, airborne particulates are transported in the upper atmosphere by turbulent diffusion and can remain aloft almost indefinitely. Recent modeling of windblown dust originating from agricultural fields (Lee et al., 1998) has shown that an accurate dust emissions rate is a critical component of the model and is often dependent upon soil properties, surface conditions, vegetation and soil moisture (Hagen, 1999; Saxton et al., 1998).

Portable wind tunnels are recognized as valuable research tools for collecting in situ soil erosion and dust emissions data from many soil types, surface and moisture conditions and vegetation levels in an efficient and timely manor. In addition, field wind tunnels provide a repeatable and controlled environment where years of equivalent naturally derived field data can be experimentally generated in the course of a few months. Pietersma et al. (1996) describe numerous wind tunnel studies that have solidified the importance and economics of their use in soil erosion research. However, Fryrear et al. (1988) identified unique problems when using portable wind tunnels in the field including: 1) the containment of particles being transported in saltation completely within the height of the boundary layer generated in the tunnel, 2) the

difficulty in duplicating a natural boundary layer over various test surfaces and 3) the difficulty in translating soil losses from the wind tunnel to equivalent soil losses in the field. Solutions to problems 1 and 2 are derived through sound aerodynamic design and construction methods for the wind tunnel test section and by utilizing various flow manipulation devices (Pietersma et al., 1996). Problem 3 has received much study (Horning et al., 1998; Fryrear, 1984; Lyles & Allison, 1981) that has produced solutions for soil loss by analytical means. In addition, these studies have shown that soil loss equations are stable as long as field and wind tunnel data are collected consistently.

PORTABLE WIND TUNNEL DESIGN AND AERODYNAMICS

Design and construction of a portable wind tunnel was achieved by senior Geological Engineering students as part of the course Geological Engineering Design II at the South Dakota School of Mines and Technology, Rapid City, SD. Research assignments were delegated to two teams, each of whom were required to submit a competing design. Team 1 was required to research and design a blowing tunnel (Fig. 1) and Team 2, a suction wind tunnel (Fig. 2). The primary difference between these two designs is in the placement of the fan. A blowing tunnel has a fan placed prior to the working section and blows air across the test surface. A suction tunnel has a fan at the end of the working section and pulls air through the front end of the tunnel and over the test sur-

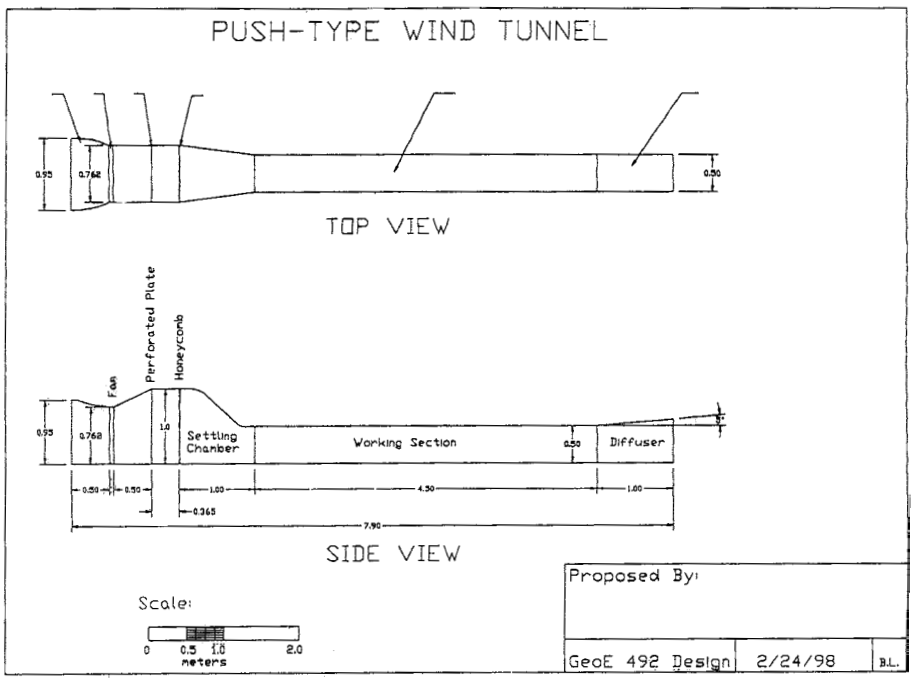

Figure 1. Design drawing of the blowing, or push type, wind tunnel.

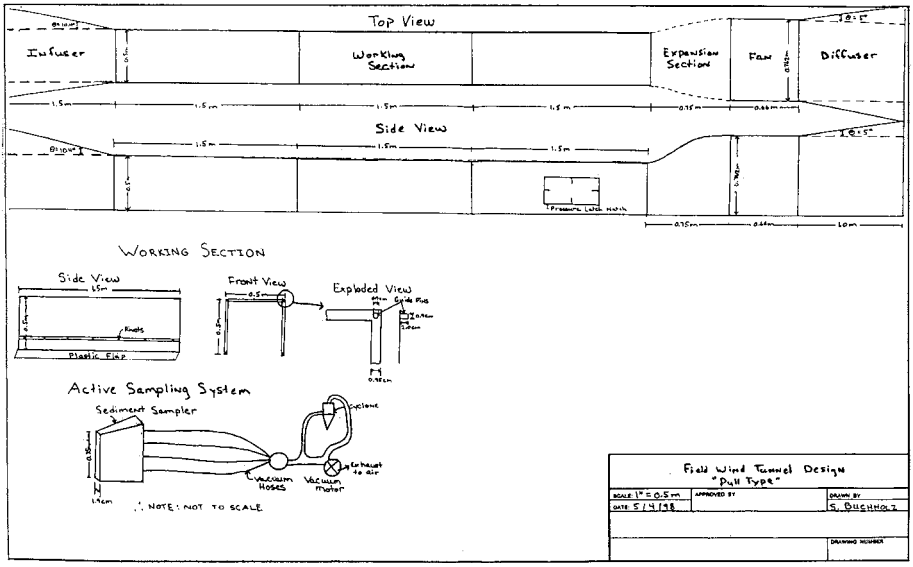

Figure 2. Design drawing of the suction, or pull-type, wind tunnel. Also shown are working section and sampler schematics.

face. As a result of fan placement, there are many advantages and disadvantages of each type, a few of which will be briefly discussed.

For example, since the working section of a suction tunnel is located upwind of the fan, air moving across it is unaltered by the fan. Therefore, uniform aerodynamic flow characteristics are achieved without the need for flow conditioning equipment and the overall tunnel length is reduced. By contrast, the fan of a blowing tunnel is located above the working section and air flowing across the test surface has been chopped by the fan which introduces severe swirl to the flow. Left in this condition, the flow across the test surface would not be representative of a natural wind and erroneous data would be collected. The problem is rectified using flow conditioning prior to the working section but requires additional equipment and tunnel length. This scenario favors a suction tunnel design. For an additional example, testing of the surface (erosion rates and velocity data) is performed after the air has moved across the test surface a sufficient length to develop a uniform velocity profile. In a suction tunnel, the measurement point is located prior to the fan which is a difficult place to access to remove samples, place equipment, etc. In a blowing tunnel, the measurement point is located just inside the tunnel exit which has easy access. This scenario favors a blowing tunnel design. Many additional considerations exist where each design factor will favor one design over the other. In short, any decision that is made must be based on both tangible (design, economics and requirements) as well as intangible factors (knowledge of operation, personal preference and expected future applications). For additional details, the interested reader is referred to an excellent book on wind tunnels and design by Rae and Pope (1984).

A major concern each design Team faced was the constraints of a budget. A limited resource base was available which narrowed the design focus toward production of the best quality research instrument allowed. As work progressed during each phase (research, design, evaluation and construction), realities imposed by the budget forced decisions to be made, often requiring 'on-the-fly' adjustment to the design. In this way, each student was exposed to not only the challenge of design but the realities of implementation of that design.

Design Criteria

Initial research by each design Team was directed toward making a detailed analysis of the literature and establishing a guiding design criteria. This phase produced numerous concerns that must be addressed in order for a research quality portable wind tunnel to be constructed, all of which can be summarized by the following list of 7 factors from Zingg (1951):

- Production of an air stream free of swirl possessing known and steady flow characteristics
- Generation of variable wind speeds that are comparable to a natural wind
- Durability
- Safety
- Sufficient size to ensure representative sampling of eroding material over field surfaces
- Possesses true portability from plot to plot within a field
- Easy assemblage and dismantling

Of these, flow characteristics, projected design performance, size and weight factors were deemed the most important criteria to be considered within budget constraints.

Aerodynamic criteria

Flow characteristics developed in a wind tunnel dictate how representative transport of eroded soil particles agree with natural processes. Therefore, a basic understanding of the fundamentals of particle transport is necessary. Particles move as a function of the interaction between stationary grains and the passing air flow. These interactions lead to three modes of particle transport, *creep*, *saltation* and *suspension* (Bagnold, 1941; Greely and Iverson, 1985). For a dry, unconsolidated surface, initial particle motion is achieved when wind forces become instantly greater than adhesive forces that attach the particle to the surface. A small number of grains are ejected into the airstream at a velocity and angle which are dependent on particle size and wind speed (Ciccone, 1988; Reeks et al., 1988). Once airborne, these particles follow long, high energy (ballistic) trajectories back to the surface. Upon impact of the saltating particle, additional particles are ejected from the surface through a process described as a 'splash function' (Unger and Haff, 1987). Each splashed particle generally follows a short, low energy trajectory, termed 'reptation. This process continues to cascade until the carrying capacity of the wind is achieved (Anderson, 1987). Both the long and short trajectory particles are moving in *salta-*

tion which accounts for approximately 75% of all eolian (wind blown) transport (Bagnold, 1941). Saltation is most pronounced for particles between 0.1 and 1.0 mm in size. For particles >1.0 mm in size, the wind force is not sufficient to pick them up and instead they are pushed and rolled along the surface by both the wind and impacting grains. These particles are moving by *creep* which accounts for <25 % of all transport. Particles whose diameter is small enough (<0.1 mm) to respond to the turbulent fluctuations in the airstream are moving in *suspension*. All three transport modes operate simultaneously given the particle size distribution of the sediment is inclusive of grains in each mode. Most soils are of this type whereas dune sands may be missing the finest particles.

Successful modeling of these transport processes depends upon the wind-generated air flow correctly representing the natural wind. This can be achieved by placing design focus on the following aerodynamic criteria as outlined by White and Mounla (1991), Raupach and Leys (1990) and Owen and Gillette (1985):

- The generated logarithmic velocity profile close to the surface should be characteristic of the natural atmosphere. This ensures realistic aerodynamic forces act on saltating grains and thus, the entire transport process.
- The stream-normal turbulence intensity in the lower boundary layer must be realistic to ensure vertical turbulent dispersion of suspended material is properly modeled.
- The flow must be spatially uniform.
- Turbulence structure, e.g., large-scale eddies, characteristic of atmospheric flow (wind gusts) should be preserved in the tunnel as an initiator of particle motion.
- Shear velocity profiles must meet aerodynamic constraints for velocity and saltation profiles to reach equilibrium conditions and assure the flow is unaffected by boundary conditions.
- For natural saltation processes to be reproduced in the tunnel, capability to introduce particles at the leading edge of the working section should be incorporated in the design.

The first five of these criteria cannot be assessed until the end of the construction phase. However, they are purely aerodynamic in nature and thus, are highly dependent upon tunnel design. Raupach and Leys (1990) state that these aerodynamic conditions are all satisfied if a well-developed equilibrium boundary layer exists near the ground over a uniformly eroding area and is sufficiently deep to contain all particle motion in the logarithmic layer. Therefore, the design Teams focused on theoretical design factors and subsequent analytical evaluations to ensure the development of a uniform flow. Most of these 5 criterion are met by construction of a uniform working section that has no regions of flow separation and having sufficient length for fully turbulent flow to develop.

The last criterion is physical and effects aspects of particle transport on the field surface being tested. As such, it was not critically analyzed during the design phase. A literature search of past research involving field wind tunnels showed that only a few tunnels (Pietersma et al., 1996; Raupach & Leys, 1990)

included particle feed mechanisms in their experiments. We concluded that unless identical comparisons were to be made with actual transport processes, particle feed would not be a necessity.

Physical wind tunnel design

Based on the above aerodynamic and design criteria, each Team submitted a full design and engineering analysis for a portable wind tunnel (Fig. 1 & 2) complete with engineering plates of joints, hinges, fan selection data and cost estimates. Evaluation of the engineering and a comprehensive design analysis were conducted by the author and the degree that each design meet with the specified criteria were noted. Discussions with the design Teams considering construction materials, functionality, aerodynamics, portability in the field and intended use led to the selection of the blowing tunnel for the constructional phase.

A major concern in the design was the field weight, specifically, the tunnel should be able to be handled easily by one person. As such, we selected 1.25 cm thick aluminum-honeycomb fiberglass composite panels as the construction materials. This material fulfilled the weight requirements plus had the additional feature of being water-proof. Total finished weight of the tunnel was <50 kg (less the fan and generator) and was built in six sections each weighing <10 kg. During construction of each section, the individual pieces were joined together using small angle brackets and the entire joint was fiberglassed.

Major tunnel components were, in order from the upwind side: infuser, fan, diffuser and flow conditioning, contraction, working section and diffuser. The working section was 0.5 m wide, 0.5 m high and 4.5 m long. Power was provided by a 6.5 kW Honda gas generator connected to a 0.62 m diameter axivane fan driven using a 5.0 Hp industrial duty electric motor. Precise velocity control was possible using a power inverter between the generator and the electric motor that allowed discrete increases of current to the motor. Using this power system, velocities in the working section could be varied from ~0.5 to >22 ms^{-1}.

All tunnel components were stored on a 1.5 x 3.0 m trailer equipped with a small 1.0 m crane. In field use, the generator remained on the trailer and the crane was used to lift and rotate the fan section into place on the field surface. The working section was separated into three sub-sections each 1.5 m long and were carried from the trailer and connected in place on the field. Sections were connected using an external frame and latch system designed specifically for this tunnel.

The tunnel has a common base which extends the entire length from infuser to diffuser. The upper surface includes a 2:1 expansion from the fan to the flow conditioning section and a 4:1 contraction back down to the working section. The diffuser has a 1.5:1 expansion at the tunnel exit. All flow paths were designed to be smooth and gradual over the necessary distance required to avoid flow separation. The first 0.25 m of the working section was equipped with a floor prior to the flow encountering the soil surface. Length of the tunnel from the upwind edge of the infuser to the downwind edge of the diffus-

er was 10.0 m.

Moving the tunnel in the field consisted of unlatching the working sub-sections from the fan, lifting it with the crane and driving forward to the next test plot. Assembly of the entire tunnel can be achieved by a single person in <30 minutes and re-assembly after a move in the field can be made in ~15 minutes.

Aerodynamic design

Aerodynamic design was a multi-step process which continued throughout the entire design phase. The initial step of the process involved calculating a wind tunnel system pressure curve for design dimensions and components utilizing the expected pressure losses in the wind tunnel as a function of airflow volume as defined using ASHRAE air duct design methodology (ASHRAE, 1981). This method consisted of calculating the flow resistance caused by the wind tunnel components (flow manipulators) and was utilized to place such components within the flow.

The second part involved designing a flow that would generate and sustain a uniform mean velocity profile with low turbulence levels (Raupach and Leys, 1990; Zingg, 1951). This was achieved by 1) providing smooth transitions to avoid flow separation and 2) passing the air through strategically positioned flow manipulators. Specific equipment used was, from the upwind direction, an infuser, guiding vanes on the fan, a perforated plate and a honeycomb. Spacing between all flow manipulators was calculated to allow for turbulence decay prior to passing through the next device.

At the upwind edge of the tunnel, smooth transition of incoming air was achieved using an infuser having a 1.5:1 contraction over a 1.5 m length. Flow diffusion occurred continuously for 0.5 m downstream of the fan blades prior to encountering the perforated plate that was located at the upwind edge of the flow manipulator section. The plate served two functions: 1) it effectively reduced the mean size of turbulent eddies and 2) it reduced turbulent fluctuations in the stream-wise velocity component (Mehta, 1977; Wigeland et al., 1977).

At the downstream end of the flow manipulation section, the flow was passed through a honeycomb which consisted of 6.35 mm diameter and 50.0 mm long plastic straws that were packed and glued together. Spacing between the plate and the honeycomb was 0.75 m which provided space for the flow to stabilize prior to entering the honeycomb. Honeycomb has been shown to be an excellent device to remove lateral mean velocity variations and reduce the scale of turbulence to a uniformly low level (Loehrke and Nagib, 1976; Scheiman and Brooks, 1981). Thus, as the airflow exited this section, it was essentially turbulence free.

The airflow then passed through a 4:1 contraction reducing to the 0.25 m^2 flow area of the working section. Thus, a high velocity laminar flow was efficiently delivered to the start of the working section where turbulence was rebuilt into the flow using a trip and a roughened floor. A canvas flap attached to the bottom edge of the working section was sealed with soil to prevent air escaping under the tunnel bottom.

VELOCITY AND DATA MEASUREMENT

Significant classroom time was spent on the characteristics of wind and turbulent boundary layers generated inside wind tunnels. Using literature and experience, we chose to measure velocity using four Pitot pressure tubes that were placed 0.5 m inside the downstream edge of the working section completely within the boundary layer. Each pressure tube was sensed by a dedicated pressure transmitter that was read, in turn, by a digital flow meter. A switch box was used to step through each tube in sequence. The flow meter was connected to a laptop PC and the data were acquired to the hard drive.

Several designs were submitted for construction of a particle sampler. However, time was a factor and this was not able to be completed as part of the overall project. Work has been initiated to construct a small, passive sediment sampling system. Concentration of fine dust particles are measured and collected using two TSI DustTrak aerosol monitors. These instruments are laser-based and measure the flight time of aerosol particles through a light beam and calculate concentration on a continual basis. Quartz fiber filters collect the particles that can subsequently be analyzed.

CONCLUSIONS

A portable field wind tunnel has been designed and constructed by senior Geological Engineering students at the South Dakota School of Mines & Technology, Rapid City, SD. The project offered these students first-hand experience in initiating a project, conducting research, performing engineering analysis and design and ultimately, seeing the design come to life through its construction.

The tunnel has an over all length of 10.0 m with a 4.5 m long working section. The implementation of a unique current control system enables flow velocities ranging from <0.5 to >22 ms^{-1} to be achieved within the working section. Simultaneous collection of eroded soil particles as well as emitted dust provide the means to characterize and compare erosion losses and dust emissions from wide varieties of soil and surface conditions on the Northern Great Plains.

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QUANTITATIVE ANALYSIS OF A SOLID SOLUTION USING FOURIER TRANSFORM INFRARED SPECTROMETRY: AN INSTRUMENTAL ANALYSIS EXPERIMENT

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ABSTRACT

When Fourier Transform Infrared (FTIR) instrumentation is used, along with careful selection of the analyte and the concentration range, quantitative infrared spectrometry is feasible (George et al. 1987, Skoog et al. 1998). For this experiment the feasibility of using FTIR for quantitative analysis of solid solutions (sample dispersed in potassium bromide and pressed into a pellet) was explored using a Thorn Smith Analyzed Unknown of potassium hydrogen phthalate (KHP). When sample pellets of similar transparency were used, the area of the peak at 760 cm^{-1} varied linearly with concentration of KHP (obeyed the Beer-Lambert Law). The experimental procedure proved to be somewhat time intensive, but results were reproducible, accurate (3% error), and precise (10% RSD). The experiment gives students experience/practice with KBr sample preparation methods, FTIR operation, peak integration methods, and linear regression analysis of a calibration curve. The experiment is amenable to either the standard calibration curve method or the method of standard addition.

Keywords

Quantitative FTIR, KBr pellet, Beer's Law, Instrumental Analysis

INTRODUCTION

The Beer-Lambert Law, A=abc, predicts that when a compound absorbs monochromatic (single-wavelength) radiation, the absorbance (A) is directly proportional to its concentration (c) and to the path length of the measuring cell (b); "a" is the absorptivity, also called the extinction coefficient or optical density, for the compound at that wavelength (e.g., Harris 1999). Applications of this law are used routinely for quantitative analysis, especially with ultraviolet and visible radiation (UV-Vis).

Theoretically the Beer-Lambert Law should also apply for infrared radiation (IR), but several practical considerations limit its use. IR spectra are more complex and in many cases more intense than UV-Vis spectra, so selection of a wavelength and appropriate concentration range are more difficult. IR source intensities are significantly lower than UV-Vis sources and IR detectors are typ-

ically less sensitive as well. This requires wider slits which means that the monochromatic wavelength stipulation is compromised. In addition, IR absorption bands are relatively narrow, so a slight shift in the wavelength selector position can have a dramatic effect on the average absorptivity (a) for the sample. Modern Fourier Transform IR (FTIR) instrumentation minimizes these problems and makes quantitative IR analysis more feasible (George et al. 1987, Skoog et al. 1998). In general, the sample used for quantitative IR analysis should have a fairly simple spectrum which contains at least one resolved peak (distinctly separated from any others) that is present in all dilutions of standards and samples in the concentration range where the Beer-Lambert Law is obeyed (George et al. 1987).

The purpose of the investigation reported here was to determine the feasibility of using FTIR spectroscopy for the quantitative analysis of a solid sample using potassium bromide as the "solvent" (KBr pellets). Solid samples present additional complications for quantitative analysis. The path length (pellet thickness) must be measured or carefully controlled to keep it constant. Light scattering from the solid and other anomalies within the solid matrix can also vary from pellet to pellet (George et al. 1987, Skoog et al. 1998). It was hoped, and subsequently proven, that standardization of the pellet making process would minimize these factors.

METHODS

All spectra were collected with a Nicolet Avatar 360 FTIR (Nicolet Instrument Corp., Madison, WI) employing 32 scans with four cm^{-1} resolution (default parameters). The sample compartment was continuously flushed with nitrogen (A-OX Welding, Sioux Falls, SD) at a flow rate of 450 mL/min. The area of the peak at 760 cm^{-1} was determined using the manual integration feature of the Nicolet EZ-Omnic software. Software defaults were used for the baseline points for integration of each peak unless a default point excluded a peak shoulder, in which case the point was manually adjusted.

For this study potassium hydrogen phthalate (KHP) was selected as the sample because a Thorn Smith Analyzed Unknown (Thorn Smith Laboratories, Beulah, MI) was available, and it proved to have a suitable IR spectrum. ACS reagent grade KHP (99.95%-100.05%, Aldrich Chemical Company, Inc., Milwaukee, WI) and Thorn Smith Analyzed KHP Unknown 277 were dried at 100°C for one hour prior to use. FTIR spectroscopic grade potassium bromide (KBr) (International Crystal Laboratories, Garfield, NJ) was dried at 120°C for 24 hours prior to use. All reagents and prepared samples were stored in a desiccator over Drierite (Hammond Drierite Company, Xenia, OH). Samples were massed on an Ohaus AP210 Analytical Plus electronic balance (Ohaus Corp., Florham Park, NJ).

Pellet preparation: Initially 0.0024 g of KHP was thoroughly mixed with 0.7784 g of KBr in a Wig-L-Bug (Crescent Dental Mfg. Co., Chicago, IL). This mixture was "diluted" to form the calibration standards by thoroughly mixing masses ranging from 0.01 g to 0.06 g with 0.21 g of KBr in the Wig-L-Bug (masses were recorded to four decimal places). Similarly, 0.0037 g of Un-

known 277 was thoroughly mixed with 0.6971 g KBr in the Wig-L-Bug. Aliquots of 0.01 to 0.06 g (recorded to four decimal places) of this mixture were each thoroughly mixed with 0.21 g of KBr in the Wig-L-Bug to form unknown pellets for analysis.

Then 0.17 g of each of these final mixtures was carefully transferred into the KBr pellet die and pressed into a pellet by applying 10,000 lb to the die for 10-15 seconds using a Carver Lab Press (Fred S. Carver, Inc., Summit, NJ). The mass of mixture used was kept constant (0.17 g) to keep the pellet thickness (path length) constant. Only pellets with a linear baseline absorbance and which were translucent and relatively homogeneous across their diameter were used in this study.

RESULTS AND DISCUSSION

This study was conducted as a part of the lab for Augustana's Advanced Analytical Chemistry course which has two four-hour lab periods each week. Since the pellet making process was somewhat tedious, it was necessary to store some of the pellets so their spectra could be obtained in a later period. A primary concern then was the stability of the sample within the pellet. It was discovered that the O-H stretch peak at 3500 cm^{-1} degraded significantly with time, even after one or two days, but the rest of the spectrum was quite stable and reproducible. Figure 1 shows this quite clearly; 1A shows the spec-

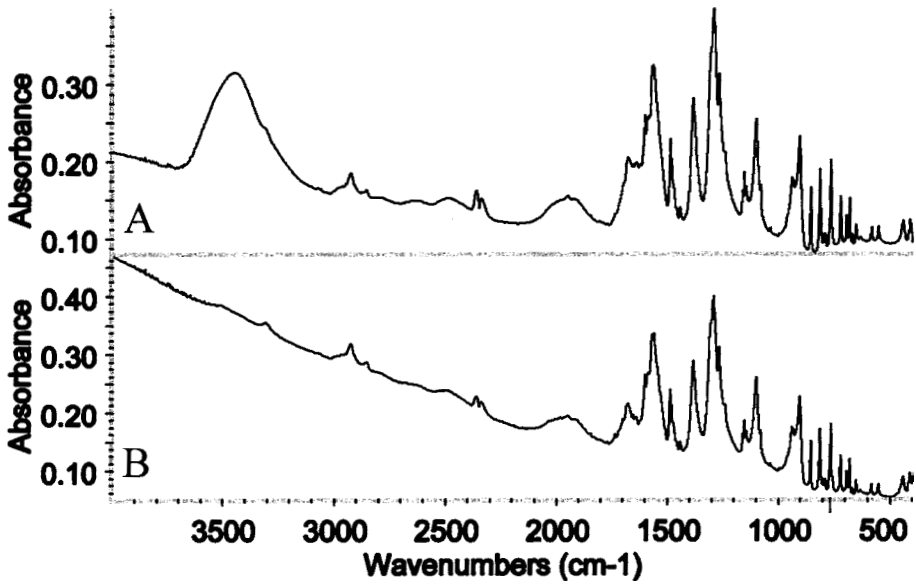

Figure 1. Sample FTIR spectrum of a KHP standard pellet (0.0554 mg KHP/g pellet). A) Spectrum obtained on the day the pellet was made. B) Spectrum obtained six months later, pellet stored in a desiccator. Marker on the x-axis indicates the location of the peak (760 cm^{-1}) used for quantitative analysis.

trum of a freshly prepared pellet of KHP standard, and 1B shows the spectrum of the same pellet after six months. It was speculated that the disappearance of the 3500 cm^{-1} peak was due to exchange of the acidic hydrogen of KHP and potassium from the KBr with subsequent outgassing of the HBr. The phenomenon was not investigated further. It should be noted that the peak (doublet) centered near 2350 cm^{-1} was due to CO_2 in the sample chamber. More extensive flushing with nitrogen eliminated this peak. However, it was found that the presence of CO_2 had no adverse effect on the quantitative analysis of KHP, so no further attempt was made to completely remove CO_2 from the sample chamber.

Figure 2 shows an expanded view of the 600 cm^{-1} to 900 cm^{-1} region of the spectra from Figure 1. This region is quite stable and reproducible over time (2A is with the fresh pellet and 2B is the same pellet six months later). After analyzing a large number of pellets, it was discovered that few of the pellets produced spectra with a flat baseline. Further analysis showed that spectra with a tilted, but linear, baseline (as in Figure 1B) were usually suitable for quantitative analysis, but spectra with a curved or zigzagged baseline were not. In addition it was noted that spectra with a nonlinear baseline (curved or zigzag) were obtained from pellets that appeared opaque or had a non-uniform appearance across their diameter. It was reasoned that the higher baseline absorbance was caused by increased light scattering for that pellet, one of

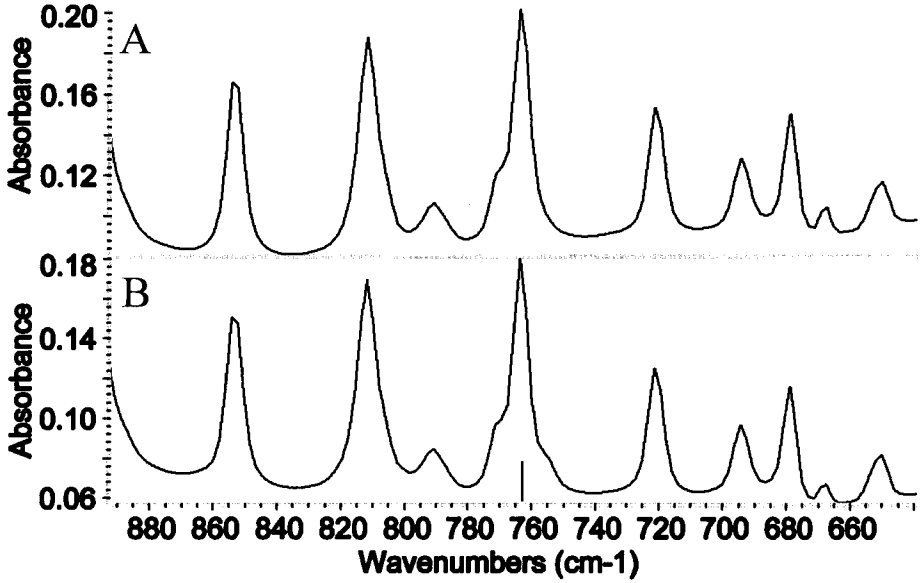

Figure 2. Spectrum of Figure 1 (KHP standard pellet) expanded around the region of interest. A) Spectrum obtained on the day the pellet was made. B) Spectrum obtained six months later, pellet stored in a desiccator. Marker on the x-axis indicates the location of the peak (760 cm^{-1}) used for quantitative analysis.

the problems associated with quantitative analysis with solid samples (George et al. 1987, Skoog et al. 1998). Only pellets which were homogeneously translucent across the majority of their diameter and which produced spectra with a linear baseline absorbance were used for the quantitative analysis. A few pellets rejected by these criteria were actually suitable for quantitative analysis (false negatives), but all of the pellets selected by these criteria proved suitable (no false positives). The visual criterium provided a simple means of preselecting pellets that were likely "to work" early in the process. The Nicolet Omnic software (full version only, not EZ) provided a baseline correction feature that may have been able to "salvage" some of the rejected pellets, however, due to the time constraints in the lab, the students did not attempt to learn how to use this feature.

Four standard KHP pellets and three pellets with Unknown 227 proved to be suitable (translucent with low baseline absorbance) for this study. The spectra of the KHP standards (Figure 2) exhibited several well-resolved peaks in the 600 cm^{-1} to 900 cm^{-1} region that should be suitable for quantitative analysis. The spectra from Unknown 227 (Figure 3) were essentially identical to that of the standard KHP (Figure 1). The 600 cm^{-1} to 900 cm^{-1} region was also similar, but some of the peaks were not as well formed in the Unknown 227 pellets (Figure 4). The peak at 760 cm^{-1} was reasonably well formed in the spectra from all seven pellets, so it was selected as the basis for the quantitative analysis. Spectra were found to be surprisingly reproducible over time. Spectra taken six months after the pellet was formed produced essentially the same peak area measurement as that from spectra of the freshly prepared pellet.

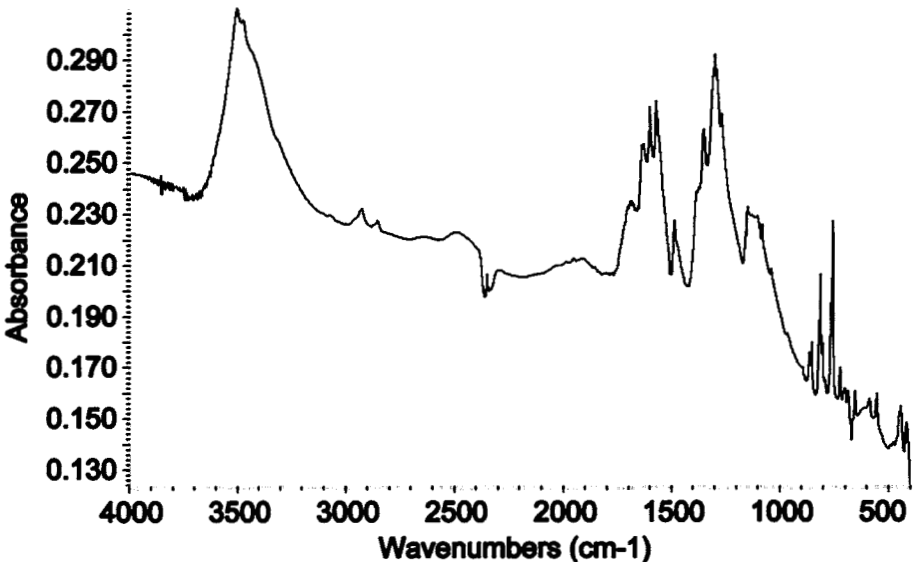

Figure 3. Sample FTIR spectrum of KHP Unknown 227 pellet obtained on the day the pellet was made.

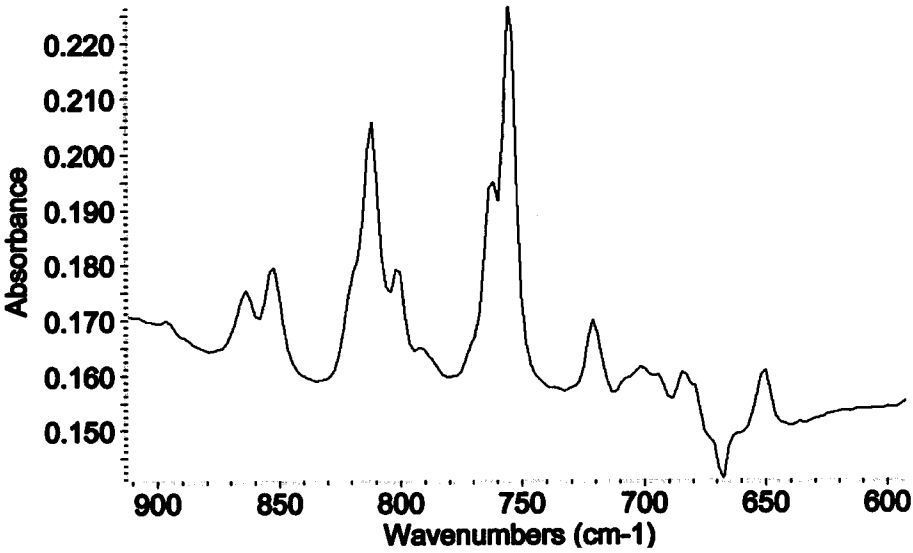

Figure 4. Spectrum of Figure 3 (KHP Unknown 227 pellet) expanded around the region of interest.

The data for the KHP standard pellets are listed in Table I. To ensure that instrumental operating conditions were constant (standard spectroscopic practice), only spectra of these pellets obtained in the same class period were used for the quantitative analysis. One of the samples was run twice to check for spectrum reproducibility. As can be seen from Figure 5, the calibration curve (peak area vs. KHP concentration) for this data was quite linear, with a slope of 1.96 g pellet/mg KHP (SD 0.4) and a y-intercept of -0.2 (SD 0.2) (essentially 0).

Table I: Data for the KHP Calibration Standards

KHP Concentration (mg KHP/g pellet)	**Peak Area (counts)**
0.0172	0.093
0.0314	0.313
0.0554	0.740
0.0653	1.029
0.0653	1.046

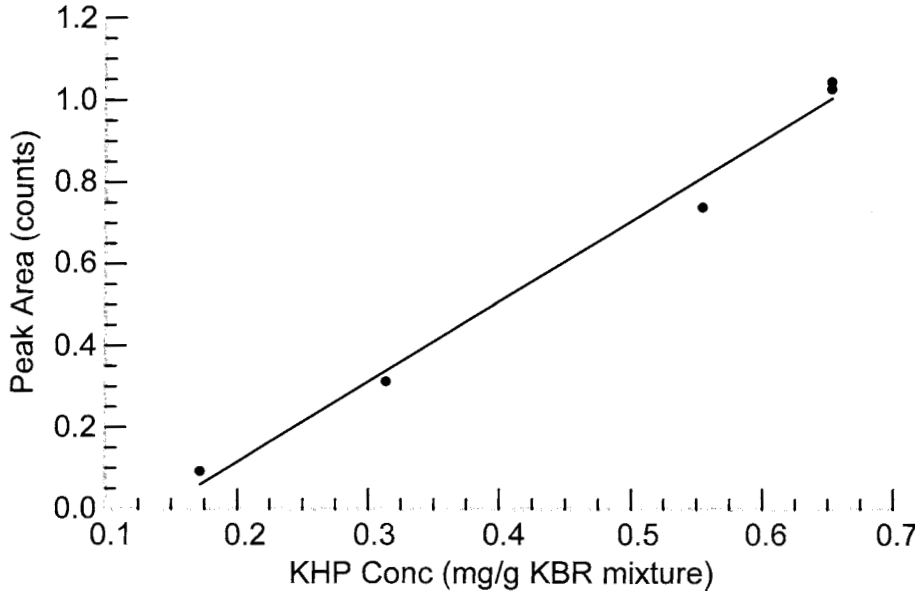

Figure 5. Calibration curve using data from Table I, slope = 1.96 g pellet/mg KHP, y-intercept = -0.2.

The data for the Unknown 227 tablets is presented in Table II. The average percentage of KHP in Unknown 227 was determined to be 55.4 % (RSD 10%). Measurements of sample masses were limited to two significant figures because of the low concentrations required to achieve adherence to the Beer-Lambert Law, so the relative standard deviation of 10% achieved here is primarily derived from uncertainty in mass measurements. The calculated percentage of KHP compares well with the accepted value of 53.62% for Unknown 227, a 3.4 % error.

The average pellet thickness (path length) measured with a caliper for the seven pellets used here was 0.52 (SD 0.01) mm, verifying that use of a consis-

Table II. KHP Unknown Data

Unk Conc (mg Unk/g pellet)	Peak Area (counts)	Calc KHP Conc (mg KHP/g pellet)	Percent KHP in Unknown 227
1.066	0.8754	0.5866	55.04%
0.9404	0.712	0.5034	54.28%
0.9288	0.776	0.5360	57.71%

tent mass of KHP/KBr mixture produces a constant pellet thickness. The variation in the thickness is about the same as the variations expected for the mass and peak area measurements (two significant figures), so, in this case, path length variability should have a minimal effect on accuracy and precision of the results. This was verified by including pellet thickness in the calculations of the percentage of KHP (peak area vs. thickness*KHP concentration). When pellet thickness was included, the percent of KHP increased slightly to 57.$_0$ % and the RSD decreased to about 4.$_7$ % (based on two unknown pellets, one pellet did not survive long enough to obtain a thickness); virtually identical results for the two significant figures expected here.

CONCLUSION

Using FTIR for quantitative analysis of solid solutions of KHP works reasonably well. With proper attention to details and careful selection of pellets, the method is accurate, precise, and reproducible. The relative accuracy (3% error) and precision (10%RSD) are in the range expected for spectrometric methods and can probably be reduced a bit further with practice.

When used as an experiment in an Instrumental Analysis course, the experiment will give students experience/practice with KBr sample preparation methods, FTIR operation, peak integration methods, and linear regression analysis using a calibration curve. Baseline correction methodology can be easily incorporated. The experiment is amenable to either the standard calibration curve method or the method of standard addition.

This lab can be quite time intensive; the students spent about twenty hours (five four-hour lab periods) collecting the necessary data for this lab; data analysis was done outside of lab time. Their experience suggests several possibilities for streamlining this experiment. One way is to predetermine an appropriate compound and the concentration range needed. Another possibility is to store and reuse the calibration standards, reducing sample preparation to the unknown samples only.

Next time this experiment is run, the use of an evacuated KBr pellet die will be investigated to see if that will increase the success rate for pellet production. The use of the baseline correction feature of the Nicolet Omnic software will also be investigated to see if that can "salvage" spectra with nonlinear baselines.

ACKNOWLEDGMENTS

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USE OF ATR AND CIRCLE CELL IN THE FTIR SPECTRA OF POLYMERS AND MICELLES

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ABSTRACT

The purpose of this research was to work out procedures for analysis of polymers in FTIR (Fourier transform infrared spectroscopy) using Attenuated Total Reflectance (ATR accessory), and to investigate micelles in FTIR utilizing Cylindrical Internal Reflectance (Circle Cell), in the context of undergraduate laboratory work.

Keywords

Attenuated Total Reflectance, Cylindrical Internal Reflectance, FTIR, polymers, micelles

INTRODUCTION

The purpose of this research was to work out procedures for analysis of polymers in FTIR (Fourier transform infrared spectroscopy) using Attenuated Total Reflectance (ATR accessory), and to investigate micelles in FTIR utilizing Cylindrical Internal Reflectance (Circle Cell), in the undergraduate laboratory. The ATR method involved internal reflections of an infrared beam within a crystal, with only slight penetration of the surface of a sample on the face of the crystal.

METHODS

The ATR crystal used was KRS-5, consisting of TlBr and TlI. With this surface analysis method, the polymer sample used could be thicker than the thin films generally needed in transmission methods of FTIR. The Circle Cell also involved internal reflections, providing an FTIR spectrum of the liquid or solution at its interface with the ZnSe rod. (Griffith, 1986). This was used to explore FTIR spectra of micelles. The instrument used was an IBM Instruments IR32 FTIR.

RESULTS AND DISCUSSION

An example of ATR on this instrument was this spectrum of a polypropylene melt.

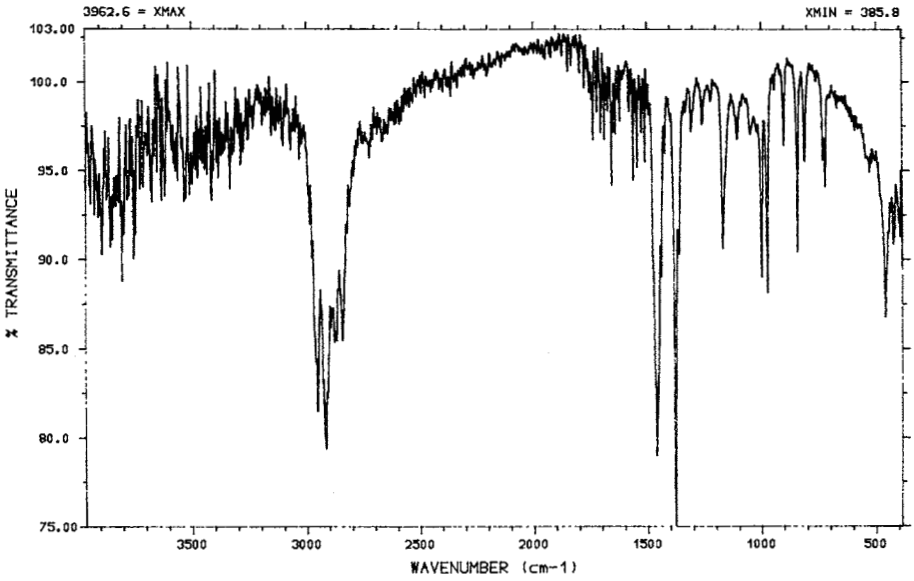

Figure 1. FTIR spectrum of a polypropylene melt using ATR

The desired result was found, in that this spectrum was matched successfully with a search of our polymer library on the FTIR.

The Circle Cell was used to explore FTIR spectra of micelles. The critical micelle concentration (CMC) was of particular interest. (Kabanov, 1999). Plots of physical properties such as conductance often show a break in slope at the CMC. (Ross, 1988). One of the systems explored here was sodium stearate in water.

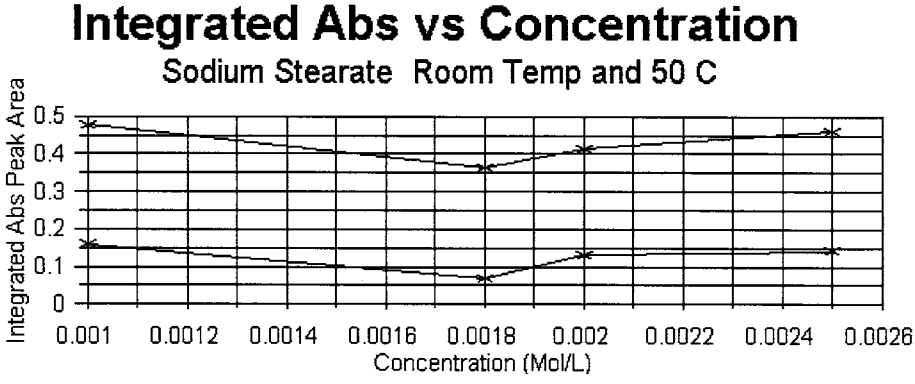

Figure 2. FTIR of sodium stearate micelles observed with Circle cell
Upper: 50°C; Lower: room temp

The result of this investigation with the Circle cell was that a break in the integrated absorbance of the C-H region occurred in the vicinity of the CMC for the system sodium stearate - water.

CONCLUSION

ATR served as a good method for determining the FTIR spectrum of a solid polymer sample. It did nor require an extremely thin layer of sample, as in the case of transmission methods of FTIR. The Circle Cell served well to measure the FTIR spectrum of surfactant materials in water. The CMC was reflected in a plot of the integrated absorbance versus concentration. Ordinary sodium chloride cells would not have been compatible with water.

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ACKNOWLEDGMENTS

Most of this research was carried out as part of the Summer Science Institute, during Summer 1998, at Augustana College.

LASER LIGHT SCATTERING FROM SILVER SOLS AND MICELLES

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ABSTRACT

The purpose of this work was to observe laser light scattering from silver sols and micelles, to prepare for possible laboratory experimental work in an undergraduate chemistry course.

Keywords

laser light scattering, silver sol, micelles

INTRODUCTION

An objective of this work was to observe laser light scattering from colloidal and micelle systems, using simple and relatively inexpensive equipment, in a manner that could be incorporated in an undergraduate chemistry course or independent study. The observations should be meaningful both qualitatively and semi-quantitatively.

METHODS

A silver sol (colloidal silver in water) was prepared by treating aqueous silver nitrate with tannin (Schlessinger, 1962). The sol was orange-brown in appearance. Qualitative observations were also made on laser light scattering from solutions of sodium lauryl sulfate in water. Materials used were reagent grade chemicals.

With silver sol in a small glass container, light scattering was observed using two low power lasers. A red laser pointer, manufactured by Quarton Inc., was a laser diode device rated with 4 mW output in the 630-680 nm wavelength range, a Class IIIA laser product. A green helium-neon laser Uniphase model 1674, was rated 0.75 mW minimum at 543.5 nm, Class IIIA. Both lasers were unpolarized.

A handheld laser power meter, LaserCheck by Coherent, had a range of 0.5 μW to 1 W. Most measurements of relative intensity were made using a simple cadmium sulfide photoresistor from Radio Shack, with an ohmmeter (multimeter).

RESULTS AND DISCUSSION

For particles which are small in comparison with the wavelength of light, and for unpolarized light, theory indicates that the intensity of scattered light is

proportional to $(1 + \cos^2\theta)$ and inversely proportional to λ^4. The usual inverse square law applies. Scattered intensity depends also on concentration, molecular weight, and (dn/dc), where n is refractive index and c is a measure of concentration. (Matthews 1985, Atkins 1998).

The relative scattered intensity was measured as a function of angle for the silver sol, for the red laser and the green laser.

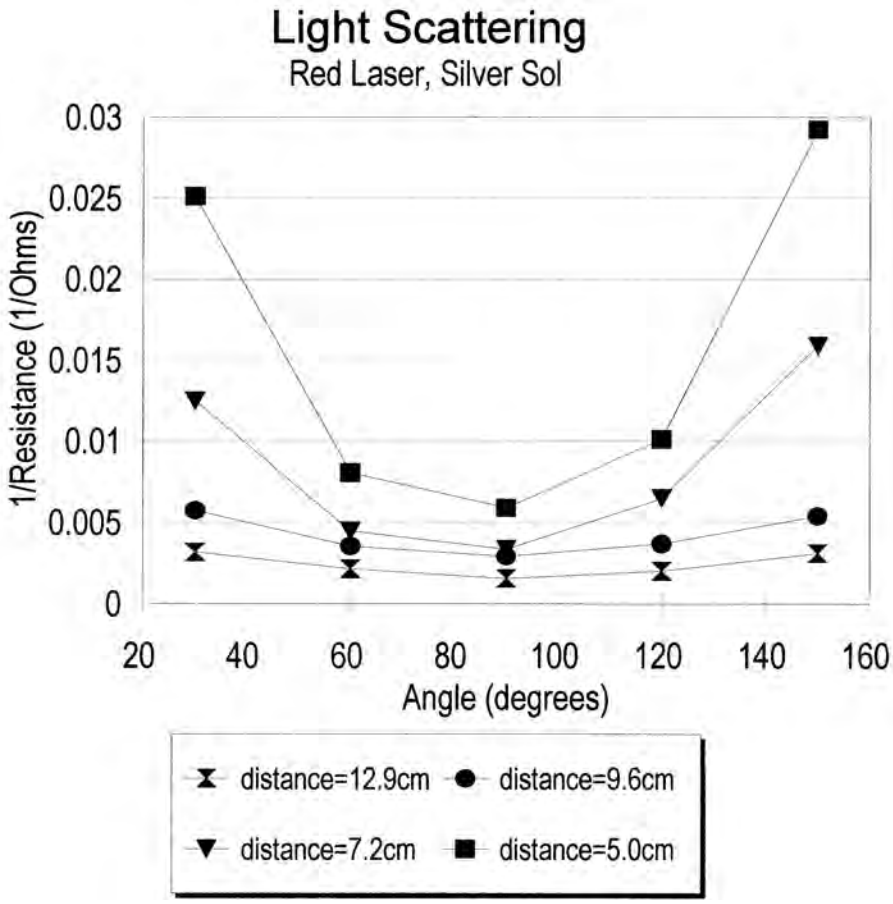

Fig. 1. Red laser light scattering from silver sol

These results were in good qualitative or semi-quantitative agreement with expectations from theory. In particular, the minimum at 90 degrees corresponds to the expected minimum in $(1+\cos^2\theta)$ for unpolarized incident light.

Qualitative observations were made on laser light scattering from solutions of sodium lauryl sulfate in water. The critical micelle concentration (CMC) of this twelve carbon chain is expected to be somewhere near that of sodium dodecanoate, which also has twelve carbons. The latter CMC is 0.024 M at 25°C

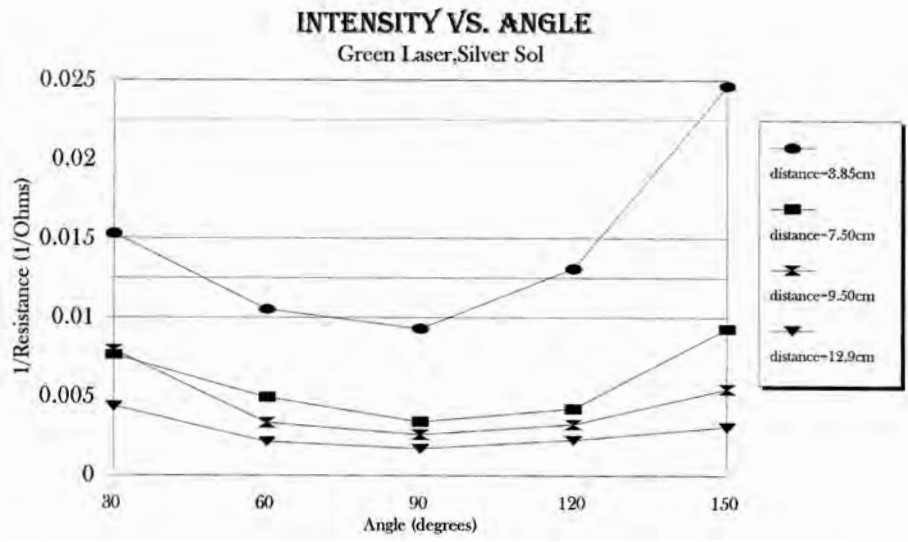

Fig. 2. Green laser light scattering from silver sol

(Ross 1988). At concentrations in the millimolar range, a distinct beam of light could be seen across the solution. Variation of intensity with angle was similar to the silver sol.

CONCLUSION

Laser light scattering from the silver sol followed the $(1 + \cos^2\theta)$ pattern expected from theory for unpolarized incident radiation, for particles small compared with the wavelength of light. This was true both for the red laser pointer (around 630-680 nm) and the green He-Ne laser at 543.5 nm. Sodium lauryl sulfate behaved similarly at sufficiently dilute solutions (millimolar range).

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ACKNOWLEDGMENTS

Most of this research was carried out as part of the Summer Science Institute, during Summer 1998, at Augustana College.

EXPLORING GLASSES AND GLAZES IN THE HISTORY OF CHEMISTRY AND THE UNDERGRADUATE LABORATORY

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ABSTRACT

The purpose of this work was to make samples of glass, related to the craft of glassmaking in the History of Chemistry. Compositions suitable for preparation at temperatures of 850ºC and 1350ºC were developed and tested. At the higher temperature, it was possible to use a higher percentage of SiO_2 in preparing glass samples.

Keywords

Glassmaking, history of chemistry, colored glass

INTRODUCTION

During Fall 1998, History of Chemistry was offered at Augustana College. GJH chose glassmaking as one of his laboratory activities, and placed this in the context of History of Chemistry. This included laboratory work which began the present study.

HISTORICAL BACKGROUND AND METHODS

The basic techniques for making glass are extremely old, and in many ways it could be said that modern techniques and processes are simply refinements of the old techniques. It is believed that the earliest objects made of glass are Egyptian beads that date back as far as 3000-2500 B.C. "The earliest glass vessels, made in Egypt during the Dynasty (1500-1350 B.C.), were made by a method that was in essence an extension of the glazing process" (Kirk-Othmer 1966). Other glass vessels that were found are believed to be of Mesopotamian origin.

Sand, limestone, and soda were the key ingredients used in early glassmaking, These ingredients made glass easy to make and manipulate, and was generally referred to as "Lime Glass." This was the most common glass because the materials were easily obtained and it could be made by using lower temperature. The "Lime Glass" was also very stable and allowed for easy resoftening for the molding and shaping processes. The one thing to remember when making this glass though is that typically, the melting point of sand is 1580ºC. That means that the more sand that is in the mixture, the higher the melting temperature will be.

The Romans added the ability to make colored glass. "They knew that specific colors could be achieved by adding particular metallic oxides to the raw materials" (Kirk-Othmer 1966). They learned that they could get a ruby red or green by adding copper, cobalt for blue, manganese for amethyst or purple, antimony for yellow, iron for green, brown, or black, and tin for an opaque white. The first small glassworks in America was made at Jamestown, Virginia, around 1609. Since then, rapid population growth and the increasing rate of urbanization has created a bigger need for glass. (Harrington, 1952).

Many different types of glass have become available over the years. Window glass is a soda-lime glass with a typical composition of73% SiO_2, 17% Na_2O (soda), 5% CaO (lime), 4% MgO, and 1% Al_2O_3. In the process of making Pyrex glass, the procedure is to use soda and borax to lower the softening point of the sand to about 800ºC. Leaded glasses are made with litharge (PbO) and are typically made into fine glassware. Laminated glass is made by placing a sheet of plastic between two layers of glass. An example of this would be the glass used for the front windshields of vehicles. Toughened glass is regular glass made by cooling the glass rapidly. Some examples of this kind of glass may be the rear or side windows of vehicles and the shower doors in a home.

A Thermolyne Type 1400 Furnace was used to heat mixtures to 850ºC. A Fisher High Temperature Furnace Model 472 was use for heating samples to 1300ºC.

RESULTS AND DISCUSSION

In the present work, representative compositions of mixtures heated to prepare glass or glazes, modified from the literature to allow lower working temperatures, around 850∞C, are as follows. (Kirk-Othmer, 1966; Corning, 1997).

Colorless soda-lime glass: 55 % SiO_2, 45% Na_2CO_3.

Cobalt blue glass: 60 % SiO_2, 35 % Na_2CO_3, 5 %$CoCO_3$.

Typically such a mixture was heated to about 850ºC for 1-2 days, and then cooled slowly.

The next stage of this work was to heat such mixtures to higher temperatures. The Fisher High Temperature Furnace Model 472 tube furnace acquired recently can safely achieve 1500ºC. So far this has been used around 1350ºC, which has made it possible to go to higher percentages of SiO_2, in the direction more nearly typical of commercial glasses. The composition range of the best looking glass at the higher temperature was typically 60-65% SiO_2, 30-35% Na_2CO_3, together with1-5% coloring material.

Producing colored glass and glazes was of particular interest. Colors of glass produced in this project, and the coloring material added, include the following. Blue glass resulted from adding $CoCO_3$, transparent amber from Fe_2O_3, and amethyst from MnO_2. Attempts to prepare red glass by adding Cu_2O resulted in teal colored glass, probably due to oxidation of copper(I) to copper(II).

The lovely blue color of cobalt blue glass is due to a relatively intense transition $t_2 \leftarrow e$ in the tetrahedral environment of $3d^7$ Co^{+2} ions in the silicate ma-

trix, as interpreted in ligand field theory. In more detail, this transition is designated $^4T_1(P) \leftarrow {}^4A_2$ (Cotton, 1999; Hush, 1968).

CONCLUSION

In the present work, small samples of glass and glazes were produced by heating SiO_2 with selected oxides and carbonates in a high temperature furnace around 850∞C, with further work around 1350ºC. Several types of colored glass were produced, including blue, amber, and amethyst. In cobalt blue glass, the color is due to a transition in the tetrahedral environment of $3d^7$ Co^{+2} ions in the silicate matrix. In ligand field theory, this transition is designated $^4T_1(P) \leftarrow {}^4A_2$ (Cotton, 1999; Hush, 1968).

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ACKNOWLEDGMENT

This work was carried out at Augustana College in the context of Chemistry 297B, Topics: History of Chemistry, during Fall 1998. It was continued as an Independent Study in Chemistry during Spring 1999.

EXPLORATIONS IN MEDICINAL CHEMISTRY THROUGH MOLECULAR MODELING WITH HYPERCHEM

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ABSTRACT

The purpose of this work was to develop and evaluate molecular modeling exercises and projects suitable for students in a course in Medicinal Chemistry.

Keywords

Molecular modeling, medicinal chemistry, acetylcholinesterase, inhibition

INTRODUCTION

Prof. Gary Earl has taught a Medicinal Chemistry course at Augustana College, and plans to do so again. Thus this project has a future and developmental orientation. The interests of medicinal chemistry overlap those of biochemistry, but include distinctive aspects such as interaction of drugs with receptor sites at the molecular level, and computer-aided drug design. (Balbes 1994).

METHODS

Tools suitable for getting started in molecular modeling at the PC level include HyperChem and PC Spartan (Caffery 1998, Hehre 1998). Chime is also useful for visualization and display on the web (Viste 1999). Isis Draw skc files are also compatible with HyperChem.

RESULTS AND DISCUSSION

Several instructive exercises were developed.

1. Select a specific protein, and download its structure, in the Protein Data Bank (Brookhaven 1999). At present the PDB includes 8677 proteins, 654 nucleic acids, and 12 carbohydrates, with additional structures added weekly.
2. Visualize the structure of the protein by two or more methods. Chime and HyperChem are particularly recommended. In this project dozens of structures were downloaded from the PDB. An example is the structure of acetylcholinesterase, which has the ID Code 2ACE in the PDB.

Figure 1. 2ACE acetylcholinesterase, Chime

Figure 2. 2ACE acetylcholinesterase, HyperChem

3. Build several smaller polypeptides in HyperChem, selecting the amino acid sequence and type of secondary structure (alpha helix or beta pleated sheet). Caffery suggests bradykinin, which is produced as a result of a wasp sting (Caffery 1998, p. 24).
4. Build a portion of a DNA double helix in HyperChem, such as CGCCGC-CGC. Note hydrogen bonds.
5. Build a small carbohydrate in HyperChem, such as the disaccharide maltose. This was readily accomplished using ChemPlus with HyperChem.

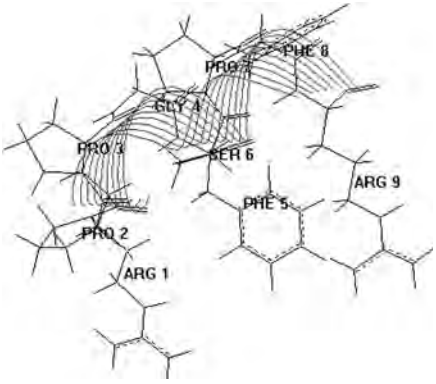

Figure 3. Bradykinin, arg-pro-pro-gly-phe-ser-pro-phe-arg alpha helix in HyperChem

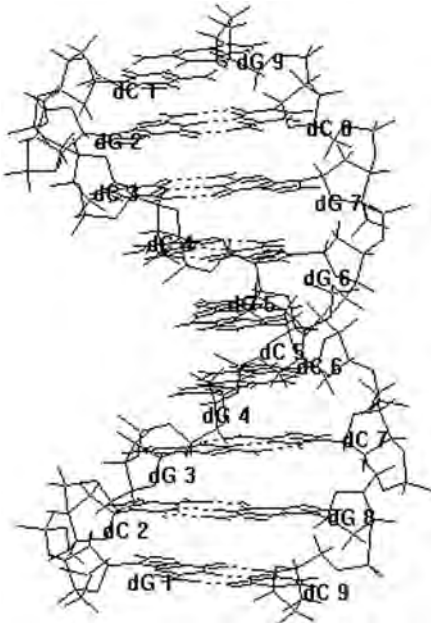

Figure 4. CGCCGCCGC

6. Carry out a small literature search to identify several compounds which are effective as general anesthetics or local anesthetics. Examples include diethyl ether, methoxyflurane, and novocaine. Build several structures in HyperChem.
7. Explore geometry optimization of the smaller structures, or of selected regions of large structures, using molecular mechanics or semiempirical molecular orbital methods such as AM1.
8. Build a small molecule such as ascorbic acid, Vitamin C. optimize geometry and carry out molecular orbital calculations in HyperChem or PC Spartan, using semiempirical methods such as AM1, or ab initio methods such as 3-21G*. Examine atomic charges, dipole moment, and a map of electrostatic potential. (Hehre 1998, p. 220).
9. Investigate a case of a medicinal drug molecule docked at a receptor site. An interesting case is provided by an anti-Alzheimer's drug docked near the active site of acetylcholinesterase (ACHE). Three such cases are available in the Protein Data Bank. Tacrine is the experimental drug bound to the ACHE enzyme in pdb1acj.ent. E2020 (Aricept) is bound to the ACHE enzyme in pdb1eve.ent. In pdb1vot.ent, Huperzine A is bound. Fig. 9 shows the residues near Aricept (E2020) in pdb1eve.ent. HyperChem was used to select the E2020 molecule and the nearby residues, and show only them. It was found that interaction of the pi electrons of aromatic por-

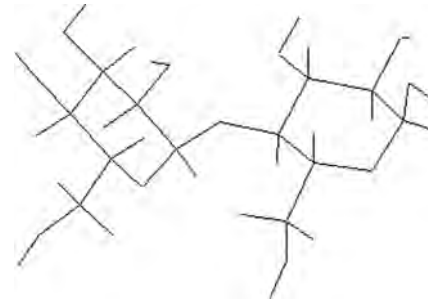

Figure 5. Maltose, α-D-glucopyranosyl-(1-4)-α-D-glucopyranose

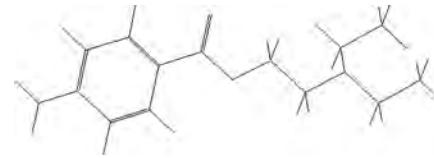

Figure 6. Novocaine

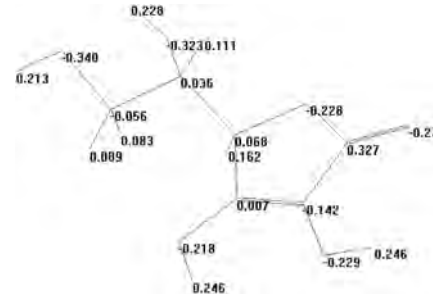

Figure 7. Vitamin C

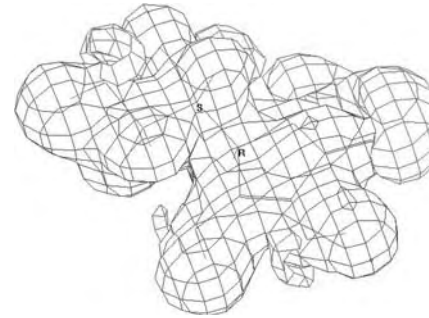

Figure 8. Vitamin C electrostatic potential

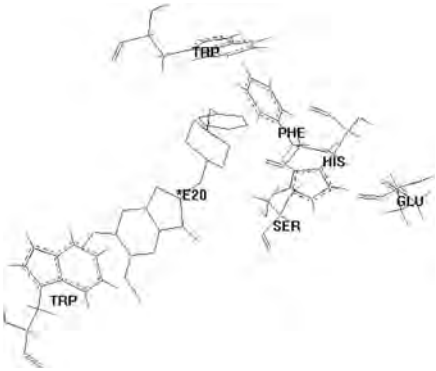

Figure 9. E2020 (Aricept) bound to acetylcholinesterase

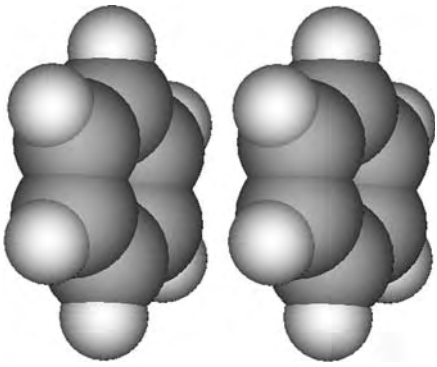

Figure 10. Two C_6H_6 in AM1

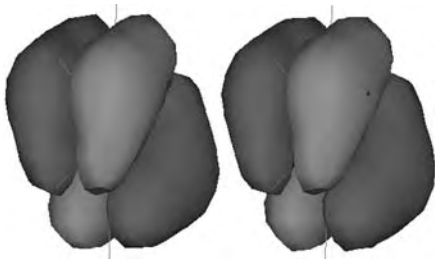

Figure. 11. HOMO of two C_6H_6 in-AM1

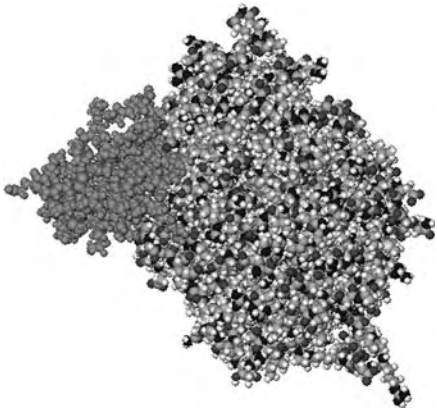

Figure 12. Snake venom on mouse ACHE, in pdb1mah.ent (HyperChem display)

tions of E2020 with nearby aromatic rings appeared to be significant in the docking of the E2020.

10. A simple system of two benzene molecules was investigated as an analog of one aspect of the docking of E2020 in ACHE. Geometry optimization was carried out through an AM1 molecular orbital calculation in HyperChem. The highest occupied molecular orbital (HOMO) is shown as well.
11. The docking of pairs of polypeptides was found to be a challenging problem of current interest. Docking two polypeptides is at the upper end of what might be a reasonable exercise in this exploration of medicinal chemistry. Some initial work has been done with GRAMM software. (Katchalski-Katzir, E. et al., 1992; Vakser, I. A., 1995, Vakser, I. A., 1998). A sample exercise related to the previous discussion is the docking of a type of snake venom on acetylcholinesterase. The experimental structure is available in the Protein Data Bank as pdb1mah.ent.

CONCLUSION

HyperChem and PC Spartan have proved to be useful tools for molecular modeling in medicinal chemistry. The work with which this project has been concerned has led to a particular interest in docking small molecules such as drugs and portions of larger molecules which can approximate receptor sites.

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ACKNOWLEDGMENT

We thank the Augustana College Department of Chemistry for software and hardware support which is essential for these molecular modeling activities.

MULTINUCLEAR FT-NMR USING THE ANASAZI EFT-60 INSTRUMENT

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ABSTRACT

The purpose of this work was to demonstrate significant examples of the multinuclear NMR capabilities of the recently upgraded Anasazi EFT-60 FT-NMR at Augustana College. Instructional examples include ^{19}F nmr spectra of NH_4 BF_4 and Na PF_6; ^{11}B nmr spectra of NH_4 BF_4; ^{31}P nmr spectra of $(CH_3O)_3P$, PF_6^-, and H_3PO_2; ^1H spectra of $(CH_3O)_3P$ and H_3PO_2.

Keywords

multinuclear nuclear magnetic resonance, spin-spin coupling

INTRODUCTION

Beginning in 1996 the nuclear magnetic resonance instrumentation at Augustana College has been upgraded from CW (continuous wave) to FT (Fourier transform). Initially a Hitachi Perkin Elmer R-24A NMR spectrometer was upgraded from CW to FT-NMR for ^1H, by Anasazi Instruments, Indianapolis, IN. In 1998 another CW instrument was acquired, a Varian EM-360A NMR spectrometer. In August 1998, Anasazi Instruments upgraded this to an Anasazi EFT-60 NMR spectrometer, providing extensive multinuclear nmr capability. This includes ^1H, ^{13}C, ^{19}F, and numerous other nuclei in the range of 11.26 to 25 MHz with the original ^1H at 60.01 MHz, such as ^{31}P, ^{11}B, ^{29}Si, ^{79}Br, ^{59}Co, and ^{23}Na. The nuclei listed have been observed in this laboratory so far. Altogether, 42 nuclei of 34 elements fall in the observable frequency range. (Drago, 1992; Pople, 1959)

METHODS

Using the Anasazi EFT-60 FT-NMR, standard pulse sequences were used to acquire free induction decay (FID) data. Fourier transform converted the spectrum from time domain to frequency domain. (Drago, 1992). Nuclei observed include ^1H, I=1/2, at 60.010 MHz; ^{19}F, I=1/2, at 56.461 MHz; ^{11}B, I=3/2, at 19.246 MHz; and ^{31}P, I=1/2, at 24.292 MHz. Samples used were reagent grade chemicals.

RESULTS AND DISCUSSION

The ^{11}B nmr spectrum of the BF_4- ion was run, showing the spin-spin coupling of the four ^{19}F with the ^{11}B nucleus.

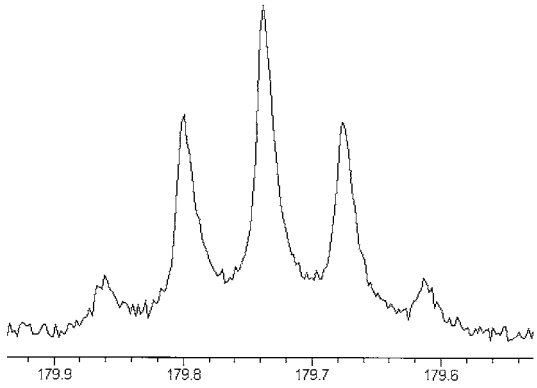


Figure 1. 11B nmr spectrum of $NH_4(BF_4)$

Note that the ^{11}B resonance is split by spin-spin coupling to the four equivalent ^{19}F in the tetrahedral BF_4^- ion. ^{19}F has I=1/2. Expected intensities are 1:4:6:4:1 in this quintuplet. The spin-spin coupling constant was J_{BF} = 1.2 Hz.

Boron has two isotopes. ^{10}B has a spin of I=3 and an abundance of 19.58%. ^{11}B has a spin of I=3/2 and an abundance of 80.42%. Its nmr frequency is 19.246 MHz, nicely within the range of our instrument. For nuclei with I > ½, there is a nuclear quadrupole moment which can be a problem, in terms of broadening spectral lines. However in high symmetry environments, specifically tetrahedral or octahedral, the nuclear quadrupole moment is not a problem. So the tetrahedral BF_4^- ion was an excellent case to study, as illustrated here.

The ^{19}F nmr spectrum of $NH_4(BF_4)$ was examined. It is expected to show a quartet of lines of equal intensity for the splitting of the ^{19}F resonance by ^{11}B with I=3/2, with J_{BF} = 1.1 Hz. ^{11}B is the more abundant isotope. The 4 line multiplet corresponds to 2I+1 = 4. ^{10}B, the less abundant isotope, has I=3 so that 2I+1 = 7 lines should occur in its multiplet. These were not quite resolved, and the chemical shift for $^{10}BF_4^-$ was slightly different than that for $^{11}BF_4^-$ in the ^{19}F nmr spectrum.

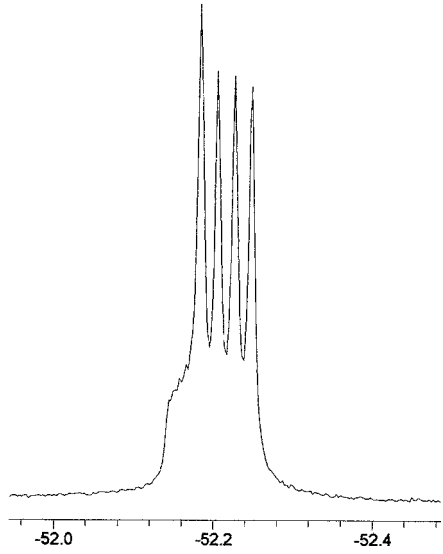


Figure. 2. ^{19}F nmr spectrum of $NH_4(BF_4)$

A good example of a ^{31}P nmr spectrum was that of $(CH_3O)3P$. The splitting of the ^{31}P resonance by the nine equivalent ^{1}H nuclei was expected to give a 10 line multiplet, with relative intensities 1:9:36:84:126:126:84:36:9:1. 8 lines were clearly visible, with the intensities of the first and last of the 10 lines too low to distinguish from noise. J_{PH} = 10.$_6$ Hz was the observed splitting.

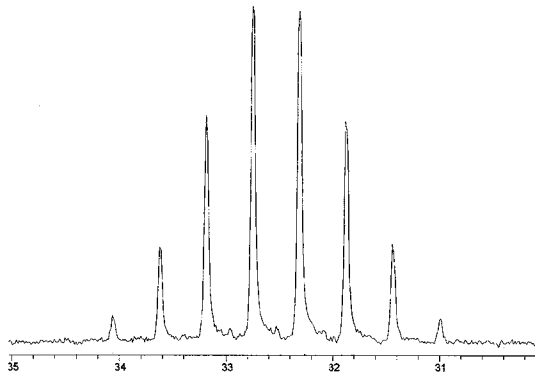


Figure 3. ^{31}P nmr spectrum of $(CH_3O)_3P$

The 1H spectrum gives JPH = 10.$_7$ Hz.

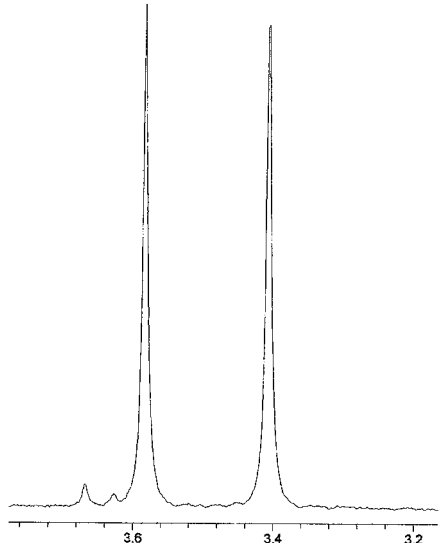
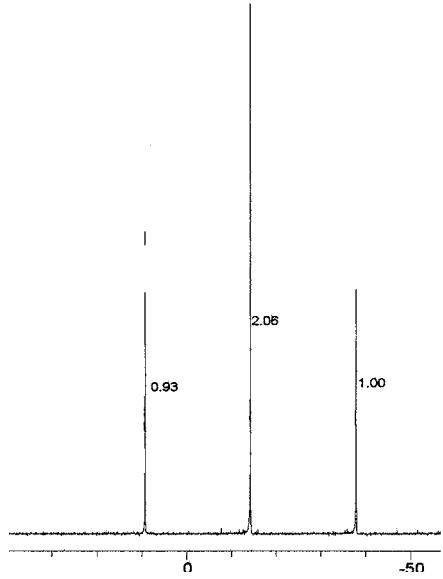


Figure 4. ^{1}H nmr spectrum of $(CH_3O)_3P$

Hypophosphorous acid, H_3PO_2 (aq) has a distinctive structure with two P-H bonds and one O-H bond. The ^{31}P nmr spectrum is a triplet, with J_{PH} = 573 Hz, a very substantial splitting.



Figrue 5. ^{31}P nmr spectrum of H_3PO_2

As a final example, two nmr spectra, for ^{31}P and ^{19}F, were observed for $NaPF_6$, with its octahedral PF_6^- ion. In the ^{31}P spectrum, the measured coupling constant was J_{PF} = 714 Hz.

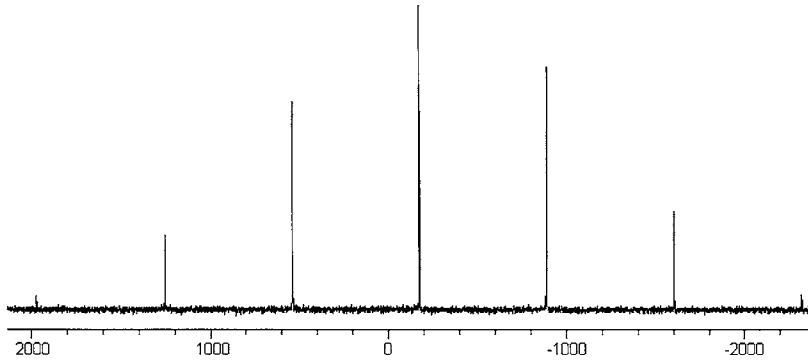


Figure 6. ^{31}P nmr spectrum of $NaPF_6$ (aq)

In the ^{19}F spectrum, the measured coupling constant was essentially the same, J_{PF} = 717 Hz.

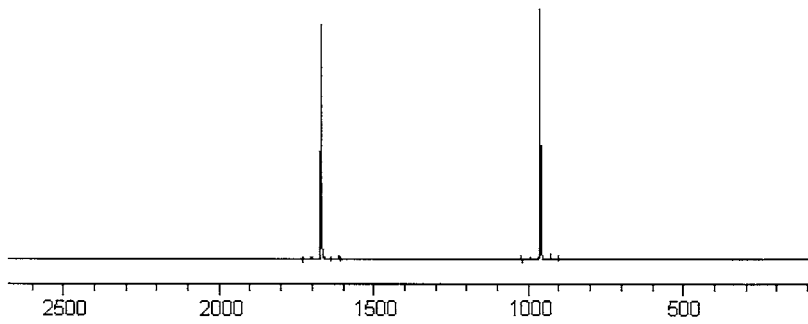


Figure 7. ^{19}F nmr spectrum of $NaPF_6$ (aq)

CONCLUSION

Significant examples of the multinuclear NMR capabilities of the recently upgraded Anasazi EFT-60 FT-NMR at Augustana College have been successfully demonstrated. Spectra have been observed thus far for ^{1}H, ^{13}C, ^{19}F, ^{31}P, ^{11}B, ^{29}Si, ^{79}Br, ^{59}Co, and ^{23}Na. Altogether, 42 nuclei of 34 elements fall in the observable frequency range of 11.26 to 25 MHz along with the vicinity of 60 MHz.

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ACKNOWLEDGMENTS

The NMR was upgraded through a 1996 research grant of Prof. Gary Earl from NSF/EPA, CHE-9613052, "Preparation of More-Biofriendly Quaternary Ammonium Compounds and their Decomposition to Useful Reagents", with matching support from Augustana College for the NMR. An Interim Leave for Arlen Viste, provided during January 1999 by Augustana College, is also gratefully acknowledged.

TADPOLE MADTOM *(NOTURUS GYRINUS)* BIOLOGY IN AN UPPER MISSOURI RIVER BACKWATER

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ABSTRACT

A tadpole madtom (*Noturus gyrinus*) population was found in Erickson Island Slough, an upper Missouri River backwater in western North Dakota. We assessed basic tadpole madtom biology to obtain baseline data that may be useful when evaluating its interactions in this backwater community. Tadpole madtoms were collected in May, July, and September of 1997 and 1998. Catch per unit effort was <5/trap net night on all sample dates, but increased by more than 4,000% from 1997 to 1998. The population was dominated by age-2 and younger tadpole madtoms. Growth rates were higher than other populations during the first two growing seasons, but decreased sharply after age 2. Food habits were similar to those reported for other populations, with tadpole madtoms primarily consuming chironomids and crustaceans. High reproductive potential, a reputation of existing at high densities, and food habits similar to other native species indicate that tadpole madtoms have the potential to become a common backwater community member and exert influence on the local aquatic system.

Keywords

tadpole madtom, *Noturus gyrinus*, backwaters, Missouri River

INTRODUCTION

The tadpole madtom *Noturus gyrinus* is widespread in eastern North America (Page and Burr 1991) and has been documented in the James, Red, and Big Sioux river systems of the Dakotas. Lee et al. (1980) reported that the tadpole madtom did not occur upstream of Gavins Point Dam in the Missouri River basin; however, North Dakota Game and Fish Department biologists collected this species from Lake Sakakawea in western North Dakota during sampling in 11 of the years between 1956 and 1994 (Hendrickson et al. 1995). We found a population of tadpole madtoms in a Missouri River backwater in northwestern North Dakota (Fig. 1), upstream of Lake Sakakawea.

Since closure of mainstem dams, the reservoir system likely inhibited additional movement of tadpole madtoms up the Missouri River. Therefore, the species may have been either present in low numbers, existing in unsampled

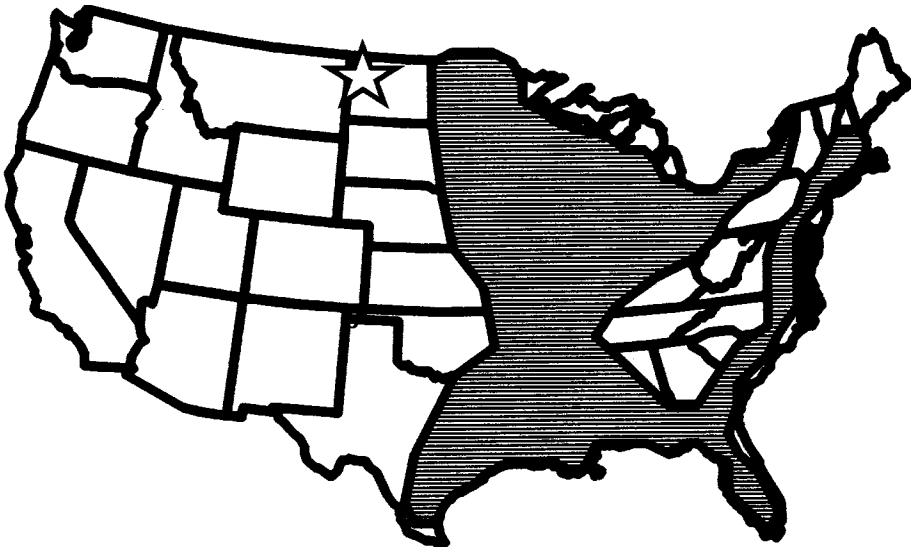

Figure 1. Historical range (shaded area) of tadpole madtoms in the United States. The Erickson Island Slough population is outside of this range (star).

habitats since a time prior to dam closures, or accidentally introduced by anglers who utilize this species as bait in many northern rivers (Eddy and Underhill 1974). Regardless of how tadpole madtoms became established in western North Dakota, the species tends to be abundant where it is found and can affect other species (Hooper 1949). Understanding the structure and function of tadpole madtom populations in regions of potentially critical habitats for native fishes of concern, such as pallid sturgeon *Scaphirhynchus albus*, blue sucker *Cycleptus elongatus*, and sicklefin chubs *Macrhybopsis meeki*, is important. Therefore, the objective for this study was to assess the biology of the species, including evaluations of relative abundance, age and size structure, growth, mortality, and food habits.

STUDY SITE

Erickson Island Slough (EIS) is a Missouri River backwater located in Williams and McKenzie counties in northwestern North Dakota. This oxbow wetland has a surface area of approximately 1,100 ha; however, surface area and mean depth vary with changes in seasonal inflow resulting from the dynamic hydrograph of the Missouri River. Due to the strong lotic influence exerted by the unregulated Yellowstone River, EIS and other backwaters in the region have maintained a reasonably historic structure and function.

METHODS

Fishes, including tadpole madtoms, were collected with trap nets in May, July, and September of 1997 and 1998 from EIS. We conducted a standard sam-

ple of 18 net nights per sample period (nine nights with nets having 0.9- x 1.8-m frames, 9.5-mm bar mesh, and 16- x 0.9-m leads, and nine nights with nets having 0.6- x 0.9-m frames, 9.5-mm bar mesh on the frames, 3-mm bar mesh on the hoops, and 7- x 0.6-m leads). When tadpole madtom catches were insufficient for food habits and aging analyses, we used additional trap net nights to increase sample size.

Tadpole madtom population density was indexed using catch per unit effort (CPUE) in 1998 and 1997. We defined CPUE as the number of tadpole madtoms per trap net night. We also wanted to compare the relative abundance of tadpole madtoms in EIS with those of other populations; however, no comparison trap net CPUE data were located.

Tadpole madtoms collected during all 1998 trap net sampling were measured to the nearest millimeter total length (TL). Ten fish per centimeter length group from the September sample were placed on ice and returned to the laboratory at South Dakota State University for age analysis. Length data were summarized into seasonal length-frequency histograms and tadpole madtoms retained in September were aged by reading annuli on cross-sections of the pectoral spine.

Left pectoral spines were grasped with a forceps and removed by pressing the spine flat against the body and rotating clockwise (Sneed 1951). Spines were stored in scale envelopes, allowed to dry, cleaned of extraneous tissue, and mounted in a wooden mold (Margenau 1982). We used clear epoxy and lined the trough (6.5 x 6.5 x 230 mm) with wax paper. Mounted spines were allowed to harden for 24-36 h. Cross sections were removed from the spine at the distal end of the basal groove using a jewelers saw (Sneed 1951) and polished with 220-grain sandpaper. Cross sections were placed in a shallow concave glass plate, covered in water (to clarify view), and viewed with a dissecting microscope. Tadpole madtoms were assigned ages based on criteria suggested by Clugston and Cooper (1960), who found that the presence of thin, clear zones under transmitted light indicated slower winter growth and wider dark zones represented faster summer growth. Spines were aged by two independent readers, discrepancies were discussed, and an age was assigned. Percent agreement between readers was calculated and an age-frequency histogram was developed.

We calculated mean TL at age for tadpole madtoms collected in September of 1998. Growth, as represented by the mean TL values, was compared with the mean TL at age for tadpole madtoms collected at other geographic locations during late August or September. Growth data from Hooper (1949) and Mahon (1977) were reported in standard length, which we converted to total length based on Becker (1983).

An annual mortality rate for tadpole madtoms from EIS was estimated using age-structure data collected in September of 1998. We used the Chapman-Robson method because Everhart et al. (1975) suggested that this method would provide a reasonable annual mortality estimate from age-structure data. Stomachs (N=30) were eviscerated from tadpole madtoms collected during each sample period in 1998. Stomach contents were assessed and seasonally quantified into percent by number (number of food items in that category/to-

tal number of items) and frequency of occurrence (number of fish containing that food category/total number of fish). Sample sizes were insufficient to assess food habits by size groups; thus, all lengths were pooled.

RESULTS AND DISCUSSION

Relative abundance

CPUE during May, July, and September of 1998 was 0.6 (SE=0.2), 0.1 (SE=0.8) and 4.6 (SE=1.4), respectively (Table 1). A high proportion of the substantial increase in the 1998 September CPUE reflects highly abundant age-0 tadpole madtoms entering the sampled population. Tadpole madtom CPUE was considerably lower in 1997 than in 1998. Two possible explanations for the higher CPUE values in 1998 are capture efficiency differences between years and increasing tadpole madtom density.

Table 1. Mean catch per unit effort (CPUE; number per trap net night) for tadpole madtoms collected from Erickson Island Slough, North Dakota in 1997 and 1998. The standard error of each mean is listed in parentheses.

Sample period	1997 CPUE	1998 CPUE
May	0.0 (0.0)	0.6 (0.2)
July	0.2 (0.1)	0.1 (0.1)
September	0.1 (0.1)	4.6 (1.4)

Lee et al. (1980) suggested that where tadpole madtoms are found, they often attain high densities. Although the September CPUE increased by more than 4,000% from 1997 to 1998, the relative abundance is still low in the EIS population. However, Hooper (1949) reported that tadpole madtoms composed 35% of the total fish biomass in Demming Lake, again supporting the contention that the species is capable of becoming abundant. The differences in CPUE between 1997 and 1998 may also be attributed capture efficiency. Peak 1997 flow exceeded 2,400 m^3/s, whereas peak flow in 1998 was approximately 850 m^3/s. The higher water levels in 1997 created habitats difficult to sample and preferred by tadpole madtoms; thus, we believe that capture efficiency was likely lower.

Size and age structure

Low sample size limited the interpretive value of the length-frequency histograms for tadpole madtoms collected in May and July of 1998. However, of

the tadpole madtoms sampled, we verified that a mode containing age-1 and age-2 madtoms was present and moved from a median of 5-cm TL in May to 8-cm TL in September (Fig. 2). By September, a second mode of madtoms with a median TL of 5 cm was present. We verified that the second mode was dominated by age-0 tadpole madtoms.

The tadpole madtoms collected in September (N=68) ranged in age from 0 to 3 (Figure 3). Hooper (1949) detected tadpole madtoms up to age 2 in Demming Lake, Minnesota and other studies have reported madtoms up to age 3 (Mahon 1977; Paruch 1979) in northern portions of their range.

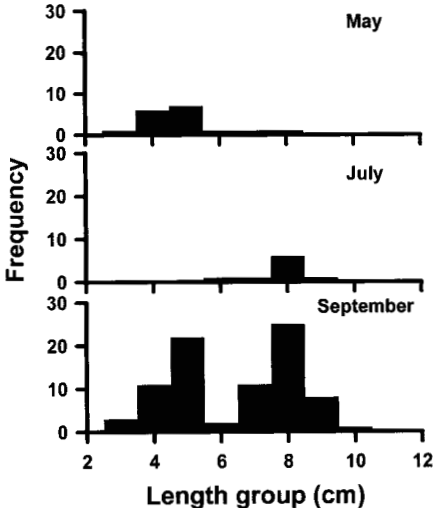

Figure 2. Size structure of tadpole madtom samples collected with trap nets from Erickson Island Slough, North Dakota during May (top), July (middle), and September (bottom) of 1998.

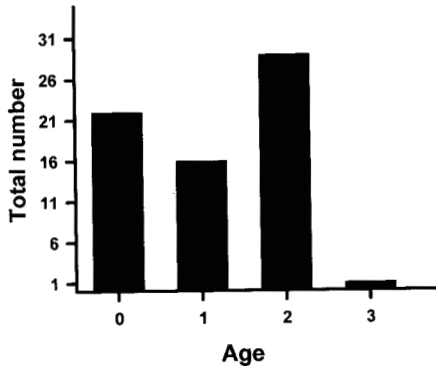

Figure 3. Age structure of tadpole madtoms collected in September of 1998 from Erickson Island Slough, North Dakota.

However, tadpole madtoms may grow older at southern latitudes. Whiteside and Burr (1986) documented age-4 tadpole madtoms from creek populations in southern Illinois.

Growth

Growth of tadpole madtoms up to fall age 1 in EIS appeared to exceed growth rates for other reported populations (Fig. 4). Tadpole madtoms typically hatch in June or July (Becker 1983). EIS age-0 tadpole madtoms attained a mean TL of 50 mm by September and added a 30-mm increment during the age-1 growing season. However, after the second growing season, growth of tadpole madtoms in EIS slowed considerably; they added only a 9-mm increment during the third growing season.

The apparent decrease in EIS tadpole madtom growth rates may be attributable to environmental conditions, energy allocation, and/or interspecific

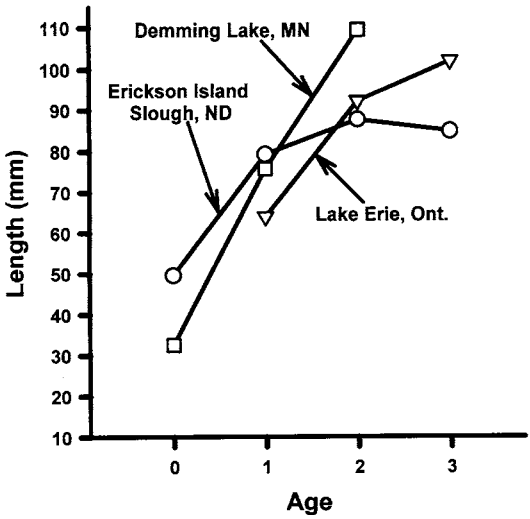

Figure 4. Mean total length at age for tadpole madtoms collected during late August and/or early September in 1998 from Erickson Island Slough, North Dakota, in 1975 from Demming Lake, Minnesota (Hooper 1949), and in 1946 from Lake Erie, Ontario (Mahon 1977).

competition for available food resources. The EIS tadpole madtom population exists on the edge of their range; therefore, environmental conditions, particularly cold, extended winters, may have a negative effect on the species. Pflieger (1975) stated that most tadpole madtoms reproductively mature at age 2. Thus, energy allocations may be moved from somatic to gonadal growth. Also, several native species that frequently utilize EIS, including river carpsucker *Carpiodes carpio*, black bullhead *Ameiurus melas*, age-0 buffaloes *Ictiobus* spp., and age-0 channel catfish *Ictalurus punctatus*, tend to consume similar prey, as will be discussed later. However, deleterious competition for food resources assumes that food availability is limiting.

Growth of age-2 tadpole madtoms in Demming Lake did not substantially decrease as did growth of EIS madtoms. The Demming Lake fish community consisted of northern pike *Esox lucius*, pumpkinseed *Lepomis gibbosus*, yellow perch *Perca flavescens*, black bullhead, and tadpole madtoms (Hooper 1949). The simple community in Demming Lake may have not experienced interspecific competition, particularly because black bullhead density was low. In EIS, juvenile black bullhead CPUE in 1997 and 1998 from EIS often exceeded 1,000/trap net night; therefore, interspecific competition was possible.

Annual mortality

Annual mortality of tadpole madtoms was estimated at 47%. Our annual mortality rate was considerably lower than the rate reported for age-1 and -2 tadpole madtoms in Demming Lake, Minnesota (97.4%; Hooper 1949), Lake Erie (77.8%; Mahon 1977), and Dutchman Creek, Illinois (67.6%; Whiteside and Burr 1986). The lower mortality rate that we report here may be skewed by the inclusion of age-0 fish and the lack of a stair-step age-frequency distribution. The age-structure histogram (Figure 3) indicates that recruitment of tadpole madtoms likely is erratic in EIS. For example, the age-2 cohort (1996 year

class) is more abundant (i.e, strong year class) than would be expected given the abundance of age-1 and age-3 fish.

Food habits

All 30 of the tadpole madtom stomachs examined contained food items. Copepoda, Diptera (>95% Chironomidae), and Plecoptera were primary diet items in May (N=11; Table 2). During July (N=7) and September (N=12),

Table 2. Summary of food items found in tadpole madtom stomachs collected from Erickson Island Slough, North Dakota, in May (n=11), July (n=7), and September N=12) of 1998. Percent by number (total number of items in each category/total number of items in all categories), and frequency of occurrence (number of fish containing food item/total number of fish) are reported.

	Percent by number			**Frequency of occurrence**		
Diet item	**May**	**July**	**September**	**May**	**July**	**September**
Plant material	0.0	10.4	16.2	0.0	14.3	33.3
Copepoda	32.5	4.4	20.0	63.6	28.6	50.0
Cladocera	2.0	3.6	1.3	0.0	14.3	16.7
Hydracarina	0.2	0	0.2	9.1	0.0	8.3
Amphipoda	0.4	0	1.0	9.1	0.0	25.0
Corixidae	4.7	6.8	1.7	45.5	57.1	8.3
Diptera	21.3	59.9	56.8	54.5	100.0	100.0
Ephemeroptera	0.7	0	0	9.1	0.0	0.0
Odonata	0.2	0	0.4	9.1	0.0	16.7
Plecoptera	22.4	10.2	1.1	81.8	57.1	16.7
Trichoptera	11.5	4.7	0.5	54.5	28.6	16.7
Fish eggs	4.0	0	0	9.1	0.0	0.0
Fishes	0	0	0.8	0.0	0.0	8.3

Diptera made up more than 55% by number of the tadpole madtom diet and Plecoptera and Trichoptera utilization decreased. Diptera were also utilized by most tadpole madtoms, occurring in 55% of the stomachs in May and 100% of the stomachs in July and September. Corixidae and Copepoda were the most persistent stomach contents among seasons and late summer diets indicated some usage of plant material and other items, such as small fish, Amphipoda, and Odonata. Regardless of sample region, tadpole madtom diets appear to be dominated by insect larvae and zooplankton. Whiteside and Burr (1986) reported that the predominant stomach contents of 267 tadpole madtoms col-

lected in southern Illinois were Diptera larvae (primarily Chironomidae) and Crustacea. Historical reports (e.g., Adams and Hankinson 1928) also indicated that tadpole madtoms primarily feed on Chironomidae and Crustacea.

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SEASONAL FOOD HABITS OF BLUEGILLS IN RICHMOND LAKE, SOUTH DAKOTA

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ABSTRACT

Although the bluegill *Lepomis macrochirus* is a popular panfish species in South Dakota, little information has been collected on seasonal trends in food habits in this geographic location. Richmond Lake, a 336-ha impoundment located in northeastern South Dakota, contained a moderate density bluegill population. We collected bluegills using trap nets in April, early June, late July, and October of 1998. Stomachs were removed from up to 20 bluegills in each of three length groups (80-149, 150-199, and >200 mm) on each date. Throughout the study, and across length groups, *Daphnia*, chironomids, and corixids made up the majority of bluegill diets. Based on percent by number and weight, bluegills in the smallest length group primarily fed on zooplankton. *Daphnia* continued to dominate by number in the larger length groups, but their importance by weight decreased, especially in later months. Bluegills in the two larger length groups principally consumed *Daphnia* in April, and switched to a diet dominated by corixids and chironomids in both June and July. No larger bluegills were collected in October.

Keywords

bluegill, *Lepomis macrochirus*, food habits, South Dakota

INTRODUCTION

The bluegill *Lepomis macrochirus* is a popular panfish often sought by anglers, and can also serve as a prey source for predatory fishes. While extensive work on food habits of other panfishes such as crappies *Pomoxis* spp. and yellow perch *Perca flavescens* has recently been completed in South Dakota (Guy and Willis 1993; Lott et al. 1996; Fisher and Willis 1997; Pope and Willis 1998), no recent work has been completed on bluegill food habits. Seaburg and Moyle (1964) found that the majority of bluegill diets in Maple and Grove lakes, Minnesota, were aquatic insects, followed by aquatic plant material. The objective of this project was to determine the seasonal food habits of bluegills in a South Dakota impoundment.

STUDY SITE

Richmond Lake is a 336-ha impoundment located in Brown County near Aberdeen, SD. The lake has a maximum depth of 8.8 m, a mean depth of 4.6 m, and is considered eutrophic to hypereutrophic based on the trophic state index (Stueven and Stewart 1996). The panfish community in Richmond Lake includes black crappie *Pomoxis nigromaculatus*, bluegill, and a low density of green sunfish *Lepomis cyanellus*. Other common fish community members include walleye *Stizostedion vitreum*, saugeye [i.e., purposeful walleye X sauger *Stizostedion canadense* hybrids (Pope et al. 1996)], channel catfish *Ictalurus punctatus*, black bullhead *Ameiurus melas*, common carp *Cyprinus carpio*, and white sucker *Catostomus commersoni*.

METHODS

Bluegills were collected from April 27–May 2, June 2–8, July 28–August 3, and October 5–9 of 1998 to determine seasonal patterns in food habits. Trap (modified fyke) nets with 13-mm bar mesh were used to capture the bluegills. Short-term sets of approximately 4-hr duration were used to reduce the extent of digestion while fish were in the nets; however, when catch per unit effort was low, nets were set overnight. Collected bluegills were measured (nearest millimeter), weighed (nearest gram) and immediately placed in an ice bath to prevent regurgitation and further digestion of stomach contents. Stomachs were removed from up to 20 bluegills per length group (80-149 mm, 150-199 mm, and >200 mm) and stored in 10% formalin until analysis in the laboratory. Lengths were taken from first 20 organisms of each prey taxon, when possible, and total wet weights were recorded to the nearest 0.01 g for the different prey taxa in each stomach.

Stomach contents were quantified using frequency of occurrence (number of fish where specific taxon was present in stomach divided by total number of fish), percentage by number (number of specific taxon divided by total number of food items present in each stomach), and percentage by weight (weight of specific taxon divided by total weight of stomach contents in each stomach). However, percent by weight is probably the most important due to its indication of caloric value of consumed food items.

Electivity of bluegills for zooplankton was determined using the linear electivity index (Strauss 1979), which is calculated as:

$$L = r_i - p_i ,$$

where r_i is the relative abundance of prey type "I" in the diet, and p_i is the relative abundance of prey type "I" in the environment. Values range from +1 to –1, with +1 indicating complete selection of a prey item, and –1 indicating complete avoidance.

Zooplankton samples were collected with a 2-m long, 75-mm diameter tube sampler. Offshore and near shore samples were taken from each of four different sites, with three samples taken from each location. Samples were fil-

tered through a 70-*u*m plankton net and preserved in 4% sucrose-formalin. In the lab, samples were diluted to 60 mL, and three 2-mL subsamples were drawn with a Henson-Stempel pipet and all organisms in the subsamples were enumerated. Total numbers were then extrapolated from the subsample data. Zooplankton densities were enumerated as number/L of filtered water, and percent composition by number was determined for electivity analysis.

Benthos samples were collected with a Ponar grab in the same manner as zooplankton samples. Samples were sifted through a mesh screen and preserved in 4% sucrose-formalin solution. Benthic organisms were identified and enumerated in the laboratory. However, this gear did not sample the entire macroinvertebrate community, so we could not determine electivity of bluegills for this food type.

RESULTS

Diet overview

A total of 116 stomachs were collected from bluegills in Richmond Lake. No bluegills from the two larger length groups (150-199 mm, and >200 mm) were sampled in October. During all sampling periods, zooplankton and macroinvertebrates were the only prey items observed in bluegill stomachs (Table 1). No fish were ever observed in bluegill stomachs. During April, macroinvertebrates and zooplankton were nearly equal in diet composition by weight for 150- to 199-mm bluegills, while zooplankton made up the majority of diet by weight for the larger (>200 mm) bluegills. Macroinvertebrates were the prevalent prey item for all three length groups in June. During July and October, macroinvertebrates were the principal diet item for bluegills >150 mm while zooplankton was the primary food item consumed by 80- to 149-mm bluegills.

Diet of 80- to 149-mm bluegills

Only four prey groups were consumed by this length group of bluegills during all sampling dates at Richmond Lake (Table 1). In April, only one bluegill was sampled and its stomach contained only macroinvertebrates (chironomids and corixids). June sampling yielded only one bluegill as well; however, its stomach showed a more equal distribution of food items. Chironomids made up the highest percentage of its diet by weight, followed by nearly equal proportions of corixids, calanoid copepods, and *Daphnia*. *Daphnia* constituted nearly 90% of the diet by weight for bluegills collected during July and October. Zooplankton commonly dominate the diet of small (e.g., <150 mm) panfish, including yellow perch (Lott et al. 1996) and white crappies (Guy and Willis 1993). In general, zooplankton tend to constitute a higher proportion of adult panfish diets in "stunted" populations compared with lower density populations (e.g., Mills and Schiavone 1982; Mills et al. 1987; Lott et al. 1996).

Table 1. Food habits by length group and date for bluegills collected from Richmond Lake, South Dakota, during 1998. Specific sampling dates can be found in the Methods. FOO = frequency of occurrence; PBN = percent by number; PBW = percent by weight; macroinvert. = macroinvertebrates.

Length (mm)	Month	N	Food item	FOO	n	PBN	Total weight (g)	PBW
80-149	April	1	Chironomidae	100.0	45	95.7	0.82	98.6
			Corixidae	100.0	2	4.3	0.01	1.4
	June	1	*Daphnia* spp.	100.0	26	36.6	0.01	20.0
			Calanoid copepods	100.0	34	47.9	0.01	20.0
			Chironomidae	100.0	9	12.7	0.02	40.0
			Corixidae	100.0	2	2.8	0.01	20.0
	August	37	*Daphnia* spp.	100.0	8225	99.7	2.40	92.3
			Calanoid copepods	5.6	2	--	--	--
			Corixidae	38.9	21	0.3	0.20	7.7
			Culicidae	5.6	1	--	--	--
			Trichoptera	5.6	1	--	--	--
	October	8	*Daphnia* spp.	100.0	494	97.4	0.24	88.9
			Chironomidae	12.5	4	0.8	0.01	3.7
			Corixidae	37.5	9	1.8	0.02	7.4
150-199	April	11	*Daphnia* spp.	100.0	5567	93.1	4.67	45.0
			Trichoptera	9.1	2	--	0.01	0.1
			Plecoptera	9.1	1	--	0.01	0.1
			Ephemeroptera	18.2	3	--	0.14	1.3
			Chironomidae	81.8	312	5.2	4.90	47.2
			Corixidae	81.8	83	1.4	0.60	5.8
			Culicidae	27.3	7	0.1	0.05	0.5
			Other macroinvert.	18.2	2	--	--	--
	June	17	*Daphnia* spp.	58.8	4746	91.6	2.31	26.3
			Trichoptera	11.8	2	--	0.01	0.1
			Ephemeroptera	17.6	7	0.1	1.10	12.5
			Chironomidae	52.9	63	1.2	1.60	18.2
			Corixidae	100.0	333	6.4	3.50	39.8
			Other macroinvert.	11.8	31	0.6	0.28	3.2
	August	12	*Daphnia* spp.	83.3	2666	90.0	0.94	26.6
			Trichoptera	41.7	14	0.5	0.30	8.5
			Plecoptera	8.3	1	--	--	--
			Chironomidae	16.7	20	0.7	0.10	2.8
			Corixidae	100.0	260	8.8	2.20	62.1
			Culicidae	16.7	2	0.1	--	
≥200	April	3	*Daphnia* spp.	67.0	2750	97.0	2.60	79.0
			Ephemeroptera	33.0	1	--	0.01	0.3
			Chironomidae	67.0	3	0.1	0.02	0.6
			Corixidae	67.0	76	2.7	0.60	18.2
			Culicidae	33.0	4	0.1	0.02	0.6
			Other macroinvert.	33.0	1	--	0.04	1.2
	June	8	*Daphnia* spp.	75.0	2573	91.1	1.45	24.5
			Trichoptera	12.5	1	--	--	--
			Ephemeroptera	12.5	2	--	0.19	3.2
			Chironomidae	25.0	10	--	0.10	1.7
			Corixidae	87.5	238	8.4	3.80	64.1
			Other macroinvert.	12.5	1	--	0.39	6.6
	August	18	*Daphnia* spp.	66.7	1553	70.7	0.55	7.5
			Trichoptera	16.7	13	0.6	0.20	2.7
			Plecoptera	11.1	2	0.1	0.20	2.7
			Chironomidae	5.6	1	--	0.10	1.4
			Corixidae	88.9	615	28.0	5.40	73.5
			Culicidae	11.1	5	0.2	0.10	1.4
			Other macroinvert.	33.3	9	0.4	0.8	10.9

Diet of 150- to 199-mm bluegills

The 150- to 199-mm bluegills had greater variety in their diet than did the smaller bluegills (Table 1). In April, *Daphnia* and chironomids dominated the diet in nearly equal proportions by weight. In June and July, corixids made up the most biomass of the diet. *Daphnia* were always a substantial part of the diet for this length group during all three sampling periods. However, the percent by weight for *Daphnia* declined from 45% in April to 26% in both June and July. *Daphnia* consumption may be a supplement to the bluegill diet in the presence of an insufficient macroinvertebrate population. We wonder if the lack of submergent aquatic macrophytes in Richmond Lake results in less diversity of aquatic insects, perhaps resulting in more reliance on *Daphnia* as a food source.

Diet of 200-mm and longer bluegills

The diet for this length group was dominated by *Daphnia* in April, and corixids in June and July (Table 1). While a number of other macroinvertebrates, such as chironomids, ephemeropterans, culicids, trichopterans, and plecopterans were consumed by these larger bluegills, these macroinvertebrates made up a small portion of the total weight of food items consumed.

Many panfish species change diets from zooplankton to larger prey items as they grow. Crappies and yellow perch may also become more piscivorous as they grow; however, most growth of adult yellow perch (Lott et al. 1996) and white crappie (Guy and Willis 1993) in South Dakota resulted from a diet of zooplankton and macroinvertebrates. Although bluegills may occasionally consume fish, they are not typically considered piscivorous (Seaburg and Moyle 1964). Thus, bluegill selection of lower numbers of larger prey leads them to feed on macroinvertebrates, but zooplankton persists as a considerable portion of their diet. We did not find any fish in the stomachs of bluegills, including the largest length group, in Richmond Lake.

Linear electivity index

Electivity of bluegills for various zooplankton taxa present in Richmond Lake was similar across length groups (Table 2). For all three length groups, during all sample dates when each was collected, *Daphnia* spp. were positively selected by bluegills. The only other positive linear electivity index value was for calanoid copepods, which were positively selected by 80- to 149-mm bluegills during June. Linear electivity index values were negative for all other zooplankton taxa collected in environmental samples in Lake Richmond. Electivity values for the 150- to 199-mm and >200-mm length groups were identical because *Daphnia* were the only zooplankton consumed by bluegills in these groups, making percent by number 100% for both length groups.

Most of the *Daphnia* consumed by bluegills in Richmond Lake exceeded 1.3 mm in diameter. The only other zooplankton group for which we collected specimens that exceeded 1.3 mm was the calanoid copepods, and individ-

Table 2. Linear electivity index values for zooplankton consumed by bluegills (BLG) by date in Lake Richmond, South Dakota, during 1998. Specific dates of sampling are included in the Methods.

BLG length (mm)	Taxon	April	June	August	October
80-149	*Daphnia*	a	0.19	0.55	0.75
	Bosmina	a	-0.01	b	-0.38
	Other Cladocera	a	b	-0.02	b
	Calanoid Copepoda	a	0.28	-0.08	-0.15
	Cyclopoid Copepoda	a	-0.03	-0.16	-0.03
	Copepoda nauplii	a	-0.25	-0.29	-0.15
	Rotifera	a	-0.19	b	-0.04
150-199	*Daphnia*	0.90	0.69	0.55	c
	Bosmina	-0.20	-0.01	b	c
	Other Cladocera	b	b	-0.02	c
	Calanoid Copepoda	-0.15	-0.22	-0.08	c
	Cyclopoid Copepoda	-0.11	-0.03	-0.16	c
	Copepoda nauplii	-0.30	-0.25	-0.29	c
	Rotifera	-0.14	-0.19	b	c
>200	*Daphnia*	0.90	0.69	0.55	c
	Bosmina	-0.20	-0.01	b	c
	Other Cladocera	b	b	-0.02	c
	Calanoid Copepoda	-0.15	-0.22	-0.08	c
	Cyclopoid Copepoda	-0.11	-0.03	-0.16	c
	Copepoda nauplii	-0.30	-0.25	-0.29	c
	Rotifera	-0.14	-0.19	b	c

a = no zooplankton consumed by this BLG length group on this date
b = this zooplankton group not collected in environmental samples on this date
c = no BLG within this length group were collected on this date

uals that exceeded this size were quite rare. Thus, bluegills in Lake Richmond were apparently selecting the largest available zooplankton. Lott et al. (1998) found that the percent of *Daphnia* that exceeded 1.3 mm in environmental samples were useful predictors of both yellow perch growth and size structure in eastern South Dakota lakes.

ACKNOWLEDGMENTS

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USE OF FTIR IN THE EXAMINATION OF QUATERNIZATION REACTIONS USING DIMETHYL CARBONATE

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ABSTRACT

The purpose of this research was to evaluate the quaternization reactions of tertiary amines and the alkylating agent dimethyl carbonate using FTIR (Fourier transform infrared spectroscopy).

Keywords

Dimethyl carbonate, methyl carbonate anion, quaternary ammonium compounds, FTIR

INTRODUCTION

Industry currently uses the highly toxic alkylating agents methyl chloride and dimethyl sulfate for the quaternization of tertiary amines. Although these alkylating agents do not survive in the final product, plant workers are at risk of accidental exposure. This research examines dimethyl carbonate as an alternative alkylating agent because of its very low toxicity rating. The original goal of this particular work was to find a means of following the quaternization reactions using FTIR. Various quaternary ammonium compounds and tertiary amines were examined. In addition, the FTIR of the methyl carbonate anion was identified.

The anion of methyl carbonate quaternaries can be replaced with the anions of various acids. A few preliminary reactions involving this conversion of the methyl carbonate anion were conducted using both weak and strong acids.

METHODS

A Parr pressure reactor was used for the quaternization reactions. Methyl alcohol was used as the reaction solvent and the headspace was purged with nitrogen gas to remove ambient oxygen. Materials used were reagent grade chemicals. Various tertiary amines were quaternized including tributylamine and tridodecylamine at reaction temperatures around 150 C and pressures around 200 psi.

Prior to FTIR analysis the methyl alcohol solvent and excess dimethyl carbonate were evaporated using a Brickmann Büchi Rotavapor apparatus. An

IBM Instruments IR32 FTIR was used to collect spectral data. Neat liquid samples were analyzed between sodium chloride plates.

RESULTS AND DISCUSSION

An example FTIR spectrum (Fig. 1) of the amine reactant and the quaternary product shows the peak overlap. This prevents using FTIR for following the extent of quaternization.

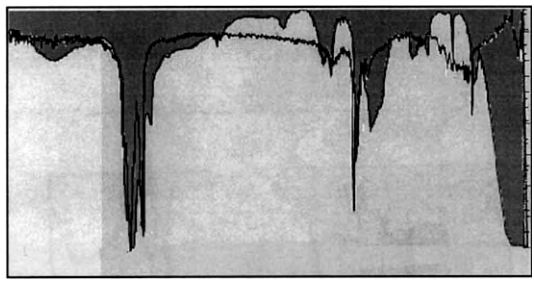

Figure 1. FTIR spectra of tridodecylmethylammonium iodide (overlay) and tridodecylamine (filled spectrum).

An FTIR spectrum of the methyl carbonate anion was not found in the chemical literature; therefore, the methyl carbonate anion was acquired by reacting sodium methoxide with dimethyl carbonate to produce the desired anion and dimethyl ether. The carbonyl peak frequency in dimethyl carbonate (~1757 cm-1) was expected to decrease due to resonance, and the carbon-oxygen bond (~1280 cm-1) frequency was expected to increase.

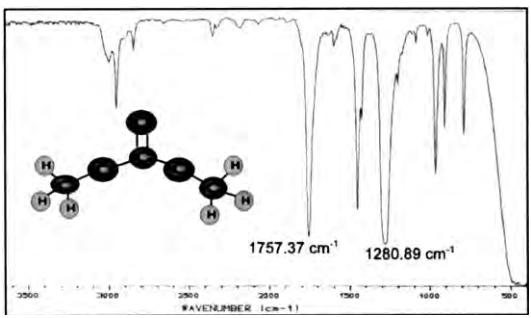

Figure 2. FTIR spectrum of dimethyl carbonate.

An example FTIR spectrum (Fig. 3) of the sodium methyl carbonate anion shows that the predicted frequency changes were accurate.

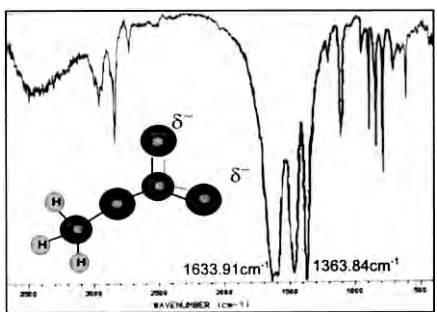

Figure 3. FTIR spectrum of the methyl carbonate anion

The possibility of using the carbonyl peak of the methyl carbonate anion for calibration was considered; however, in our hands the methyl carbonate anion converts to hydrogen carbonate in the presence of water. An example FTIR spectrum (Fig. 4) of a methyl carbonate quaternary product after exposure to water shows peaks matching those of hydrogen carbonate (Fig. 5). Therefore, it was concluded that FTIR was not a feasible means of following the extent of reaction.

The next portion of the work involves making new quaternaries possessing different anions. The methyl carbonate quaternary was treated with various acids to form new compounds via the following reaction:

$$Quat^{+}\,Anion + H^{+}\,New\ Anion \rightarrow Quat^{+}\,New\ Anion + CO_2 + Methanol$$

Conversions with strong acids go to completion. An example of a strong acid product is the following sulfate quaternary (Fig. 6).

Conversions with weak acids are problematic in that the reaction does not go to completion. The methyl carbonate anion acts as a weak base which does not react well with a weak acid. Therefore, the methyl carbonate quaternary was converted to the hydroxide quaternary using calcium hydroxide. The basicity of the hydroxide anion (pH ~14) thus allows for a complete reaction using a weak acid. An example of this process for the lactate quaternary is shown in figures 7, 8, 9, and 10. A partial anion conversion resulted using only lactic acid (Fig. 7); however, complete conversion was achieved via the hydroxide quaternary intermediate (Fig. 9).

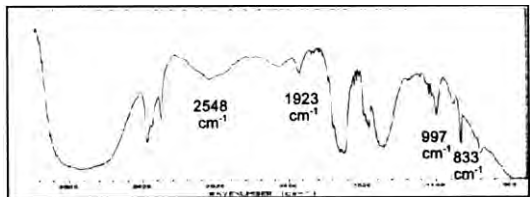

Figure 4. FTIR spectrum of Tributylmethylammonium hydrogen carbonate.

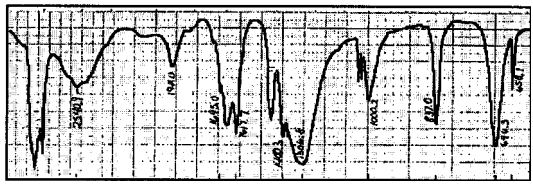

Figure 5. FTIR spectrum of sodium hydrogen carbonate from Aldrich literature.

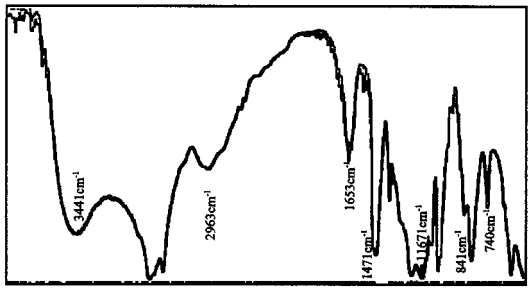

Figure 6. FTIR spectrum of Tributylmethylammonium sulfate.

CONCLUSION

Infrared spectroscopy is not a good method for following quaternization reactions of tertiary amines and dimethyl carbonate. The conversion of methyl carbonate quaternaries to hydroxide quaternaries provides a means by which many new quaternaries can be made.

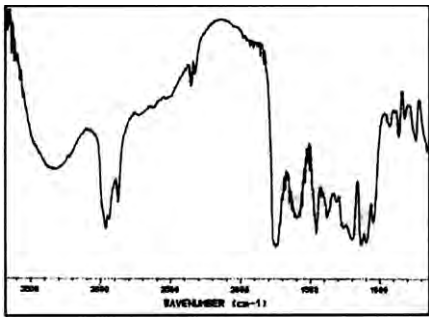

Figure 7. FTIR spectrum of Tributylmethylammonium lactate made without the hydroxide quaternary intermediate.

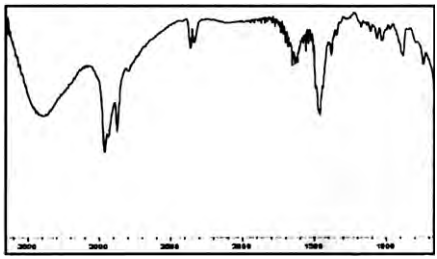

Figure 8. FTIR spectrum of the Tributylmethylammonium hydroxide quaternary intermediate.

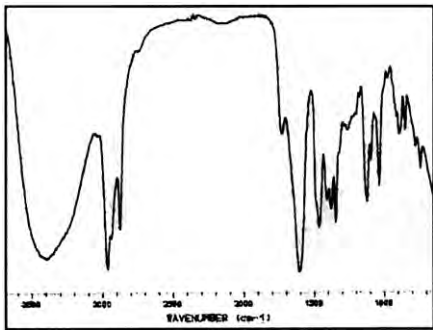

Figure 9. FTIR spectrum of Tributylmethylammonium lactate made via the hydroxide quaternary.

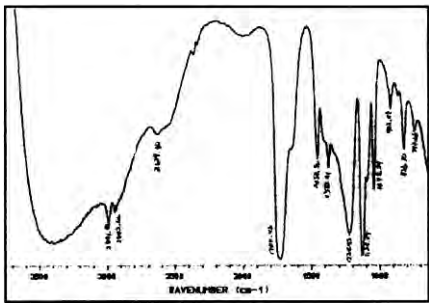

Figure 10. FTIR spectrum of Lactic acid.

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GENERIC PROGRAMMING IN C++

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ABSTRACT

The ability to write generic functions and classes is becoming increasingly important in software development and maintenance. The programming language C++ supports several fundamentally different paradigms, one of which is generic programming. While there are literally hundreds of books discussing the language C++, not many of them cover the subject of generic programming. It is the goal of this paper to demonstrate some of the basic skills in writing and using generic components in C++ by stepping through a detailed example. We achieve genericness in our example by using the template class and operator overloading features provided by C++.

Keywords

Generic Programming, Template Classes, Operator Overloading

1. INTRODUCTION

The ability to write generic functions and classes is becoming increasingly important in software development and maintenance. The programming language C++ supports several fundamentally different paradigms, one of which is generic programming [1]. Although many would agree that it is vital for students to know how to create generic programs and understand the importance of reusable and portable software, many of the leading textbooks on C++ provide little treatment on the subject of generic programming [2, 3, 4, 5, 6, 7, 8, 9]. A literature search using keywords "generic programming", "operator overloading", and "generic templates" produced only a few references that are somewhat related to writing generic functions and templates [10, 11, 12]. It is the goal of this paper to introduce some of the basic concepts of generic programming by presenting a detailed example which makes use of the template class and operator overloading features provided by C++.

We begin by presenting a very specific class (intlist) which supports a list of integers in section 2. Note that if a list of real numbers is needed, class intlist cannot be used since it is specifically designed to work with integers only. The programmer could write another class called doublelist to support a list of real numbers. However, since intlist and doublelist are essentially the same, doing this would be a waste of programming effort. To overcome this difficulty, Section 3 discusses how we can rewrite intlist to make a generic list class (list<T>), which can support a list of any data type T. The implementation of

a generic class such as list<T> typically assumes certain properties about the data type T. If the data type T supplied by the client program does not satisfy these properties, the list<T> class will not behave properly. Section 4 describes situations where the data type T does not satisfy the requirements set forth by list<T>. Finally, in section 5, we explain how the data type T should be prepared so that it can be used with list<T>.

2. A SPECIFIC CLASS – INTLIST

Figure 1 shows the specifications of class intlist, which supports a list of integers. Since the focus of this paper is to discuss the techniques in writing generic programs, the operations supported by class intlist are very rudimentary. Figure 2 shows a client program that uses intlist. Note that the client program does not need to know how intlist is implemented in order to use it. Figure 3 gives the output generated when the client program is run. Figure 4 gives the implementation of intlist.

class intlist:
A list of integers.

Object instantiation:
intlist x;

Public Member Methods:

a. Desc : Constructor to initialize a list of integers.
Usage : intlist (void);
Pre : None.
Post : *this initialized to empty.

b. Desc : Copy constructor to constructs a list of integers as a copy of another intlist.
Usage : intlist (const intlist& x);
Pre : x is the intlist to be copied.
Post : *this initialized as an exact copy of x.

c. Desc : Inserts an element to the end of an intlist.
Usage : intlist& insertlast (int x);
Pre : x is the integer to be inserted.
Post : x inserted to the end of *this.
*this returned.

d. Desc : Deletes the last element of an intlist.
Usage : intlist& deletelast (int& x);
Pre : x is where the deleted element will be put.
*this is not empty.
Post : Last element is deleted from the intlist and placed in x.
*this returned.

e. Desc : Checks whether an intlist is empty.
Usage : bool empty (void);
Pre : none.
Post : Returns true if *this is empty and false if it is not.
f. Desc : Assigns a copy of an intlist to *this.
Usage : const intlist& operator=(const intlist& x);
Pre : x is the intlist to assign to *this.
Post : *this contains an exact copy of x.
*this returned.
g. Desc : Destructor to destroy an intlist.
Usage : ~intlist (void);
Pre : None.
Post : *this destroyed.

Global Friend Functions:
h. Desc : Outputs an intlist to an output stream.
Usage : ostream& operator<< (ostream& os, const intlist& x);
Pre : x is the intlist to be printed.
os is the output stream.
Post : Each element of x is outputted to os on separate lines.
os returned.

Figure 1. Specification of class intlist.

```
#include "intlist.h"
#include <iostream.h>
void main (void)
{
    intlist x;
    x.insertlast(1);
    x.insertlast(2);
    cout << "The integers are: \n" <<x;
}
```

Figure 2. A simple client program which uses intlist – mytest.cpp.

```
The integers are:
1
2
```

Figure 3. The output generated when mytest.cpp is run.

```
#ifndef intlist_h_
#define intlist_h_
#include <iostream.h>

class intlistnode
{
     int data;
     intlistnode *link;
friend class intlist;
friend ostream& operator<<(ostream&, const intlist&);
};

class intlist
{
     intlistnode *head;
public:
     intlist(void);
     intlist(const intlist& x);
     intlist& insertlast(int x);
     intlist& deletelast(int& x);
     bool empty(void) const;
     const intlist& operator=(const intlist& x);
     ~intlist(void);
friend ostream& operator<<(ostream&, const intlist&);
};

intlist::intlist(void)
{
     head=NULL;
}
intlist::intlist(const intlist& x)
{
     intlistnode *temp=x.head;
     head=NULL;
     while (temp!=NULL)
     {
          insertlast(temp->data);
          temp=temp->link;
     }
}

intlist& intlist::insertlast(int x)
{
     intlistnode *temp, *q=head;
     temp=new intlistnode;
     temp->data=x;
     temp->link=NULL;
```

```
    if (q==NULL) head=temp;
    else {
            while ((q->link)!=NULL)
                q=q->link;
            q->link=temp;
        }
    return *this;
}

intlist& intlist::deletelast(int& x)
{
    if (empty()) cout<<"Empty list.  Deletelast failed.\n";
    else {
            intlistnode *temp = head;
            if ((temp->link)==NULL)
            {
                x=temp->data;
                head=NULL;
            }
            else
            {
                while ((temp->link->link)!=NULL)
                    temp=temp->link;
                x=temp->link->data;
                delete temp->link;
                temp->link=NULL;
            }
        }
    return (*this);
}

bool intlist::empty(void) const
{
    return (head==NULL);
}
const intlist& intlist::operator=(const intlist& x)
{
    if (this == &x) return *this;
    while (head!=NULL)
    {
        intlistnode *temp=head;
        head=head->link;
        delete temp;
    }
    intlistnode *temp=x.head;
    head=NULL;
    while (temp!=NULL)
```

```
    {
        insertlast(temp->data);
        temp=temp->link;
    }
    return (*this);
}

intlist::~intlist(void)
{
    while (head!=NULL)
        {
            intlistnode*temp=head;
            head=head->link;
            delete temp;
        }
}

ostream& operator<<(ostream& os, const intlist& x)
{
    intlistnode *temp=x.head;
    while (temp!=NULL)
    {
        os<<(temp->data)<<endl;
        temp=temp->link;
    }
    return os;
}
#endif
```

Figure 4. The implementation of class intlist – intlist.h.

3. A GENERIC LIST CLASS—LIST<T>

The problem with class intlist is that it can only be used to support a list of integers and not a list of any other data type. We can always write other classes that support lists of other data types. Doing so, however, is a waste of effort and time since the implementation of these classes will be essentially the same. This is where the C++ template class feature comes to the rescue. A list for any type of data becomes possible using the template class. We will rewrite class intlist to make it a generic class, called list<T>, which can be used for a list of any data type T. Figure 5 gives the specification of class list<T>. Figure 6 shows a client program that uses list<T>. Figure 7 shows the output generated when the client program is run. Figure 8 gives the implementation of list<T> (the line numbers are added so that we can reference them in section 5).

class list<T>:
A list of elements of any data type T.

Object instantiation:
list<T> x; //T can be any built-in type like int or double, or it can be any user-defined type

Public Member Methods:
//Omitted here since they are similar to those listed in Figure 1.

Global Friend Functions:
//Omitted here since they are similar to those listed in Figure 1.

Figure 5. Specifications of class list<T>.

```
#include "listt.h"
#include <iostream.h>
void main (void)
{
      int n;
      list<int> x;
      x.insertlast(1);
      x.insertlast(2);
      cout << "The integers are: \n" <<x;
      x.deletelast(n);
      cout<< "The deleted element is: "<< n <<endl;
      cout << "The integers are: \n" <<x;

      double d;
      list<double> y;
      y.insertlast(1.1);
      y.insertlast(2.2);
      cout << "The real numbers are: \n" <<y;
      y.deletelast(d);
      cout << "The deleted element  is: "<<d<<endl;
      cout << "The real numbers are: \n" <<y;
}
```

Figure 6. A simple client program which uses list<T> -- mytest2.cpp.

```
The integers are:
1
2
The deleted element is: 2
The integers are:
1
The real numbers are:
1.1
2.2
The deleted element is: 2.2
The real numbers are:
1.1
```

Figure 7. The output generated when mytest2.cpp is run.

```
1.  #ifndef _listt.h_
2.  #define _listt.h_
3.  #include <iostream.h>
4.  template <class T>
5.  class list;
6.  template <class T>
7.  class listnode
8.  {
9.        T data;
10.       listnode *link;
11. friend class list<T>;
12. friend ostream& operator<<(ostream&, const list<T>&);
13. };
14. template<class T>
15. class list
16. {
17. private:
18.       listnode<T> *head;
19.       listnode<T> *makenode(T x);
20. public:
21.       list(void);
22.       list(const list& x);
23.       list& insertlast(T x);
24.       list& deletelast (T& x);
25.       bool empty(void) const;
26.       const list& operator=(const list& x);
27.       ~list(void);
28. friend ostream& operator<<(ostream&, const list&);
29. };
20. template<class T>
```

```
21. list<T>::list(void)
22. {
23.       head=NULL;
24. }
25. template<class T>
26. list<T>::list(const list& x)
27. {
28.       listnode<T> *temp=x.head;
29.       head=NULL;
30.       while (temp!=NULL)
31.       {
32.             insertlast(temp->data);
33.             temp=temp->link;
34.         }
35. }
36. template<class T>
37. listnode<T> *list<T>::makenode(T x)
38. {
39.       listnode<T> *temp;
40.       temp= new listnode<T>;
41.       temp->data=x;
42.       temp->link=NULL;
43.       return temp;
44. }
45. template<class T>
46. list& list<T>::insertlast(T x)
47. {
48.       listnode<T> *temp, *q = head;
49.       temp= makenode(x);
50.       if (q==NULL) head=temp;
51.       else  {
52.                   while ((q->link)!=NULL)
53.                         q=q->link;
54.                   q->link=temp;
55.               }
56.       return (*this);
57. }
58. template<class T>
59. list& list<T>::deletelast(T& x)
60. {
61.       if (empty()) cout<<"Empty list.  Deletelast failed.\n";
62.       else {
63.                   listnode<T> *temp = head;
64.                   if ((temp->link)==NULL)
65.                   {
66.                         x=temp->data;
67.                         head=NULL;
68.                   }
```

```
69.                 else
70.                 {
71.                     while ((temp->link->link)!=NULL)
72.                     temp=temp->link;
73.                     x=temp->link->data;
74.                     delete temp->link;
75.                      temp->link=NULL;
76.                  }
77.             }
78.       return (*this);
79. }
80. template<class T>
81. bool list<T>::empty(void) const
82. {
83.       return (head==NULL);
84. }
85. template<class T>
86. const list& list<T>::operator=(const list& x)
87. {
88.       listnode<T> *temp=x.head;
89.       if (this==&x) return *this;
90.       (*this).~list();
91.       while (temp!=NULL)
92.       {
93.             insertlast(temp->data);
94.             temp=temp->link;
95.       }
96.       return *this;
97. }
98. template<class T>
99. list<T>::~list(void)
100. {
101.      while (!empty())
102.      {
103.            listnode<T> *temp=head;
104.            head=head->link;
105.            delete temp;
106.      }
107. }
108. template<class T>
109. ostream& operator<<(ostream& s, const list<T>& x)
110. {
111.      listnode<T> *temp=x.head;
112.      if (x.head==NULL) s<<"empty list!!"  <<endl;
113.      else  while (temp!=NULL)
114.            {
115.                  s<<temp->data<<endl;
```

```
116.                temp=temp->link;
117.            }
118.      return s;
119.  }
120.  #endif
```

Figure 8. The implementation of class list<T> – listt.h.

4. HOW GENERIC IS LIST<T>?

Though this new generic class list<T> seems to be fine as far as reusability is concerned, one needs to be careful when using it. Otherwise, very common data types, such as a simple struct or char*, will cause errors as illustrated in section 4.1 and 4.2.

4.1 list<item>

Suppose the manager of a grocery store would like to keep track of each grocery item by storing the name and price of every item in the store. Figure 9 gives an example of such a client program.

```
#include <iostream.h>
#include <fstream.h>
#include "listt.h"

struct item
{
    char name[100];
    double price;
};

void main(void)
{
    item temp;
    list<item> itemlist;

    strcpy(temp.name, "apple");
    temp.price=0.99;
    itemlist.insertlast(temp);
    strcpy(temp.name, "orange");
    temp.price=0.49;
```

```
    itemlist.insertlast(temp);
    cout<<itemlist;
}
```

Figure 9. A simple client program which uses list<T> -- grocery.cpp

Though grocery.cpp seems uncomplicated, attempting to compile and run this program reveals that it will not even compile. The problem is that when the compiler tries to instantiate list<item>, it encounters a statement in list<item> that it does not know how to handle. This statement is:

```
115.        s<<temp->data<<endl;
```

The reason is obvious: the compiler cannot find a function named << which can be used to output a struct item to an ostream object.

4.2 list<char *>

A careless attempt to use list<T> for a list of char* objects creates even more problems. Figure 10 shows an example of a client program using such a type. Figure 11 shows the output generated when the client program is run.

```
#include <iostream.h>
#include <string.h>
#include "listt.h"
void main (void)
{
    char name[21];
    list<char *> names;

    strcpy(name, "Johnson");
    names.insertlast(name);
    strcpy(name, "Paulson");
    names.insertlast(name);
    cout <<names;
}
```

Figure 10. A client program which uses list<T> -- mytest3.cpp.

Paulson
Paulson

Figure 11. The output generated when mytest3.cpp is run.

Although mytest3.cpp compiles, the output is not what the programmer has intended. Walking through the program reveals that when insertlast is called for the first time to insert name to the list, the value of name is inserted. However, the value of name is the address of the buffer where "Johnson" is stored. Then when insertlast is called for the second time to insert name to the list, the address of the same buffer is inserted into the list. Since the buffer now contains "Paulson", when the list is printed, "Paulson" is printed out twice.

5. DOCUMENTATION OF A GENERIC CLASS

The reason that list<char *> and list<item> do not work is not because list<T> is written incorrectly. It is because list<T> failed to provide proper documentation to inform its users how to prepare class T for use with list<T>. A generic class should have two parts: the set of generic instructions that defines the class and the set of requirements that its argument types must satisfy. To discover what the set of requirements are, the programmer who implements the generic class must go through the set of generic instructions and examine those operators that manipulate objects of type T. Operators must be overloaded if they do not work with data type T objects (which will cause the compilation of, in this case, list<T> to fail), or when the default behaviors of those operators are not what the client program wants (which will cause, in this case, list<T> to behave strangely). Let us examine the list of instructions of list<T> in order to come up with the set of requirements. Figure 12 shows the instructions which directly manipulate an object of type T as well as the operators which might need to be overloaded in order to have those instructions to work properly on objects of type T. Figure 13 then shows the revised specification of list<T>.

	Statement	Operator
32.	insertlast(temp->data);	copy constructor
40.	temp= new listnode<T>;	constructor
41.	temp->data=x;	operator=
49.	temp= makenode(x);	copy constructor
66.	x=temp->data;	operator=
73.	x=temp->link->data;	operator=
74.	delete temp->link;	destructor
90.	(*this).~list();	destructor
93.	insertlast(temp->data);	copy constructor

105.	delete temp;	destructor
115.	s<<temp->data<<endl;	operator<<

Figure 12. Instructions of list<T> that directly manipulate objects of type T.

class list<T>:
A list of elements of any data type T.

Requirements on the data type T:
The user must overload, for objects of type T, the following operators:

a. operator =, if the default bit copy is not appropriate for objects of type T,
b. operator<<, if the default insertion << is not appropriate for objects of type T,
c. constructor, if the default constructor is not appropriate for objects of type T,
d. copy constructor, if the default copy constructor is not appropriate for objects of type T,
e. destructor, if the default destructor is not appropriate for objects of type T.

Object instantiation:
list<T> x; //T can be any built-in type like int or double, or it can be any user-defined type

Public Member Methods:
//Omitted here since they are similar to those listed in Figure1.

Global Friend Functions:
//Omitted here since they are similar to those listed in Figure1.

Figure 13. Specification of class list<T>.

Following this documentation, it is possible to use list<T> for any data type T. As examples, we will redo list<item> in section 4.1 and list<char *> in section 4.2. For the data type item, since it is a struct that contains a char array and a double, the only operators among the five specified in the requirements of list<T> which we need to overload is the operator<<. Figure 14 shows the modified data type item and Figure 15 shows the output generated when the client program is run.

```
#include <iostream.h>
#include <string.h>
#include "listt.h"
struct item
{
    char name[100];
    double price;
};
ostream& operator<<(ostream& os, const item& x)
{
    os << x.name << '  ' << x.price;
    return os;
}
void main(void)
{
    item temp;
    list<item> itemlist;

    strcpy (temp.name, "apple");
    temp.price=0.99;
    itemlist.insertlast (temp);
    strcpy (temp.name, "orange");
    temp.price=0.49;
    itemlist.insertlast (temp);
    cout<<itemlist;
}
```

Figure 14. A simple client program which uses list<T> -- revised grocery.cpp

```
apple 0.99
orange 0.49
```

Figure 15. The output generated when revised grocery.cpp is run.

For the data type char*, let us look at the problem again. The client program wants a list of char pointers to point at individual strings. The client program in Figure 10 fails because the pointers in list<char*> are pointing at the same buffer. Therefore, to get list<char*> to work, we must somehow dynamically allocate individual char buffers for the char pointers in the list to point at. Doing so requires that all five operators specified in the requirements of list<T> to be overloaded. Of course, we cannot change the semantics of the

operator= on a built-in type such as char*. Therefore, we must package the pointer inside a struct, and supply member functions for the struct. Figure 16 shows the modified struct type and Figure 17 shows the output generated when the client program is run.

```
#include <iostream.h>
#include <string.h>
#include "listt.h"
struct charstar
{
     char *name;
     charstar (void)
     {
          name=NULL;
     }
     charstar (const charstar& x)
     {
     name=new char [strlen(x.name)+1];
     strcpy (name, x.name);
     }
     const charstar& operator=(const charstar& x)
     {
          if (this==&x) return *this;
          delete [ ] name;
          name=new char [strlen(x.name)+1];
          strcpy (name, x.name);
          return *this;
      }
     ~charstar (void)
          {
          delete [ ] name;
          }
};
ostream& operator<<(ostream& os, const charstar& x)
{
     os<<x.name;
     return os;
}
void main (void)
{
     charstar n;
     list<charstar> names;
```

```
    n.name=new char[30];
    strcpy (n.name, "Johnson");
    names.insertlast (n);
    strcpy (n.name, "Paulson");
    names.insertlast (n);
    cout <<names;
}
```

Figure 16. A client program which uses list<T> -- revised mytest3.cpp.

```
Johnson
Paulson
```

Figure 17. The output generated when revised mytest3.cpp is run.

6. CONCLUSION

Although there are literally hundreds of books discussing the language C++, not many of them cover the subject of generic programming. Few people would disagree that generic programming is an important subject and students should be taught how to write generic components. However, since only a small number of references in the literature cover the subject, students might get the wrong idea that generic programming is a subject that requires a great deal of programming experience to master. We hope that this paper has shown readers that the basic concepts and skills of generic programming are not difficult. This paper has also pointed out the importance of any generic component to provide complete documentation so that it can be properly reused by others.

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LIPOPHILIC MRI CONTRAST AGENTS AS POTENTIAL MARKERS FOR CARNIVORE POPULATION STUDIES

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ABSTRACT

Radioisotope tagging is a commonly used technique in studying carnivore populations. Recent reports of the utility and metabolic fate of lipophilic MRI contrast agents have demonstrated that the lanthanide complex is excreted safely and completely, largely through the biliary system (feces).

The objective of this project is to synthesize a close structural and chemical mimic of current lipophilic Magnetic Resonance Imaging (MRI) contrast agents and determine the potential of the Europium-chelate complex as a tracking agent.

A ligand structurally analogous to the proprietary MRI ligands reported was synthesized. The europium-chelate complex was then synthesized by literature methods. Pellets were prepared from polylactic acid (PLA) mixed with the complex. PLA is a common support for tagging and marking, and decomposes in tissues with a well defined rate. Two swift fox (Vulpes velox) were implanted subcutaceously in the upper shoulder region. Fecal samples were collected from one day before implantation daily for 20 weeks. The fecal samples analyzed for Eu by AA (graphite furnace). The Eu concentration in each sample was determined by calibration curve and plotted vs. time since implantation (days). The Eu was excreted from day 2 to day 4 for both fox. This is significantly less than the normal decay of PLA and due to incomplete mixing of the complex and PLA. The plot also shows that the concentration of the Eu complex in each tablet was insufficient for long term tracking purposes. A higher amount of complex is desirable for increased signal to noise, but toxicity levels must be monitored, especially under these chronic conditions.

Keywords
Population density, carnivore, tagging, marking, lanthanide, MRI contrast agent

BACKGROUND

Radioisotope tagging is a commonly used technique in studying carnivore population density, movement in home ranges, energetic rates, and interaction among individuals (Pelton and Marcum 1977, Crabtree et al. 1989, Brady et al. 1973). This method has advantages over more traditional mark-recapture or

mark re-observe techniques such as larger sample size and no need to recapture animals to collect data (Crabtree et al. 1989). Public opinion in opposition of the release of radioisotopes into the environment drives the search for non-radioactive markers and tags for animal population study.

Radioisotopes are mixed with biodegradable polymers and formed into implants that slowly release the radioisotope into the animals system as the polymer hydrolyses under physiological conditions (Crabtree et al. 1989, Brady et al. 1973). The polymer extends the length of the marking and tracking period to approximately six months, the minimum required for adequate tracking study (Brady et al. 1973). Radioisotopes are detected in the feces over this period. The most common radioisotopes used for population density study are ^{65}Zn and ^{54}Mn (Shirley et al. 1988). Many isotopes are limited in their use due to short body retention times. Calculations based on the comparison of marked and unmarked fecal samples allow conclusions to be made on population sizes, density and behavior.

Several magnetic resonance imaging (MRI) contrast agents have been developed to be liver specific (Muhler et al. 1991, Pavone et al. 1990, Weinman et al. 1991). These compounds have in common, lipophilic groups that enhance the fat solubility of the MRI agent. These compounds are excreted primarily through the biliary system (feces). The body retention time of these compounds has been determined to be on the order of several days. The toxicity of these compounds has also been determined and is in the range of 5-11 g/kg body weight. These compounds are therefore very inert and are expected to exhibit no long term toxicity effects.

The objective of this project is to synthesize a close structural and chemical mimic of current lipophilic Magnetic Resonance Imaging (MRI) contrast agents and determine the potential of the Europium-chelate complex as a tracking agent. This project was designed as a proof of concept experiment to which to base further work and funding opportunities.

STUDY AREA

Two Swift foxes (Vulpes velox) captured by the Kansas Department of Game and Parks were transferred to the South Dakota State University Research Farm in Brookings, SD. These foxes were kept in pens, to facilitate fecal collection, and fed a simulated natural diet of different roadkill such as muskrat, white tailed deer and ring-necked pheasants mixed with a small amount of dog food.

METHODS

Synthesis

The synthesis is outlined in Figure 1. Diethylene triamine was derivatized with a bromo-p-methoxyacetophenone (1 equiv.) in dimethylformamide at reflux. The mono derivatized diamine in the reaction mixture was used without isolation or purification. t-Butyl-bromoacetate was added to the reaction mix-

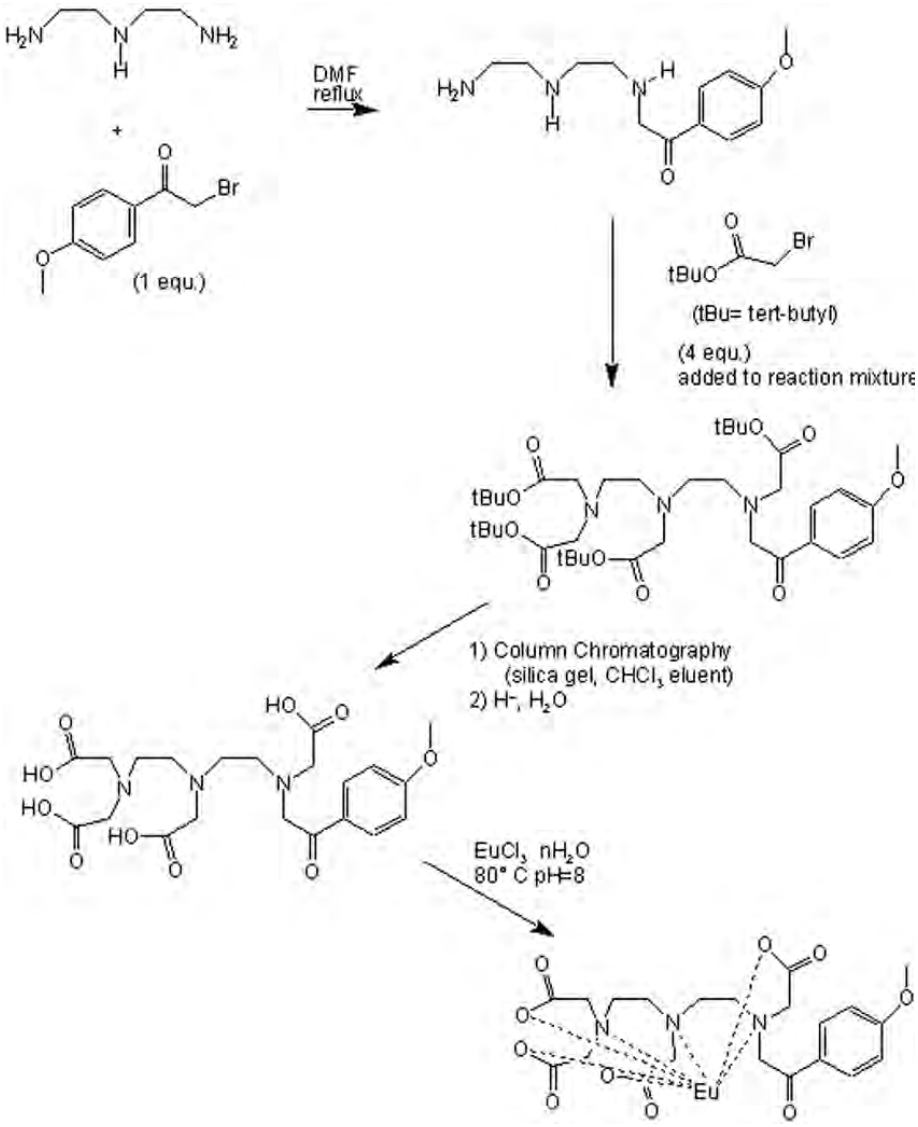

Figure 1. Synthesis of the ligan-metal complex.

ture to derivatize the remaining nitrogen positions. Silica gel chromatography with chloroform as the eluent was used to separate the products in the reaction mixture. Preliminary characterization was performed (NMR, IR) to determine that the desired product was obtained. Acid hydrolysis resulted in the desired ligand. The ligand was characterized by NMR and IR. The complex was prepared by heating a solution of the ligand in water at pH 8 to 80° C. A solution of europium chloride in water was added slowly. Complexation was complete in minutes. The solution was heated with stirring for 2 hours. The europium-chelate complex was obtained by evaporation of water by heat or

lyophilization. The presence of europium was determined by the paramagnetic effect on the ligand NMR signal.

The Eu-chelate complex (20mg Eu/tablet-kg determined for average animal kg) was mixed with polylactic acid (PLA) in an ethanol suspension, stirred, and the solvent evaporated. The mixture as a rubbery mass was cut into 3 cm long pieces and placed into a stainless steel mold. The tablets were annealed at 100° C for 30 minutes. The tablet was removed from the mold and cut into three pellets. The pellets were separately bagged. The amount of Eu used was determined from 90% the recommended dose for a MRI experiment.

Implantation and Sample Collection

Two swift fox (Vulpes velox) were implanted subcutaceously in the upper shoulder region. Fecal samples were collected from one day before implantation daily for 20 weeks. The fecal samples were collected, bagged, labeled and frozen (-80° C). The fecal samples were oven dried and weighed immediately prior to analysis.

Analysis

The samples were prepared for atomic emission analysis by dissolving 0.5g of selected samples in concentrated nitric acid (6 ml). The sample/acid mixture was warmed in a hood and 1ml of concentrated perchloric acid was added. The mixture was heated at 200° C until the mixture cleared and white plumes were observed. The samples were cooled and diluted to 100 ml with nanopure water. Calibration standards were prepared by adding a weighed amount of EuCl3.6H2O to a solution of nitric (4% w/w) and perchloric acid (1% w/w). This ensured the same acid composition of the sample and calibration standards. The concentration of Eu in the standards was 500, 200 and 100 ppb. The solutions, sample and standard, were analyzed by graphite furnace atomic emission with a standard Eu hollow cathode lamp. The Eu concentration was determined for each sample by comparing the experimental value (in counts) to the calibration curve derived from the standard measurements. The average of three measurements was used to determine the sample data value. The concentration of Eu in the sample was plotted vs. time since implantation (days).

RESULTS AND DISCUSSION

The Eu was excreted from day 2 to day 4 as seen in Figure 2. This is significantly less than the normal decay of PLA. Analysis of the remaining tablets showed that the Eu complex was not mixed throughout the tablet, but concentrated on the surface. The excretion of the Eu complex therefore more closely followed the normal metabolic path. The PLA used was in a form difficult to grind and did not allow complete mixing of the complex throughout the polymer. This is a mechanical problem, easily solved with suitable grinding equipment. The plot also shows that the concentration of the Eu complex

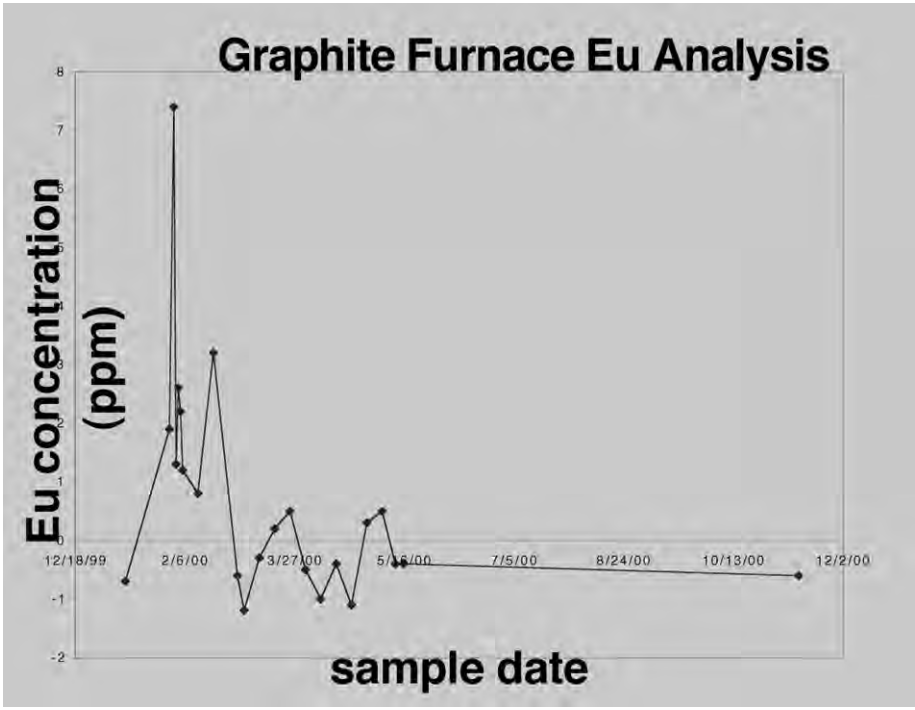

Figure 2. Chart of Eu excretion as a function of date.

in each tablet was insufficient for tracking purposes over extended (six months) time periods. The level of Eu spread over a longer time period would not be distinguishable from the background. The low dose of Eu incorporated into each tablet was sufficient for detection under routine (short time period) analysis conditions. A higher amount of Eu, and therefore the complex, is required for increased signal to noise, but toxicity levels must be monitored, especially under these long term conditions.

SUMMARY AND CONCLUSIONS

MRI contrast agents are useful for animal tracking studies and can replace radioactive tags currently used. Further work and support of this project is warranted in order to fully develop this new class of tags and markers. Specifically, the toxicity of the complex in the polymer matrix must be determined. Conceivably, a much higher level of complex than the LD_{50} can be tolerated due to the slow release of the complex by the polymer. A lower dose may be sufficient, but any dose near the toxic level can have adverse effects. A systematic investigation using animal models of the dose response to higher complex levels in the polymer matrix will determine the optimum complex concentration and excretion levels. Commercial or new complexes may then be used for full tracking and marking studies.

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ADVANCES IN ANCIENT DNA RETRIEVAL

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ABSTRACT

For the last few decades, molecular biologists have been attempting to retrieve ancient DNA for study, but have encountered difficulties such as contamination due to sugar-derived cross-linked proteins (Maillard products), human interaction and microorganisms, just to name a few. It has been discovered that pyrolysis gas chromotography-mass spectrometry (Py-GC-MS) can predict how successful DNA extraction will be from ancient samples by identifying oxidative base damage and the presence of Maillard reaction products (Poinar *et al.* 1998). Handt and his colleagues found that agarose gel electrophoresis and ethidium bromide staining can be used to see a difference between contaminated and pure nucleic acids (Handt *et al.* 1994). Recently, scientists have discovered special methods to crack the cross-linked proteins from the Maillard reaction that restrain DNA with the use of N-phenacylthiazolium bromide (PTB) (Vasan *et al.* 1996). Improved purification techniques have been developed to reduce contamination and produce clearer amplification products. Difficulties still persist, but these methodological advances have the potential to release previously inaccessible information from ancient samples. DNA extraction from coprolite (ancient feces) specimens can be used to establish genetic fingerprinting, kinship and parasitic load, as well as territorial domain, population and diet of extinct species. It will now be possible to determine if a reduction in genetic diversity actually precedes extinction which will enable scientists to acquire new strategies to limit the ongoing erosion of biodiversity.

Key Words

Ancient DNA, PCR, PTB, coprolite, archeology, evolution, contamination

The development of the polymerase chain reaction (PCR) by Kary Mullis in 1983 (Mullis 1990) opened new doors in the study of DNA. There has been

an increased interest in the fields of ecology, evolution, archeology, paleontology, anthropology as well as many others enabling scientists to analyze DNA from many unexplored origins (Paabo 1989; Higuchi & Wilson 1989).
One of the most rewarding sources of genetic information is feces. Ancient feces (coprolite) provides a wide range of applications like diet, parasitic load, evolution, phylogeny, taxonomic morphology and genetic variation within species. With coprolite analysis, it may be possible to determine why species became extinct thousands of years ago by reconstructing the events that led to their extinction. Changes in climate and geography are being studied to determine if they significantly altered the ecological niches occupied by the extinct species. Questions related to changes in gene pool and genetic diversity regarding extinction can now be addressed by DNA analysis (Paabo 1989). If researchers can identify the circumstances that led to extinction, the odds of saving our currently endangered species will increase.

Ancient DNA research is definitely a new and exciting branch of science, but it does not come without drawbacks or hardships. Ancient specimens are especially sensitive to postmortem modifications in their DNA sequences, which lessens the odds for interpretation (Paabo 1989). Even in arid climates, DNA found in non-replicating, inactive cells can experience hydrolytic damage, which causes deamination of bases, depurination and depyrimidination. Additionally, oxidative processes can occur because of ionizing radiation or the development of free radicals (Papoulis *et al.* 1995). In all of the studies conducted to investigate ancient DNA damage, it has been determined that the size of the DNA molecule is reduced 100 base pairs or more and resolutely damaged by oxidation (Handt *et al.* 1994). It is also possible for ultraviolet irradiation or alkylation to contaminate DNA samples, but these are unlikely because most of the samples are buried beneath soil or rocks or found in caves (Hoss *et al.*1996).

Extracts of ancient DNA have been removed from sources that are thousands or even millions of years old, but because of low copy rates in nuclear DNA, mitochondrial (mt) DNA has been used. When Handt and his associates analyzed mitochondrial DNA from the Tyrolean Ice Man, they encountered difficulties such as deletions, insertions, and substitutions. They attempted to use the PCR with specific primers for single copies of DNA, but the endogenous DNA was too degraded and failed to produce usable results. Even when they finally did amplify and clone the mt DNA, the sequences from individual extracts produced many different sequence types indicating contamination from postmortem microorganisms or humans during discovery or collection (Handt *et al.* 1994).

Matthias Krings and his colleagues encountered difficulties differentiating mt DNA sequences of the Neandertal fossil found in western Germany from contemporary human DNA. This is an expected problem when working with ancient DNA because of the extensive handling of the specimens. This type of contamination is a major obstacle in this type of research because Krings was trying to determine if a phylogenic relationship exists between Neandertals and humans (Krings *et al.*1997).

Svante Paabo has been very successful working with ancient DNA from a 100,000 year-old Neandertal and may have brought one of the greatest anthropology controversies to a close. He was able to successfully amplify and clone this type of mt DNA and in doing so, revealed 27 differences between the DNA from Neandertals and humans (there are approximately 8 variations between humans) (Friend 1997). This implies that Neadertals did not evolve into modern humans, even if they did coexist. Paabo is continuing to retrieve sequences from the Neandertal to strengthen this theory (Hotz 1997).

Another complication of amplifying ancient DNA stems from recombinant PCR products caused by "jumping PCR." This occurs when there is contamination from UV light, apurinic sites or breaks in the strands of DNA. When using the PCR, this type of damage in the template can cause the extending primer to "jump" to a different template. When the polymerase comes to the end of the template strand, it may insert an adenosine. Now the polymerase can jump to another molecule because of the absence of the primer and create a recombination product. As a result of this activity, incorrect sequences are created. It is expected that adenosine or thymidine residues will occupy the damaged sites because in ancient DNA, pyrimidines are most often missing or damaged (Paabo *et al.*1990).

PCR is a tool that allows scientists to amplify fragments of DNA for closer examination by duplicating the natural processes by which DNA replicates itself *in vitro*. The PCR essentially consists of three basic steps: denaturing, annealing, and adding polymerase. DNA is arranged in the shape known as the double helix. In order to unwind and split the two strands of DNA, a temperature ranging from 90-96ºC is required. Next, the primers, consisting of four nucleotides, bind to the template molecule (the one to be copied) by annealing. The order of the primer's nucleotides must be identified so they can correspond to the template. The third step involves the addition of the DNA polymerase (Mullis 1990). The polymerase's role is to read the template molecule and match it with the complementary nucleotides. Polymerase can be obtained from the thermophilic (heat loving) bacterium, *Thermus aquaticus* (*Taq* polymerase). An enzyme that is not stable at high temperatures would separate during the denaturing step. As a result, polymerase would need to be added for each independent cycle (Mathews, van Holde 1996).

Upon completion the system is denatured and cooled so the primers can hybridize and bind to another single strand of DNA. Once this has occurred, the temperature is raised so the polymerase can take over. This is an exponential process that creates millions of copies of DNA in a few hours. A test tube, reagents and a heat source are the equipment that is needed to conduct the PCR. Since the PCR progresses from heating to cooling to heating in rapid succession, machines are used to regulate time and temperature automatically (Fig. 1) (Mullis 1990).

It is recommended to use two or more extracts and treat them with exactly the same primers. Product comparison will be better suited to reveal contamination (Hoss *et al.* 1996). A high number of successive cycles of the PCR is sometimes necessary to create at least one usable DNA template. Additionally, it is advised to run amplifications that vary in length ranging from 100-500

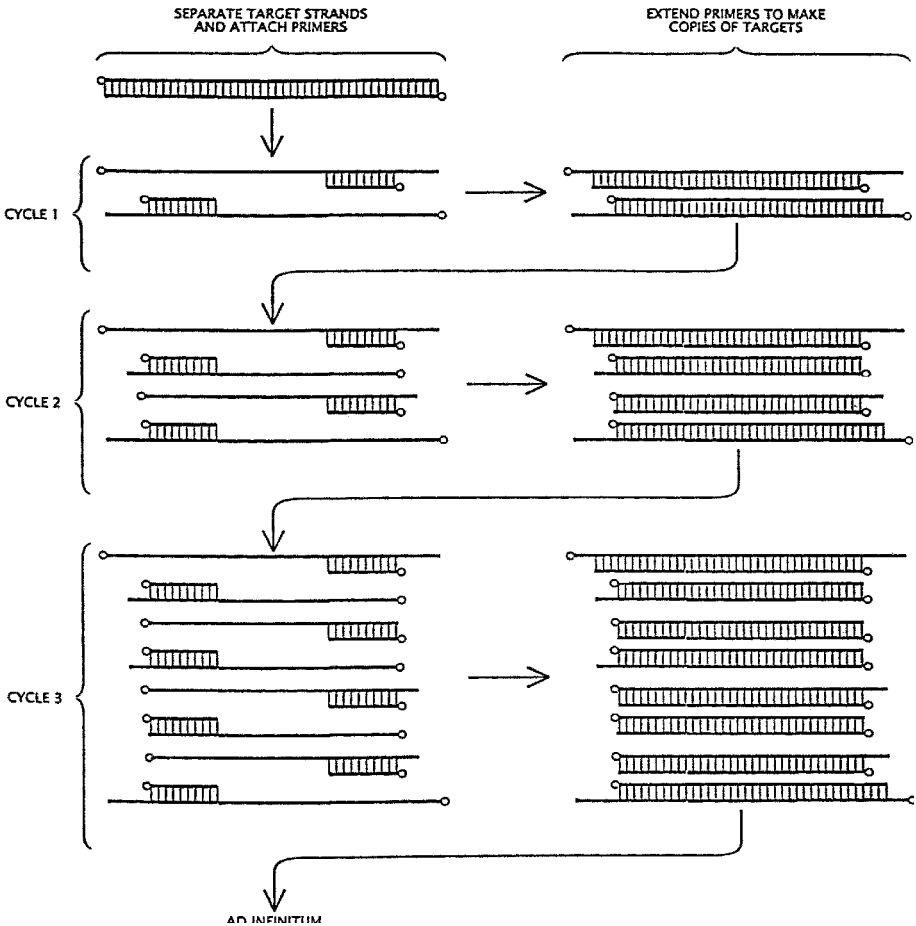

Figure 1. The Polymerase Chain Reaction (Mullis 1990).

base pairs combined with various sets of control amplifications to insure contaminating DNA is absent. Due to the elevated sensitivity of the PCR, DNA can be amplified from specific regions of a sequence. The negative aspect of this sensitivity is that the PCR can also amplify damaged sites characterized by additions of ambiguous bases or recombination products from "jumping PCR." It can also be expected that damaged sites due to lesions or deletions will slow the replication or DNA molecules damaged by inter- or intramolecular crosslinks will inhibit the replication. The polymerase is unable to read and/or match these damaged sites causing the slowed or inhibited reaction (Paabo *et al.* 1990).

As mentioned previously, feces may be one of the most rewarding sources of DNA. Coprolite (ancient DNA) contains evidence identifying diet, parasitic load (Poinar 1998), territory and familial relationship (since each animal has a unique genetic fingerprint) (Kohn & Wayne 1997). It is possible to reconstruct some aspects of the environment at the time the animal occupied the area.

This can be done by using accelerator mass spectroscopy and radioactive dating of the sample DNA and then comparing the age and location of the coprolite with geological surveys. These surveys can identify the contour of the land, some of the types of plants present and location of some water sources.

Poinar and his colleagues used this method for the coprolite from the *Nothrotheriops shastensis* (shasta ground sloth) who's remains were found in Gypsum Cave in Nevada (Harrington *et al.*1934). The sample was determined to be 19,875 ± 215 years old (Ua 11835) using accelerator mass spectrometry. Macromolecular analysis and pyrolosis gas chromatography-mass spectrometry (Py-GC-MS) were used to identify the plant components of the coprolite (Fig. 2a. Symbols are: reverse triangle for pyrolysis products, triangle for hemicellulose, diamonds for amino acid products, G for guaiacol, S for syringol and Po for phenol.). The graph revealed abundant amounts of pyrolysis polysaccharide products such as, hemicellulose and cellulose. Lignin was also identified and exhibited only relative amounts of oxidation. The presence of lignin and polysaccharides especially, hemicellulose may indicate high levels of chemical preservation. The significant amount of syringol indicates angiosperm as the major component of the coprolite. Vinylphenol derivatives are indicators of monocotyldonous lignins.

They also compared the unique inserts of the amplified sequences from a chloroplast gene encoding a subunit of ribulose-biphosphate carboxylase (rbcL) to 2300 rbcL sequences in the GenBank database. Eventually, they concluded the sloth's diet consisted of lilies, capers, grasses, mustards and grapes. It seemed strange to find grapes in the diet of the sloth, because this kind of grape grows near water and the closest water sources (identified via geological surveys) were 6-12 miles away (Poinar *et al.* 1998).

Head space GC-MS can help predict the success of amplification of an ancient DNA sample. The GC-MS can determine the chemical composition of the coprolite and can indicate the presence of Maillard products, sugar derived protein crosslinks (Fig. 2b). Alkylpyrazine, furanone, and furaldehyde groups that can be seen on the graph are heterocyclic compounds formed during the advanced stages of the Maillard reaction (Poinar *et al.*1998). The alkylpyrazines are distinct indicators of this reaction because they are not naturally occurring compounds (Evershed *et al.* 1987).

The Maillard reaction occurs in three phases. In the first stage of this reaction, the free amino group of the amino acid (or protein) and carbonyl group of reducing carbohydrate undergo condensation (Mauron 1981). The amino acids and α-dicarbonyl compounds (collectively known as carbinolamine) experience dehydration via β-elimination to form a Schiff base or imine. This is generally the rate limiting step of the Schiff base formation (Loudon 1995). The Schiff base experiences a rotational change to an analogous N-substituted glycosylamine.

The presence of water determines the direction of the initial phase of the reaction, because of glycoslyamine's instability and isolation difficulty. If water is present at this point in the reaction, N-glycosylamine will rearrange into its initial form, sugar and amino acid. If a weak acid is present, the Amadori rearrangement becomes dominant.

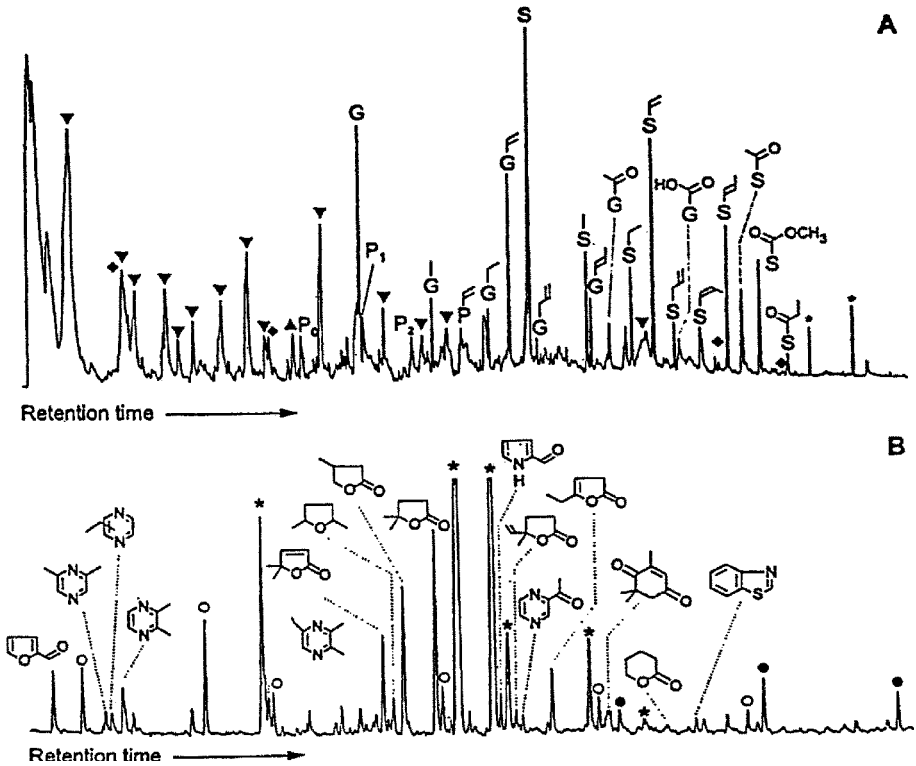

Figure 2. **a)** Total reconstructed ion chromatogram of the pyrolysate (610ºC for 10 sec.) from coprolite derived from an extinct ground sloth, presumably the *Nothrotheriops shastensis*. **b)** Partial desorption head-space GC-MS total ion chromatogram for the volatile compounds within the coprolite (Pionar *et al*. 1998).

In the second stage of Maillard product formation, an Amadori product (AP) -dione enolizes at carbons 1 and 2 and then experiences dehydration via β-elimination to an (AP) -ene-dione. The addition of protein and water produces 3-deoxyhexosone. Water is once again removed resulting in furaldehyde derivatives.

There is an alternate pathway within the second stage of Maillard reaction. First of all, the (AP)-dione enolizes at carbons 3 and 2, respectively and then loses the amine group. This forms a methyl dicarbonyl intermediate that is cleaved into dicarbonyls, keto-aldehydes, C-methyl-aldehydes and reductones (Mauron 1981).

The final stage of the Maillard reaction is characterized by the formation of the brown melanoidin products which is why it is sometimes called the enzymatic browning reaction or the carmelization reaction. The products from the second stages are capable of reacting with one another or other dehydration derivatives to form heterocyclic compounds such as alkylpyrazines, furanones and furaldehydes (Poinar et al. 1998).

Since this reaction has the potential to form intermediates when the pH is not optimal for the Amadori rearrangement, it is reasonable to assume that over extended periods of time that the aggregation of intermediate compounds will ultimately reorganize to produce substantial amounts of products (Lee & Cerami 1987). Maillard researched the relationship between time and temperature and determined that the rate of reaction and temperature are directly proportional. Maillard and other scientists concluded that temperature is the most important variable in terms of success. In 1949 it was demonstrated that in the caesin-glucose reaction, the rate of amino-nitrogen degradation increases 40,000 times when the temperature is raised 80ºC (Mauron 1981).

It is being speculated that the accumulation of these intermediate products may take place within the nucleus of the cell. It is known that Schiff bases and Amadori products exist on all proteins, so perhaps, a cytoplasmic DNA binding protein could transport the sugar-protein complex into the nucleus. The binding protein may remain bound to the sugar may remain inactive until the conditions are favorable to expedite the reaction with DNA (Lee & Cerami 1987).

Once the Maillard products have reached the final stage, they have evolved into inter-twined cross-links with a molecular weight greater than 1000. The meshwork of alkylpyrazines, furaldehydes and furanones contains nitrogen and is biologically and chemically inactive. The products are soluble (first stage), somewhat soluble (advanced stages), or insoluble (final stage) in water (Mauron 1981) which may help to protect the DNA from hydrolytic and post-mortem microbial contaminations and modifications (Stokstad 1998).

Concentrated extracts of ancient DNA are generally brown in color because of contaminating Maillard products. If the extract is going to be examined with agarose gel electrophoresis and ethidium bromide staining, it is recommended to take a picture of the sample before and after staining. When pure nucleic acids are subjected to gel electrophoresis and staining, a light violet color should be emitted. Maillard product contaminants generally give off a blue color under UV light, but if they have been analyzed with gel electrophoresis and staining, they may be mistaken for pure nucleic acids. Occasionally, contaminants and nucleic acids will glow after exposure to ethidium bromide that makes comparison difficult (Paabo 1990).

Recently, Vasan and his colleagues discovered that N-phenacylthiazolium bromide (PTB) could bind to the cross-linked proteins that form on the collagen of diabetic rats. These cross-linked proteins are formed by a process known as non-enzymatic glycation which is the addition of a sugar-derived carbonyl group to a free amine. Over time these products rearrange and are called advanced-glycation end-products (AGEs). Once formed the AGEs bind to macromolecules trapping the amino acids. AGEs are comparable to Maillard products because they both are products of rearrangement reactions involving a-dicarbonyl intermediates and sugar derived protein cross-links.

Figure 3 indicates the pathway for the formation of the Amadori product (AP)-ene-dione triacetyl product of its 1,2-enol form. The AP-ene-dione is unstable and difficult to isolate because of its for affinity amine based nucleophiles. The first step involves the removal of water from carbon 4 via b-elim-

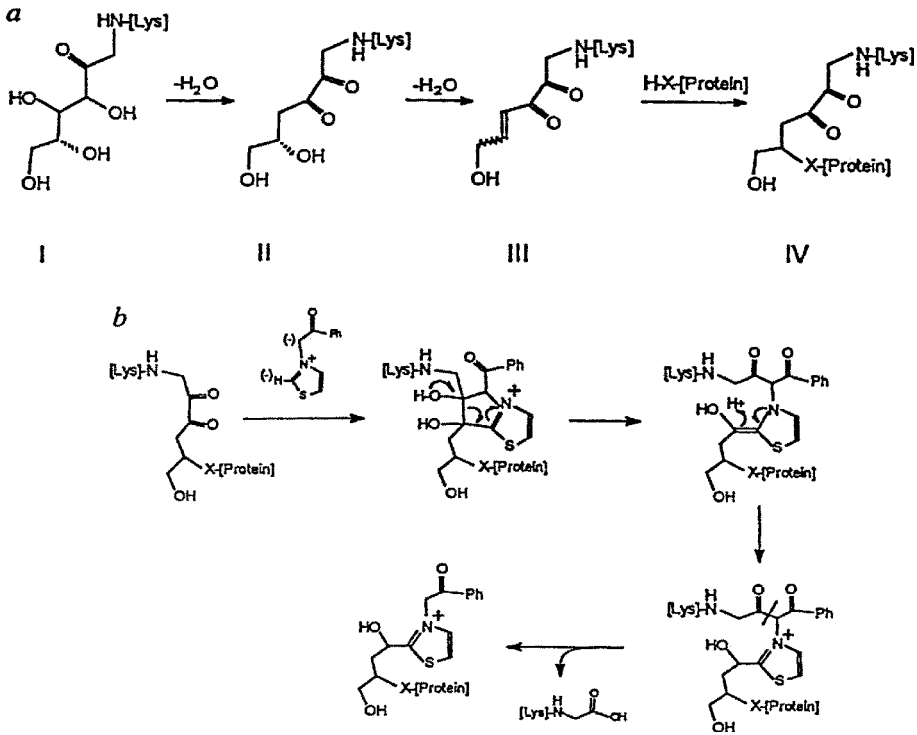

Figure 3. **a)** The formation of glucose-derived protein cross-links from Amadori products. **b)** The proposed reaction for the cleavage of an AP-ene-dione derived, protein-protein crosslink with N-phenacylthiazolium bromide (PTB) (Vasan *et al.* 1996).

ination to form 1,4-dideoxy-1-alkylamino-2,3-hexodiulose (AP-dione). The second step is another dehydration reaction at carbon 5 resulting in the formation of 1,4,5-trideoxy-1-alkylamino-2,3-hexulos-4-ene (AP-ene-dione). Molecule IV represents the enolized form. Part b indicated the structures involved in the cleavage of the C-C bond between the two carbonyl groups. The 2 nucleophilic centers of the PTB molecule react with the 2 carbonyl groups to produce a 5-membered ring causing a tetrahedral formation at the carbonyl carbons. It is this configuration that initiates the automatic severing the bond of the α-diketone (Vasan *et al.* 1996)

In light of these new findings, Hendrik Poinar decided to test PTB on the ancient DNA extracted from the *Nothrotheriop shastensis*. He used agarose gel electrophoresis to analyze a "153 base pair (with primers) mitochondrial amplification for the 12sRNA gene" (Fig. 4). "Lanes: 1 & 12, 1-kb ladder (Gibco BRL, Bethesda, Maryland); 2 & 11, PCR blanks; 3 & 4, 1 and 10 mM PTB added before organic extraction; 5 & 6, 1 and 10 mM added after organic extraction but before silica purification; 7, extraction without PTB and without silica purification (note lack of primer dimers indicating inhibition of the enzyme); 8, extraction without PTB; 9 blank extraction with PTB; 10 blank extraction with-

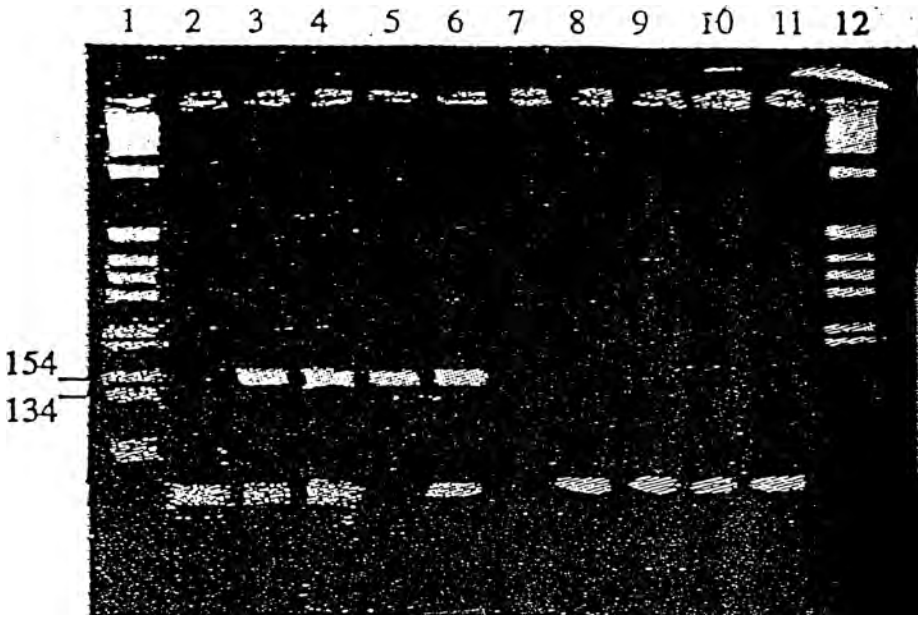

Figure 4. Agarose gel electrophoresis of a 153-bp (with primers) mitochondrial amplification for the 12s rRNA gene (Poinar *et al.* 1998).

out PTB. Numbers on side indicate sizes (base pairs) of marker bands." For every extract treated with PTB, PCR products were evident. When PTB was not used, there were no PCR products. The figure also shows that there is a direct relationship between the amount of PTB used and the strength of the amplification of the DNA (Poinar 1998).

PTB is synthsized by heating a solution of 1 M phenacyl bromide and 1 M thiazole in ethanol at reflux for 2 hours. When the heating is complete, the solution must be cooled and the precipitate must be filtered. When the solution is completely cooled a crystalline structure will form. The crystals must be heated to 222-223°C (melting point of PTB) to produce a liquid form (Vasan *et al.*1996).

Nucleic acids are often extracted from ancient sources by using proteinase K and phenol. This often leads to contaminating components that repress the activity of the *Taq*. polymerase during the PCR. The researcher must dilute the extract until it becomes responsive to the PCR (Handt 1994). A new purification technique has been developed to access amplifiable DNA and remove contemporary contaminating DNA.

Hoss and Paabo tried this technique with a 25,000 year-old bone sample from *Equus hemionus*, a member of the horse family. When the bone sample has been drilled out, it is ground into a fine powder in a freezer mill under liquid nitrogen (DNA preservation is better at lower temperatures. The rate of chemical decay of the nucleotide bases decreases 10-25 times when the temperature of their environment drops 20∞C (Hoss et al.1996)). The extraction buffer used in this purification technique contains 10 M guanidinium thio-

cyanate (GuSCN), 0.1 M Tris-HCl, 0.02 M EDTA, and 1.3% Triton X-100. After incubation and centrifugation, the supernatant is removed and added to more buffering solution and a silica suspension. After this incubation, the silica forms a pellet that is removed and washed with a buffering solution of GuSCN and Tris-HCl. The pellet is washed in ethanol twice and acetone once. The pellet is kept at 56ºC for drying. When this is completed, the nucleic acids can be eluted and stored in water at -20ºC.

Because of the high binding capacity of the silica and buffers (especially the GuSCN) to DNA, the probability of contamination from contemporary DNA is increased. To prevent this from happening, the buffers are preincubated with silica for a few hours. This will bind the contaminating nucleic acids. After centifugation the supernatant is recovered and stored in a dark area. To be certain that there are no silica particles remaining is the extract, it should be centrifuged at 12,000 rpm for 2 minutes prior to running the PCR (silica particles inhibit the PCR).

This is a fast and easy technique to use when extracting ancient DNA. It has a high extraction efficiency as seen in Figure 4 and can be used for a large number of samples. Hoss and Paabo used this technique on 50 different bone samples and the *Taq*. polymerase was not inhibited once (Hoss & Paabo 1993).

There are two additional factors that characterize the amplification of ancient DNA. It is practically impossible to acquire long amplification sequences from the PCR. But, if overlapping primers are used, longer products can be achieved.

The second characteristic of ancient DNA is the inverse relationship between the length of the amplification products and the efficiency of amplification (Handt *et al*. 1994). By using the DNA sequences from the 12S rRNA gene (sequences can be found at www.sciencemag.org/feature/data/981503.html), this relationship can be demonstrated. This can be calculated by counting the deletions, insertions and ambiguous bases and determining the average occurrence of variation per 100 base pair within the number of clones used per template. By graphing the frequency of change in the altered bases per 100-bp against the number of base pairs within the fragment, an inverse relationship can be observed (Fig. 5).

When the relationship is directly proportional between the length of the amplification products and frequency of change, the DNA templates have always been contaminated with modern DNA (Handt 1994).

All of these methods are useful in producing pure, amplifiable ancient DNA sequences, but there are certain criteria that need to be met to insure that the sample is, in fact of ancient origin. The laboratory where the sample DNA is to be worked with needs to be entirely separate from any other laboratories where DNA research is being done. The lab must also be cleaned daily with a 5% sodium hyperchlorite solution and irradiated with UV light. It is also necessary for the lab to have its own independent ventilation system. To protect the sample from human contamination, it is essential to wear protective clothing and use equipment and reagents solely for the purpose of working with that type of DNA for that research project. Two extraction controls, taken from different regions of the sample at different times, and at least one PCR control

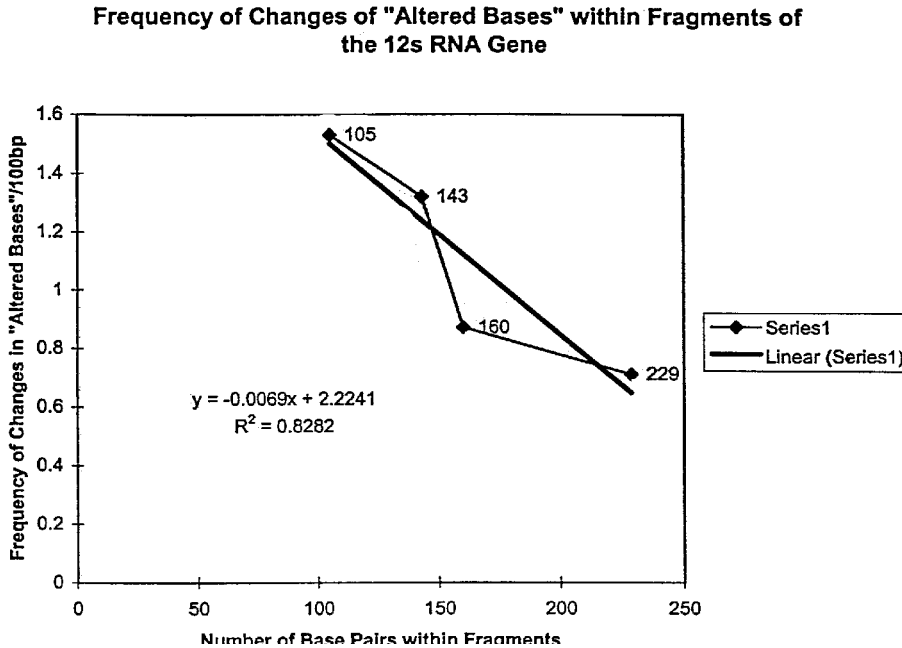

Figure 5. The graph indicates the inverse relationship between length of the amplification products and the amplification efficiency of the PCR.

should be used to screen for contamination. The PCR results should still be the same, and if not, it should be stated in the article. "The sequence should make phylogenetic sense." For example, if DNA from a lion is being studied, the genetic sequence obtained should have some characteristics in common with the genetic sequence obtained from a cheetah. The amplification length and efficiency should be inversely proportional in ancient DNA (Figure 6). If these conditions are met, it will be a reasonable assumption that the DNA in question is that of ancient origin (Handt *et al.* 1994).

There are many challenges regarding ancient DNA, but if the obstructions can be alleviated, the tasks will be rewarding. Research in ancient DNA is relatively new and evolving study since the polymerase chain reaction was developed less than two decades ago.

Research by Kohn and Wayne used feces to establish genetic fingerprinting, stress levels, kinship and reproductive patterns, as well as territorial domains, population and diet of the endangered Brenta bears in Europe (Kohn & Wayne 1997). Hoss and his associates also used feces as the source of their information from the Brenta bears so that it was not necessary to interfere with the animals normal activities or disrupt their normal environment (Hoss *et al.* 1992). Findings such as these may help scientists prevent the extinction of other endangered species by monitoring activity via feces (Kohn & Wayne 1997).

There are vast amounts of information found in feces, contemporary or ancient. It will now be possible to determine if a reduction in genetic diversity actually precedes extinction. If we can reconstruct the past, it will be possible

to more accurately determine why animals like the Shasta ground sloth became extinct and why others survived. With the growing interest and increased accessibility of ancient DNA, the long-lived questions regarding evolution and extinction may be answered. Perhaps, these answers will enable scientists to acquire new strategies to limit the ongoing erosion of biodiversity.

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HABITAT AREA REQUIREMENTS OF WETLAND BIRDS IN WESTERN SOUTH DAKOTA

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ABSTRACT

In 1996, surveys were conducted in 168 wetlands to evaluate the influence of habitat area on bird use of wetlands in western South Dakota. Wetland birds were surveyed using 18-m (0.1-ha) fixed radius circular-point counts. An average of five species (SE = 0.30; range 0-17) occupied semipermanent and seasonal wetlands. Semipermanent wetlands with intermediate emergent vegetation coverage were used by a greater number of species ($P < 0.05$) than wetlands with more or less vegetation. Small wetlands were predominantly occupied by area-independent species (e.g., red-winged blackbird [*Agelaius phoeniceus*], blue-winged teal [*Anas discors*]) whose presence was unrelated to wetland area. In contrast, larger wetlands contained area-dependent species (e.g, eared grebe [*Podiceps nigricollis*], American bittern [*Botaurus lentiginosus*]) whose occupancy rates increased with increasing wetland area. We suggest that managers consider habitat area requirements in wetland creations and restorations when wetland bird productivity is the management goal.

INTRODUCTION

Area of individual forest patches (Robbins et al. 1989, Pearson 1993), woodlots (Martin 1980, 1981) and prairie remnants (Samson 1980, Herkert 1994) is known to influence habitat suitability of these sites for avian species. Pioneering research by Brown and Dinsmore (1986) has shown that wetland bird species richness also is often greater in larger than in smaller marshes of northwest Iowa. Recent research in the glaciated region of eastern South Dakota has indicated that wetland isolation and the abundance of upland grassland habitat also are related to bird use of prairie wetlands (Naugle 1997, Naugle et al. In Press).

Wetland ecosystems in the glaciated prairie pothole region of the United States and southern Canada provide the principal breeding and foraging habitats for more than one-third of North America's avifauna. In less than a century, however, wetland losses due to agricultural activities and urbanization have been extensive and widespread, with losses near 45% in eastern South Dakota and exceeding 90% in northwest Iowa and western Minnesota (Tiner 1984, Dahl 1990). Recent declines in wetland bird numbers coupled with continued loss of habitat have heightened concerns over the future of midwestern wetland bird populations (e.g., Haig et al. 1998). Although conservation planners realize that wetlands in the glaciated portion of eastern South Dakota still constitute the majority of available wetland habitat, the importance of natural and created wetlands in western South Dakota has been relatively unexplored (but see Brewster et al. 1976, Weber and Flake 1982, Rumble and Flake 1983). The objective of this study was to evaluate the habitat area requirements of waterfowl and nongame birds using semipermanent and seasonal wetlands in the unglaciated region of western South Dakota.

STUDY AREA AND METHODS

Study Area

Our study area encompassed western South Dakota from the Missouri River west to the Montana and Wyoming borders. Western South Dakota is primarily rangeland comprised of native mixed-grass prairie grasses. Small grain farming represents a major and increasing source of land use change throughout the region. Wetlands in western South Dakota are characterized as a mixture of isolated natural and man-made ponds typically found at much lower densities compared to wetlands in the glaciated region of eastern South Dakota. We randomly selected semipermanent (n = 100) and seasonal (n = 68) wetlands (Stewart and Kantrud 1971) for study using 7.5' National Wetland Inventory maps within western South Dakota, excluding the forested Black Hills and Badlands physiographic regions.

Bird Surveys

Survey methodology followed an established sampling protocol that has been used extensively to survey wetland birds (Brown and Dinsmore 1986, Hemesath and Dinsmore 1993, VanRees-Siewert and Dinsmore 1996). Species lists were compiled for breeding wetland bird species seen or heard during 8-minute surveys (Scott and Ramsey 1981, Fuller and Langslow 1984) within 18-m (0.1 ha) fixed radius circular-plots (Reynolds et al. 1980, Edwards et al. 1981). We defined breeding species as those nesting in herbaceous vegetation within seasonal and semipermanent wetlands. Species that foraged in wetlands but which nested elsewhere (e.g. great blue herons [*Ardea herodias*] and double-crested cormorants [*Phalacrocorax auritus*]) were excluded. In addition to recording conspicuous birds that were seen or heard during 8-minute surveys, we played tape recordings of Virginia rail (*Rallus limicola*), sora

(*Porzana carolina*), least bittern (*Ixobrychus exilis*) and American bittern (*Botaurus lentiginosus*) calls to elicit responses from these secretive species (Marion et al. 1981, Johnson and Dinsmore 1986, Gibbs and Melvin 1993). A 3-minute, continuous-loop tape (Library of Natural Sounds, Cornell Laboratory of Ornithology, Ithaca, New York 14850) consisting of 25 seconds of male territorial vocalizations of each of four species, interspersed with five seconds of silence, was played for two minutes at each circular plot. The third minute of calling repeated 10 seconds of vocalizations of each species, interspersed with five seconds of silence. Recordings were played during the 3-5 minute period of each 8-minute survey. Vocalizations of soras included "kerwee" and whinny calls. Both "kiddick" and grunt calls of Virginia rails were broadcast.

Number of circular-plots used in surveys increased with increasing wetland area and complexity of wetland vegetation (Brown and Dinsmore 1986). A maximum of four circular-plots was used to survey birds in each wetland. Plots were dispersed throughout the wetland to facilitate sampling within multiple types of vegetation. Coverage of the total wetland area varied from nearly 100% in small wetlands to <1% in large wetlands. When no vegetation was present, circular-plots were placed near the wetland edge and birds were surveyed before approaching the wetland. Species detected outside of circular-plots during surveys or while moving between plots were recorded (Brown and Dinsmore 1986, Hemesath and Dinsmore 1993). We also traversed wetland perimeters to ensure that each species present was recorded. Wetlands were classified as used by a particular species if we observed adults, active nests or young. Surveys were conducted when birds were most active (sunrise to 10:00 hr and 18:00 hr to sunset [Verner and Ritter 1986]). Surveys were not conducted during rainy or windy (≥24 km/h) days.

Habitat Area and Wetland Vegetation Characteristics

Area of surveyed wetlands was estimated using a dot grid on NWI 7.5' maps. Percent vegetated wetland area was estimated visually using a modification of the Daubenmire scale (Bailey and Poulton 1968). Percent vegetated wetland area was recorded into the class intervals: 1) <1%; 2) 1-5%; 3) 6-25%; 4) 26-50%; 5) 51-75%; 6) 76-95%; 7) >95%. We also recorded number of dominant emergent vegetation types comprising ≥10% of the vegetated wetland area.

Data analysis

Occupancy rates of breeding bird species in semipermanent and seasonal wetlands were calculated as the number of wetlands used by a particular species divided by the number of surveyed wetlands. Species with <10 occurrences were excluded from analyses. Wetland bird species richness (i.e., number of species in the assembledge studied) was calculated for wetlands with variable coverage of emergent vegetation (0-5%, 6-75%, 75-100%) to evaluate whether wetlands with intermediate vegetation cover were used by a greater number of species than wetlands with either more or less emergent

vegetation. Differences in species richness on wetlands that varied in emergent vegetation coverage were evaluated with the Kruskal-Wallis test (Wilkinson 1997). Results from the vegetation coverage analysis were used to restrict our use of survey data to wetlands with intermediate cover-to-water ratios (6-75%) to minimize the influence of vegetation coverage from our ensuing analysis of wetland bird habitat area requirements. Occupancy rates were calculated within 5 wetland size classes (<1, 1-2.9, 3-9.9, 10-20, >20 ha) to evaluate the influence of habitat area on bird use of semipermanent and seasonal wetlands. Species were subjectively grouped into 3 categories (area-dependent, possibly area-dependent and area-independent) describing area-dependency relationship with wetland size. Occupancy rates of area-dependent species increased with increasing wetland area compared in contrast to area-independent species whose occupancy rates were little affected by wetland area.

RESULTS AND DISCUSSION

Bird Use of Western South Dakota Wetlands

Semipermanent and seasonal wetlands in western South Dakota provided breeding habitat for a diverse assemblage of wetland bird species. We recorded an average of 5 wetland bird species (SE=0.30; range 0-17) occupying semipermanent and seasonal wetlands. Waterfowl (i.e., blue-winged teal [*Anas discors*], mallard [*Anas platyrhynchos*], gadwall [*Anas strepera*]) and blackbirds (i.e. red-winged [*Agelaius phoeniceus*] and yellow-headed blackbird [*Xanthocephalus xanthocephalus*]) were the most common wetland birds occupying both wetland classes (Table 1). Each species occurred more frequently in semipermanent than seasonal wetlands, with the exception of Wilson's phalarope (*Phalaropus tricolor*) and sora that occupied a greater proportion of seasonal than semipermanent wetlands (Table 1). Occupancy rates exceeded 20% for northern pintail (*Anas acuta*) and northern shoveler (*Anas clypeata*) in seasonal wetlands (Table 1).

Influence of Vegetated Wetland Area on Bird Species Richness

Among the 100 semipermanent wetlands surveyed, those with intermediate coverage of emergent vegetation (5-75%) were used by a greater number ($P < 0.05$) of species (Kruskall-Wallis Test, $\chi 2 = 8.04$, df = 2, $P = 0.01$) than wetlands with either more (>75%) or less (<5%) vegetation (Fig. 1). Similar relationships between the wetland bird species richness and vegetated wetland area found in this study have been widely reported (e.g., Weller and Spatcher 1965, Gibbs et al. 1991, Murkin et al. 1997). Kaminski and Prince (1981, 1984) found that higher dabbling duck densities and species diversity were related to the effects that vegetation-water interspersion had on forage availability and isolation space for breeding pairs. In the present study, a 7-fold increase in the occurrence of gadwall (59% versus 8%) in semipermanent wetlands with intermediate cover-to-open water ratios (6-75% vegetated) compared to wetlands lacking open water areas (>76% vegetated) attested to the importance of habi-

Table 1. Proportion of semipermanent and seasonal wetlands occupied (% Occurrence) by 19 wetland bird species in western South Dakota, 1996.

	% Occurrence	
	Semipermanent (n = 100)	Seasonal (n = 68)
Red-winged Blackbird (*Agelaius phoeniceus*)	88	81
Blue-winged Teal (*Anas discors*)	76	62
Mallard (*Anas platyrhynchos*)	70	62
Gadwall (*Anas strepera*)	49	47
Yellow-headed Blackbird [a]	47	28
American Coot (*Fulica americana*)	32	13
Northern Shoveler (*Anas clypeata*)	29	25
Northern Pintail (*Anas acuta*)	29	24
Wilson's Phalarope (*Phalaropus tricolor*)	28	44
Pied-billed Grebe (*Podilymbus podiceps*)	22	3
Virginia Rail (*Rallus limicola*)	21	4
Canada Goose (*Branta canadensis*)	20	10
Black Tern (*Chlidonias niger*)	18	7
Redhead (*Aythya americana*)	18	7
Ruddy Duck (*Oxyura jamaicensis*)	16	4
Sora (*Porzana carolina*)	15	22
Marsh Wren (*Cistothorus palustris*)	15	7
American Bittern (*Botaurus lentiginosus*)	15	4
Eared Grebe (*Podiceps nigricollis)*	11	4

[a] (*Xanthocephalus xanthocephalus)*

tat interspersion to breeding dabbling ducks. Bird use of open (<5% vegetated) and closed (>76% vegetated) wetlands (Fig. 1) reflects the divergent life history requirements of breeding nongame wetland species. Wilson's phalaropes prefer foraging sites that include more open water habitats or those dominated by thin-stemmed emergent plants (Colwell and Jehl 1994). In contrast, increased use of closed wetlands by American coot (*Fulica americana*) and sora probably reflects their need for nest-building materials and concealment cover (Johnson and Dinsmore 1986, Alisauskas and Arnold 1994, Conway 1995). Wetland bird species richness was not related to emergent vegetation coverage in seasonal wetlands (Kruskall-Wallis Test, $\chi 2 = 0.93$, df = 2, P = 0.93).

Wetland Bird Habitat Area Requirements

Habitat area requirements presented in this study were not influenced by vegetated wetland area because we restricted our use of species occurrence data to semipermanent wetlands with intermediate cover-to-water ratios (6-75%)

(Weller and Spatcher 1965, Weller and Fredrickson 1974, Brown and Dinsmore 1986). Wetland area influenced composition of breeding wetland bird communities surveyed in western South Dakota. Small semipermanent wetlands were predominantly occupied by area-independent species (e.g., red-winged blackbird and blue-winged teal [Table 2]) whose occurrence was unrelated to wetland area. In contrast, larger wetlands contained area-dependent species (i.e., eight species; see Table 2) whose occupancy rates increased with increasing wetland area. Canada geese (*Branta canadensis*) and eared grebes (*Podiceps nigricollis*), which did not occur in semipermanent wetlands <10 ha in area, were the most area-dependent species in this study (Table 2). No species was classified as area-dependent in seasonal wetlands because occupancy rates of the smallest seasonal wetlands (<0.5 ha) were higher than those of semipermanent wetlands (Tables 2 and 3). Six species (Table 3) were classified as possibly area-dependent in seasonal wetlands because proportion of occupied wetlands increased with increasing habitat area.

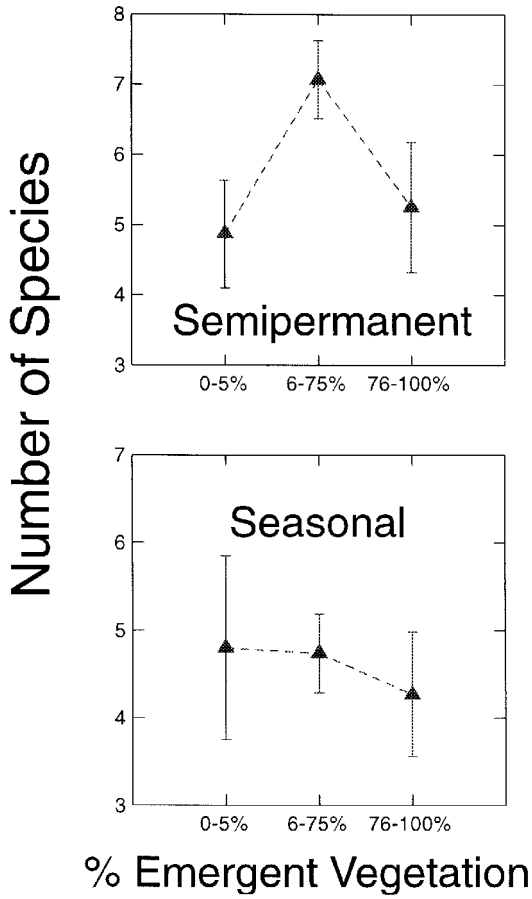

Figure 1. Relationship between wetland bird species richness and percent vegetated wetland area for semipermanent and seasonal wetlands in western South Dakota, 1996. Semipermanent wetlands with intermediate vegetation coverage (6-75%) were used by the greatest number of species ($P < 0.05$). Species richness was not related to emergent vegetation in seasonal wetlands ($P > 0.90$).

In western South Dakota wetlands and in Iowa marshes (Brown and Dinsmore 1986), species occurrence rates increase with increasing wetland area, but the rate of increase declines as the wetlands become larger. For example, the occurrence of five area-dependent species that were absent from semipermanent wetlands <3 ha in area (Table 2) increased sharply in 3-9.9 ha wetlands, but then decreased or increased more slowly before reaching a max-

Table 2. Occupancy rates of 19 wetland bird species in 5 size classes of semipermanent wetlands in western South Dakota, 1996.

	Wetland Area (ha)				
	<1	1-2.9	3-9.9	10-20	>20
Area-dependent species[a]					
Pied-billed Grebe (*Podilymbus podiceps*)	13	25	40	40	45
Northern Pintail (*Anas acuta*)	7	8	40	40	45
Redhead (*Aythya americana*)			40	10	55
Black Tern (*Chlidonias niger)*			30	50	36
Marsh Wren (*Cistothorus palustris*)			20	30	55
American Bittern (*Botaurus lentiginosus*)			20	10	64
Eared Grebe (*Podiceps nigricollis*)				10	56
Canada Goose (*Branta canadensis*)				40	73
Species possibly area-dependent[b]					
Wilson's Phalarope (*Phalaropus tricolor*)	7	17	50	40	27
Ruddy Duck (*Oxyura jamaicensis*)	7		50	10	36
Virginia Rail (*Rallus limicola*)	7		20	60	64
Area-independent species[c]					
Red-winged Blackbird (*Agelaius phoeniceus*)	80	92	100	100	100
Blue-winged Teal (*Anas discors*)	80	67	90	100	100
Mallard (*Anas platyrhynchos)*	53	67	100	90	91
Yellow-headed Blackbird [d]	33	42	90	70	82
Gadwall (*Anas strepera*)	33	42	90	60	82
Northern Shoveler (*Anas clypeata*)	20	42	40	40	18
American Coot (*Fulica americana*)	13	33	80	50	64
Sora (*Porzana carolina)*	13	8	20		36

[a] Linear relationship between occupancy rates and wetland area.
[b] Possible relationship between occupancy rates and wetland area.
[c] Occupancy rates little affected by wetland area.
[d] (*Xanthocephalus xanthocephalus*).

imum in wetlands >20 ha in area. Although a single large wetland (i.e., >20 ha) may not always be the best management strategy, habitat area requirements should be considered in future management decisions. Data from this study indicate that wetlands in western South Dakota that consistently provide habitat for the most area-dependent wetland bird species are approximately 20-25 ha in area. Although research designed to assess the importance of land-

Table 3. Occupancy rates of 9 wetland bird species in 5 size classes of seasonal wetlands in western South Dakota, 1996.

	Wetland Area (ha)				
	<0.5	0.5-1.9	2-4.9	5-10	>10
Species possibly area-dependent[a]					
Mallard (*Anas platyrhynchos)*	48	33	73	89	100
Blue-winged Teal (*Anas discors*)	43	53	55	89	100
Gadwall (*Anas strepera*)	35	20	64	78	70
Wilson's Phalarope (*Phalaropus tricolor*)	30	20	64	44	90
Northern Pintail (*Anas acuta*)	13	13	18	33	66
Northern Shoveler (*Anas clypeata*)		27	36	33	60
Area-independent species[b]					
Red-winged Blackbird (*Agelaius phoeniceus*)	70	80	91	89	90
Yellow-headed Blackbird [c]	13	27	27	44	50
Sora (*Porzana carolina*)	13	7	27	56	30

[a] Possible relationship between occupancy rates and wetland area.
[b] Occupancy rates little affected by wetland area.
[c] (*Xanthocephalus xanthocephalus*)

scape structure on prairie wetland birds is yet to be conducted (but see Rumble and Flake 1983), studies in northwest Iowa (Brown and Dinsmore 1986) and eastern South Dakota (Naugle 1997, Naugle et al. 1999) indicate that wetland isolation and the abundance of grassland habitat in uplands surrounding wetlands influence wetland bird occurrence. We suggest that managers constructing or restoring wetlands in western South Dakota consider the habitat area requirements of wetland birds when bird productivity is the management goal. We further recommend that studies be initiated to further identify local and landscape-level variables influencing habitat suitability for breeding wetland birds in western South Dakota.

ACKNOWLEDGMENTS

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PROTECTION AGAINST BOVINE VIRAL DIARRHEA VIRUS (BVDV) OF CALVES VACCINATED WITH A BOVINE HERPESVIRUS-1 (BHV-1)-BVDV RECOMBINANT

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ABSTRACT

A recently developed recombinant bovine herpesvirus 1 (BHV-1) virus containing the envelope protein gp53 of bovine viral diarrhea virus (BVDV) type 1 was assessed for its ability to protect against BVDV in calves. Four calves were vaccinated intranasally with the recombinant BHV-1-BVDV vaccine and did not exhibit any clinical signs following vaccination. The vaccine virus was recovered from all vaccinated calves on days 8 through 10. Twenty-eight days after vaccination, the four vaccinated and four control calves were challenged with the type 1 BVDV, NY-1. All calves had slight temperature elevations but the clinical signs were more severe in the control calves. The platelet counts were depressed in the control calves. Prior to challenge, neither group had BVDV serum neutralizing antibody. Following challenge, the vaccinated calves developed higher serum antibody levels indicating a secondary immune response. Calves were euthanized and tissues were taken weeks following infection. No latent BHV-1 virus was detected from the trigeminal ganglion of any of the vaccinated calves. The recombinant BHV-1 virus vaccine containing a single BVDV protein provided partial protection against BVDV infection. This recombinant virus replication appeared to be restricted to nasal passages.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BHV-1) infections are a major source of respiratory and reproductive problems in cattle (Fields et al., 1996; Ludwig, 1983). Live attenuated vaccine strains of BHV-1 are effective and have been used safely in cattle for several years (Bello et al., 1992). BVDV vaccines that control persistent infections, which occurs in animals that are infected in utero and results in lifelong viral shedding, are nonexistent. Modified-live BVDV vaccines are available, but they are not safe to use in pregnant animals. Inactivated BVDV vaccines can be used in a preg-

nant animal, but they are inconvenient and ineffective at controlling persistent infections (Baker, 1995).

Bello et al. (1992) determined BHV-1 is useful as an expression and vaccine vector. Whetstone et al. (1992) determined the BHV-1 thymidine kinase (TK) gene was non-essential for BHV-1 reactivation in cattle. This led to the development of the BHV-1-BVDV recombinant virus vaccine. The glycoprotein 53 (gp53) of BVDV is the major target for neutralizing antibody against BVD virus. The gp53 from BVDV was inserted into the TK locus of a TK-- BHV-1 genome. This work was done by Dr. L.J. Bello and Dr. W.C. Lawrence of the University of Pennsylvania (personal communication).

The use of the BHV-1-BVDV recombinant virus vaccine in pregnant cattle to control persistent infections of the fetuses is an important and promising endeavor. This preliminary vaccine trial using calves is a model for future trials to protect pregnant cows against BVDV infection.

MATERIALS

The vaccine virus used was the recombinant BHV-1 (v1V31) containing gp53 of the NADL strain of BVDV type 1 from the University of Pennsylvania in Philadelphia, PA. The cells used for all assays were bovine turbinate (Bt) cells. The challenge virus used was the BVDV NY-1 from the National Veterinary Services Laboratory in Ames, IA. BVD Singer type 1 and BVD A125 type 2 strains of virus were used for the serum neutralization assays which were performed by the Serology section of the Animal Disease Research and Diagnostic Laboratory (ADRDL) at South Dakota State University (SDSU).

Eight male, BHV-1 and BVDV negative, Holstein calves were used in the study. The calves were four to five months old and ranged in weight from 200 to 300 pounds. Two groups of calves were used, a vaccinated group and a control group, with each group consisting of four animals. The calves were initially housed first in an open enclosure with calf hutches and later moved to an open-front barn in a small pasture enclosure.

METHODS

The vaccinated calves were intranasally inoculated with $10^{7}TCID_{50}$/2ml of recombinant BHV-1-BVDV vaccine on day 0. Nasal samples were taken day 0 to 14 to monitor for recombinant BHV-1-BVDV excretion. Clinical signs were taken every day. The clinical signs included respiration, attitude, nasal discharge, temperature, and diarrhea. The clinical signs were rated on a 0 to 4 point scale, (i.e. normal nasal discharge=0, whereas thick or crusted nasal discharge=4). Serum samples were taken every seven days starting at day 0 to monitor for seroconversion to recombinant BHV-1-BVDV.

The vaccinated and control group calves were intranasally inoculated with $10^{7.9}TCID_{50}$/2ml of BVDV on day 28 of the study. Nasal samples were taken days 28 through 35 to monitor for BVDV excretion. Clinical signs were taken every day with the same categories and ratings as the pre-challenge period. Serum samples were taken every seven days to monitor for seroconversion to

BVDV. Whole blood was taken days 28 through 38 to count the number of whole blood cells and platelets.

Calves were vaccinated and/or challenged intranasally. The calves were secured in a headgate and halter. A small plastic biosafety bag was placed over their muzzles to induce hypoxia, and increase respiration rate and inspiration volume. The bag was removed and the vaccine/challenge was administered with a Chromist TLC aerosol unit (Gelman Sciences) for one minute to each nostril. The bag was again placed over the calves' muzzles and hypoxia was induced. The halter was removed and the calves were released.

The calves were euthanized at the end of the study. The tissues harvested were: tonsil, spleen, thymus, testes, Peyer's patches, trigeminal ganglion, and medistineal, mesenteric, retropharyngeal, and subiliac lymph nodes.

The calves antibody levels for BHV-1 and BVDV were determined by a virus neutralization assay (Manual of Standards for Diagnostic Tests and Vaccines, 1992). The titers of the vaccine and virus were determined using the Kärber method $TCID_{50}$ assay (Kärber, 1931).

The nasal samples taken after vaccination and challenge were collected and virus isolations were performed. The secretions were collected by aspiration using a vacuum pump. 0.5 ml of nasal secretions were added to 4.5 ml of 1% FBS media. This mixture was vortexed, centrifuged and filter sterilized in 0.45 mm filters. Duplicates were plated at 1:5 serial dilutions on Bt cells. The BHV-1 isolation plates after vaccination were read four days after inoculation and the cytopathic effect (CPE) was recorded. Seven days after the inoculation, the cells on the BVDV isolation plates were fixed with 20% acetone, 80% PBS and 0.02% BSA. The plates were dried overnight and frozen at -70°C. Later a BVDV immunoperoxidase test was performed on the cells to detect BVDV in the samples (Saliki, 1997).

White blood cell and platelet counts were performed by the Clinical Pathology section at the ADRDL of SDSU from the whole blood.

The calves were all euthanized by electrocution and \ tissue samples were taken and were frozen in -80°C. Virus isolations were performed on these samples.

RESULTS

All of the calves had sero-converted to BVDV type 1 by day 56 of the study or 28 days after the challenge. Although all of the calves sero-converted, the response peak of the serological titers of the vaccinated calves was faster and more pronounced than the control calves response (Fig. 1). Calf #4 values were excluded from the data because of a high and outlying BVDV type 2 titer on a single day.

BHV-1 was recovered 8 and 10 days post-vaccination (Fig. 2). BVDV was not detected from any nasal samples after challenge (data not shown).

The post-vaccination temperatures were normal and clinical scores for post-vaccination were negligible (Fig. 3 & 4). The post-challenge temperatures of the vaccinated and control calf groups were similar (Fig. 5). The post-chal-

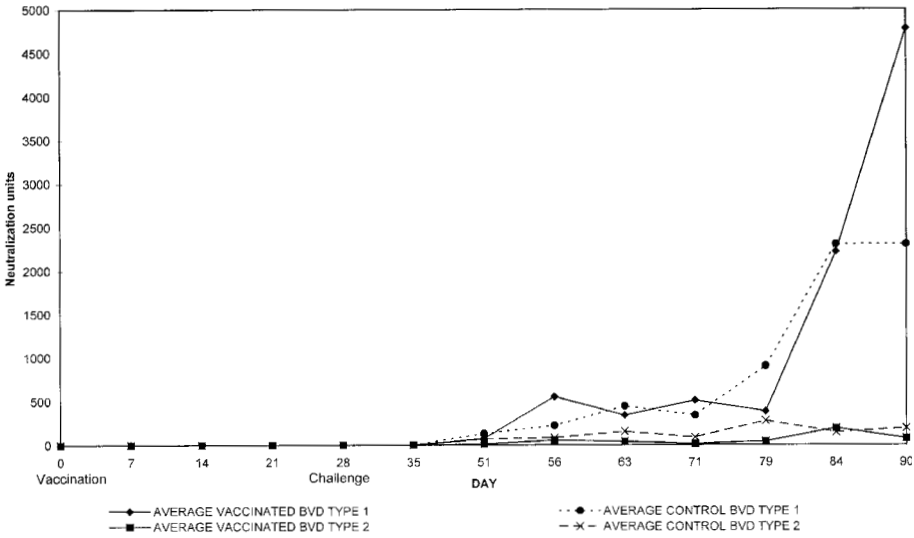

Figure 1. Singer "BVDV-1" and A125 "VBDV-2" average neutralizing antibody titer in sera of vaccinated and control calves.

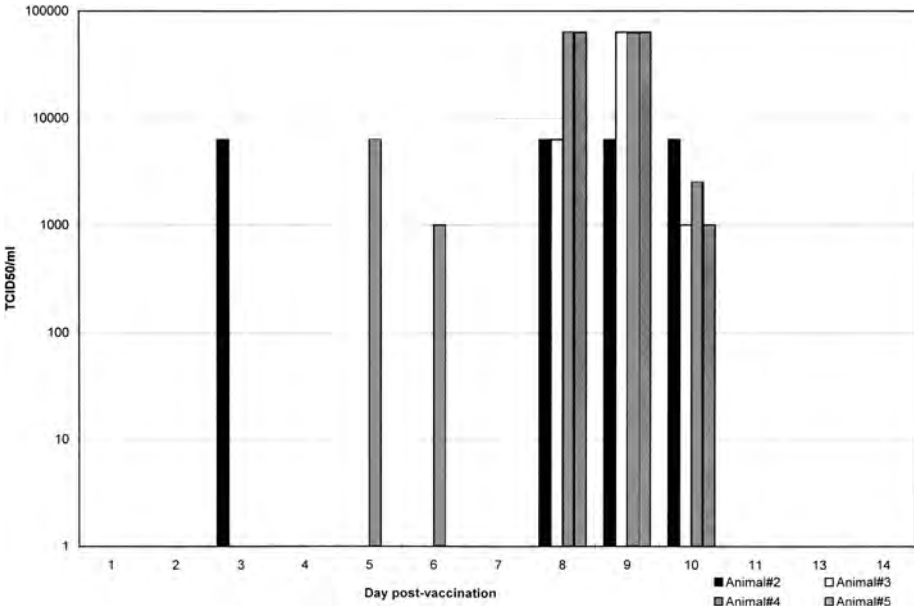

Figure 2. Recombinant BHV-1 shedding in nasal secretions after intra-nasal inoculation.

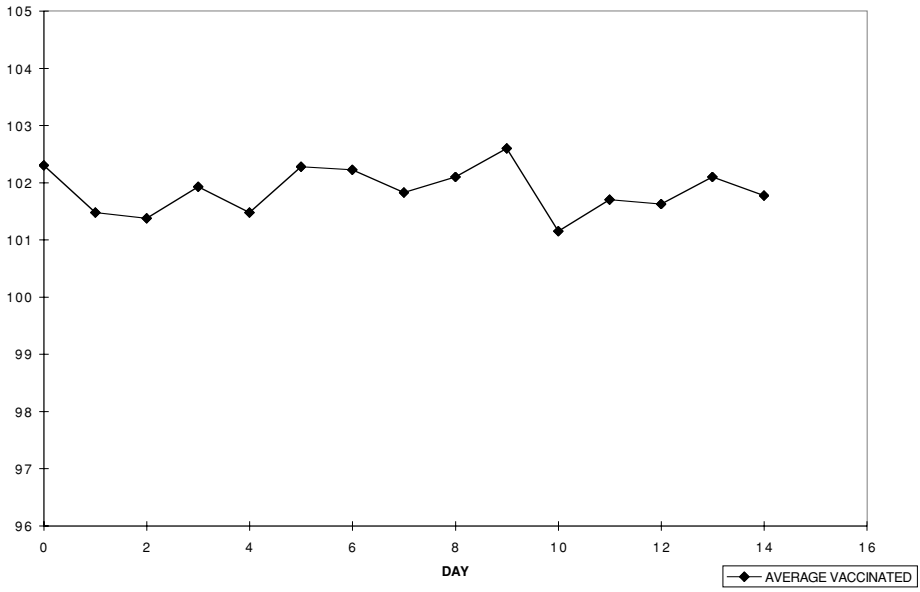

Figure 3. Post-vaccination average calf rectal-temperature values.

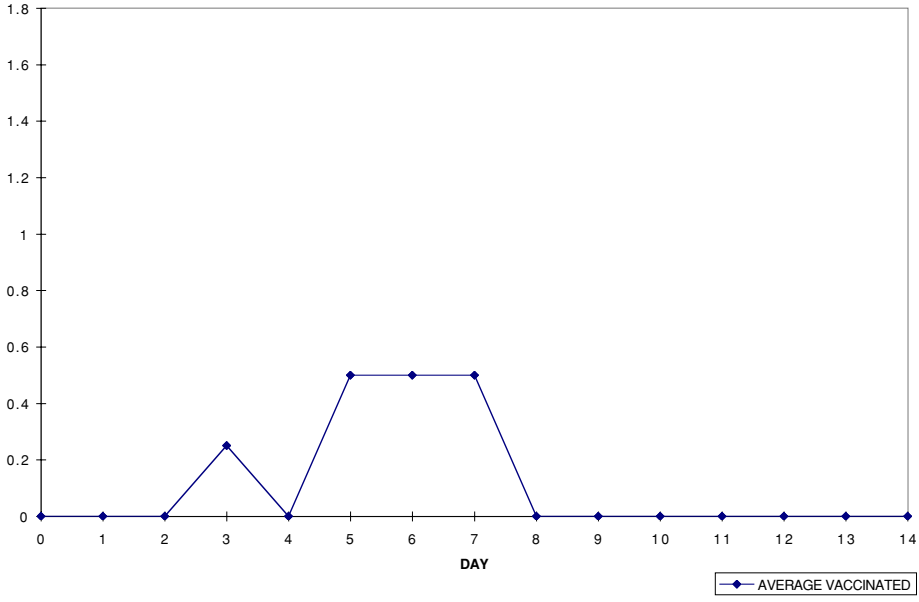

Figure 4. Average post-vaccination calf clinical scores.

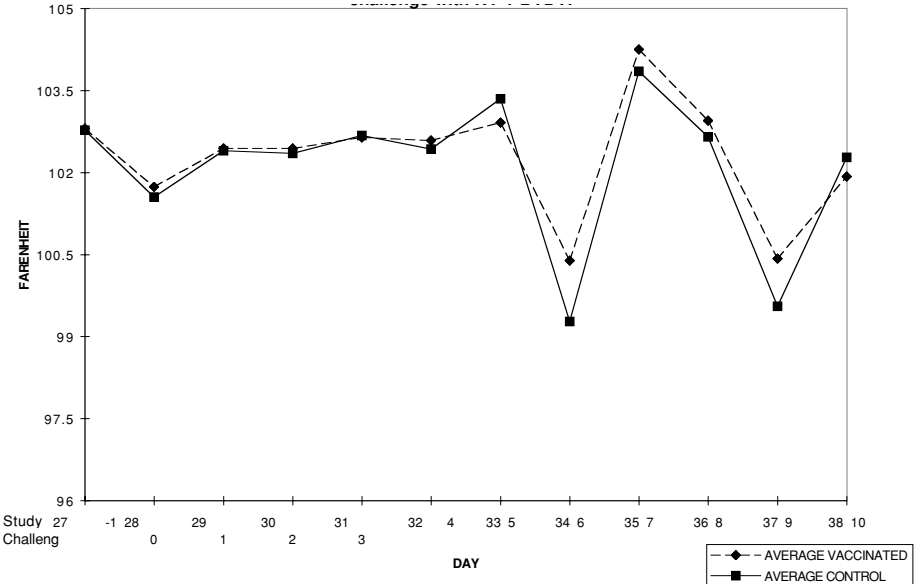

Figure 5. Average calf rectal temperature values1 day pre-challenge and 10 days post-challenge with NY-1 BVDV.

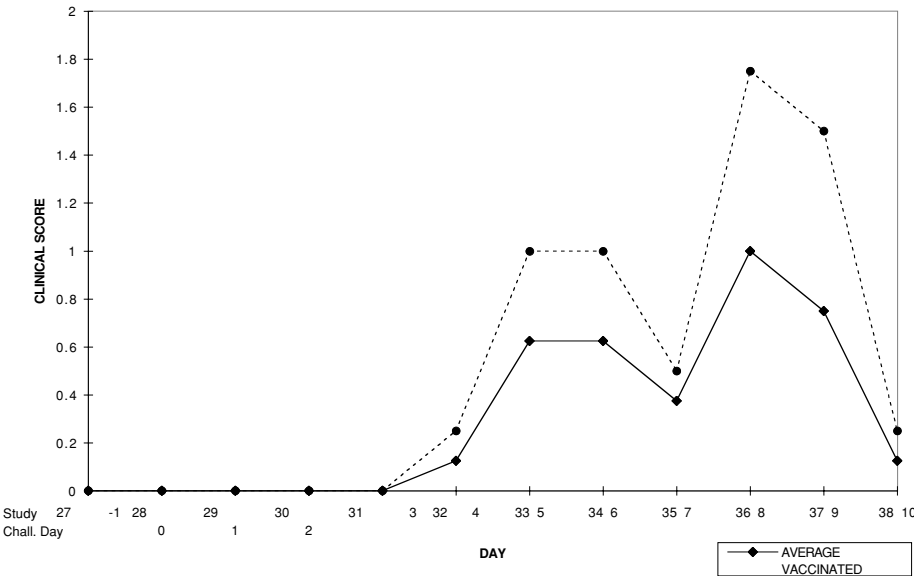

Figure 6. Average calf clinical scores following challenge with NY-1 BVDV.

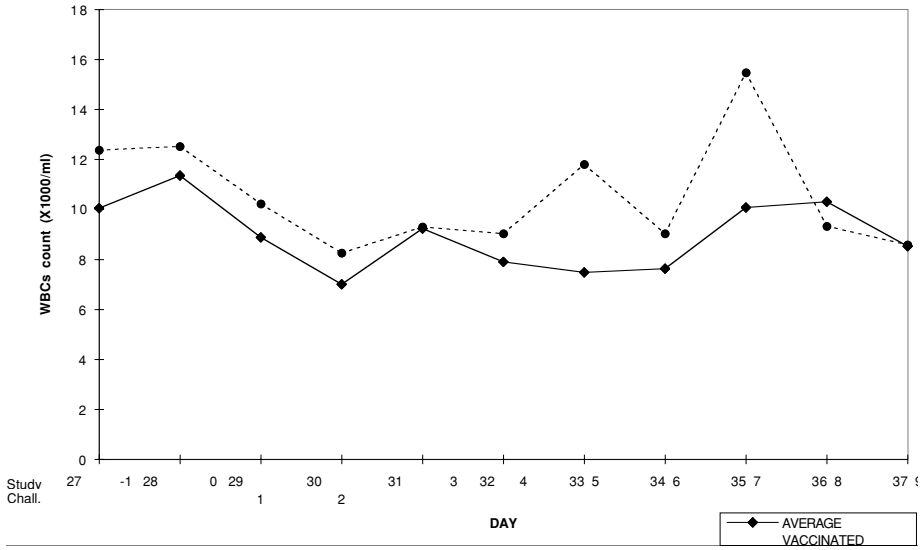

Figure 7. Average calf-white blood cells counts following challenge with NY-1 BVDV.

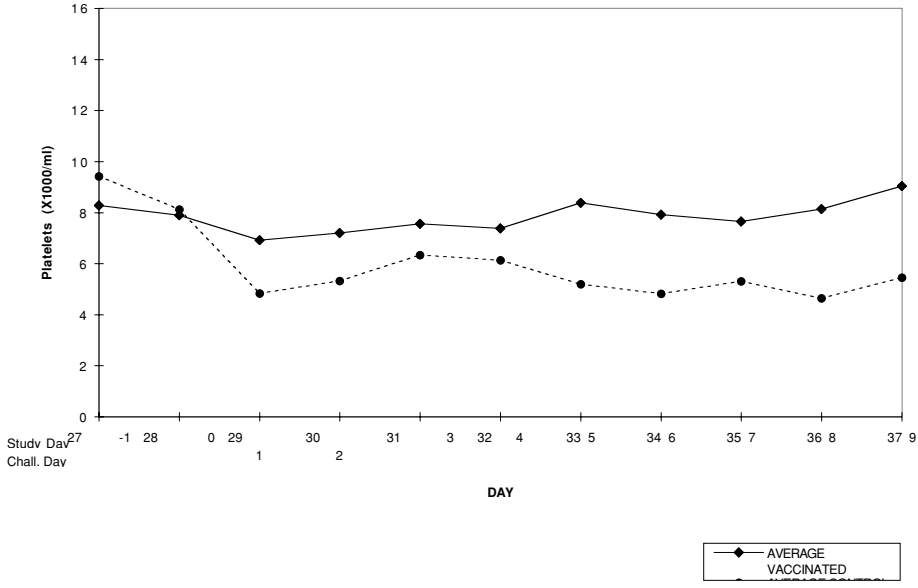

Figure 8. Average calf-blood platelets counts following challenge with NY-1 BVDV.

lenge clinical scores showed an increase in signs during day 5 to 6 and day 8 to 9 (Fig. 6).

The white blood cell count of the vaccinated calf group remained normal throughout the trial and the control calf group's count was slightly higher and increased on day 35 (Fig. 7). The platelet count of the vaccinated calf group remained normal after challenge, while the control calf group's count was depressed (Fig. 8).

No latent BHV-1 was detected from the trigeminal ganglion of any of the vaccinated calves.

DISCUSSION

The recombinant BHV-1-BVDV was safe to use in calves. The calves showed minimal reactions to the vaccine when it was administered to them as measured by body temperatures and clinical scores. These minimal symptoms coincided with the detection of the recombinant BHV-1-BVDV shedding in the nasal secretions of the vaccinated calves (Fig. 2). This indicated that local replication occurred. Interestingly, no apparent systemic replication occurred as no latency could be detected in the trigeminal ganglia during the necropsy.

After challenge the vaccinated animals exhibited less clinical disease than the controls. The clinical signs were less severe in the vaccinated animals and the clinical pathology results indicated there was no effect on the vaccinated animals.

Serum neutralizations indicated the development of neutralizing antibody titers to BVDV type 1, but not to the BVDV type 2 (Fig. 1). This shows the specificity of type 1 gp53 for type 1 and not for BVDV type 2 . After the challenge there was a significant increase in the vaccinated BVDV type 1 titers compared to the control BVDV type 1 titers. This represents a secondary immune response in the vaccinated animals, showing the vaccine had induced memory cell development to BVDV. Work continues to evaluate the nasal secretions antibody levels.

In conclusion, the calves vaccinated with recombinant BHV-1-BVDV virus vaccine showed no adverse reactions to the vaccine. The vaccine did replicate locally in the respiratory tract and there were noticeable differences in the post-challenge clinical scores, temperatures, and WBC and platelet counts of the vaccinated and control calves. The recombinant BHV-1-BVDV vaccine did provide partial protection against a BVDV challenge. This study suggests a trial with pregnant cows would provide significant information towards creating a successful vaccine against BVDV persistent infections in cattle.

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OLD BLACK HILLS PONDEROSA PINES TELL A STORY

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ABSTRACT

A single ponderosa pine tree found in the central Black Hills of South Dakota revealed its age of more than 700 years by its tree rings taken from coring in 1992. The purpose of this study was to examine historic climatic patterns from the 13th century through most of the 20th century as inferred from ring widths of this and other nearby trees. The steep, rocky site where this tree was found helped this tree and its cohorts survive fires and other damaging agents.

Our analysis indicated that both dry and wet spells of longer duration and magnitude than the 1930's drought have occurred previously. For example, since 1600, the 1930's "dust bowl" drought ranks 4th in severity. Further, if additional evidence supports the single tree record, the 1930's drought may be of even less significance.

INTRODUCTION

The use of dendrochronological techniques to study drought history of regions has been available much of this century. Two of the pioneers include A.E. Douglass (1914 and 1919) and H.C. Fritts (1976). Meko (1982) used these techniques to study drought history of the western Great Plains (1640-1977). He suggested that the severity of the 1930's drought was over-shadowed by droughts of the 1750's, 1820's, and 1860's. The purpose of the present study is to get a glimpse of even earlier droughts using tree core data collected in 1992 and to explore their severity and periodicity, if any, and to extend the earlier work of DeGaetano and Miller (1990).

DATA ANALYSIS

Two tree-ring chronologies of ponderosa pine (*Pinus ponderosa*) from the central and southern Black Hills of South Dakota gathered during 1991-1992 are examined. The collection, development, and overall reliability of the data are described in Sieg *et al.* (1996). The oldest tree dates to 1281 A.D., while the most reliable period of the chronology covers approximately 400 years, starting from 1600, when at least five independent tree-ring samples were available. The data set consists of detrended tree-ring widths from 1281-1991.

There is more variability of the tree-ring widths in the first few hundred years of the chronology when compared to the latter half of the chronology due in part to the gradual increase in sample size. In order to account for variability due to sample size, the tree-ring time series was incrementally standardized from 1281-1393, 1394-1597, 1598-1681, 1682-1768, and 1769-1991. Each sub-period contained 1-2, 3-4, 5-8, 9-15, and 16-22 tree samples, respectively. The choice of the sub-periods was based both on having close to 100 years in each epoch and subjective consideration of the variability in the original time series. After this operation was performed, the collective time series was standardized again to ensure continuous Gaussian characteristics. A similar procedure has also been performed on the Southern Oscillation index data to account for differences in monthly variability (Wilks 1995).

Similar to other studies (Stockton and Meko 1983; DeGaetano and Miller 1990; Sieg *et al.* 1996), precipitation from September of the year prior to tree growth to August of the year of tree growth was chosen to correlate with tree-ring width. Precipitation data were obtained from: (i) Hill City (1956-1990, in the central Black Hills); (ii) Rapid City (1889-1991, along the eastern Black Hills); and (iii) a climate region both surrounding the Black Hills and including part of the southern Black Hills (1889-1990, cluster "C" in Figure 8 of Bunkers *et al.* (1996) — herein denoted BLKHLS).

RESULTS AND DISCUSSION

The one-year correlations were nearly identical among the three precipitation records and the tree-ring widths, but the five-year average (precipitation and tree-ring width) correlations were notably stronger using both the Rapid City and BLKHLS region precipitation (Table 1). The one-year correlation coefficients are comparable to those found in the previous studies mentioned above. The relationship between the September to August precipitation and the tree ring indices suggests that the tree-ring data can be used as a proxy for seasonal precipitation for the period of record with precipitation data (e.g., Figs. 1a, b). Extrapolation to earlier years assumes: (1) a similar growth-precipitation relationship, and (2) a similar role (or lack thereof) of temperature dependence. The extrapolation necessarily includes years when temperature extremes could have played a significant role during the Little Ice Age, 1550-1870 (Pielou, 1992).

Other studies typically reconstruct the annual (September-August) precipitation via multiple linear regression using a combination of the tree growth

Table 1. Correlation between September-August precipitation and tree-ring width.

	Hill City	Rapid City	BLKHLS Region
1-year	0.56	0.56	0.53
5-year	0.59	0.70	0.70

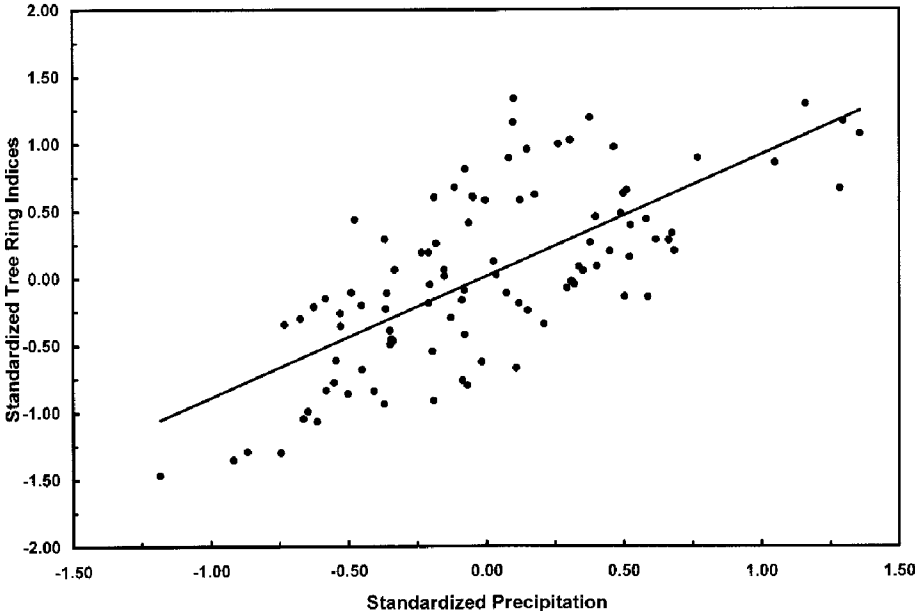

Figure 1a. Scatter plot of the 5-year, September-August, Rapid City standardized precipitation versus the 5-year standardized tree-ring indices.

during year T-1, T, and T+1. Yet other studies have reconstructed the Palmer drought severity index (PDSI) using similar tree-growth variables as predictors (Stahle and Cleaveland 1988). In the current study, no significant additional information could be gained by performing a multiple linear regression such as these for the period of record; rather, the annual (September-August) precipitation was best explained solely by tree growth during year T (i.e., a simple correlation). Furthermore, we have examined the 5-year-average annual (September-August) precipitation since this has the advantage of highlighting periods of extended drought, and also benefits from the stronger correlations (Table 1, Fig. 1b). This study also differs from previous studies in that we examine the standardized precipitation, and not the actual precipitation. Since September-August precipitation from the selected sites is approximately Gaussian, this facilitates interpretation of the twice-standardized tree-ring widths during each year in terms of probability. Recently, a drought index (the Stan-

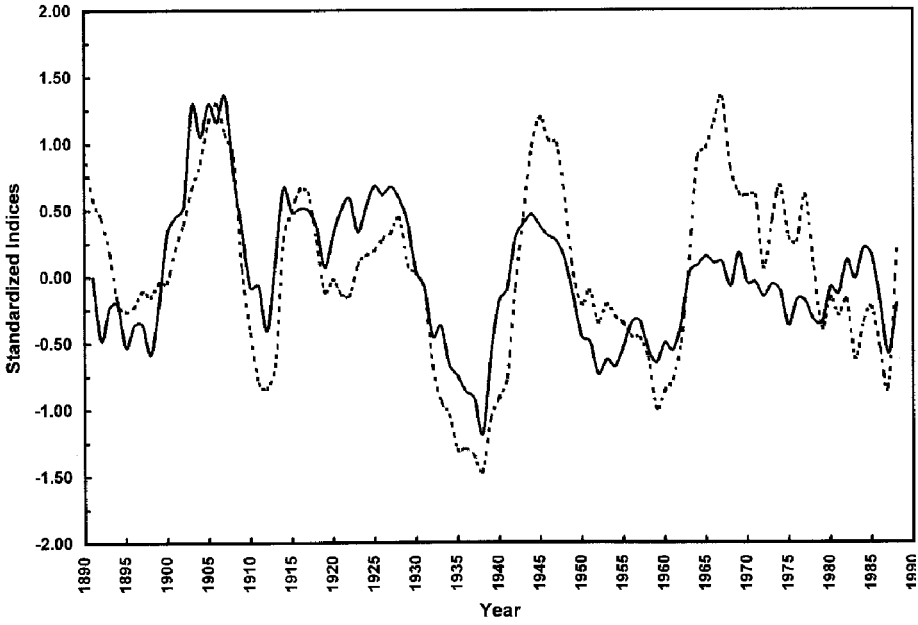

Figure 1b. Plot of 5-year, September-August Rapid City standardized precipitation data (dashed) and the 5-year standardized tree-ring indices (solid).

dardized Precipitation Index) has been developed which takes an approach similar to ours in monitoring and assessing drought (Hayes *et al.* 1999).

The twice standardized tree ring index values are shown in Figure 2 (a table of actual values is given in Appendix A). The number of trees in the sample is also indicated in Appendix A, which must be considered when discussing the climatic features suggested by this chronology.

A spectral analysis of the tree-ring chronology is shown in Figure 3. The search for cycles that reappear in time revealed 40% of the variance is explained by high-frequency (<10

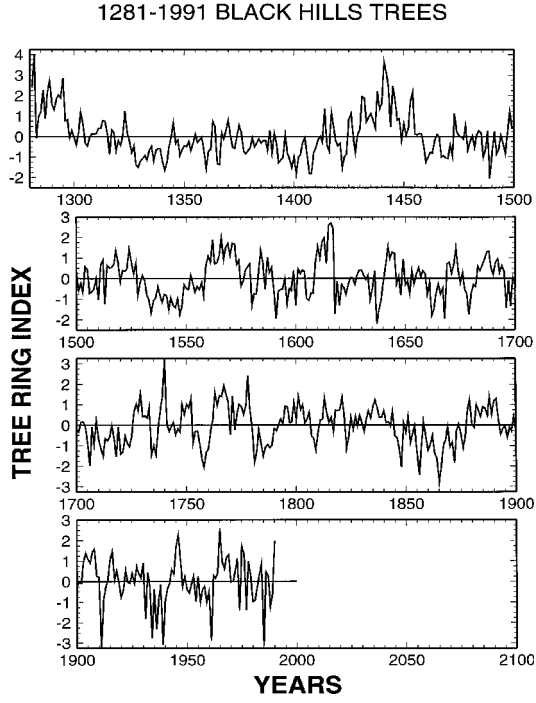

Figure 2. Twice standardized tree ring indices shown as standard deviation from the mean for years 1281-1991.

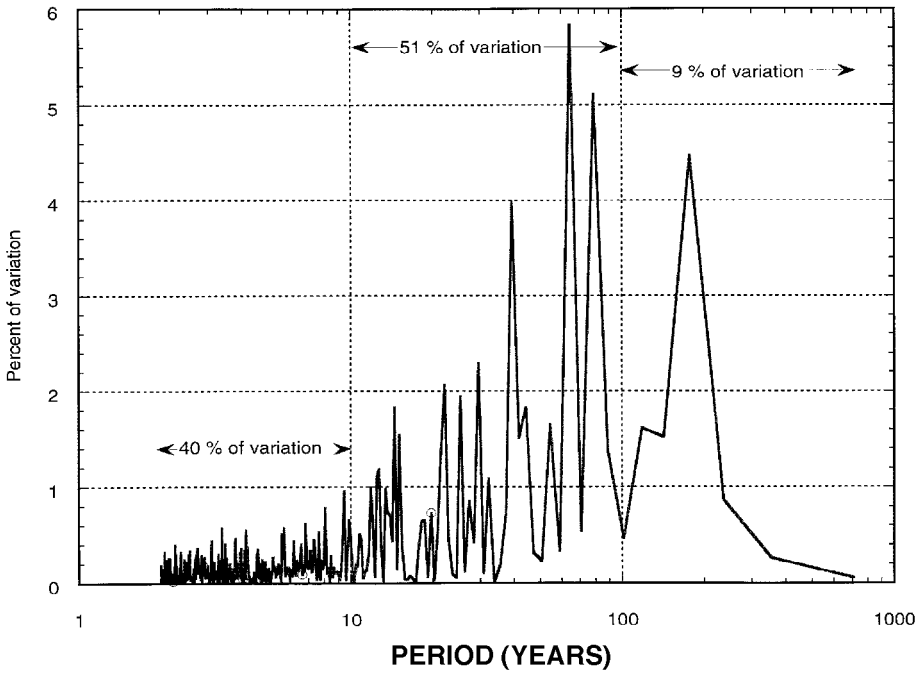

Figure 3. Spectral analysis representation of the tree-ring chronology given in Appendix A.

years) noise (i.e. year to year deviations); and 51% of the variance is distributed over periods from 10-100 years. Of note is the absence of an 11-year cycle which would correspond to the sunspot cycle. Other cycles, although weak, are 22-year (3%), 26-year (<3%), 29-year (>3%), 39-year (5%), 44-year (<3%), 54-year (<3%), 64-year (6%), 79-year (7%), and 178-year (9%). All of these values are very weak in their explanation of periodicity of the variability in the tree-ring indices. We cannot say with any confidence that there exists any relationship between these periodicities and other physical or astronomical indicators.

The early years of the single tree showed good growth as might be expected of a relatively young ponderosa pine, but the weather was most likely very favorable for its early development given its relatively large tree-ring indices (Fig. 2). In the early 1300's the tree appeared well established, but its narrow rings suggest that conditions were not favorable for growth the first one hundred years. This agrees with other regional studies by Bryson and Murray (1977) which showed a general drying of the climate across the South Dakota-Iowa region as revealed by changes in living habits of the indigenous peoples, especially in northwest Iowa. The tree-ring data shown in Figure 2 may reflect an extended dry period from 1300 to 1424 (ninety years of index values were < 0.00). Bryson and Baerreis (1968) suggest this dry period was likely associated with an expansion in the westerlies in the northern hemisphere as a whole, thus restricting the flow of Gulf of Mexico moisture flow northward in-

to the Dakotas and Iowa. A subsequent change to more favorable climate likely occurred in the 1400's, about 1430's through 1450 or so, as shown by the tree-ring indices from this one tree. Additional tree-ring samples extending back into the 1200's are needed to determine if these trends are consistent throughout the region.

Later on, the period 1792 through 1844 was a 5-decade period of lesser variability in the severity of wet and dry years; it appeared as the most stable climate period in this 711-year record—*a sign of little variability in the general circulation over the region*. 1845-1877 was a period with extreme drought, as has been noted in other studies across the Great Plains (e.g., Woodhouse and Overpeck 1998).

Finally, the remainder of the graph follows closely the known official precipitation record as measured at Rapid City beginning in 1888. It is characterized by a slight dry period from 1893 through 1902, a wet spell through 1910, a drought from 1911-1913, the major drought of the 1930's, another downward trend in growth in the 1950's, and yet another in 1988 which corresponds with the occurrence of several major forest fires in the Black Hills.

Table 2 shows the rank, estimated magnitude, and duration of the major dry and wet spells as suggested by the examination of this tree ring chronology. The magnitude of the droughts and wet spells are estimated by summing the consecutive negative and consecutive positive standardized index values, respectively, in the years shown. This summary helps us to put recent droughts in perspective. Since 1600, the 1930's "dust bowl" drought ranks 4th (note that using this summation of indices method of estimated magnitude of the drought, the 1930's was about 50% as severe as the worst drought); the 1950's drought ranked 12th. The 15-year drought associated with "The Great American Desert" period, 1859-1873, ranked 3rd.

Should additional samples support the record inferred by the single tree extending into the 1200's, the 21-year dry period from 1531 through 1551 could be the most severe drought in this 711-year chronology. By comparison, the 1930's "dust bowl" drought of the present century ranked 7th.

SUMMARY

The data from the Black Hills trees suggest dry and wet periods of similar magnitude to the 1930's drought have occurred since the late 1200's. One measure of drought indicates that the 1933-1942 period was only 50 percent of the magnitude of the 1531-1551 period, and ranks seventh overall. Caution should be used, however, when interpreting the early part of the tree-ring record as a sample size of one tree is likely not adequate to infer climatic patterns, and variables other than precipitation may be influencing the growth.

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Table 2. Duration and magnitude estimates of 15 dry and 15 wet spells.

	Dry Periods*				Wet Periods**			
Rank	**Sum of indices**	**Years**	**No. Years**	**% of MAX**	**Sum of indices**	**Years**	**No. Years**	**% of MAX**
1	–20.15	1531-1551[a]	21	100.0	29.03	1429-1448[a]	20	100.0
2	–18.30	1325-1344[a]	20	90.8	23.3	1284-1297[a]	14	80.3
3	–16.62	1859-1873	15	82.5	19.15	1559-1574[a]	16	66.0
4	–14.70	1397-1411[a]	15	73.0	15.57	1609-1617	9	53.6
5	–13.25	1710-1725	16	65.8	10.37	1762-1769	8	35.7
6	–10.34	1780-1791	12	51.3	9.14	1882-1892	11	31.5
7	–10.08	1933-1942	10	50.0	8.92	1683-1695	12	30.0
8	–8.76	1753-1761	9	43.5	8.15	1792-1806	15	28.1
9	–8.40	1660-1668	9	44.7	7.89	1903-1910	8	27.2
10	–6.48	1580-1598[a]	9	32.2	7.57	1962-1969	8	26.1
11	–5.98	1852-1857	6	29.7	7.09	1773-1779	7	24.4
12	–5.96	1956-1961	6	29.6	6.13	1832-1842	11	21.1
13	–5.44	1467-1472[a]	6	27.0	6.10	1726-1733	8	21.0
14	–5.30	1377-1388[a]	12	26.3	5.97	1943-1947	5	20.6
15	–5.00	1637-1640	4	24.8	5.66	1641-1645	5	19.5

* "narrow rings" ** "wide rings"

[a]sample size <5 trees, and is likely not adequate to reliably infer precipitation patterns.

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APPENDIX A

Twice-Standardized Tree-Ring Indices

NO. OF TREES	YEAR	STD2	NO. OF TREES	YEAR	STD2	NO. OF TREES	YEAR	STD2
1	1281	2.40	1	1321	-0.42	1	1361	-0.72
"	1282	4.02	"	1322	0.07	"	1362	-0.64
"	1283	-0.09	"	1323	1.25	"	1363	0.74
"	1284	0.95	"	1324	0.20	"	1364	0.50
"	1285	1.20	"	1325	-0.22	"	1365	-1.33
"	1286	2.28	"	1326	-0.78	2	1366	-1.39
"	1287	0.87	"	1327	-0.48	"	1367	0.18
"	1288	2.16	"	1328	-1.39	"	1368	-0.13
"	1289	2.73	"	1329	-1.51	"	1369	0.28
"	1290	1.58	"	1330	-1.20	"	1370	0.78
"	1291	1.31	"	1331	-1.08	"	1371	0.04
"	1292	1.85	"	1332	-0.93	"	1372	-0.54
"	1293	2.03	"	1333	-1.15	"	1373	-0.58
"	1294	1.90	"	1334	-0.69	"	1374	0.00
"	1295	2.86	"	1335	-0.49	"	1375	0.54
"	1296	0.78	"	1336	-1.19	"	1376	0.19
"	1297	0.82	"	1337	-0.68	"	1377	-0.72
"	1298	-0.06	"	1338	-0.60	"	1378	-0.85
"	1299	0.30	"	1339	-0.60	"	1379	-0.72
"	1300	-0.05	"	1340	-1.23	"	1380	-0.57
"	1301	-0.41	"	1341	-1.66	"	1381	-0.08
"	1302	0.21	"	1342	-1.26	"	1382	-0.51
"	1303	1.19	"	1343	-0.52	"	1383	-0.34
"	1304	0.65	"	1344	-0.04	"	1384	-0.13
"	1305	-0.32	"	1345	0.68	"	1385	-0.32
"	1306	-0.46	"	1346	-0.35	"	1386	-0.20
"	1307	-0.02	"	1347	-0.19	"	1387	-0.19
"	1308	0.13	"	1348	-0.94	"	1388	-0.67
"	1309	0.13	"	1349	-0.66	"	1389	0.31
"	1310	0.14	"	1350	-0.46	"	1390	-0.80
"	1311	0.40	"	1351	-0.50	"	1391	0.16
"	1312	0.40	"	1352	-0.26	"	1392	-0.36
"	1313	0.78	"	1353	-0.69	3	1393	-1.28
"	1314	0.75	"	1354	-0.33	"	1394	-1.16
"	1315	0.16	"	1355	0.11	"	1395	-1.03
"	1316	-0.88	"	1356	-0.29	"	1396	0.05
"	1317	0.32	"	1357	-0.16	"	1397	-0.84
"	1318	0.21	"	1358	-0.04	"	1398	-1.09
"	1319	-0.69	"	1359	-0.72	"	1399	-1.53
"	1320	-0.20	"	1360	-1.57	"	1400	-1.03

NO. OF TREES	YEAR	STD2	NO. OF TREES	YEAR	STD2	NO. OF TREES	YEAR	STD2
3	1401	-1.83	4	1446	1.76	4	1491	0.24
"	1402	-1.02	"	1447	0.81	"	1492	-0.84
"	1403	-0.89	"	1448	0.87	"	1493	-0.65
"	1404	-0.28	"	1449	-0.17	"	1494	0.05
"	1405	-0.07	"	1450	0.62	"	1495	-0.34
"	1406	-0.95	"	1451	0.08	"	1496	-0.83
"	1407	-1.83	"	1452	0.41	"	1497	0.23
"	1408	-1.83	"	1453	1.70	"	1498	1.23
"	1409	-0.82	"	1454	2.16	"	1499	0.42
"	1410	-0.54	"	1455	0.13	"	1500	0.38
"	1411	-0.15	"	1456	0.09	"	1501	-0.58
"	1412	0.00	"	1457	0.16	"	1502	-0.23
"	1413	-0.27	"	1458	0.14	"	1503	-0.66
"	1414	1.36	"	1459	-0.43	"	1504	0.58
"	1415	-0.40	"	1460	-1.26	"	1505	0.43
"	1416	-0.72	"	1461	-1.01	"	1506	-0.72
"	1417	1.17	"	1462	-0.77	"	1507	-0.63
"	1418	0.56	"	1463	-0.81	"	1508	-0.50
"	1419	-0.21	"	1464	-0.20	"	1509	-0.03
"	1420	-0.46	"	1465	0.10	"	1510	-1.02
"	1421	-0.33	"	1466	0.00	"	1511	0.64
"	1422	-1.53	"	1467	-1.01	"	1512	0.89
"	1423	-1.00	"	1468	-0.93	"	1513	-1.24
"	1424	-0.86	"	1469	-1.05	"	1514	0.64
"	1425	0.85	"	1470	-1.08	"	1515	0.51
"	1426	1.11	"	1471	-0.41	"	1516	0.55
"	1427	-0.06	"	1472	-0.96	"	1517	0.69
4	1428	-0.80	"	1473	1.11	"	1518	1.33
"	1429	0.09	"	1474	0.24	"	1519	0.76
"	1430	0.33	"	1475	0.05	"	1520	-0.15
"	1431	1.95	"	1476	-0.02	"	1521	0.38
"	1432	1.89	"	1477	-0.13	"	1522	0.36
"	1433	0.72	"	1478	0.51	"	1523	0.46
"	1434	0.93	"	1479	-0.65	"	1524	1.41
"	1435	1.12	"	1480	-0.25	"	1525	0.78
"	1436	0.81	"	1481	0.02	"	1526	0.22
"	1437	0.43	"	1482	-0.52	"	1527	0.76
"	1438	2.19	"	1483	0.08	"	1528	-0.82
"	1439	1.34	"	1484	0.30	"	1529	-0.19
"	1440	1.61	"	1485	0.19	"	1530	0.14
"	1441	3.66	"	1486	-1.02	"	1531	-0.09
"	1442	3.09	"	1487	-0.60	"	1532	-0.81
"	1443	2.50	"	1488	0.28	"	1533	-1.02
"	1444	0.46	"	1489	-2.06	"	1534	-1.65
"	1445	2.47	"	1490	-0.83	"	1535	-1.09

NO. OF TREES	YEAR	STD2	NO. OF TREES	YEAR	STD2	NO. OF TREES	YEAR	STD2
4	1536	-0.93	4	1581	-0.74	8	1626	0.02
"	1537	-0.41	"	1582	-0.76	"	1627	-0.28
"	1538	-0.80	"	1583	0.24	"	1628	0.16
"	1539	-1.16	"	1584	1.36	"	1629	0.41
"	1540	-0.77	"	1585	0.74	"	1630	0.41
"	1541	-0.84	"	1586	-0.51	"	1631	0.17
"	1542	-1.51	"	1587	1.04	"	1632	-0.04
"	1543	-0.93	"	1588	0.29	"	1633	0.16
"	1544	-1.31	"	1589	0.54	"	1634	-1.03
"	1545	-1.41	"	1590	-0.57	"	1635	-0.47
"	1546	-1.01	"	1591	-1.96	"	1636	0.37
"	1547	-1.74	"	1592	-0.60	"	1637	-2.20
"	1548	-1.39	"	1593	-0.54	"	1638	-1.55
"	1549	-0.29	"	1594	-0.50	"	1639	-1.08
"	1550	-0.47	"	1595	-0.03	"	1640	-0.17
"	1551	-0.52	"	1596	-1.22	"	1641	0.63
"	1552	0.16	"	1597	-0.49	"	1642	1.55
"	1553	-0.21	"	1598	-0.57	"	1643	0.98
"	1554	-0.66	"	1599	0.39	"	1644	1.28
"	1555	-0.38	"	1600	-1.32	"	1645	1.22
"	1556	-0.52	5	1601	0.47	"	1646	-0.27
"	1557	-0.21	"	1602	0.23	"	1647	0.09
"	1558	-0.82	"	1603	0.41	"	1648	-0.81
"	1559	0.62	"	1604	0.33	"	1649	0.96
"	1560	1.08	"	1605	-0.98	"	1650	-0.31
"	1561	0.79	"	1606	-1.05	"	1651	-0.13
"	1562	1.88	"	1607	-0.69	"	1652	-0.73
"	1563	0.70	"	1608	-0.71	"	1653	0.37
"	1564	0.75	"	1609	0.76	"	1654	-0.26
"	1565	1.10	7	1610	1.56	"	1655	0.20
"	1566	2.01	"	1611	1.00	"	1656	0.53
"	1567	0.92	"	1612	1.80	"	1657	0.09
"	1568	1.50	8	1613	1.98	"	1658	0.37
"	1569	1.71	"	1614	0.72	"	1659	0.23
"	1570	1.13	"	1615	2.60	"	1660	-0.76
"	1571	1.72	"	1616	2.71	"	1661	-0.18
"	1572	1.68	"	1617	2.44	"	1662	-1.80
"	1573	0.68	"	1618	-1.72	"	1663	-1.53
"	1574	0.88	"	1619	-0.13	"	1664	-0.72
"	1575	-0.37	"	1620	-1.29	"	1665	-0.27
"	1576	-0.11	"	1621	-0.29	"	1666	-0.75
"	1577	0.42	"	1622	-0.43	"	1667	-0.47
"	1578	0.55	"	1623	-0.77	"	1668	-1.96
"	1579	0.72	"	1624	-0.30	"	1669	0.70
"	1580	-1.30	"	1625	0.05	"	1670	0.78

NO. OF TREES	YEAR	STD2	NO. OF TREES	YEAR	STD2	NO. OF TREES	YEAR	STD2
8	1671	0.25	10	1716	-0.01	15	1761	-0.24
"	1672	0.70	"	1717	-0.67	"	1762	0.31
"	1673	1.47	"	1718	-1.53	"	1763	1.59
"	1674	0.45	"	1719	-0.09	"	1764	0.96
"	1675	-0.21	"	1720	-1.41	"	1765	1.44
"	1676	0.29	11	1721	-1.27	"	1766	1.41
"	1677	-0.54	"	1722	-0.55	"	1767	1.96
"	1678	-0.72	"	1723	-0.86	16	1768	1.56
"	1679	-1.75	"	1724	-1.03	"	1769	1.14
9	1680	-0.70	12	1725	-0.55	"	1770	-0.47
"	1681	-0.29	"	1726	0.66	"	1771	1.44
"	1682	-0.38	"	1727	1.05	"	1772	-0.23
"	1683	0.59	"	1728	0.71	17	1773	0.51
"	1684	0.40	"	1729	1.57	"	1774	1.02
"	1685	0.66	"	1730	0.42	"	1775	0.93
"	1686	0.94	"	1731	0.46	"	1776	0.54
"	1687	1.31	"	1732	0.36	"	1777	0.95
"	1688	1.32	"	1733	0.87	"	1778	2.42
"	1689	0.53	"	1734	-1.43	"	1779	0.72
"	1690	0.19	"	1735	-1.00	"	1780	-0.21
"	1691	0.78	"	1736	-1.43	"	1781	-1.80
"	1692	0.95	"	1737	-0.13	"	1782	-1.25
"	1693	0.30	"	1738	0.67	"	1783	-0.23
"	1694	0.62	"	1739	1.41	"	1784	-0.86
"	1695	0.33	"	1740	3.27	"	1785	-1.51
"	1696	-1.44	13	1741	0.05	"	1786	-1.06
"	1697	-0.10	"	1742	-0.30	"	1787	-0.94
"	1698	-1.34	"	1743	-0.09	"	1788	-1.08
"	1699	0.06	14	1744	0.13	18	1789	-0.97
"	1700	-0.24	15	1745	-0.46	"	1790	-0.16
"	1701	-0.33	"	1746	-0.17	"	1791	-0.27
"	1702	0.15	"	1747	-0.34	"	1792	0.05
"	1703	0.19	"	1748	1.00	"	1793	0.32
"	1704	-0.05	"	1749	0.54	"	1794	0.07
"	1705	-0.85	"	1750	1.02	19	1795	0.94
10	1706	-1.97	"	1751	0.85	"	1796	0.92
"	1707	-0.10	"	1752	1.31	"	1797	0.13
"	1708	-0.90	"	1753	-0.86	"	1798	0.15
"	1709	0.18	"	1754	-0.29	"	1799	1.08
"	1710	-0.68	"	1755	-0.27	"	1800	0.27
"	1711	-1.14	"	1756	-0.91	"	1801	1.41
"	1712	-1.51	"	1757	-1.67	"	1802	0.76
"	1713	-0.63	"	1758	-2.06	"	1803	0.99
"	1714	-0.75	"	1759	-1.32	"	1804	0.12
"	1715	-0.57	"	1760	-1.14	"	1805	0.29

NO. OF TREES	YEAR	STD2	NO. OF TREES	YEAR	STD2	NO. OF TREES	YEAR	STD2
19	1806	0.65	21	1851	0.41	22	1896	-0.58
"	1807	-0.54	"	1852	-0.84	"	1897	-0.09
"	1808	-0.61	"	1853	-0.44	"	1898	-0.27
"	1809	-1.19	"	1854	-0.03	"	1899	0.42
"	1810	-0.33	"	1855	-1.30	"	1900	-0.25
"	1811	0.25	"	1856	-2.42	"	1901	-0.04
"	1812	0.30	"	1857	-0.95	"	1902	-0.08
"	1813	1.33	"	1858	0.18	"	1903	0.94
"	1814	0.62	"	1859	-0.51	"	1904	1.39
"	1815	0.15	"	1860	-1.21	"	1905	1.10
"	1816	0.39	"	1861	-0.96	"	1906	0.92
"	1817	-0.30	22	1862	-0.50	"	1907	1.46
"	1818	0.74	"	1863	-1.34	"	1908	1.59
"	1819	0.71	"	1864	-1.63	"	1909	0.28
"	1820	0.76	"	1865	-2.78	"	1910	0.21
"	1821	1.36	"	1866	-1.97	"	1911	-3.27
"	1822	0.33	"	1867	-0.96	"	1912	-0.92
"	1823	-1.14	"	1868	-0.74	"	1913	-0.31
"	1824	-0.56	"	1869	-0.04	"	1914	0.07
"	1825	0.38	"	1870	-1.05	"	1915	1.08
"	1826	-0.18	"	1871	-0.81	"	1916	1.47
"	1827	0.44	"	1872	-1.84	"	1917	0.09
"	1828	-0.03	"	1873	-0.28	"	1918	0.55
"	1829	0.50	"	1874	0.06	"	1919	-0.04
20	1830	0.11	"	1875	-0.42	"	1920	-0.75
"	1831	-0.31	"	1876	-1.10	"	1921	-0.41
"	1832	0.31	"	1877	-0.51	"	1922	0.53
"	1833	0.70	"	1878	0.68	"	1923	-0.02
"	1834	0.37	"	1879	1.25	"	1924	-0.07
"	1835	0.91	"	1880	0.05	"	1925	0.39
"	1836	1.22	"	1881	-0.10	"	1926	-0.06
"	1837	0.83	"	1882	1.00	"	1927	0.74
"	1838	0.39	"	1883	0.74	"	1928	0.40
"	1839	0.39	"	1884	0.43	"	1929	0.19
"	1840	0.71	"	1885	0.90	"	1930	0.91
21	1841	0.13	"	1886	0.84	"	1931	-1.80
"	1842	0.17	"	1887	0.49	"	1932	0.44
"	1843	-0.04	"	1888	1.41	"	1933	-0.20
"	1844	0.66	"	1889	0.56	"	1934	-2.76
"	1845	-0.52	"	1890	1.19	"	1935	-0.38
"	1846	-0.59	"	1891	1.30	"	1936	-2.33
"	1847	-1.19	"	1892	0.28	"	1937	-0.84
"	1848	-2.04	"	1893	-0.43	"	1938	-0.15
"	1849	0.09	"	1894	-0.16	"	1939	-3.07
"	1850	-0.34	"	1895	-0.05	"	1940	-0.95

NO. OF TREES	YEAR	STD2
22	1941	-0.34
"	1942	-0.06
"	1943	0.60
"	1944	0.37
"	1945	1.71
"	1946	2.27
"	1947	1.02
"	1948	-0.23
"	1949	0.23
"	1950	-0.39
"	1951	-0.55
"	1952	-0.09
"	1953	0.26
"	1954	-0.95
"	1955	0.26
"	1956	-0.99
"	1957	-0.36
"	1958	-0.24
"	1959	-1.01
"	1960	-0.48
"	1961	-2.88
"	1962	0.30
"	1963	0.17
"	1964	0.42
"	1965	2.60
"	1966	0.98
"	1967	0.63
"	1968	1.16
"	1969	1.31
"	1970	-0.02
"	1971	+0.02
21	1972	0.56
"	1973	1.14
"	1974	-1.39
"	1975	1.72
"	1976	1.35
"	1977	-1.38
"	1978	0.99
"	1979	0.33
"	1980	-0.97
20	1981	-0.91
"	1982	-0.25
"	1983	0.32
"	1984	0.88
18	1985	-3.15
18	1986	0.48
"	1987	0.27
16	1988	-1.20
"	1989	-0.59
15	1990	1.99
13	1991	

FACTORS INFLUENCING AGE RATIOS OF MALE MALLARDS HARVESTED IN EASTERN SOUTH DAKOTA

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ABSTRACT

We analyzed harvest age ratios (immatures:adult) for male mallards (*Anas platyrhynchos*) (n = 185) shot by waterfowl hunters during September - November 1996 from 10 counties in eastern South Dakota. Our objectives were to evaluate within-year temporal trends in male mallard age ratios and to determine if hunting method, area hunted, and hunter effort influenced these age ratios. Chi-square goodness-of-fit tests were used to determine age-specific differences in male mallards harvested within and among the 3 sample periods (Period 1 = 28 Sept. - 13 Oct., Period 2 = 14 - 30 Oct., Period 3 = 31 Oct. - 16 Nov.). Goodness-of-fit tests also were used to determine influence of hunting method on age of harvested male mallards and differences in hunter effort by area. There was no difference (P = 0.2) in the number of harvested adult male mallards across sample periods, however, a significant (P < 0.01) sample period effect was documented for immature male mallards. Over twice as many adult than immature male mallards were harvested during the last sample period. Male mallard age ratios declined (1.19, 0.83, 0.48) across the 3 sample periods defined for this study. More male mallards of both age classes were harvested using decoys over-water than any other method. Participants in this study spent more time hunting on private than on public land. Although we recognize that immature waterfowl are considered more susceptible to harvest, other factors may contribute to differences in age-specific harvests at a smaller spatial scale.

INTRODUCTION

Duck harvest regulations were historically driven by mallard population indices (Martin et al., 1979) and more recently are derived from a matrix incorporating number of May ponds and mallard population indices (i.e., adaptive harvest management; Johnson et al., 1993; Nichols et al., 1995). A variety of factors influence annual mallard production including breeding population size, age and breeding experience, body condition, nesting cover, wetland conditions, local weather patterns, and predation (Johnson et al., 1992). One method of assessing mallard recruitment is to determine the proportion of juveniles from the harvest (March and Hunt, 1978; Kaminski and Gluesing, 1987; Raveling and Heitmeyer, 1989). Estimates of harvest age ratios are obtained

annually from duck wings sent in by a random sample of waterfowl hunters (Geissler, 1990). Annual estimates (1961 - 95) of male mallard age ratios in the Central Flyway averaged 0.66 immatures:adult, the lowest ratio among the four flyways. Within the Central Flyway, South Dakota had the second highest male mallard age ratio (0.93 immatures:adult) during this same period (Sharp, 1997). The low harvest age ratio obtained for male mallards in the Central Flyway is an interesting phenomena considering the Prairie Pothole Region is an important breeding area for continental duck populations (Smith et al., 1964).

Data obtained from waterfowl hunters are commonly used in waterfowl ecology studies and for management of waterfowl populations. However, data from hunter-killed birds may be biased due to differential vulnerability attributed to age (Martin et al., 1979) and sex (Olson, 1965), flock size (Olson, 1965; Dufour and Ankney, 1995), species (Stott and Olson, 1972), brightness of plumage (Metz and Ankney, 1991), and body condition (Greenwood et al., 1986; Reinecke and Shaiffer, 1988; Dufour et al., 1993; Heitmeyer et al., 1993). Hunter selectivity likely plays an important role accounting for some of the differential vulnerability of hunted waterfowl (Metz and Ankney, 1991; Gleason and Jenks, 1997). Our objectives were to evaluate within year temporal trends in male mallard harvest age ratios in relation to hunting method, area hunted, and hunter effort. We predicted that male mallard age ratios would differ throughout the season with more immatures harvested early compared to late season. We further predicted that hunting method also would influence age ratios with a higher proportion of immatures harvested over decoys compared to other hunting methods.

STUDY AREA

The study area included mallards collected from 10 counties in eastern South Dakota. Wetland demography in eastern South Dakota is characteristic of the glaciated prairie pothole region with a relatively large number of permanent lakes, and semi-permanent, seasonal, and temporary wetlands (Brewster et al., 1976; Johnson and Higgins, 1997). Waterfowl distribution on the study area has been relatively consistent from year-to-year depending on wetland conditions and local weather patterns. Early in the hunting season individuals, pairs, late broods, and small flocks are scattered across the study area using seasonal and semipermanent wetlands (Gleason pers. obs.). As the season progresses, harvest pressure (Kirby et al., 1989) and low temperatures (Rakowicz et al., 1996) force the birds off smaller wetlands onto larger, open water lakes and rivers.

South Dakota hunters are regulated by a daily-bag system with restrictions on certain species and sexes. During the 1996 - 97 waterfowl season, the daily bag limit was 5 ducks of which no more than 1 could be a female mallard. In South Dakota, there was a 60-d regular season (i.e., ducks) and opening date for the north zone occurred on 28 September. Most hunters participating in this study hunted in the middle zone with the season opening on 5 October and closing on 3 December. Daily shooting times for all waterfowl species in South Dakota was one-half hour before sunrise (CST) to sunset.

METHODS

Mallard wings were collected opportunistically from hunters in eastern South Dakota (28 September - 16 November). Ages of male mallards were determined by cloacal examination (Hochbaum, 1942) if the whole carcass was obtained or by wing plumage (Carney, 1992). Mallard wings were aged based on the color, shape, and wear of the tertails, scapulars, and coverts (Carney, 1992). The greater tertial coverts are a good indicator of age for both sexes to at least 1 March (Hopper and Funk, 1970). Since collection for this study was completed by mid-November, we considered our aging techniques to be accurate for this study. Mallards were assigned to one of three periods: early (28 Sept. - 13 Oct.), mid (14 Oct. - 30 Oct.), and late (31 Oct. - 16 Nov.) depending on when they were shot (Rakowicz et al., 1996). Time periods were defined at the end of the study with the late period occurring after the first freeze on 30 October.

Harvest age ratios were calculated for the three periods by dividing the number of immatures by the number of adults. We also separated the harvest by hunting method (i.e., decoying over water, decoying over land, pass shooting, jump shooting) and area hunted (i.e., public vs. private) and documented temporal and between-area differences in hunter effort. For this study, hunter effort was defined as the amount of time spent in pursuit of waterfowl on a specified area or during a specified time period. Kill rate was determined for the three sample periods and the two hunting areas. For this study, kill rate was the number of mallards harvested divided by the number of hours hunted. Chi-square goodness-of-fit tests (Wilkinson, 1990) were used to determine age-specific differences in male mallards harvested within and among the three sample periods, to determine influence of hunting method on age of harvested male mallards and differences in hunter effort by area. Chi-square Goodness-of-fit tests also were used to evaluate temporal and spatial influences on the number of male mallards harvested by method.

RESULTS

A total of 185 (85 immatures, 100 adults; overall age ratio = 0.85) male mallards were collected during the early (38 immatures, 32 adults), mid (34 immatures, 41 adults), and late (13 immatures, 27 adults) seasons (Fig. 1). Age ratios declined (1.19, 0.83, 0.48) across the three sample periods. There was no difference ($\chi^2 = 3.02$, df = 2, $P = 0.2$) in the number of adult males harvested during the three periods, however, there was a significant ($\chi^2 = 12.73$, df = 2, $P = 0.002$) temporal difference in the number of immature males harvested. Within a sample period, age ratios differed only for the late period when significantly ($\chi^2 = 4.90$, df = 2, $P = 0.03$) more adults were shot. For this study, the majority of male mallards were shot in Brookings and Kingsbury counties (Table 1) with mallard harvest increasing on weekends (Fig. 2).

A large proportion (85.95%) of males were collected by decoying over-water (n = 92) and land (n = 67). In contrast, pass shooting and jump shooting accounted for only 21 and 5 male mallards, respectively. For both age class-

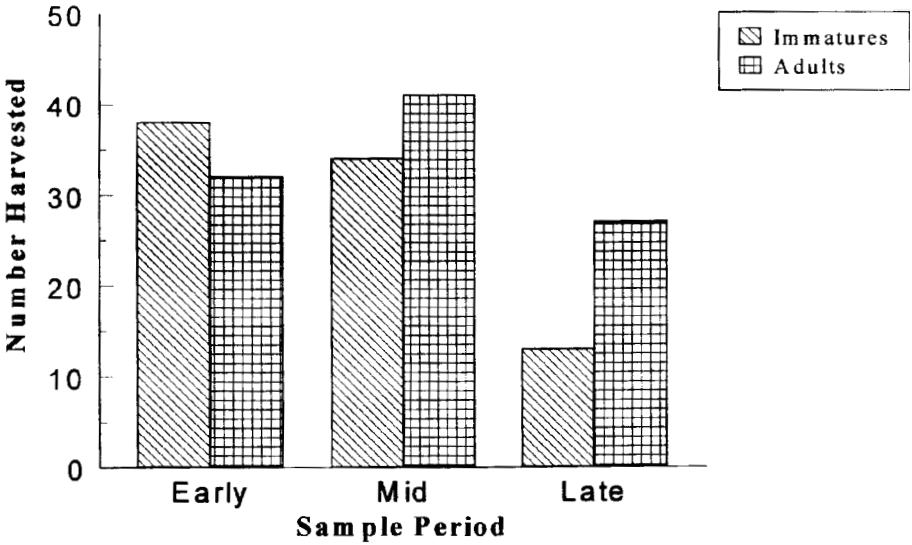

Figure 1. Number and age of male mallards harvested during the 3 sample periods defined for this study.

Table 1. Distribution of male mallards harvested by waterfowl hunters participating in this study, 1996.

County	**Number Harvested**
Brookings	55
Brown	7*
Clark	2
Hamlin	3
Hand	2
Kingsbury	86
Lake	6
Marshall	18
McPherson	1*
Moody	5
Total	185

* A total of 8 male mallards was harvested in the north zone, of which 3 occurred on opening weekend (28 - 29 September).

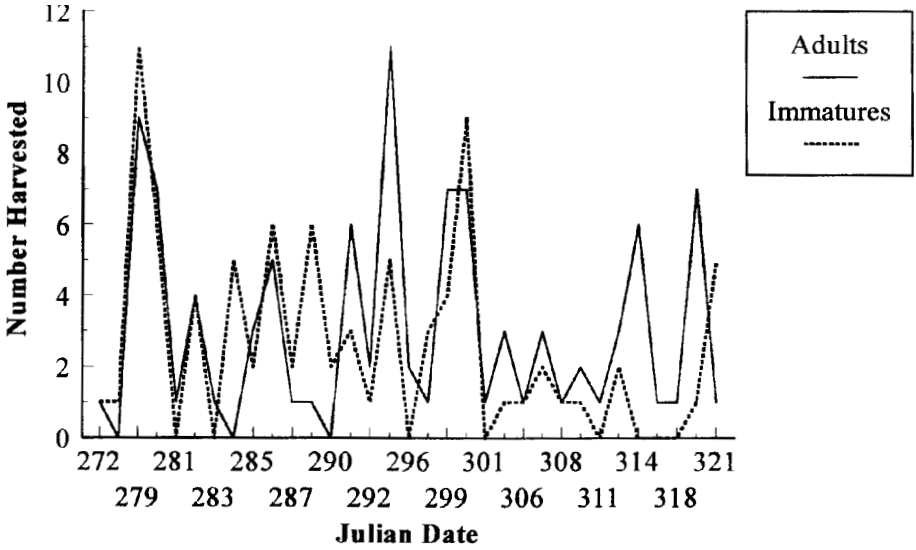

Figure 2. Harvest chronology by age for male mallards collected during this study.

es, significantly ($P < 0.01$) more mallards were harvested by decoying over-water than by other methods. Age ratios for the four hunting methods averaged across time periods were 0.70 (decoys over-water), 1.03 (decoys over-land), 0.75 (pass shooting), and 4.00 (jump shooting), respectively. However, no age differences ($P > 0.09$) were detected when comparing male mallards collected using the four hunting methods. Harvest age ratios for the two decoy methods combined was 0.87, which was similar to the overall age ratio of 0.85. Early period age ratios obtained using decoys over-land was 2.33 compared to 0.43 during the late period (Table 2). Neither pass nor jump shooting provided sufficient sample sizes for determining age ratios by period. Similarly, data were not sufficient to determine temporal differences in area-by-method age ratios.

Hunters participating in this study spent a total of 33 days afield; 20 weekdays and 13 weekend days. The highest single day harvest (n = 20, 9 adults and 11 immatures) for male mallards occurred on opening day of the middle zone (October 5). Daily totals (all hunters combined) for adult and immature male mallards were 3.0 + 0.51 (SE) and 2.6 + 0.48 (SE)/day, respectively. Approximately 55% (n = 102) of the total male mallard harvest occurred on weekends. Hunter effort was relatively constant for the first and second periods (66.5 and 72.5 hrs.), but declined considerably during the last period (45 hrs.). Total effort of waterfowl hunters on both private and public lands was 184 hours. Eighty-two percent (151.5 hours, period $\bar{x} = 50.5$ hrs.) of the total hunting effort occurred on private land, compared to 18% (32.5 hours, period $\bar{x} = 10.83$) on public land (Table 3). More time ($P < 0.01$) was spent hunting on private than on public land irrespective of the sample period hunted. No difference ($\chi^2 = 1.96$, df = 2, $P = 0.38$) was detected in hunter effort by sample

Table 2. Number and age ratio of male mallards harvested during the 3 sample periods (1 = 28 September - 13 October, 2 = 14 October - 30 October, 3 = 31 October - 16 November) using the 4 hunting methods (1[A] = decoys over-water, 2[B] = decoys over-land, 3[C] = pass shooting , 4[D] = jump shooting) defined for this study.

Sample Period	Number Harvested	Age Ratio	Method
1	47	0.88	1
1	20	2.33	2
1	0	0*	3
1	3	2.00	4
Total	70	1.18	
2	34	0.70	1
2	27	1.07	2
2	12	0.50	3
2	2	0*	4
Total	75	0.83	
3	11	0.22	1
3	20	0.43	2
3	9	1.25	3
3	0	0*	4
Total	40	0.48	

[A] Age ratio obtained by decoying over-water = 0.70, [B] Age ratio obtained by decoying in the field = 1.03, [C] Age ratio obtained by pass shooting = 0.75, [D] Age ratio obtained by jump shooting = 4.00
* Unable to determine age ratios from this sample.

Table 3. Hunter effort (in hours) by area (1 = Public land, 2 = Private land) for the 3 sample periods (1 = 28 September - 13 October, 2 = 14 October - 30 October, 3 = 31 October - 16 November) defined for this study.

Sample Period	Hunter Effort	Area
1	15.0	1
1	51.5	2
Total*	66.5	Combined
2	15.5	1
2	57.0	2
Total	72.5	Combined
3	2.0	1
3	43.0	2
Total	45	Combined
Overall	184	

* A total of 3 male mallards were harvested in the north zone on opening weekend (28 - 29 September).

period for individuals on private land, however, less time (χ^2 = 10.563, df = 2, P = 0.005) was spent hunting on public land during the late period.

Overall, kill rate (# mallards shot/hr.) declined slightly across the three sample periods (Period 1 = 1.05, Period 2 = 1.03, Period 3 = 0.88). Kill rates (+ SE, all four methods combined) varied by area hunted with the highest average harvest (1.08 + 0.11) occurring on private land. In comparison, kill rate on public land averaged 0.85 + 0.33. No temporal differences (χ^2 = 2.947, df = 2, P = 0.229) were found for male mallards collected on public areas, however, a significant (χ^2 = 9.554, df = 2, P = 0.008) sample period affect was documented for male mallards shot on private land. Temporal differences (P < 0.0001) existed in number of male mallards collected on private and public lands.

DISCUSSION

Temporal Differences in Age Ratios

Our results indicate a consistent temporal pattern of harvest for male mallards shot by hunters in eastern South Dakota. As predicted, age ratios obtained for this study declined (1.19, 0.83, 0.48) (Table 2) during the 1996 - 97 waterfowl season. Analogous to our results, harvest data obtained from the Harvest Surveys Section of the U. S. Fish and Wildlife Service (USFWS) resulted in age ratios of 1.32 (72 males), 1.25 (81 males), and 0.86 (65 males) for the same periods and counties defined for this study. Sample sizes for our data and the USFWS were relatively similar, however, the sample of wings obtained by the USFWS is a random and representative sample of waterfowl hunters (Geissler, 1990). Conversely, hunters participating in our study likely represent a small contingent of South Dakota's serious waterfowl hunters. The number of males harvested by period were relatively similar for the first two sample periods, but declined during the late period (Figure 1). Similarly, age ratios were not different for the first two sample periods, but significant differences occurred during the late period with a greater number of adults than immatures being shot (Table 2). For this study, kill rate declined across the three sample periods with the lowest take occurring during the late period when age ratios were also the lowest.

It is well established that immature waterfowl are more vulnerable to hunting than are adults (Bellrose et al., 1961; Geis, 1972; Munro and Kimball, 1982) and in some cases, males are more vulnerable to hunting than are females (Anderson, 1975; Burnham et al., 1984; Nichols et al., 1990; Metz and Ankney, 1991). Trost et al., (1987) found that waterfowl harvest is a direct function of both hunter numbers and hunter success. At the same time, there exists a relationship between waterfowl abundance and subsequent harvest (Trost et al., 1987). Taking into account age-specific differences in vulnerability, the age ratio for a target population is simply the proportion of immatures in that population. In our case, locally raised immature males are more available and hence more likely to be shot early in the season than later (Jessen, 1970; March and

Hunt, 1978; Clark et al., 1988, Kirby et al., 1989). Two reasons may explain why age ratios obtained later in the season were lower. First, local weather patterns (Nichols et al., 1983; Jorde et al. 1984; Nichols and Hines, 1987) and hunting pressure (Jessen, 1970; March and Hunt, 1978) may have forced the majority of locally hatched immatures to disperse or migrate from natal marshes. Second, an influx of migratory mallards with lower age ratios occurred later in the season (March and Hunt; 1978). During this study, a cold front occurred on 31 October, causing a reduction in the availability of open water. This likely caused a movement of immature male mallards off the study area. However, large flocks of field-feeding mallards (presumably adults), remained on the study area until the last week of November (Gleason pers. obs.).

Hunting Method as an Influence on Age Ratios

For this study, over 85% of male mallards were harvested using decoys, both over-water and in the field (Table 2). Age ratios obtained by averaging both decoying methods (0.87) was similar to the overall age ratio (0.85). Age ratios averaged across time periods for these two methods were 0.70 and 1.03. In comparison, age ratios averaged across time periods for pass shooting and jump shooting were 0.75 and 4.00. Our results do not support the prediction that a greater proportion of immatures would be harvested using decoys compared to other methods. However, the similar or higher age ratios obtained from males shot by pass and jump shooting is likely an artifact of small sample sizes. Very few mallards were shot using these two hunting methods. We believe that given adequate and representative samples of male mallards collected from the various hunting methods, results would support our prediction that male mallard age ratios shot over decoys would be higher than those obtained from other hunting methods.

Gleason and Jenks (1997) documented the importance of decoying as a favored method among South Dakota natural resource agency personnel participating in waterfowl hunting. Similarly, Humburg et al., (1988) identified decoying birds over water as the preferred method of waterfowl hunters responding to a survey in Missouri. On a continental basis, more waterfowl are likely shot over decoys than by any other method. Age ratios obtained from ducks shot over decoys may overestimate local recruitment in hunted waterfowl populations. Conversely, using harvest age ratios as an index to recruitment on a large geographic scale (i.e., flyway level) may in fact underestimate actual age ratios in the population (see Rakowicz et al., 1996). It has been documented that the use of decoys to entice birds within shooting range may evoke a feeding response by passing ducks (Weatherhead and Ankney, 1984). Because immature ducks are subordinate (Hepp, 1989) and possibly in poorer condition (see Table 1, Dufour et al., 1993), they must spend more time searching for food and unoccupied foraging habitats, thus increasing their exposure to hunters (Bain, 1980; Munro and Kimball, 1982; Weatherhead and Ankney, 1985). A condition bias from hunter-shot waterfowl was first established by Bain (1980) for canvasbacks (*Aythya valisineria*) and redheads (*Aythya amer-*

icana). Recent studies on mallards were consistent with these results supporting the assertion that mallards shot by hunters using decoys were in poorer condition (i.e., less mass or mass/wing length) than mallards collected by other means (Greenwood et al., 1986; Hepp et al., 1986; Reinecke and Shaiffer, 1988; Dufour et al., 1993; Heitmeyer et al., 1993).

Hunting Effort as an Influence on Age Ratios

During the season, waterfowl hunters participating in this study spent more time hunting on weekdays than on weekends. However, approximately 55% of all male mallards harvested during this study were shot on weekends (Fig 2). Of the total mallards shot during this study, 19% were shot on opening weekend of the middle zone. This higher harvest may reflect the fact that hunter numbers for the entire hunting public increased on weekends (Fig. 1; Gleason and Jenks, 1997) forcing ducks to spend more time flying in search of secluded marshes away from hunting pressure. Most of the ducks collected during this study were shot during the first 30 days of the season. This result is important considering that early in the season immature male mallards are likely more available to be shot. Similar to our results, Martin and Carney (1977) documented that a major portion of waterfowl harvested during their study occurred during the early part of the hunting season and attributed this high early season harvest to relatively mild weather conditions and high hunter numbers. Gleason and Jenks (1997) demonstrated that approximately 50% of hunter effort occurred during the first month of the season and that effort declined through time.

Hunter effort differed depending on the period and area hunted (Table 3). Nearly 82% of the total time was spent hunting on private land. Conversely, Gleason and Jenks (1997) determined that public land was the most frequently hunted area (56.3%) for waterfowl hunters participating in their survey. Average kill rates were higher for male mallards collected on private versus public lands. A temporal effect was documented when comparing numbers of male mallards shot on private versus public lands. The results we obtained likely reflects a willingness of participants in this study to seek permission to hunt waterfowl on private lands. Waterfowl use of private lands on the study area likely increased during the hunting season. Possible reasons for this shift in waterfowl distribution include, 1) an increase in hunting pressure or disturbance on public areas and subsequent dispersal to secluded marches on private land, 2) a decrease in availability of natural aquatic plants and seeds consumed by waterfowl on public areas, or 3) a dietary shift in foods consumed by waterfowl (Jorde et al., 1983; 1984). One of the highest single period age ratios (2.33) obtained during this study was for mallards collected in the first sample period using decoys over-land. Male mallards shot using this method were likely responding to decoys placed on a food source such as corn or millet.

A variety of factors can influence the harvest of waterfowl (Trost et al., 1987) and understanding biases associated with hunter-shot birds is important.

Our results indicate a decline in numbers of immature male mallards harvested throughout the season. Similarly, we found that the number of adults and immatures harvested differed by hunting methods and area hunted. We agree with Rakowicz et al., (1996) that caution should be used when interpreting harvest age ratios as an index to recruitment. Hunter numbers and a differential availability of ages and sexes during the season directly influence mallard harvest. This understanding is critical since information on mallards continues to be used as the foundation for managing duck populations in North America.

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OVER-WINTER CONDITION CHANGES IN FEMALE RING-NECKED PHEASANTS DURING TWO MILD WINTERS

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ABSTRACT

Supplemental winter food plots are a common management tool used to enhance the over-winter survival and condition (body and lipid masses) of ring-necked pheasants (*Phasianus colchicus*). However, beneficial effects on winter condition are not well documented in wild populations. We examined the effects of prior winter condition and access to winter corn food plots on spring condition levels of ring-necked pheasants by measuring the body mass and estimating lipid mass and percent lipid of 73 females transplanted to Moody County, South Dakota during winters 1994-95 and 1995-96. Measurements were repeated the following spring on the 37 survivors. Spring body mass and lipid mass were directly related to the winter values ($\underline{r}_p$ = 0.576, $\underline{P}$ < 0.001; and $\underline{r}_p$ = 0.549, $\underline{P}$ = 0.001, respectively). An inverse relationship existed between lipid mass change and percent lipid change and the winter values ($\underline{r}_p$ = -0.623, $\underline{P}$ < 0.001; and $\underline{r}_p$ = -0.597, $\underline{P}$ < 0.001, respectively). Females with winter food plots within their winter home range did not have larger body or lipid masses than females without access to winter food plots during two winters with low snow accumulation.

INTRODUCTION

Seasonal changes in ring-necked pheasant body weight are primarily due to changes in lipid mass (Anderson 1972, Kabat et al. 1956). Increased winter mass losses in penned ring-necked pheasants were positively associated with longer mass recovery periods and lower maximum mass reached later in the year (Gates and Woehler 1968). Likewise, captive ring-necked pheasants with greater lipid stores and body mass were more resistant to stress (Kabat et al. 1956). Lipid stores can be used for maintenance during periods of inclement weather or lowered food availability, thus enhancing survival. Lipids are also utilized during the reproductive season, at the end of which hens are at their lowest mass and are least able to withstand applied stress (Kabat et al.

1956). Based on weights of wild female ring-necked pheasants, Edwards et al. (1964) postulated that reproductive success could be influenced by winter severity and subsequent spring condition of females.

Food plots can benefit the condition levels of wintering northern bobwhites (*Colinus virginianus*). Northern bobwhites using food plots (corn, sorghum, and wheat) in Kansas had increased body and lipid mass and more metabolizable energy in their crops compared to individuals not using food plots (Robel et al. 1974). Birds in both groups preferred to consume native food items when possible. During periods when preferred native foods were scarce, individuals with access to food plots were able to turn to easily metabolized cultivated grains whereas those without access to food plots used native foods that were more difficult to metabolize. Northern bobwhites within 600 m of food plots consumed 14,838 cal/day (33%) more food than needed for existence requirements; those > 900 m from food plots only 86 cal/day (<1%) more (Robel et al. 1974). Northern bobwhites eating easily metabolized common broomweed (*Xanthocephalum dracunculoides*) seeds had greater lipid reserves than those eating low energy green vegetation (Leif and Smith 1993), an indication that easily metabolized grains in food plots could improve overwintering condition. Female ring-necked pheasants in South Dakota utilizing wild foods or soybeans in the winter had smaller lipid reserves than those eating corn or sorghum. Similarly, females without access to food plots had lower reserves than those with access (Bogenschutz et al. 1995).

We hypothesized that winter condition influences spring condition of wild female ring-necked pheasants, as it does in penned individuals. We also hypothesized that females with access to supplemental winter food plots would enter the reproductive season in higher condition than those without access.

STUDY AREA AND METHODS

The study was conducted in Moody County, South Dakota, from December 1994 through April 1996. The area was characterized as a prairie pothole landscape with cropland dominated by row crops, primarily corn and soybeans. Climate differed between winters, with 1994-95 being warmer than 1995-96 (Fig. 1). Snow cover on agricultural fields was minimal both winters and cover was readily available. Core study areas ($\underline{n}$ = 3) were chosen on the basis of road access, landowner cooperation, and presence of high quality winter cover, primarily dense cattail (*Typha* spp.) stands. Wetlands comprised approximately 16% of the core study areas, grasslands (including pastures and Conservation Reserve Program fields) 18%, and cropland 53%. Corn food plots (1.2 - 2.0 ha) were leased from cooperators until 1 April of each year; these food plots were located within 300 m of the cover.

Female ring-necked pheasants (19 adults, 54 juveniles) were captured from a high density population in Beadle County, South Dakota during winter (late November through mid January) with baited funnel traps, mist nets, pointing dogs (Dumke and Pils 1973), and nightlighting (Labisky 1968) and transplanted to the study sites within two days of capture. Ring-necked pheasants were present in moderate densities on the study areas, but due to landscape features

they were difficult to capture and were not used in this study. Bursal depth (Linduska 1943) was used to determine age. Mass was measured to the nearest gram. Lipid content was estimated with an EM-SCAN/TOBEC® Model 3152 scanner (EM-SCAN Inc., 3420 Constitution Dr., Springfield, IL 62707) (Purvis et al. 1999).

Necklace-mounted radio transmitters (150 - 152 MHz, 15 g) were placed on each bird prior to release. A truck-mounted, four-element dual yagi system was used to monitor the birds at distances of ≤ 1.6 km. Each bird was located three times a week from 1 January to 30 March 1995 and 1 December 1995 to 30 March 1996; three azimuths were recorded whenever possible. Ninety-five percent home range isopleths were estimated using the adaptive kernel method with CALHOME (Kie et al. 1996). In late March and early April of each year portable receivers and nets (Dumke and Pils 1973) were used to locate and recapture survivors (n = 37). All birds were then weighed, scanned, and re-released onto the core areas within 24 hours of capture.

Analysis of variance was used to test for the effect of age and year on winter total mass, lipid mass, and percent lipid. The effect of age, year, and the presence of a food plot within the home range on spring and over-winter change values were tested with analysis of covariance; the respective winter mass or lipid content value was added as a covariate. Elapsed days between measurements was also added as a covariate to the over-winter change models. If dependence between winter and the spring or over-winter change values was found, Pearson's product-moment correlation was used to find the direction of dependence. A significance level of $\underline{P} \leq 0.05$ was used for all tests. All statistics were calculated using SAS (SAS Institute, Inc. 1988).

RESULTS AND DISCUSSION

Winter body mass, lipid mass, and percent lipid (Table 1) differed by year ($\underline{P} < 0.001$ for all). Winter body mass differed by age ($\underline{P} = 0.002$). Spring body mass (Table 2) was dependent on winter body mass ($\underline{P} < 0.001$), lipid mass change on winter lipid mass ($\underline{P} < 0.001$), and percent lipid change on percent winter lipid ($\underline{P} < 0.001$). Spring body mass and lipid mass were correlated to the winter values ($\underline{r}_p = 0.576$, $\underline{P} < 0.001$; and $\underline{r}_p = 0.549$, $\underline{P} = 0.001$, respectively). A negative correlation existed for lipid mass change and winter lipid mass ($\underline{r}_p = -0.623$, $\underline{P} < 0.001$) and percent lipid change and percent winter lipid ($\underline{r}_p = -0.597$, $\underline{P} < 0.001$). As would be expected, high condition levels in winter led to better condition levels the following spring. Larger animals have been noted to have greater stress resistance than small animals when other factors were held equal (Kendeigh 1945, Phillips et al. 1932, Tester and Olson 1959). While no effect from age was found on spring body mass or body mass change, adults had greater ($\underline{P} = 0.040$) spring lipid mass as compared to juveniles. This may be due to their greater size and/or larger lipid reserves in adults at the start of winter.

Besides affecting the ability to resist stress, mass and nutrition levels are known to affect reproduction in birds. Laying in ring-necked pheasants on limited intake was 9% that of controls and was delayed by one month because re-

productive development was retarded by the lowered nutritional plane of the individuals (Breitenbach et al. 1963). Increased winter mass loss delayed laying in ring-necked pheasants (Barrett and Bailey 1972, Gates and Woehler 1968). Mallards (*Anas platyrhynchos*) on restricted diets had smaller clutches in the first nest; furthermore, female mass at nest initiation affected individual egg and duckling masses (Dubovsky and Kaminski 1994). Lipid reserves of female mallards in North Dakota have been correlated with clutch size (Krapu 1981). Pre-breeding condition has also been found to affect reproduction in red grouse (*Lagopus lagopus scoticus*) (Jenkins et al. 1967) and blue grouse (*Dendragapus obscurus*) (Zwickel and Bendell 1967). In short-lived species such as the ring-necked pheasant, the population-level effects of maintaining over-winter condition may be more important in reproduction than in reducing winter mortality.

The presence of a food plot within the 95% home range had no effect ($P \geq 0.43$) on spring body mass, lipid mass, percent lipid, and the over-winter changes in these variables. The winter of 1994-95 could be characterized as mild for this area, while winter 1995-96 was colder than normal (National Oceanic and Atmospheric Administration 1994-1996). However, accumulated snow on agricultural fields was low both years and waste grains and native seeds would have been readily available. We hypothesize that food plot utilization was unnecessary for maintenance of condition during this study. Some birds had home ranges within 300 m of a food plot, yet were never located within it. Ring-necked pheasant use of food plots in Iowa was related to the severity of the winter (Riley 1992). In a Minnesota winter with persistent deep snow, wild turkeys (*Meleagris gallopavo*) with access to corn food plots were able to maintain their body mass while those without access lost body mass (Porter et al. 1980). No effect of food plots on body mass was found during a mild winter. Alternatively, ring-necked pheasants may be adjusting density to the food supply so that similar lipid levels are maintained between areas (Dowell 1982).

Although no effect from food plots was found in this study, the lack of snow cover may have made them unnecessary for maintenance of condition levels. This study was to have continued through spring 1997, but deep snow cover (≥ 60 cm), periodic blizzard conditions, and unusually low temperatures caused a loss of 43 of 47 radio-marked birds during the period 9 December 1996 to 3 February 1997. From 1 January 1997 all birds had a food plot within their home range, and examinations of opportunistically found birds leads us to believe that standing patches of corn (i.e., food plots and unharvested fields) were virtually the only food source for the surviving population. In these harsh conditions, food plots would be expected to have a greater impact than that found for the previous two years of our study.

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Table 1. Winter body mass, estimated lipid mass, and estimated percent lipid in South Dakota female ring-necked pheasants, 1994-95 and 1995-96.

Year	Age	n	Winter		
			Mass (g)	Lipid (g)	%Lipid
1994-95	A	3	1041 ± 27[a]	111 ± 43	10.6 ± 3.8
	J	19	982 ± 70	99 ± 28	10.0 ± 2.7
1995-96	A	16	908 ± 53	58 ± 32	6.3 ± 3.3
	J	35	844 ± 75	46 ± 20	5.4 ± 2.2

[a] Mean ± standard deviation.

Table 2. Effects of a food plot within the 95% home range on spring and over-winter change of body mass, estimated lipid mass, and estimated percent lipid in South Dakota female ring-necked pheasants, 1994-95 and 1995-96.

Year	Age	Food	n	Spring			Over-Winter Change		
		Plot		Mass (g)	Lipid (g)	%Lipid	Mass (g)	Lipid (g)	%Lipid
1994-95	A	Y	1	1042	88	8.5	6	-4	-0.4
		N	1	1075	103	9.6	58	22	1.6
	J	Y	3	917±97	63±35	6.8±3.3	-42±64	-33±13	-3.4±1.2
		N	9	925±48	77±32	8.2±3.3	-79±68	-33±37	-2.5±3.4
1995-96	A	Y	2	936±45	79±27	8.4±2.5	21±22	-4±5	0.7±0.3
		N	7	981±119	60±37	5.9±3.0	60±98	16±51	1.2±4.6
	J	Y	7	931±99	51±14	5.6±1.5	63±25	-5±16	-0.8±1.6
		N	7	910±90	34±25	3.8±2.5	59±29	-4±21	-0.8±2.2

[a] Mean ± standard deviation.

A BIOLOGICAL ASSESSMENT OF FOUR NORTHERN BLACK HILLS STREAMS

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ABSTRACT

The health of Whitewood Creek, which has a history of degradation from mining activities, was evaluated using a rapid bioassessment technique for benthic macroinvertebrates, and by determining fish and anuran species richness. Data from two reaches of Whitewood Creek were compared with data from two reaches in Bear Butte, Spearfish, and Crow creeks. Macroinvertebrate communities were similar between reaches in each stream and between 1997 and 1998 ($P > 0.05$), but seasonal differences were found. In August, the macroinvertebrate assessment scores were similar among streams (Whitewood Creek score = 38; reference creek score = 36), but in May, the Whitewood Creek score declined to 16, whereas the score for reference creeks remained 36. Five of seven components of the assessment score for Whitewood Creek declined in May, especially components using counts of ephemeroperans, plecopterans, and trichopterans. Anuran species richness ranged from three to four species depending on stream. There were five fish species in Whitewood Creek, seven in Spearfish Creek, eight in Crow Creek, and 22 in Bear Butte Creek. The invertebrate community may be impacted by contaminants leached from mine tailings by spring floodwaters.

Keywords

Black Hills, invertebrates, streams, bioassessment, mining, anuran, fish.

INTRODUCTION

Past mining practices in the Black Hills of South Dakota often damaged stream habitat and water quality. From 1878 through 1970, about 15 kg of mercury and 140 kg of cyanide were discharged to the Whitewood Creek daily (Hesse et al. 1975, Rahn et al. 1996). In the 1950s, Whitewood Creek was a "legally" polluted stream (Stewart and Thilenius 1964), and was later a "su-

perfund" site (Lineburg and Lawrensen 1993, Homestake Mining Company 1996). Tons of tailings remain in the lower Whitewood Creek basin on the Missouri Plateau, which extends from the foot of the Black Hills to the confluence with the Belle Fourche River (Fox Consultants, Inc. 1984, Marron 1992). Contaminants (e.g. arsenic, mercury) from the tailings may be harmful to the aquatic biota (Brown 1972, Bergeland et al. 1976, Cain et al. 1988, U.S. Geological Survey 1988, 1989), but there has been no assessment of aquatic communities in the Plateau reach of Whitewood Creek.

Fish and benthic macroinvertebrate communities have been studied in Black Hills streams before they leave the Black Hills physiographic province and enter the Missouri Plateau. Analysis of the benthos has been done to evaluate impacts of municipal waste, recreational property development, and mining (Jurgens 1968, Drewes 1984). Lechner (1986) found 71 macroinvertebrate taxa in streams in the central portion of the Black Hills. Fisheries surveys have been conducted routinely to gather data for recreational fisheries management (Meester 1999). The fish community is usually made up of about six species; several salmonids and longnose dace (*Rhinichthys cataractae*), mountain sucker (*Catostomus platyrhynchus*) and white sucker (*C. commersoni*). The amphibians and reptiles of the Black Hills have not been studied recently (Peterson 1974).

Our study was preceded by a cursory study of the benthic community in Whitewood Creek that suggested the presence of a stressor (Duffy, unpublished data). Our objective was to compare the macroinvertebrate, fish, and anuran communities in the Missouri Plateau portion of Whitewood Creek with those of three near by creeks (Bear Butte, Spearfish, and Crow) where mining has not occurred.

STUDY REACHES

Bear Butte, Spearfish, and Crow creeks were chosen as reference streams because they are near Whitewood Creek, and are in the same geologic and climatic setting (Fig. 1). Sampling was done on each reference stream and on Whitewood Creek in spring and fall of 1997 and 1998 at two 200-m-long reaches that were located away from tributaries, bridges and roads. Both reaches were on the Missouri Plateau; reach one was located where the streams enter the prairie

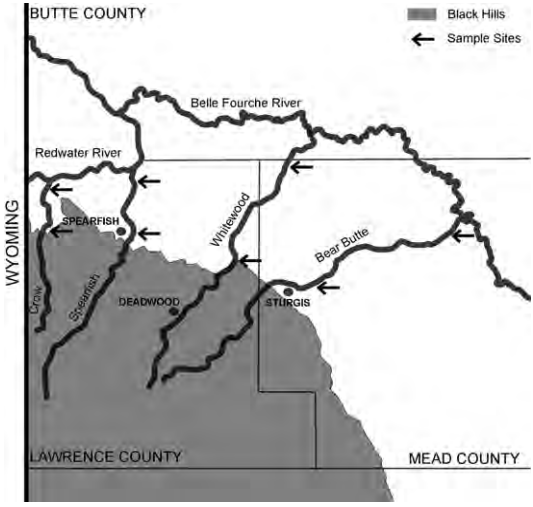

Figure 1. Map of the northern Black Hills showing four streams and study sites on the Missouri Plateau.

and reach two was downstream near the confluence with the receiving stream (Belle Fourche or Redwater rivers).

Reaches were dominated (50%-80%) by cobble and gravel substrate (Table 1). Each stream had peak flows in April, May and June and lowest flows in the fall and winter. Daily discharge at the time of sampling ranged from 117 cubic feet per second in May on Spearfish Creek to 0.65 cubic feet per second in August on Crow Creek. Dissolved oxygen was higher in May (8.1-9.2 mg/l) than in August (3.4-5.3 mg/l). Specific conductance ranged from 320–1080 *u*S at upper reaches to 430-2300 *u*S at downstream reaches. There was evidence of habitat disturbance (i.e. irrigation, grazing, physical channel alterations) at the upstream reach on Crow Creek, but the reach was cautiously included because study reaches were limited by access and landowner cooperation.

Parameter	Bear Butte Site 1		Bear Butte Site 2		Whitewood Site 1		Whitewood Site 2		Spearfish Site 1		Spearfish Site 2		Crow Site 1		Crow Site 2	
Month	May	Aug	May	Aug	May	Aug	May	Aug	May	Aug	May	Aug	May	Aug	May	Aug
Water Temp. (^{O}C)	8.3	19.5	18.1	23.4	9.6	16.1	11.8	23.4	9.2	10.2	14.6	13.6	14.2	14.5	11	16.5
Conductivity (uS)	1080	1030	2200	2300	710	1100	770	1300	320	300	430	630	490	570	1200	1430
Dissolved Oxygen(mg/l)	9.1	5.3	8.6	3.4	9.2	4.6	8.4	4.6	8.1	5.6	8.4	4.7	8.3	4.87	8.56	4.75
Ph	8.9	8.0	8.1	7.7	8.4	8.1	8.6	8.1	8.8	8.4	8.4	8.2	8.1	2.95	8.12	7.98
Discharge (CFS)	1.8	3	11	3.9	58	28.5	46.5	27.4	117	103	94.5	105	2.7	.65	7.55	6.82
Boulder (%)	5	5	5	5	10	10	5	5	15	15	5	5	5	5	0	0
Cobble (%)	45	45	20	20	35	35	20	20	65	65	10	10	40	40	10	10
Gravel (%)	30	30	35	35	40	40	40	40	15	15	15	15	30	30	10	10
Sand (%)	20	20	30	30	10	10	25	25	5	5	65	65	20	20	40	40
Silt (%)	0	0	10	10	5	5	10	10	0	0	5	5	5	5	40	40
Turbidity (NTU)	3.1	1.5	25.6	20.6	5.7	5.4	7.5	2.3	2.6	1.9	4.3	5	3	3.77	4.21	3.53

Table 1. Stream characteristics recorded in 1998 from two Missouri Plateau sites each on Bear Butte, Whitewood, Spearfish, and Crow creeks, South Dakota. Site 1 is upstream from Site 2 on each stream.

METHODS

Benthic macroinvertebrate sampling: Surber samples were collected at five riffle sites along the midline of the downstream half of the 200-m reach. Five submerged snags were sampled by placing a 500-um-mesh net over a 1-m-portion of the snag and removing the enclosed portion with a saw. Samples were preserved in 95% ethanol. Floatation was used to separate organisms from sample residue (Anderson 1959, Mangum 1991). Each sample was separated three times by mixing with 800 ml of distilled water and 120 mg of table salt (specific gravity = 1.12), allowed to settle for 3 min, and filtered through a 500-um-mesh sieve (Brinkman and Duffy 1996, Rosillon 1987, Anderson 1959, Lackey and May 1971)). Rose Bengal die was added to make the organisms more visible. Invertebrates were identified to family. Total invertebrate numbers were compared among reaches, seasons, years, and creeks using analysis of variance (PROC CATMOD, SAS Institute 1989).

Invertebrate community metrics: The macroinvertebrate community was analyzed using a modified rapid bioassessment protocol with seven metrics se-

lected from Plafkin et al. (1989). We substituted the number of mollusk species for the number of scraper-filter feeding species because we identified organisms only to the family level, and feeding group differences are usually assigned at the genus level. Each metric has a specific value as an indicator of stream water quality:

- Taxon richness: the number of taxa in a sample reflects the health of the community, and generally increases with increasing quality of water and habitat.
- EPT index: the number of taxa in the orders Ephemeroptera, Plecoptera, and Trichoptera summarizes the presence of orders sensitive to pollution, and usually increases with increasing water quality.
- Hilsenhoff biotic index (HBI) is the overall tolerance of the community calculated by by using tolerance values (Plafkin et. al. 1989) for each taxon. The HBI is calculated as: $HBI = \Sigma(x_i t_i)/n$ where x_i = the number of individuals with a taxon, t_i = the tolerance value of a taxon and n = the total number of organisms in the sample. Tolerance values (range = 0 to 10) increase as water quality decreases.
- EPT/Chironomid ratio: the ratio of the EPT organisms to midge larvae (family Chironomidae), which are usually more pollution tolerant that EPT organisms, indicates stress if the EPT/Chironomid ratio is low.
- Percent contribution by dominant taxon: the total number of organisms in the dominant taxon is an indication of community balance. Communities dominated by few species indicate environmental stress, therefore increases in this percentage indicate stress.
- Percent shredders: shredders feed on course particulate organic matter (CPOM). The number of shredders declines when the CPOM is contaminated because of effects on the microbial communities colonizing the CPOM or on the shredders directly (Merritt and Cummins 1996).
- Mollusk species richness: number of mollusk species may indicate stress because mollusks are intolerant of metal contaminants.

Differences in metrics among streams, reaches, months and years were analyzed using analysis of variance (PROC GLM, SAS Institute 1989). Total stream scores were the sum of individual metric scores assigned a value of 0, 2, 4, or 6 according to the degree of impairment (Newman, 1999). Scores for reference creeks were averaged for comparison to the Whitewood Creek score, which allowed us to assign Whitewood Creek to a biological condition category (Table 2).

Fish sampling: Fish were collected from the upstream half of each 200-m reach, where riffle, run and pool habitats were present. Block nets (4.7-mm mesh) were placed at the lower and upper ends of the 100-m reach before sampling with pulsed, direct-current, backpack electrofishing. We determined electrofishing time by a timer on the equipment. When flows or conductivity precluded electrofishing, we collected fish with a seine (4.7-mm mesh) that reached from bank to bank. Fish were identified and released. Data were reported as catch-per-unit-effort, which was number of fish collected per hour of electrofishing or number of fish collected per 100-m seine haul.

% Comparison to reference score	Biological condition	Score interpretations
>83	Not impaired	Comparable to pristine conditions
54-79	Slightly impaired	Community structure less than expected. Composition lower than expected due to loss of some intolerant forms. Percent contribution of tolerant forms increases
21-50	Moderately impaired	Fewer species due to loss of most intolerant forms. Reduction in EPT index.
<17	Severely impaired	Few species. If high densities of organisms, then dominated by one or two taxa.

Table 2. Interpretation of total stream assessment scores developed using benthic macroinvertebrate rapid bioassessment protocols of Plafkin et al. 1989.

Amphibian sampling: We searched the riparian zone along both banks of a 100-m section of stream using a hand net to search under rocks, logs, and debris (Bayless 1978). Amphibians were identified to species, and released. Amphibian call surveys were also conducted in each reach in 1998 just after sunset. We sat at each site for 15 min and recorded calls by species.

RESULTS

We found 15 to 17 invertebrate orders representing 46 to 53 families, depending on stream. The most common taxa were Ephemeroptera, Trichoptera, Diptera, Plecoptera, Coleoptera, and Odonata in decreasing order of magnitude. The total number of individuals varied widely among streams, reaches, years, and months. A significant 4-way interaction precluded further statistical analysis for main effects on the total number of individuals. However, a trend was apparent (Figure 2). The number of invertebrates was usually lower in Whitewood Creek than in other creeks, especially in the spring. In May of both years, Whitewood Creek had fewer invertebrates at each reach than did the reference reaches, with the exception of Bear Butte Creek in 1998.

When raw data were converted to metrics for calculating stream assessment scores, six of seven metrics indicated degraded conditions in Whitewood Creek in the May (Table 3). The exception was the percent shredders, which had a higher value in Whitewood Creek compared to reference streams (a low-

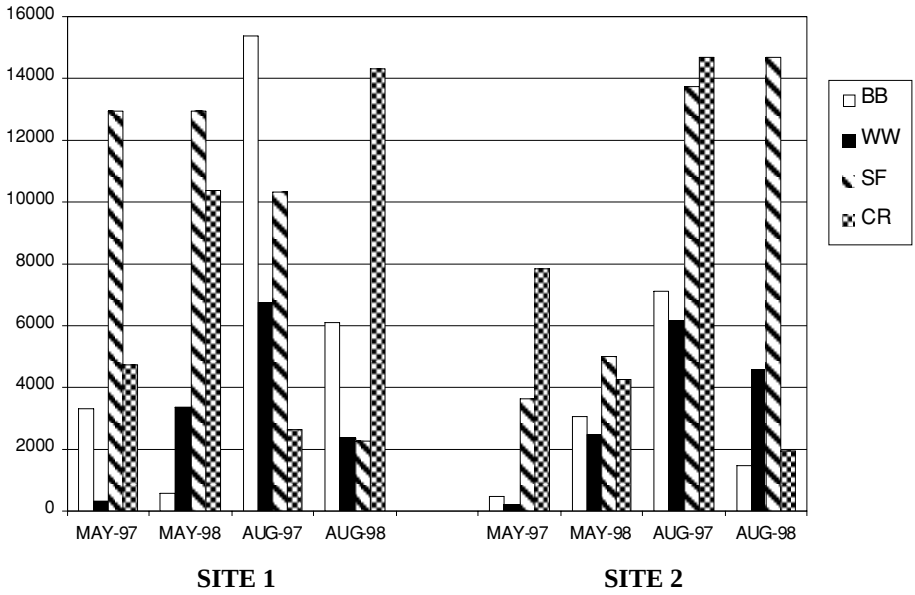

Figure 2. Total number of benthic macroinvertebrates collected from two sites on Bear Butte (BB), Whitewood (WW), Spearfish (SF), and Crow (CR) creeks in May and August of both 1997 and 1998.

Month	Stream	Percent Dominant Taxa +	Percent Shredders -	HBI +	Mollusk Richness -	Species Richness -	EPT/ Chironomid -	EPT Index -
				Average Metric Values				
May	Whitewood	73.1	74.5	6.77	1	33	0.382	5.25
	Bear Butte	61.0	62.5	6.51	2	44	0.513	7.75
	Spearfish	53.0	34.9	4.70	5	42	4.58	10.3
	Crow	57.4	67.9	6.04	3	51	0.464	9.50
August	Whitewood	34.6	26.0	4.86	3	40	3.32	9.75
	Bear Butte	35.7	30.0	5.07	3	44	2.93	8.75
	Spearfish	47.5	51.6	5.66	3	38	0.895	7.50
	Crow	46.2	51.9	5.71	3	40	1.56	9.75
				Least-Squares Means				
May	Whitewood	73.09	74.52	6.77	1.00	17.50	0.38	5.25
	Bear Butte	61.02	62.55	6.51	1.25	23.25*	0.51	7.75
	Spearfish	52.98	34.88*	4.70*	2.50	26.00*	4.58*	10.25*
	Crow	57.43	67.90	6.04	2.00	27.50*	0.46	9.50*
August	Whitewood	34.59	25.97	4.86	1.63	21.25	3.32	9.75
	Bear Butte	35.72	29.96	5.07	1.50	23.25*	2.93	8.75
	Spearfish	47.52	51.58	5.66	0.00	20.75	0.89*	7.50
	Crow	46.19	51.92	5.71	2.00	23.50*	1.56*	9.75

Table 3. Values of rapid bioassessment protocol metrics for 1997 and 1998 data for Bear Butte, Whitewood, Spearfish and Crow creeks. A "+" or "-" indicate the direction the metric takes when conditions become stressful. Post comparison of means test (lsd) results for the stream by month interaction for each metric are indicated. "*" indicates statistical significance (Pr>|T|) between Whitewood Creek and the respective reference stream.

er value for this metric indicates stress). Stream by month interaction was significant (P<0.05) for the HBI index, total species richness, and EPT/Chironomidae ratio. Post-comparison of least-square means showed the same trends found in the metric averages (Table 3). Scores from Spearfish Creek were significantly (P< 0.05) better than those from Whitewood Creek in May for percent shredders, HBI, species richness, EPT/chironomid ratio, and EPT index.

The final stream score for Whitewood Creek was similar (38) to the average score for the reference streams (36) in August (Table 4). However, in May the score for the reference streams remained 36, but the score for Whitewood Creek dropped to 16. Important changes were decreases in species richness, EPT index, and EPT/Chironomid ratio, and an increase in percent contribution by dominant taxon.

	Metric value		% Comparison		Assigned score	
Metrics	Whitewood	Reference	Whitewood	Reference	Whitewood	Reference
			August 1997 and 1998			
Species Richness	40	41 (38-44)	98	100	6	6
EPT/Chironomid Ratio	3.3	1.8 (0.9-2.9)	185	100	6	6
HBI	4.9	5.5 (4.9-5.7)	113	100	6	6
% Contrib. Dom. Taxon	35	43 (35-48)	35	43	2	0
Shredders/Total (%)	26	45 (26-52)	58	100	6	6
Mollusk Richness	1.8	1.6 (2-5)	111	100	6	6
EPT Index	9.8	8.7 (7.5-9.8)	112	100	6	6
Total -					38	36
			May 1997 and 1998			
Species Richness	33	46 (42-51)	72	100	4	6
EPT/Chironomid Ratio	0.4	1.9 (0.5-4.6)	21	100	0	6
HBI	6.8	5.8 (4.7-6.5)	85	100	4	6
% Contrib. Dom. Taxon	73	57 (53-61)	73	57	0	0
Shredders/Total (%)	75	55 (35-68)	132	100	6	6
Mollusk Richness	1	1.9 (2-5)	52	100	2	6
EPT Index	5.3	9.2 (7.8-10.3)	57	100	0	6
Total -					16	36

Table 4. Metric values, percent comparison, and bioassessment scores for Whitewood Creek and three reference streams (Spearfish, Bear Butte and Crow Creeks) for August and May 1997 and 1998. The reference scores are means (range).

Whitewood Creek had the lowest fish species richness (five) compared to seven in Spearfish, eight in Crow, and 22 species in Bear Butte creeks (Table 5). Fish communities were similar between seasons and years but downstream reaches in each creek had more species than did upstream reaches. Brown trout and white sucker were found in all four creeks, but catch-per-unit-effort was always lower in Whitewood Creek than in reference creeks (Table 5). However, the same trend was not apparent for the other three fish species that Whitewood Creek had in common with some reference streams.

	Bear Butte		Whitewood		Spearfish		Crow	
Family, species, and scientific name	1	2	1	2	1	2	1	2
Catostomidae								
Longnose sucker, Catostomus catostomus	0.84					2.6	11.0	4.4
Mountain sucker, C. platyrhynchus						0.9		
Shorthead redhorse, Moxostoma macrolepidotum		3.1						
White sucker, C. commersoni	60.6	5.4	4.4	2.9		5.3		28.0
Centrarchidae								
Black crappie, Pomoxis nigromaculatus		0.4						
Green sunfish, Lepomis cyanellus		5.3					0.9	4.4
Orangespotted sunfish, L. humilis		0.3						
Cyprinidae								
Common carp, Cyprinus carpio	6.7	0.8						
Creek chub, Semotilus atromaculatus	38.7	0.4	13	85				
Emerald shiner, Notropis atherinoides		0.3						
Fathead minnow, Pimephales promelas	1.7	12.8				46		0.9
Flathead chub, Platygobio gracilis		1.6						
Longnose dace, Rhinichthys cataractae	251	0.8	131	129				20.6
Red shiner, Cyprinella lutrensis		36.5						
Sand shiner, Notropis stramineous		7.1						
Western silvery minnow, Hybognathus agyritis		4.6						
Cyprinodontidae								
Banded killifish, Fundulus diaphanus		0.1						
Ictaluridae								
Black bullhead, Ameiurus melas		0.1	0.6					
Channel catfish, Ictalurus punctatus		0.4						
Stonecat, Noturus flavus		2.5						
Percidae								
Yellow perch, Perca flavescens		0.1						
Salmonidae								
Brook trout, Salvelinus fontinalus	5.9				33		0.9	
Brown trout, Salmo trutta	16.8			1	283	33	319	28.0
Rainbow trout, Oncorhynchus mykiss					4.8			1.5

Table 5. Fish catch per unit effort (fish per hour electrofishing) for two sites on four eastern Black Hills streams, 1997-98.

All five anuran species known to the region were encountered (Fischer et al. 1999). Three species were found in all creeks: western chorus frog (*Pseudacris triseriata*), northern leopard frogs (*Rana pipiens*), and Woodhouse's toad (*B. woodhousei*). The Great plains toad (*Bufo cognatus*) was found only in Whitewood Creek, and the plains spadefoot toad (*Scaphiopus bombifrons*) was found only in Bear Butte Creek.

DISCUSSION

The low number of invertebrates, brown trout, and white suckers in Whitewood Creek, and the low stream assessment score for Whitewood Creek indicate stressful conditions compared to reference streams, especially in May. The assessment score for Whitewood Creek in the spring was 44% of the average for the reference streams, thus placing Whitewood Creek in the biological condition category of "moderately impaired" (Table 2). The most likely stressors are toxins (e.g. arsenic, copper, mercury) from mine tailings in the riparian zone (Marron 1989, Isom 1978). Arsenic has been detected in surface water, ground water, vegetation, and wildlife in the Whitewood Creek basin (Bergeland et al. 1976, Stach et al. 1978, Cain et al. 1988, Callender and Robbins 1993, U.S. E.P.A. 1971, 1973, 1989, U.S. Geological Survey 1995).

The EPT index and the EPT/Chironomid ratio suggested impairment in Whitewood Creek during spring flooding. The EPT components of the index may be good indicators of mine waste impacts. Horn (1993) found the EPT index and the EPT/Chironomidae ratio to be good indicators of water quality in Chalk Creek, Colorado where mine tailings were present. Chironomidae tend to become increasingly dominant in the benthic community as heavy metal

concentration increases (Ferrington 1987) perhaps because they are more resistant to metal toxicity than mayflies. The data also suggest some impairment at reach one of Crow Creek, where we suspected impacts from irrigation activities and grazing.

We assumed that the seasonal changes in the invertebrate community would be similar among creeks. Seasonal fluctuations occur because of life cycle dynamics and scouring flows (Somer and Hassler 1992, Merritt and Cummins 1996). However, communites recover quickly from disturbance (Barton and Wallace 1978), which may explain why there were no differences among creeks in August after contaminants were flushed or resettled.

The paucity of fish in Whitewood Creek also indicates degraded habitat or water quality. The longnose sucker, which is on the state's threatened fishes list, was found in reference streams, but not in Whitewood Creek. Fish species richness in each stream was higher at the second reach than the first because the streams change from cold-water to warm-water prairie streams. The community at the lower reach includes species usually found in either the Belle Fourche or Redwater rivers (Doorenbos 1998).

Schooner and Sate (1984) found that some arsenic in Whitewood Creek waters was derived from eroding banks, bed, and alluvium, and found transport of particulate arsenic at scouring flows. We suggest that flooding may inundate mine tailings on the flood plain and carry toxins into the creek in the spring. The contaminants markedly change invertebrate communities in the spring, and probably have subtle long-term negative impacts on both invertebrate and fish communities.

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Abstracts of Senior Research Papers

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The 84th Annual Meeting

of the

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STRAWBERRY CLOVER AND ITS USE IN THE AMELIORATION OF CADMIUM CONTAMINATED SITES

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ABSTRACT

Cadmium is a toxic heavy metal found naturally in soils throughout the Midwest as well as in sites of mining, processing, and manufacturing. Two proposed methods for cleaning up these sites involve the use of cover crops to ameliorate the soil. These crops may be used to change the soil characteristics making the cadmium unavailable to crops. In smaller sites, cover crops that would take up high levels of cadmium could be grown and harvested, physically removing the cadmium from the soil. Strawberry clover (*Trifolium fragiferum* L.) is a legume found throughout the world and is related to the red clover (*Trifolium pratense*) and the white clover (*Trifolium repens* L.), common to this area. It is a very hardy plant with the ability to tolerate saline and alkaline soils as well as flooding, making it a good prospect for reclamation purposes. A research project was developed to examine accessions of strawberry clover representing worldwide populations. Seeds from selected accessions were planted in the greenhouse for comparison of morphological variation. Strawberry clover accessions were also grown hydroponically to examine differences in cadmium uptake. The ability of strawberry clover roots to change rhizosphere pH and take up cadmium was examined using culture tubes containing nutrient agar, a moderate level of cadmium and a pH indicator dye. After 2 and 5 weeks of root growth, the agar was examined colorimetrically to determine the effect the root had on media pH. After 5 weeks the seedlings were removed, dried and weighed and the cadmium levels were measured. The results suggested a negative correlation between root dry weight and rhizosphere pH after 2 weeks growth. These findings also suggested the possibility of a negative correlation between rhizoshpere pH and cadmium uptake and prompted a final study of the same nature in which pH and cadmium concentrations in seedlings will be measured after 2 weeks of growth. This relationship would prove useful in screening other plants for reclamation purposes.

MORE BIOFRIENDLY QUATERNARIES: SYNTHESIS OF DIHYDROGENATEDTALLOW METHYL AMMONIUM METHYL CARBONATE AND REPLACEMENT OF THE ANION WITH OTHER ANIONS

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ABSTRACT

Quaternary ammonium compounds are widely used chemicals with many diverse applications. From use as surfactants in hair care products to lubricant additives in internal combustion engines, quaternary ammonium compounds are intertwined in the culture of the modern developed country.

Previously, though, to make these compounds placed the industrial worker at risk of exposure to toxic alkylating chemicals such as dimethyl sulfate, DMS, and methyl chloride. It has been noted that minimally toxic DMC could potentially be used to alkylate tertiary amines to produce ammonium salts.[1] However, unfavorable reaction conditions such as pressures up to 500 p.s.i have caused industry to look elsewhere for synthetic reagents.

The research described here had its goal in making reaction of a tertiary amine with the minimally toxic dimethyl carbonate, DMC under industrially favorable conditions. More specifically, dihydrogenatedtallow methyl amine and DMC, were solvated in methanol and reacted in a Parr pressure reactor to obtain the methylated methyl carbonate quaternary amine. It was found through NMR spectrometry that a 10:9:1 DMC: methanol: dihydrogenatedtallow methyl amine reaction mixture would come to completion after 7 hours at pressures just above 100 p.s.i.

NMR spectrometry was used to follow the quaternization. Samples of the homogenous mixture were taken from a side port on the reactor every hour. These samples were solvated in $CDCl_3$ with TMS, and proton spectra were obtained. The methyl protons directly bonded to the nitrogen atom in the tertiary amine exhibited a distorted triplet resonance around 2.2 ppm downfield of TMS.[2] Distortion was explained in terms of the nuclear quadrupole moment of the nitrogen nucleus. The methyl protons bonded to the nitrogen atom in the positively charged quaternary amine were shifted and resonated around a value of 3.4 ppm downfield of TMS (Fig. 1). This more symmetric tetrahedral environment served to partially quench the quadrupole moment of the nitrogen nucleus allowing greater resolution of the triplet splitting pattern. Through the integration of these peaks, and evaluation of the resonance due to the tertiary amine methyl group protons in relation to baseline resolution, complete reaction time was determined in terms of NMR sensitivity.

Figure 1. PNMR specturm of the partially quaternized amine reaction mixture.

The possibility of synthesizing a quaternary analog of this amine which would posses a hydroxide anion was also explored. This product may not only serve in industrial processes such as circuit-board etching, but could possibly provide a means of producing other analogs by circumventing the problem of reaction of the hydrogen carbonate anion, a weak base, with an acid. Three methods, reaction with $Ca(OH)_2$, reaction with NaOH, and exchange with an ion exchange resin, were then studied and compared in relation to their ability to produce the hydroxide analog. Qualitative results and infrared spectroscopy (Fig. 2) were used to evaluate the effectiveness of these methods. These qualitative results were found to favor the process of ion exchange with a resin.

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2. Hanson, Michael; *NMR Analysis of Quaternary Ammonium compounds, May 29 to July 28, 1997*; July 28, 1997.

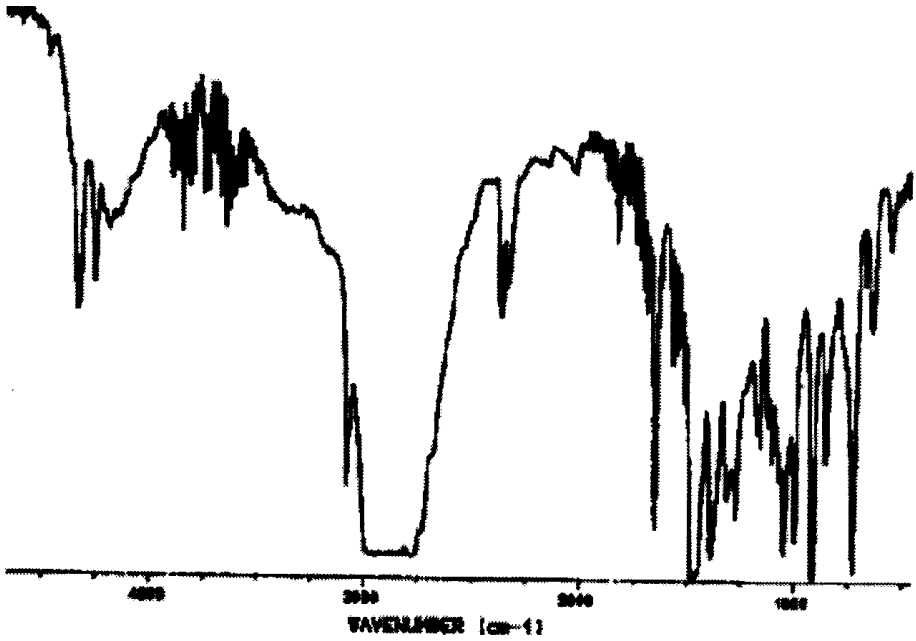

Figure 2. FT-IR spectrum of the quaternary compound after reaction with $Ca(OH)_2$

QUATERNIZATION OF DIHYDROGENATEDTALLOW METHYL AMINE, THE SYNTHESIS OF ESTER AMINES, AND THE QUATERNIZATION OF THESE ESTER AMINES

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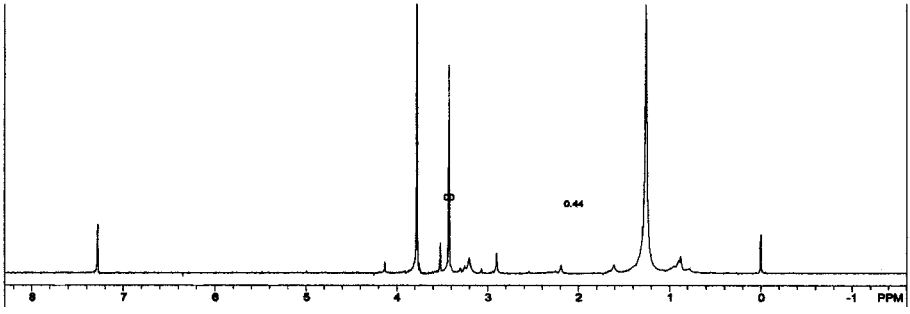
ABSTRACT

The purpose of this research was to become more familiar with the reaction conditions and properties of methyl carbonate quats. A series of replicate reactions were run inside a pressure reactor using dihydrogenatedtallow methyl amine as the model amine for the research project and dimethyl carbonate in the presence of methanol to produce the dialkyl dimethyl quat with a methyl carbonate anion.

$$(C_{18}H_{37})_2NCH_3 + CH_3OCOOCH_3 \rightarrow (C_{18}H_{37})_2N^+(CH_3)_2\ ^-OCOOCH_3$$

This reaction was run under an oxygen-free atmosphere. An FT-NMR instrument was used to monitor the rate of the reaction by analysis of peak area ratios.

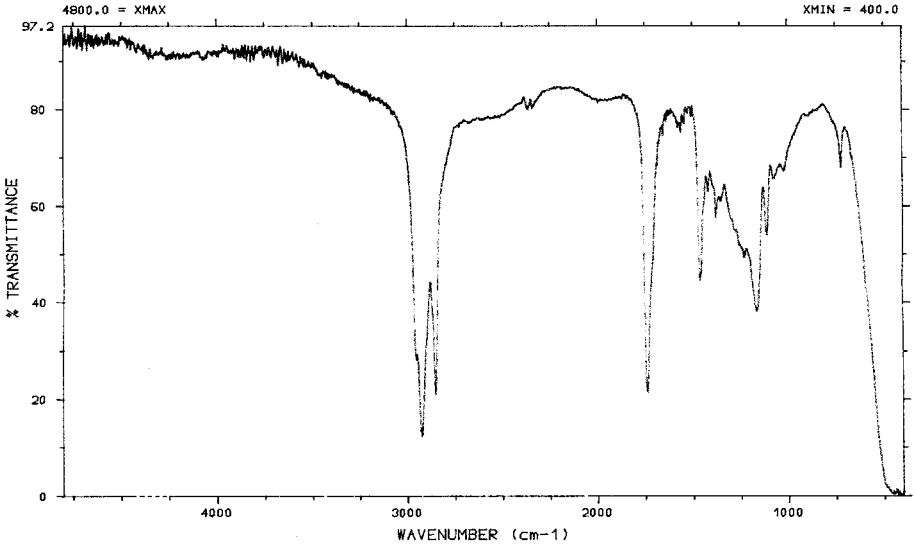


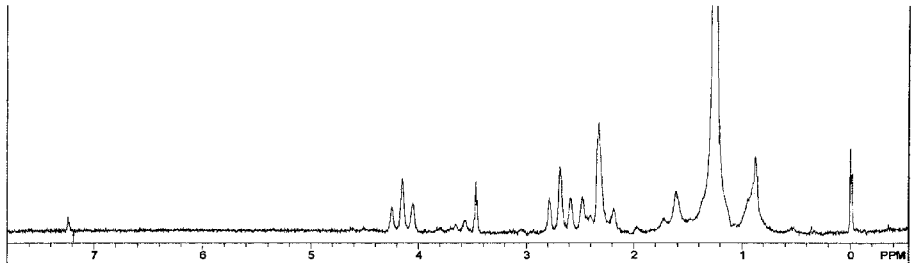
The reaction ratio of dimethyl carbonate to amine was 10:1, respectively. Research was done to see if the dimethyl carbonate / methanol mixture recovered from a quaternization reaction could be recycled in future reactions.

Other experimentation was done to synthesize new ester amines using N-methyldiethanolamine and lauric acid with sulfuric acid as a catalyst in the reaction:

$$C_{11}H_{23}COOH + (HOCH_2CH_2)_2NCH_3 \rightarrow (C_{11}H_{23}COOCH_2CH_2)_2NCH_3$$

This reaction was also run under an oxygen-free atmosphere in a pressure reactor. The ester product was characterized by FT-IR and FT-NMR, as shown respectively.

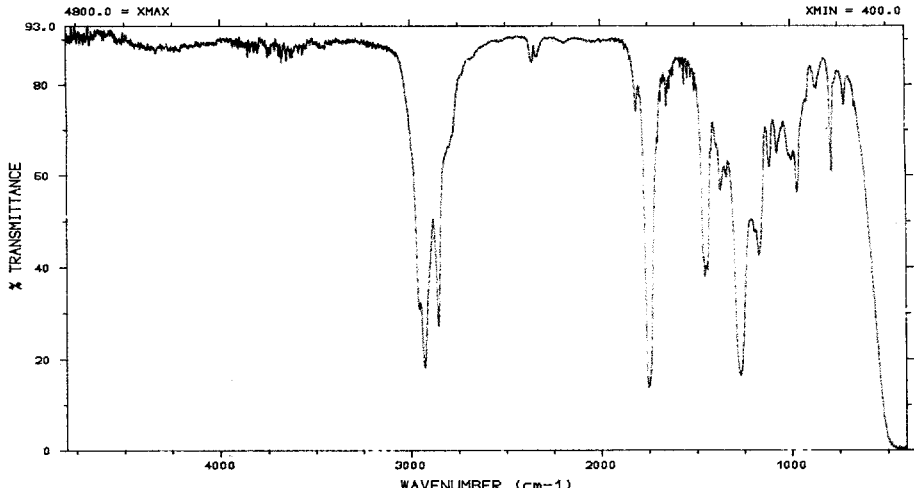




The reaction of lauric acid to amine was 2.3:1, respectively. The ester amine was quaternized using dimethyl carbonate in the presence of methanol in the reaction:

$$(C_{11}H_{23}COOCH_2CH_2)_2NCH_3 + CH_3OCOOCH_3 \rightarrow (C_{11}H_{23}COOCH_2CH_2)_2N^+(CH_3)_2 \ ^-OCOOCH_3$$

As usual, this reaction was also run under an oxygen-free atmosphere, with the reaction ratio of dimethyl carbonate to amine being 10:1, respectively. An FT-IR instrument was again used to characterized the ester quat product.



Continued research investigated the corrosiveness of both the dialkyl dimethyl and ester quats in stainless steel beakers.

CONCLUSION

It was determined that the quaternization of the dihydrogenatedtallow methyl amine went to completion in 7 hours at a temperature between 135° - 140° C. Also, the dimethyl carbonate / methanol mixture recovered from a reaction could be reused in another quaternization reaction. Results from the synthesis of the ester amine yield that the reaction time is about 4 hours at a temperature between 160° - 165° C. Quaternization of this ester amine is thought to be complete after 8 hours at a temperature range of 95° - 100° C. Both the dialkyl dimethyl and ester amine quats were found to be non-corrosive in stainless steel containers.

ACKNOWLEDGMENTS

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DEVELOPMENT OF AN HPLC METHOD FOR THE ANALYSIS OF QUATERNIZATION REACTIONS OF TERTIARY AMINES WITH DIMETHYL CARBONATE

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ABSTRACT

The authors have been developing an environmentally friendly method of quaternizing tertiary amines using dimethyl carbonate as the alkylating agent for possible use in industry. The facet of the study reported here is the development of an HPLC method for quantitatively following the quaternization reactions. Reaction products from two reactions were analyzed; the preparation of tributylmethylammonium methyl carbonate and dihydrogenatedtallowdimethylammonium methyl carbonate. Baseline separation and quantitation of the quaternary product and the amine precursor were achieved using ion exchange HPLC. Figure 1 shows an example chromatogram of the separation of dihydrogenatedtallowmethylamine (~ 3.7 min) and dihydrogenatedtallow- dimethylammonium methyl carbonate (~ 8.7 min).

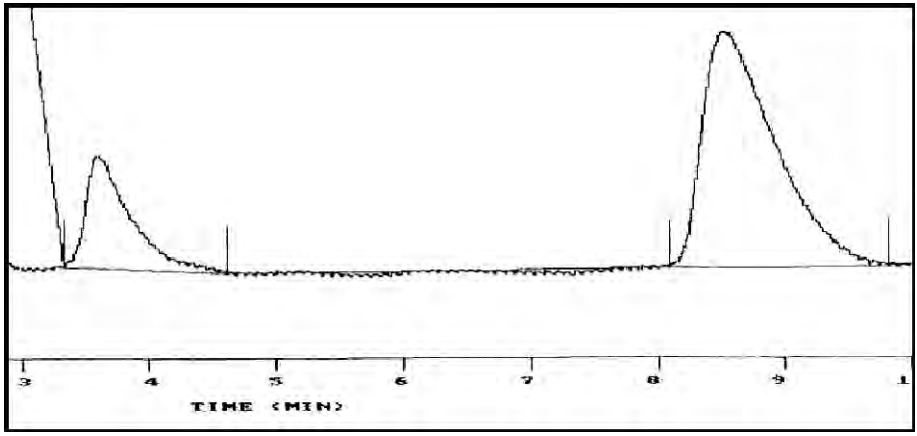

Figure 1. HPLC separation of dihydrogenatedtallowmethylamine and dihydrogenatedtallowdimethylammonium methyl carbonate.

GENETIC ANALYSIS OF A PULLULAN GENE IN AUREOBASIDIUM PULLULANS

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ABSTRACT

Aureobasidium pullulans, a saprophytic fungus, grows on plant leaves. The fungus produces a polysaccharide called pullulan, which is important in various industrial applications. Pullulan can be used as a coating for time-release drugs, a biodegradable agent in plastics, a computer display binding agent, a colonic bacterial stimulant, an adhesive, and an ingredient in many other products. *A. pullulans* does not naturally produce large quantities of pullulan, which is one explanation of why it is expensive and industry cannot feasibly use it in products. Furthermore, the United States does not currently produce pullulan in mass quantities. Thus, our goal is to find a pullulan gene and create pullulan over-producers to overcome this shortfall. If *A. pullulans* produced large quantities, the price would dramatically decrease and companies would use it in their products. It would also have a large economic impact in South Dakota since the fungus could be grown in ethanol plants without expensive equipment. Pullulan would also increase the corn and soybean markets, as well as create jobs. Several probable genes have been identified through PCR (polymerase chain reaction) comparison between non-producers and *A. pullulans*; the corresponding bands were extracted from agarose gels. The extracted bands were then transformed into *Escherichia coli* cells and the plasmid DNA was isolated. The DNAs were then transformed into a non-pullulan producing yeast strain to determine if pullulan production was restored. The protoplasts were plated onto various media. The colonies that appeared to have restored pullulan were replated to fresh media, as well as sequenced. Results of the pullulan experiment have shown that all three bands may play a role in pullulan production. One larger band is currently being confirmed for pullulan production, as it appears that the band is a pullulan gene. In conclusion, presumptive pullulan genes have been identified and are currently being sequenced.

BODY WEIGHT AND OVARIAN FUNCTION IN AY/A MICE

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ABSTRACT

The lethal yellow mutation (Ay) of the mouse agouti locus provides researchers with an animal model that is useful for the investigation of how caloric intake and body fat affect fertility. The lethal yellow mutant shows enhanced feed efficiency with respect to fat. Although in the homozygous condition, this gene is indeed lethal and arrests development of the embryo, heterozygous individuals (Ay/a) survive and express the lethal yellow syndrome (LYS). The LYS includes a yellow coat color, development of adult obesity, and decreased reproductive success. If the amount of fat in the diet is increased, the degree of obesity is greater and reproductive efficiency is severely affected. Previous studies have shown decreased estrous cyclicity, mating success, uterine weights, ovulation rates, and levels of Follicle Stimulating Hormone in obese Ay mice.

Ovaries were removed from lethal yellow (Ay/a) and control black (a/a) mice that had been maintained on low fat diets until three weeks before initiation of the study. At that time the mice were fed diets with 10% fat content instead of the maintenance diets with 4% fat content. The concept of "flushing" with higher calorie diets is believed to enhance the rate of estrous cycles and ovulation. Each female was caged with a black male, and vaginal smears were taken every morning to determine the stage of estrous. When the females reached proestrous, they were sacrificed, weighed, and the ovaries removed. Five micrometer sections were made of the ovarian tissue and the sections were stained with eosin and hematoxylin. The ovaries were examined for evidence of growing follicles, Graafian follicles, and corpora lutea.

Three age groups were studied: 90, 180, and 270 days. In each age group, the yellow mice were 4-6 grams heavier than the black controls. All ovaries appeared to be in proestrous, with growing follicles present and corpora lutea from the previous cycle. At 90 days of age, there was no difference between ovaries of the yellow mice and the black controls, each averaging about 16 new growing follicles per ovary. By 180 days of age, ovaries of black mice contained an average of 7 growing follicles and ovaries of yellow mice only 3 growing follicles. A great deal of scar tissue (corpora albicans from previous

ovulations) occurred in ovaries of the yellow mice. By 270 days of age, ovaries of both black and yellow mice contained an average of about 5 growing follicles, and the remaining ovarian tissue was mostly scar tissue. Both black and yellow mice appeared to be approaching the end of ovarian function.

The apparent accelerated senescence of the ovaries of the lethal yellow mice at 180 days of age may be due to the increased body fat of the yellow mice or to a more direct expression of the mutant gene in the ovaries of the yellow mice. Since the yellow mice were only about 4 grams heavier than the black controls, the difference may not be due to body fat. Ovaries from mice in the same age groups kept on high (10%) fat diets will be examined to see if early senescence is more pronounced with higher body fat.

CHARACTERIZATION OF LAKE LITTORAL ZONE HABITATS AND INVERTEBRATE COMMUNITIES IN NORTHERN GLACIATED PLAINS AND NORTHWESTERN GLACIATED PLAINS ECOREGIONS

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ABSTRACT

The objective of this project was to characterize lake littoral habitat and macroinvertebrate community structure among two ecoregions in eastern South Dakota. Water temperature, conductance, dissolved oxygen, pH, substrate particle size, vegetation biomass, shoreline slope and invertebrate samples were collected from twelve lake basins in the Northern Glaciated Plains (NGP, n=8) and Northwestern Glaciated Plains Ecoregions (NWGP, n=4). Five random locations were sampled from each basin during the growing season on one date in 1996 and two dates in 1997 and 1998. Over half of all habitat and invertebrate measurements displayed greater coefficients of variability from NGP sites. Higher percentages of cobble and boulder substrate were found in lake littoral areas of NGP basins while silt and clay fractions were found in greater proportions from NWGP basins. Vegetation dry weight and ash free dry weight biomass were significantly greater in NWGP basins. Total invertebrate abundance ranged from 0 to 9235 individuals per 3 minute sweepnet (mean = 1739). Insecta and Annelida were numerically most abundant, contributing on average 61% and 11% of total numbers, respectively. Invertebrate total abundance was not found to vary significantly between ecoregions. However, Insecta and Nematoda abundances were greater from NGP sites and Mollusca abundance was greater from NWGP sites. Results of this effort demonstrate significant differences in littoral habitat and invertebrate community structure between these two landscape regions.

CHARACTERIZATION OF WATER QUALITY AND PHYTOPLANKTON DYNAMICS IN THE COOLING POND OF THE OTTERTAIL POWER PLANT, MILBANK, SD

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ABSTRACT

Ottertail Power Plant maintains a 350-acre artificial pond to provide cooling water to the boilers within their plant. Over the past several years, plant managers have witnessed frequent blooms of noxious algae, fish kills, fish stunting and odor problems within the pond and calcium carbonate build-up on structures within the plant. The objective of this project was to develop baseline data describing current physical, chemical and biological conditions within the cooling pond. Characteristics measured were water temperature, dissolved oxygen, specific conductance, total hardness, nitrate and ammonia nitrogen, total phosphorus, water transparency, chlorophyll a and total and relative abundance of phytoplankton.

Water temperatures varied seasonally by site and temperatures were highest and most variable near the plant effluent structure. Total hardness (mean = 1699, range = 1250 to 2070 mg/L as $CaCO_3$) and specific conductance (mean = 4315, range = 3151 to 5847 uS/cm) also varied seasonally and both were highest near the effluent structure. Total phosphorus concentrations (mean = 0.93, range = 0.32 to 2.16 mg/L) were two to five times higher than measured ammonia (mean = 0.44, range = 0.13 to 3.48 mg/L as N) and nitrate (mean = 0.31, range = 0.10 to 2.6) nitrogen combined. Chlorophyll a (mean = 33.7, range = 0 to 352.4 ug/L) and Secchi depth (mean = 64.7, range = 25 to 120 cm) were found to be highest during the winter months. Total phytoplankton cell counts averaged 31,088 cells/ml and ranged from 13,392 to 66,423 cells/ml. Diatoms, green algae and euglenophytes were found in greater abundance during the winter months (mean = 11,043, range = 0 to 43,101 cells/ml) while cyanobacteria predominated during the warmer summer months (mean = 11,030, range = 0 to 28,709 cells/ml) at all sites. Calcium carbonate precipitation appears to be a function of high primary production and high calcium concentrations during the months of December to February. High nutrient concentrations and low nitrogen to phosphorus ratios appear to favor cyanobacteria during the summer months.

Results of this effort provide a baseline against which future changes can be measured within the cooling pond. Data collected from this effort will be used to guide cooling pond management.

INFLUENCE OF ELEVATION AND WEED ECOLOGY ON SUPERVISED CLASSIFICATION PROCEDURE USING A GEOGRAPHIC INFORMATION SYSTEM

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ABSTRACT

Herbicides were applied to 96% of the U.S. corn and soybean acres in 1997. The majority of production fields are treated as a single unit with a single, uniform herbicide application. Site-specific application, matching the herbicide needs to weed problems of an area, is now possible using global positioning systems (GPS) and injection-type sprayers. At present, intensive ground-based sampling best identifies application zones. Aerial imagery holds much promise for increasing weed-scouting efficiency. Images can be used "as is" to distinguish anomalous areas or analyzed further with geographic information systems (GIS) software that use a classification procedure to aid in identification of specific problems. Computer analysis was rapid, but problems were noted with differentiation of mixed cover types. The goal of this project is to improve the accuracy of the computer classification by incorporating weed ecology into program training procedures. In previous work, it has been noted that certain weed species are prevalent on hilltops while others are more prevalent in footslope positions. Weed grid sampling data for three years (1995, 1996, and 1998) were used to compare mean weed densities in elevation zones for three weed species, Canada thistle (*Cirsium arvense*), kochia (*Kochia scoparia*), and common ragweed (*Ambrosia artemisiifolia*). Sampling was performed on a 160-acre field using a 100 x 50-foot grid. The elevation range of the field was 50 feet from the lowest point to the highest point. Analysis revealed that Canada thistle had higher mean densities within the higher relative elevation zones, and kochia and common ragweed had higher mean densities within the lower elevation zones. By separating data into relative elevation zones and incorporating known weed ecological factors into search parameters, accuracy of weed identification was improved by ten percent using elevation-delineated classification compared with field wide assessment procedures.

THE EFFECT OF THE REMOVAL OF LIPIDS FROM SOIL ON THE GROWTH OF FAST PLANTS

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ABSTRACT

Virgin soil isolated from an area near Yankton was sieved and excess debris removed. The soil was Soxhlet-extracted in chloroform for 24 hours to remove the lipids and lipid-like molecules. Fast-plants (Brassica rapa) were grown in the lipid-free soil and in three other soils as controls: whole soil, a chloroform-slurry soil allowed to air-dry, and commercial potting soil. Each soil type was placed in 32 individual containers (8 "quads"), and a single Fast-plant seed was placed in each container. Plants grown in each soil type were followed from germination to death, with parameters such as timing of germination, leaf production, flower and fruit production, and plant height (at various stages) and final biomass being recorded.

In general, plants grown in the air-dried chloroform-slurry soil were least productive, slowest developing, and showed the most deformities. However, the performance of the plants in the lipid-free soil was nearly indistinguishable from the performance of the plants grown in whole soil, and plants grown in both of these treatments performed differently from the plants grown in commercial potting soil.

We conclude that although lipids are an integral component of soil contributing to the hydrophobic nature of humic materials and may allow contaminants to partition into the organic matter of soil, their removal seems to have little effect on the growth and development of Fast plants.

ENCOURAGING THE ESTABLISHMENT OF A NATIVE GRASS VEGETATION ON UNGRAZED RANGELAND IN SOUTHEASTERN SOUTH DAKOTA

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ABSTRACT

Much rangeland in South Dakota is dominated by a non-native vegetation. The pre-settlement prairie vegetation, dominated by a combination of tall-grass species, has largely been displaced by a vegetation dominated by Eurasian introductions. In this experiment, we sought to replace the non-native vegetation, dominated by smooth brome and leafy spurge, with a native grass vegetation composed of a mixture of big bluestem, little bluestem, switchgrass, Indian grass, and sand lovegrass over a four acre treatment area in southeastern South Dakota

Another objective of the study was to attempt to reduce the cover and vigor of leafy spurge, which is considered a noxious weed in rangelands. Typically, treatments of rangeland to control leafy spurge involve the application of chemical herbicides in increasing amounts with each subsequent application.

In the treatment area smooth brome was reduced from 80% cover to 9% cover after two years. Leafy spurge cover had been reduced from 11% to 3% cover, and among the taller native grasses the leafy spurge plants were very much less vigorous, such that by the second year the spurge plants did little flowering in the treatment area. The reduction of spurge was accomplished with a minimal application of herbicide in the first two years, and with no herbicide application since.

REPORT ON PLANS FOR THE DEVELOPMENT OF THE NORTHERN GREAT PLAINS ENVIRONMENTAL RESEARCH NETWORK

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ABSTRACT

The Northern Great Plains Environmental Research Network (NGPERN) is being planned to consolidate comprehensive surface environmental measurement capabilities in 3 focussed research sites representing different types of environments within South Dakota. These sites are being developed initially by researchers at the South Dakota State University and the South Dakota School of Mines and Technology, with collaboration from a variety of landowner groups, industrial groups, and government agencies. Instrumentation will be installed at these sites for long-term monitoring of critical environmental and ecological parameters. These will include meteorological variables, soil moisture and temperature, and fluxes of moisture, various chemical species, and energy. Instrumentation will include sonic anemometers, fast-response CO_2 and humidity flux instrumentation, a relaxed eddy accumulator to determine fluxes of organic gases, real-time trace-gas samplers for flux estimates, and a variety of analytical instrumentation including gas chromatographs and ion-mobility analyzers. Remote monitoring of the characteristics of these sites will be performed as well using dedicated airborne multispectral sensors and LANDSAT imagery from the EROS Date Center. This basic infrastructure can be augmented for more specialized studies as interest develops.

One site will be in the wetlands of the prairie pothole region of eastern South Dakota. The two remaining sites will be in the Black Hills region, one in shrubland and the other in a Ponderosa pine habitat. These sites will serve as foci for long-term environmental and ecological monitoring and study, focussing on issues related to long-term environmental stability of the Great Plains in general, and those portions of the Plains in South Dakota, in particular. Each site will have a unique set of characteristics and serve as the focus for studies related to different goals. Initially, the main focus at the prairie pothole site, which will be the first site established, will be studies that will serve as the basis for development of land-use management strategies that maximize

yields and agricultural sustainability while minimizing environmental impacts. The focus at the Black Hills shrubland site will be wildlife habitat, while that in the Black Hills Ponderosa pine site will be forest productivity and the carbon and energy budgets of mature pine forests.

Support for development of these sites is currently being sought through the NSF EPSCoR program, NASA, EPA, and other agencies with interests in long-term monitoring and the understanding of processes responsible environmental change. Collaborators are sought for any studies that can make use of the base facilities to be established at these sites.

ASSESSING THE ECONOMIC IMPORTANCE OF NEMATODES IN COW-CALF PAIRS IN EASTERN BROOKINGS COUNTY – A PILOT STUDY

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ABSTRACT

Cattle nematodes are known to adversely affect production parameters such as weight gains and reproduction efficiencies. Quantifying these losses under grazing conditions has been a difficult task, and has involved the use of "twin pastures" with one pasture containing parasitized cattle and the other pasture with cattle that are kept free of nematode parasites. For this reason, losses from these parasites in South Dakota can only be estimated based upon a few other studies conducted in neighboring states. The purpose of this project was to develop an inexpensive and easy "single-pasture" method to measure production losses from internal nematodes in cow-calf pairs during the summer grazing season. Four cattle herds from a single cattle producer in eastern Brookings County were selected for this study. The four herds consisted of 320 Angus and cross-bred Angus cow-calf pairs grazed on four separate pastures. The ages of the cows and the stocking density differed slightly among each of the herds. The study started during the second week in June, and ended approximately 100 days later. Within each herd, approximately 10% of the cows-calves pairs were injected with the recommended dosage of Dectomax (Pfizer Animal Health, Inc.) three times during study period to protect them from intestinal nematode. The remaining pairs served to keep larval nematode pasture levels high and also served as the group controls. Cow and calf weight gains and cow pregnancy rates were determined at the end of the study, and multiple regression analysis was used to separate out known confounders such as pasture conditions, calf gender and calf starting weight. After controlling for these confounders, steer calves from the treated pairs gained 18 pounds more than paired calves from the untreated group, and heifer calves from the treated pairs gained 14 pounds more than those in the untreated group ($p = 0.02$). These weights are a valid estimate of the losses due solely to internal parasites in these cattle, since all other variables within each herd (e.g. pasture condition and external parasite pressure) were equal. Pregnancy rates between the two groups were not significantly different.

FURTHER CHARACTERIZATION OF ADULT EXCRETORY/SECRETORY ANTIGENS

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ABSTRACT

Echinococcus multilocularis is a parasitic tapeworm found in canines (definitive host) which completes its life-cycle in the livers of rodents (intermediate host) producing hundreds of protoscoleces in a multi-chambered hydatid cyst. Canines become infected with the adult stage if an infected rodent is ingested. Rodents develop hydatid cysts if eggs are ingested from fecal matter excreted from infected canines. Through the course of this life-cycle, protoscoleces from the cysts of rodents pass through the stomach and into the intestine of the canines. Once in the intestines, protoscoleces will develop into the adult stage. The tegument (outer most syncytial layer) provides protection while the tapeworm is in the harsh environment of the canine intestine. This tegument will continually be sloughed off which provides a possible source of excretory/secretory (E/S) antigens. Other sources of E/S antigens include excretory products and reproductive fluids secreted from the genital pore. Antibodies developed against adult E/S antigens have been incorporated into a fecal ELISA for diagnosing canine *E. multilocularis* infections in dogs. In a previous study, E/S antigens from adult and protoscolex antigen sources were analyzed to determine if shared antigens exist between the two developmental stages. It was found that nine major adult proteins bound to anti-protoscolex antibodies, and were therefore shared between the two developmental stages. Molecular weights for four of the protoscolex proteins were similar those of adult antigens. These shared E/S antigens may represent a safer and cheaper source of "adult antigens" for production of antibodies for a fecal ELISA.

While *E. multilocularis* is located in the intestinal tract, they will be continually in contact with enzymes whose function is to primarily breakdown proteins. The carbohydrates attached to the proteins of the adult worms may add stability while the worms are present in the intestines of canines. Proteins with higher carbohydrate composition will have a higher stability over proteins with less carbohydrates attached. Therefore, the purpose of this study is to further characterize E/S antigens of adult *E. multilocularis* tapeworms by their carbohydrate composition with the use of lectins. Crude E/S antigen samples were applied to SDS-Polyacrylamide Gel Electrophoresis to isolate the proteins. These proteins were either stained with Coomassie Blue or transferred to Im-

mobilon-P transfer membranes (PVDF; Millipore corporation Bedford, MA) by a Western Blot technique. Once the proteins were transferred to the membranes, they were incubated first in BSA (bovine serum albumin) for 30 min to block non-specific sites, then with biotinylated lectins for 1 hr, with a horseradish-peroxidase-streptavidin solution for 30 min and finally with a DAB substrate kit (Vector laboratories Burlingame, Ca) until the bands appears. Seven major and 4 minor glycoproteins were detected with the lectin concanavalin-A; 7 of these glycoproteins also bound to the anti-protoscolex antibodies. Nine major and 3 minor glycoproteins bound to succinylated wheat germ agglutinin; 6 of these glycoproteins also bound to the anti-protoscolex antibodies. Six proteins from post-2,500g pellet fraction of the adult tegument bound to the anti-protoscolex antibodies, confirming that at least a portion of the E/S antigens were of tegumental origin.

DIETARY RECONSTRUCTION OF HUMAN BONE SAMPLES FROM EARLY POPULATIONS IN THE CENTRAL COLUMBIAN ANDES, CA. 7000-4000 BP, WITH THE USE OF STABLE CARBON AND NITROGEN ISOTOPES.

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ABSTRACT

Thirty-one human skeletal samples representing eight burial sites from early populations in the central Columbian Andes (Paleo-Indian and early Formative Periods, ca. 7000-4000 BP) were analyzed for carbon and nitrogen stable isotopes. Collagen and bioapatite extractions were completed on the samples, and the isotopic composition was obtained with isotopic ratio mass spectrometry. Collagen, the primary component of the organic phase of bone, is the rubbery protein that forms cartilage, tendons, and the matrix of bones. Bioapatite, with carbon as carbonate, is in the mineral phase of calcified tissues. With respect to paleodietary analysis, collagen $\delta^{13}C$ values reflect primarily the protein intake of the diet, and apatite $\delta^{13}C$ values reflect the diet portion metabolized as fuel. Isotopic composition of a sample is measured as the ratio of one isotope to another and compared to a universally accepted standard. The method of carbon and nitrogen isotope analysis is based on the observation that these signatures are transferred along food webs in predictable ways. Variable fractionation takes place in plants during photosynthesis, with each of two photosynthetic pathways discriminating more or less against ^{13}C. This variation is reflected in the tissues of the consumers, and the original ratios are altered only by the loss of some ^{13}C during fractionation at each level in the food chain.

Differences between collagen and apatite $\delta^{13}C$ values (known as collagen-apatite spacing, designated Δ) vary with trophic level as a result of the significance of different macronutrients (proteins, carbohydrates, lipids) within individual diets. The collagen of both herbivores and carnivores reflects the growth (protein synthesis) aspect, and the apatite of each reflects the source of metabolic energy of their respective diets. The spacing helps define the degree of herbivory or carnivory of the animal's diet. The degree to which an animal is either carnivorous or herbivorous can also be estimated using nitrogen isotopes, $\delta^{15}N$ which increase 3 per mil per trophic level. General reconstruction of animal diets is based on the tissue $\delta^{13}C$, some knowledge of feeding patterns, and an understanding of the C3 and C4 plants of its habitat or proximity to the ocean.

The Columbian samples were originally interpreted from collagen and apatite data. Isotopic results do show significant differences among the burial sites. Two of the sites, Soacha and a site of unspecified origin (N/A), show enriched $\delta^{13}C$, $\delta^{15}N$, and small Δ. This may signify dependence on greater maize or marine dietary sources. The reduced Δ suggests dependence on marine dietary sources. These results will assist anthropologists and archeologists develop more complete dietary reconstructions and cultural understandings.

SEED WEIGHTS AND GERMINATION OF TWO POPULATIONS OF AMERICAN LOTUS

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ABSTRACT

Seeds of American lotus (*Nelumbo lutea*) from Iowa and Florida were tested for differences in seed weights and germination rates. Our assumption was that Iowa populations of American lotus may be adapted to germinate at lower temperatures than Florida populations. Seeds were weighed individually and mean weights were tested for significant differences. For germination tests, seeds were first scarified by placing twenty-seed lots in 500 ml of concentrated sulfuric acid and stirring for two hours followed by rinsing 5 minutes in running water. Seeds were then placed in plastic germination trays of water with a group of ten seeds per container. Containers were placed in a growth chamber at 25, 20, 15, and 5C, with 12 hr of light and dark. Seeds were checked daily for germination, and the number of days required for seeds to germinate under each temperature regime was recorded. PROC MIXED in SAS was used to determine if there were any significant differences between the Florida and Iowa seeds with respect to weight and germination rates at the different temperatures. Preliminary tests showed some significant differences in seed weights and germination rates between Iowa and Florida populations.

AGOUTI PROTEIN AND MELANOCORTIN INTERACTIONS IN AY/A MICE

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ABSTRACT

Interactions between agouti protein, αMSH (Melanocyte Stimulating Hormone), and melanocortin cell surface receptors (Mc1r) are fundamental to our understanding of agouti gene regulation. MSH and agouti protein compete for sites on Mc1r; the results of this competition dictates patterns of yellow and black pigment production within hair follicle pigment cells. We used exogenous MSH and Ay/a mice to determine how excess MSH in the presence of excess agouti protein (Ay/a mice) alters cell signaling and subsequent pigmentation. We conducted two experiments: 1) exogenous MSH injections in both Ay/a and control black (a/a) mice and 2) reciprocal serum injections between histocompatible Ay/a and a/a mice. In both instances we tested the hypothesis that MSH and agouti protein would, by virtue of their actions on Mc1r (melanocortin receptor 1), elevate and dampen tyrosinase activity (TH), respectively. TH activity measured in pmol dopa/hour/ug protein is a key indicator of pigmentation. When excess MSH was injected subcutaneously to Ay/a and a/a mice undergoing hair regeneration, TH activity exhibited the predicted pattern (3-4 units of TH in a/a per unit of TH in Ay/a) except in the case of the des-MSH-treated groups. In contrast to B16 melanoma cells (Ramasastry, 1997, Ph.D. Dissertation), hair bulbs treated with des-MSH exhibited a slight decline in TH activity. Upon reciprocal injections of sera of Ay/a and a/a mice beneath the dorsal skins of Ay/a and a/a mice undergoing hair bulb regeneration, TH activities were 62.3±9.4 U (a/a controls), 48.1±8.0 U (a/a experimentals), 16.3±2.0 U (Ay/a controls), and 12.6±2.8 U (Ay/a experimentals). Although equivocal, these data suggest the presence of pigmentation regulatory factors, not necessarily agouti protein, in sera of both Ay/a and a/a mice.
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A BREEDING POPULATION OF VIRGINIA'S WARBLERS IN THE SOUTHWESTERN BLACK HILLS

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ABSTRACT

The known breeding range of Virginia's Warbler (*Vermivora virginiae*) is scattered throughout southwestern North America in appropriate habitats, ranging from southern Idaho and south-central Wyoming south to northern Sonora and Chihuahua. Within this range, breeding habitat consists of dense brush on mountain slopes with interspersed taller vegetation. Here we report a breeding population of Virginia's Warblers from Custer County, South Dakota in the southwestern Black Hills. The habitat in which warblers occurred in this area consisted of a shrubby understory of skunkbush sumac (*Rhus aromatica)* and mountain-mahogany (*Cercocarpus montanus*) with interspersed or adjacent Rocky Mountain juniper (*Juniperus scopulorum*) and ponderosa pine (*Pinus ponderosa*). We used fixed-width (25 m) line transect censuses and mist net sampling during the breeding season (25 May-9 July 1998) to document the abundance and breeding condition of Virginia's Warblers in this habitat. Line transects yielded overall raw density estimates of 8.3 birds km^{-2} and an overall relative abundance (birds per kilometer-long transect) of 1.5 birds/transect. Virginia's warblers were detected on each of 10 kilometer-long transects established in this habitat and relative abundances for individual transects ranged from 0.43-3.75 birds/transect. Male warblers captured by mist net (n = 22) had well developed cloacal protuberances and females (n = 5) had well-developed brood patches or eggs within the reproductive tract. These features strongly suggest breeding in these habitats. In addition, we found an active nest with four nestlings on 3 July 1998, thus documenting breeding for Virginia's Warbler in South Dakota. This population represents the northeasternmost breeding population of Virginia's Warblers and extends the known breeding range of this species by over 200 km.

BREEDING BIRD RICHNESS AND ABUNDANCE IN PINE-JUNIPER-SHRUB HABITAT IN THE SOUTHWESTERN BLACK HILLS

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ABSTRACT

Pine-juniper-shrub habitat with a shrubby understory of skunkbush sumac (*Rhus aromatica)* and mountain-mahogany (*Cercocarpus montanus*) and interspersed or adjacent Rocky Mountain juniper (*Juniperus scopulorum*) and ponderosa pine (*Pinus ponderosa*) is restricted in South Dakota to small areas in Custer and Fall River Counties in the southwestern Black Hills. These habitats have received very little ornithological investigation. We used fixed-width (25 m) line transect censuses and mist net sampling during the breeding season (25 May-9 July 1998) to document the abundance and breeding condition of birds in this habitat. Line transects yielded an overall raw density estimate of 411.7 birds km^{-2} and a mean (±SD) relative abundance (birds per kilometer-long transect) of 56.9 ± 12.8 birds/transect. Overall abundance increased significantly ($P<0.01$) over the study period from 43.7 birds/transect in late May to 68.2 birds/transect in early July. This probably results both from the recruitment of juveniles into the population and from the appearance of irruptive species in the habitat. We detected 54 species on censuses and 21 species by mist net sampling, for a total of 56 species, 52 of which were potential breeders in this habitat. Of these 52 species, 19 (36.5%) were Neotropical migrants, 18 were permanent residents (34.6%), and 15 were temperate-zone migrants (28.8%). Nest searches were conducted concurrently with mist net sampling but at different sites. A total of 30 nests was found representing 15 different species. Of these 30 nests, 16 nesting attempts were complete by the end of the study period and seven of these (43.8%) were successful (i.e., fledged at least one young). Only one of the 26 nests with visible contents was parasitized by cowbirds, despite relatively high abundance of cowbirds in this habitat, suggesting relatively low rates of nest parasitism. Thus, a diverse assemblage of birds occupies this habitat during the breeding season, many of which are Neotropical migrants showing population declines. This suggests that this restricted habitat in South Dakota is an important breeding locale for a number of species.

DISCRIMINATION BETWEEN TWO VARIETIES OF SOYBEAN [GLYCINE MAX (L.) MERR.: FABACEAE] USING NUCLEAR RIBOSOMAL INTERNAL TRANSCRIBED SPACER DNA SEQUENCES

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ABSTRACT

Glycine max (L.) Merr. cv. "Virginia" is a cultivar that was maintained at both of the soybean germplasm collection facilities: in Urbana, Illinois and in Stoneville, Mississippi. Rather than maintain two independent collections of this cultivar, it was decided to transfer the Stoneville collection to Urbana. The Stoneville collection was given the designation "Virginia S." Both "Virginia" and "Virginia S" cultivars were field grown and evaluated for similarities. On the basis of standard morphological characteristics it was determined that the two cultivars were identical. The purpose of our project was to use nuclear ribosomal internal transcribed spacer DNA sequences to determine the degree of similarity between these two putative cultivars. Seeds were obtained from the soybean germplasm collection facility in Urbana, Illinois and grown in a growth chamber. Secondary leaf material was used to extract total DNA, using the CTAB method. Symmetric polymerase chain reactions were performed yielding DNA fragments of approximately 700 base pairs in length, as verified by agarose gel electrophoresis. DNA fragments were purified using an active matrix of silica beads and chaotropic salts. Purified DNA fragments were sequenced using a Perkin Elmer ABI 377 sequencer. Analysis of the sequences obtained from each putative cultivar indicated that they were identical for every base pair of the 700 base pairs. Based on the morphological analysis of previous workers and on nuclear ribosomal internal transcribed spacer regions, we conclude that *G. max* (L.) Merr. cv. "Virginia" and *G. max* (L.) Merr. cv. "Virginia S" represent identical taxa.

DIFFERENTIAL EFFECTS OF HABITAT DISTURBANCE ON THE GENETIC STRUCTURE OF TWO CLOSELY RELATED BEETLE SPECIES

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ABSTRACT

The tortoise beetle *Physonota helianthi* (Coleoptera: Chrysomelidae) feeds on sawtooth sunflower (*Helianthus grosseratus*) in our area. This native species is now restricted to roadsides, ditches, and natural areas that have not been disturbed by agricultural activity. Another tortoise beetle species, *Chelymorpha cassidea*, feeds on opportunistic weeds that rapidly colonize disturbed areas (e.g. field bindweed (*Calystegia sepium*) and creeping jenny (*Convolvulus arvensis*)). Both tortoise beetle species lay eggs in large groups (~25 individuals/mass) providing an opportunity to compare genetic variability within families (i.e. among sibs), within patches (i.e. among masses within a field), and across the landscape (i.e. among patches). Specifically, we want to know how the fragmentation of sawtooth sunflower patches has impacted the genetic structure of *P. helianthi* and if *C. cassidea*, feeding on the more common hosts, shows less genetic structure. We used allozyme frequencies to evaluate the impacts of host plant fragmentation on the population genetic structure of both tortoise beetle species. We also wanted to determine the mating structures of the two species. Specifically, we wanted to detemine if females mate once (resulting in full sibs within an egg mass) or multiply (half sibs within an egg mass).

Relatively higher levels of genetic variability existed among patches than within patches for *P. helianthi* while genetic structuring of *C. cassidea* was most apparent within patches. *Physonota helianthi* females were most often multiply mated while *C. cassidea* females appeared to mate with a single male. These differences may be related to the differential effects of disturbance on host plant distributions and differences in the life histories of the two tortoise beetles.

ROW SPACING EFFECTS ON THE GROWTH AND YIELD OF HYBRID CORN (*ZEA MAYS* L.)

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ABSTRACT

Traditionally, corn (*Zea mays* L.) has been grown in rows spaced from 30 to 40 inches apart in South Dakota. Interest in corn planted in narrow (less than 30 inch) rows is developing as a potential method to increase the yield of hybrid corn by maximizing sunlight, water and nutrient absorption. The objective these experiments was to determine the effect of narrow row spacing on corn growth and yield in South Dakota.

Five full season genotypes were used in the preliminary 1996 experiment year. Ten genotypes ranging in relative maturity from 70-104 days were used in the following 1997 and 1998 years. Experiments were conducted at four different environmental locations in Eastern South Dakota. Growth data was measured at the Brookings location.

In the 1996 experimental year, row spacing did not significantly affect grain yield across locations. The 15-inch row spacing increased yield by 2.4% when averaged across nine environments and the years of 1997 and 1998. The increase in yield was highly variable between locations with a highly significant row spacing x location interaction. Narrow row spacing increased leaf area exposure for sunlight absorption. The increased leaf area index provided by narrow rows benefited the mid-season maturing hybrids more than full and early-season hybrids.

Plant height was reduced with narrow row spacing over the three years tested. Root capacitance was reduced in 1997 and 1998 with narrow row spacing. This may indicate differences in root size or structure between row spacing treatments. Stalk lodging was not affected by row spacing in 1996. The following two years pooled across hybrids resulted in a 2.64% increase in stalk lodging with narrow row spacing. Stalk lodging was affected by the environment and hybrid tested. Percent crude protein and harvest percent grain moisture were not affected by row spacing.

The effects of narrow row spacing on yield were generally small. Yield advantages from narrow rows was not consistent between years at most locations. This influence of years and locations minimizes the potential of 15-inch row spacing as a management technique for improving and/or stabilizing grain yield for farmers in South Dakota where full season maturing corn hybrids are planted.

DIET RECONSTRUCTION OF HERBIVORES FROM MONGOLIA USING STABLE ISOTOPES OF CARBON

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ABSTRACT

This project was undertaken to aid the reconstruction of herbivore diets using stable isotopes of carbon, focusing primarily on isotopic fractionation of carbon in C_3 and C_4 plants. C_3 and C_4 plants are distinct photosynthetic pathways with physiological differences in carbon dioxide uptake. This difference results in a variation of $\delta^{13}C$ signal with respect to type. When herbivore tissues were analyzed for carbon isotopes, differences in the signal between C^3 and C^4 plants were seen. The $\delta^{13}C$ signals obtained resembled three dietary types: predominately C^3 plant diet, mixture of C^3 and C^4 plant diet, or predominately C^4 plant diet.

Samples were grouped according to species and Aimag. Aimags are administrative units similar to our counties in the U.S. The entire sample set consisted of three hundred cancellous bones of five species, horse, sheep, goat, camel, and cattle (*Equus caballus, Ovis* sp., *Capra hircus, Camelus bactrianus,* and *Bos taurus*), and one hundred and forty wool samples (*Capra hircus* and *Ovis* sp.). Samples were processed to extract and partially purify collagen (bone gelatin), bioapatite (organic carbonates), and lanolin free wool. These organic samples were then automatically combusted, quantitatively analyzed for carbon and nitrogen concentrations, and then analyzed using a VG Isotech Isotope Ratio Mass Spectrometer SIRA Series II. The mean $\delta^{13}C$ values for each species grouped by Aimag was then determined for further statistical analyses. The species mean values for $\delta^{13}C$ ranged from -20.3 ‰ for sheep to -18.5 ‰ for camels. Animals grouped by Aimag showed a similar range of $\delta^{13}C$ values, -21.8 ‰ to -17.9‰. This data, along with ancillary climate data for each Aimag, was then used to see if the variation was significantly different by species or across Aimags and to see if climate factors had an effect.

Statistical analysis techniques, ANOVAs, and step-wise multiple regressions were performed to examine how each species was affected as to generate a predominately C_3 signal or mixed C_3 /C_4 signal. From these statistical analyses, it was determined that sheep (*Ovis* sp.) and goat (*Capra hircus*) signal did not vary significantly, but other species varied significantly from sheep and goat. Isotopic values differed significantly across Aimags. Statistical techniques also tested for the significance of each climate factor. The climate factors for each Aimag included annual and growing season factors that impacted plant growth. Multiple regressions showed climate factors had no significant impact on specie $\delta^{13}C$ values, with the exception of camel (*Camelus bactrianus*) and horse (*Equus caballus*). In both cases, temperature was significant with respect to growing seasons.

This particular study allowed us to examine the factors involved in reconstructing herbivore diets by means of carbon isotopes. The study also showed how climate factors and distribution of species over a large area significantly affected C_3 /C_4 dietary makeup.

ALTERATION OF MONOCYTE-DERIVED MACROPHAGE FUNCTION BY BOVINE HERPESVIRUS-1 INFECTION IN VITRO

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ABSTRACT

Bovine herpesvirus 1 (BHV-1) is a major cattle pathogen causing bovine respiratory and reproductive tract diseases. BHV-1-associated immunosuppression has been demonstrated *in vitro* and *in vivo*. The mechanism of immunosuppression is poorly understood. To determine whether infection of macrophages by BHV-1 may play a role in virus-induced immunosuppression, the ability of monocyte-derived macrophages (MDM) to support BHV-1 replication was assessed. In addition, the effects of BHV-1 on expression of surface molecules, phagocytic activity and immunoregulatory function were examined. BHV-1 can replicate in MDM *in vitro*. However, yields of infectious virus were low. The percentage of macrophages expressing the surface antigens CD11b, CD14, MHC class I and MHC class II was significantly decreased after BHV-1 infection. The phagocytic activity and production of radical oxygen in BHV-1-infected MDM were decreased. In contrast, BHV-1 stimulated production of IL-12 and TNF-a. Thus, exposure of MDM to BHV-1 appears to stimulate their immunoregulatory functions, whereas the microbicidal activity and expression of several important functional molecules on macrophage surface seem to be severely diminished.

VAGAL LOBE STRUCTURE IN DIFFERENT SPECIES OF CATOSTOMID FISHES

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ABSTRACT

The vagal lobe is an enlargement of the dorsal surface of the hindbrain of fishes that have a well-developed sense of taste. In some goups of fishes, e.g. carps (cyprinids) and suckers (catostomids), the vagal lobe is very complex in structure and can comprise 25-30% of the total mass of the brain. The cellular organization within the lobe of these fishes is laminar, with different layers consisting of sensory nerve fibers entering the lobe, interneurons connecting different layers with each other or with different regions of the brain, and motor neurons with fibers that innervate musculature involved in feeding behaviors. Members of the cyprinid family, mostly carp and goldfish, have been studied extensively, but very little is known about the cytological structure of the vagal lobe of catostomid species. The suckers provide an interesting model for study of the vagal lobe because they are primarily a North American group of fishes that has diverged over the past 30-50 million years to inhabit various bodies of water from large lakes and rivers to small, mountainous headwaters. This allows one to ask evolutionary questions about the functional role of the vagal lobe by correlating anatomical differences between species with differences in their ecology and evolutionary history.

Several species of catostomids were used in this study: white sucker (*Catostomus commersoni*), longnose sucker (*Catostomus catostomus*), mountain sucker (*Catostomus platyrhynchus*), river carpsucker (*Carpiodes carpio*), and smallmouth buffalo (*Ictiobus bubalus*). Formaldehyde-fixed specimens were acquired from various ichthyological collections, and the brains were dissected out, dehydrated, and embedded in paraffin for sectioning. Serial sections were cut through the hindbrain of each specimen (5-25 um) and mounted on slides. Each series of slides was stained either for cell bodies (Nissl protocols) or for neuronal processes (Bodian silver-staining) and the different species were compared microscopically.

The species studied possessed subtle differences in vagal lobe cytoarchitecture, although the gross morphology and general construction were conserved across the species. While relative size of the vagal lobe is smaller in species that are adapted for smaller, rapidflowing streams, our results suggest a reduction in the complexity of the lamination is not correlated to the habitat. There are species differences in relative sizes of the sensory and fiber layers of the lobe, and in the orientation of motor cells in the deeper layers, but the phylogenetic significance of these differences are still being investigated.

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CHUTE STRESS PHASE II

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PURPOSE

The purpose of this project was to determine if working stress in cattle could be reduced if the cattle were worked with longer intervals of time between workings (worked daily, every 3 days, every 7 days, every 10 days, every 14 days, every 17 days). If the stress was reduced, it would also be determined over which interval stress was reduced the most.

PROCEDURE

Sixty yearling calves were randomly sorted out of feeder calves. These sixty calves were then sorted into six groups of ten. Each group was then designated to be worked six times with one of the chosen intervals of time. These intervals were: daily, every 3 days, every 7 days, every 10 days, every 14 days, and every 17 days. Each group was then put into separate yards to avoid stress from being sorted for each working. Each group was also treated as much alike as possible.

When the groups were worked, each calf was separately run into a cattle working chute. When each calf was in the chute, their stress was measured by temperature, heart rate, and breathing rate. The temperature was measured in degrees Celsius with a rectal animal thermometer. The heart rate was counted in beats per minute by using a stethoscope placed between the front, left "elbow" and “shoulder”. The breathing rate was measured in respirations per minute by watching and counting the rise and fall of the chest.

As each group finished being worked for its sixth time, they were allowed to return to their normal lives.

CONCLUSION

In conclusion, the purpose of this project was to determine if working stress in cattle could be reduced if the cattle were worked with longer intervals of time between workings and over which interval the stress was most reduced. It was found that working stress in cattle could be reduced if the cattle were worked with longer intervals of time between workings. It was also found that every group’s stress appeared to be reduced. The last working measurements were lower than the first working measurements. The group that was worked every 7 days had the best stress reduction.

TAKING THE EARTH'S PULSE

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PURPOSE

The purpose of this project is to create a simple operating magnetometer. A magnetometer is a sensitive instrument that measures fluctuations in the Earth's magnetic field using powerful magnets to pick up magnetic ripples in the ionosphere. This project also explores any connection between the magnetometer's readings and the environment.

PROCEDURE

To begin the project, a magnetometer was constructed using rare-earth magnets, ordinary pane glass, a nylon filament, common mirror, black vinyl tape, four "doughnut magnets," a laser, and a wooden base. Once the instrument was built and properly set up so that the rare-earth magnets were nulled by the "doughnut magnets," it could be used to show micropulsations in the Earth's magnetic field. These micropulsations were observed and recorded several times. They were also observed while the instrument was placed in the presence of radio transmissions. The observations were then analyzed to find a conclusion.

CONCLUSION

In conclusion, the magnetometer constructed in this project properly measured micropulsations of the Earth's magnetic field. The micropulsations occurred spontaneously and with no particular cause or order, other than magnetic ripples in the ionosphere. This was what the instrument was designed to measure. However, environmental factors do not affect the magnetomer's readings. Neither any sort of weather or even the presence of radio transmissions affected the fluctuations of the magnetometer. Therefore, it is concluded that the instrument is a simple, functional way to measure micropulsations of the Earth's magnetic field.