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Academy Editors

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CODEN:
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ARKANSAS ACADEMY OF SCIENCE

VOLUME XXXVIII
1984

ARKANSAS ACADEMY OF SCIENCE
DEPT. OF NATURAL SCIENCE
MONTICELLO, ARKANSAS 71655

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Dwight M. Moore, 1932-33, 64
Flora Haas, 1934
H. H. Hyman, 1935
L. B. Ham, 1936
W. C. Munn, 1937
M. J. McHenry, 1938
T. L. Smith, 1939
P. G. Horton, 1940
I. A. Willis, 1941-42
L. B. Roberts, 1943-44
Jeff Banks, 1945
H. L. Winburn, 1946-47
E. A. Provine, 1948
G. V. Robinette, 1949
John R. Totter, 1950
R. H. Austin, 1951
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Delbert Swartz, 1953
Z. V. Harvalik, 1954
M. Ruth Armstrong, 1955
W. W. Nedrow, 1956
Jack W. Sears, 1957
J. R. Mundie, 1958
C. E. Hoffman, 1959
N. D. Buffaloe, 1960
H. L. Bogan, 1961
Trumann McEver, 1962
Robert Shideler, 1963
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James H. Fribourgh, 1966
Howard Moore, 1967
John J. Chapman, 1968
Arthur Fry, 1969
M. L. Lawson, 1970
R. T. Kirkwood, 1971
George E. Templeton, 1972
E. B. Whitlake, 1973
Clark McCarty, 1974
Edward Dale, 1975
Joe Guenter, 1976
Jewel Moore, 1977
Joe Nix, 1978
P. Max Johnston, 1979
E. Leon Richards, 1980
Henry W. Robison, 1981
John K. Beadles, 1982
Robbin C. Anderson, 1983

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The Arkansas Academy of Science recognizes the support of the following institutions through their Institutional Membership in the Academy.

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EDITORIAL STAFF

Cover: Arkansas Mastodon dug up by Frank Reynolds in 1936. Figure 3.2 in Archaeology of the Central Mississippi Valley by Dan F. and Phyllis A. Morse, Academic Press (p. 52).

In Memory of

DR. DAVID A. BECKER

and

DR. DWIGHT M. MOORE

ARKANSAS ACADEMY OF SCIENCE

Volume XXXVIII **1984**

Proceedings

Secretary's Report

MINUTES OF THE SIXTY-EIGHTH ANNUAL MEETING — 6-7 APRIL 1984

FIRST BUSINESS MEETING

Dr. Paul Sharrah, President, opened the meeting and introduced Dr. James Halligan, interim Chancellor of UAF, who welcomed the Academy to the Fayetteville campus.

President Sharrah recognized Dr. Robbin Anderson who reminded the membership of the Science Education Symposium. Dr. William Evans, local arrangements chairman, summarized the meeting events.

President Sharrah recognized Dr. David Chittenden, Secretary, who presented the minutes of the 67th annual meeting as printed in the *Proceedings*.

Dr. Sharrah then recognized Dr. Arthur Johnson, Treasurer, who presented the following Financial Report.

FINANCIAL STATEMENT

March 11, 1983 to March 12, 1984

1.	Annual Meeting, UCA, April 1-2, 1983		
	a. Registration	$ 1,040.00	
	b. Banquet Tickets	643.75	
	Total Meeting Income	1,683.75	$ 1,683.75
2.	Individual Memberships		
	a. Regular (286)	2,860.00	
	b. Sustaining (31)	417.00	
	c. Sponsoring (6)	250.00	
	d. Life (2)	400.00	
	e. Associate (8)	40.00	
	Total Individual Memberships	3,967.00	3,967.00
3.	Institutional Dues ( )		1,200.00
4.	PROCEEDINGS, Subscriptions		1,112.50
5.	PROCEEDINGS, Page Charges		1,940.00
6.	PROCEEDINGS, Miscellaneous Sales		172.19
7.	BIOTA Receipts		52.00
8.	Arkansas Collegiate Academy of Science		53.86
9.	Interest (First State Bank & Trust Co.)		
	a. TOTAL Account	$ 154.26	
	b. Certificates of Deposit	213.58	
	c. Savings Account	13.24	
	Total Interest	381.08	381.08
TOTAL INCOME			$10,562.38

1.	PROCEEDINGS, Publication and Distribution		
	a. IRS (#127)	$ 26.65	
	b. Jones Truck Lines (#131)	30.35	
	c. Phillips Litho Co., Inc. (#133)	2,700.00	
	(#136)	1,733.28	
	(#140)	800.00	
	d. Ark. State Dept. of Finance and Administration (#146)	1.40	
	Total	$ 5,291.69	$ 5,291.69
2.	Meeting Expenses (UCA Statement)		
	a. Postage and Mailing	$ 34.18	
	b. Duplication of Programs	324.44	
	c. Name Tags	26.21	
	d. Refreshments	192.65	
	e. Banquet	803.25	
	f. Cash Handling Discrepancy	5.75	
	Total	$ 1,386.48	1,386.48
3.	Awards		
	a. Rebecca Sue Sample (#129)	$ 35.00	
	b. Timothy Jay Groseclose (#130)	30.00	
	c. Ark. Jr. Acad. Sci. (#144)	200.00	
	Total	$ 265.00	265.00
4.	Operating Costs		
	a. Treasurer's Office		
	1) Coleman's Office Supply (#128)	$ 18.85	
	(#130)	13.73	
	2) Postage (#139)	20.00	
	3) Instaprint (#143)	15.60	
	b. Secretary's Office (#134)	125.00	
	(#149)	100.00	
	c. Newsletter		
	1) UALR Biol. (#141)	44.56	
	(#147)	14.58	
	2) Copy Cat (#142)	114.40	
	(#148)	114.97	
	d. Ad Craft of Arkansas (#132)	62.25	
	e. Ark. Collegiate Acad. Sci. (Insuff. Funds)	53.86	
	f. BIOTA Printing (#135)	86.14	
	g. Nat. Assoc. Acad. Sci. (#137)	25.00	
	h. Douglas James (#145) Overpaid	10.00	
	i. Ark. Lib. Comm. Ret. Sales Tax(#150)	4.50	
	Total	$ 823.44	823.44
TOTAL EXPENSES			$ 7,766.61

Dr. Rick McDaniel, Editor of the *Proceedings*, presented a short synopsis of this year's edition and then moved that $500 be appropriated for editorial assistance and $120 for travel during the preparation of Volume 38. The motion will be voted on in the Second Business Meeting. (The motion was seconded.)

Dr. John Rickett, Editor of the Newsletter, reported that two editions of the Newsletter will again be published next year. He moved that $335 be appropriated for the Newsletter in 1984-85. The motion was seconded. It will be voted on in the Second Business Meeting.

The Nominating Committee presented the following candidates for office:

For Vice President: Dr. Ed Bacon - UAM
Dr. Gary Tucker - ATU

For Secretary: Dr. Walt Godwin - UAM

John Peck, Chairman of the Arkansas Science Talent Search, reported that first prize in the 1984 Search had been won by Deborah Renay Fisher of Sheridan and the second place prize by Jon Anthony Davenport of White Hall. It was moved that the Academy continue to provide $35 for first prize and $30 for second prize for the 1985 Search. The motion was seconded. It will be voted on in the Second Business Meeting.

Leo Paulissen, Chairman of the Committee on Endowments, reported that initial contacts had been made with potential contributors and that the committee was optimistic that some funding was forthcoming. Efforts will be made to cover the state at a later date.

Marie Arthur, Director of the Junior Academy, reported that interest had increased in the Junior Academy and thus the number of regions had been increased from four to eight. A motion was made to continue the support of the Junior Academy at a level of $200. The motion was seconded. The motion will be voted on in the Second Business Meeting.

Art Johnson presented the report of Michael Rapp, Director of the Arkansas Science Fair Association. It was moved that the Academy again appropriate $100 for the support of the Science Fair. The motion was seconded. It will be voted on in the Second Business Meeting.

It was announced that the Academy had two invitations for the location of the 1986 meeting, from Ouachita Baptist University and from the University of Arkansas at Little Rock. The invitations were referred to the Meeting Site Committee. The membership was reminded that the 1985 meeting would be held at the University of Arkansas at Monticello.

President Sharrah adjourned the First Business Meeting.

SECOND BUSINESS MEETING

President Sharrah recognized David Chittenden, Secretary, who made the following motion.

> I move that the Minutes of the 67th Annual Meeting, published in the 37th *Proceedings* of the Arkansas Academy of Science be approved as written.

The motion was seconded and passed.

It was moved and seconded to accept the Treasurer's Report. Dr. Arthur Fry presented the following report from the Audit Committee.

> The Audit Committee has examined the financial records of the Academy and finds them to be in good order. All receipts and expenditures are adequately accounted for and the books are in balance.

It was moved and seconded to accept the report of the Audit Committee. This motion passed. The motion concerning the Treasurer's Report passed.

The motions, made at the First Business Meeting, concerning funding were passed. These included the appropriations for

Junior Academy of Science
Arkansas Science Fair Association
Science Talent Search
Proceedings
Newsletter

Tom Palko reported that 108 papers had been submitted for the Junior Science and Humanities Symposium. Fifteen were read and five won a trip to the University of Wisconsin-LaCrosse.

Gary Heidt reported that the Undergraduate Awards has been won by

Robert Roundtree - UALR -
Comparative Study of the Diving Physiology of Reptiles

William Bednar - UAF - Irradiation of Dienes

Ballots were distributed for the vote for the offices of Vice President and Secretary. Those elected were

Vice President - Edmond Bacon
Secretary - Walt Godwin

The Meeting Site Committee recommended Ouachita Baptist University as the site for the 1986 meeting since OBU will be celebrating their centennial in that year. The Committee expressed its hope that UALR will extend an invitation for the 1987 meeting.

Leo Paulissen, reporting for the Biota Survey, announced that there were new checklists on mosses and beetles.

J. Kenneth Beadles, chairman of the Resolutions Committee, moved the adoption of the following resolution.

Be it resolved:

> The members of the Arkansas Academy of Science express their gratitude to William L. Evans, President-Elect of the Academy, and to the other members of the local arrangements/program committee, Walter L. Manger and Lester C. Howick - for the precise planning of the meeting and for their efficient implementation of those plans. Also, the Academy thanks P. M. Johnston and other members of the faculty for their hospitality. Appreciation is expressed to the citizens of Fayetteville and the administration of the University of Arkansas for the use of the Continuing Education Center.
>
> The Academy appreciates the efforts of each standing committee for their contributions to a very smooth and eventful year. To be noted are Robbin Anderson, Leo H. Bowman and Neal D. Buffaloe (Science and Mathematics Education), Joe M. Guenter, Jewel Moore and E. E. Dale (Nominating Committee), John Peck and Leo J. Paulissen (Arkansas Science Talent Search), Gary Heidt (Undergraduate Awards), Michael Rapp and Robert T. Kirkwood (Science Fair), Art Fry, J. H. Fribourgh and M. L. Lawson (Audit Committee), and Leo Paulissen, George Harp, Gary Heidt, Henry Robison, Arthur Johnson, and Gary Tucker (Arkansas Biota Survey Committee), Leo Paulissen (Endowment Committee), and George Templeton and Joe Guenter, (Meeting Site Committee).
>
> The Academy appreciates the efforts of the section chairman and recognizes that they play an important role in conducting the meeting. To be noted are Ken Beadles (Aquatic and Environmental), Gary Tucker (Botany), Neil

Secretary's Report

Allison (Chemistry I), Robert Steinmeier (Chemistry II), Victor Vere (Geology), Peggy Dorris (Invertebrate Zoology), A. L. Barron (Microbiology and Immunology), John A. Sealander (Vertebrate Zoology), James L. Wickliff (Botany II), John Sorenson (Parasitology and Biomedicine), Neal Buffaloe (Science Education) and Gary Heidt (Vertebrate Zoology II). The members of the Academy rely heavily upon the officers to implement the programs. We extend our thanks to Paul C. Sharrah, President; William L. Evans, President-Elect; David Chittenden, Secretary; Gary Heidt, Vice President; Arthur Johnson, Treasurer; V. Rick McDaniel, *Proceedings* Editor; John Rickett, Newsletter Editor; and to Henry Robison as Historian.

We also express our gratitude to the directors of the various science activities which are supported by the Arkansas Academy of Science: Robert Wright, Pat Howerton, Mike Rapp, and Robert Kirkwood, Co-directors of the Arkansas State Science Fair; Tom Palko, Director of the Junior Science and Humanities Symposium; and to Wayne Everett, Liaison Officer for all supported activities.

Special thanks are extended to Derek Sears for presenting the public lecture "Meteroites of Arkansas".

We also express our thanks to Sargent-Welch and Advanced Scientific Inc. for exhibiting some of their products at this sixty-eighth meeting of the Arkansas Academy of Science.

The motion was seconded and passed.

William Evants presented a plaque to the Secretary on his retirement from office.

President Sharrah turned the gavel over to President-Elect Evans. President Evans adjourned the Second Business Meeting.

Respectfully submitted,

David M. Chittenden
Secretary

REGULAR MEMBERS

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Michael W. Rapp	University of Central Arkansas	Duane C. Wolf	University of Arkansas
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PROGRAM
Arkansas Academy of Science

Sixty-eighth Annual Meeting

UNIVERSITY OF ARKANSAS

Fayetteville, Arkansas

Meeting concurrently with sessions of:

The Collegiate Academy of Science

Friday, 6 April

SENIOR AND COLLEGIATE ACADEMIES -- Registration

SENIOR ACADEMY -- Executive Board Meeting

SENIOR ACADEMY -- First General Business Meeting

Lunch

SENIOR AND COLLEGIATE ACADEMIES -- Registration

SENIOR AND COLLEGIATE ACADEMIES -- Paper [Concurrent Sessions]:

Aquatic & Environmental I
Botany
Chemistry I
Chemistry II
Geology
Invertebrate Zoology
Microbiology & Immunology
Vertebrate Zoology

SENIOR AND COLLEGIATE ACADEMIES -- Banquet

POST BANQUET SPEAKER -- Mr. Randall Sabine, EPA, Dallas

Saturday, 7 April

SENIOR AND COLLEGIATE ACADEMIES -- Registration

SENIOR AND COLLEGIATE ACADEMIES -- Papers [Concurrent Sessions]:

Aquatic & Environmental II
Botany II
Chemistry III
Parasitology & Biomedicine
Science Education
Vertebrate Zoology II

ARKANSAS SCIENCE TALENT SEARCH

SENIOR ACADEMY -- Second General Business Meeting

SECTION PROGRAMS

[Papers marked with * are presentations by Collegiate Academy members]

AQUATIC AND ENVIRONMENTAL I

Session Chairperson: John K. Beadles

SELECT BIOLOGICAL ASPECTS OF THE YELLOWCHEEK DARTER, *Etheostoma moorei* RANEY AND SUTTKUS.
Roland E. McDaniel and George L. Harp, Arkansas State University, AR 72467 and Henry W. Robison, Southern Arkansas University, Magnolia, AR 71753.

TEMPERATURE PREFERENCE AND TOLERANCE OF GRASS CARP *(Ctenopharyngodon idella).*
Marvin L. Galloway & Raj V. Kilambi, Department of Zoology, University of Arkansas, Fayetteville, AR 72701.

GROWTH OF LARGEMOUTH BASS FROM HEATED FLINT CREEK RESERVOIR IN NORTHWEST ARKANSAS.
Marvin L. Galloway & Raj V. Kilambi, Department of Zoology, University of Arkansas, Fayetteville, AR 72701.

EFFECTS OF COMMERCIAL FISH REMOVAL ON SPORT FISH POPULATIONS IN TWO ARKANSAS RESERVOIRS.
Tommie Crawford, Arkansas Game and Fish Commission, No. 2 Natural Resources Drive, Little Rock, AR 72205.

FURTHER DISTRIBUTION RECORDS OF *Fundulus chrysotus* AND *Fundulus notti.*
Alan D. Price, Julia L. Reid, V. Rick McDaniel and C. Renn Tumlison, Department of Biological Sciences, Arkansas State University, State University, AR 72467.

REPRODUCTIVE SUCCESS OF CHANNEL CATFISH IN A FARM SITUATION IN MISSISSIPPI.
William W. Stephens, Sandling and Stephens' Fisheries, Rt. 1, Box 232, Silver City, MS 39166. Larry W. Dorman, Extension Assistant Fisheries Specialist, University of Arkansas Cooperative Extension Service, Drawer D., Lonoke, AR 72086.

DISTRIBUTION AND EFFICIENCY OF HYDROCARBON-OXIDIZING BACTERIA IN A FRESHWATER RESERVOIR.
Carol H. Smedley, Jimmy D. Bragg and Aubrey B. Gosnell, Henderson State University, Arkadelphia, AR 71923.

THE INFLUENCE OF DeGRAY RESERVOIR ON ZOOPLANKTON POPULATIONS IN THE CADDO AND OUACHITA RIVERS.
Ralph B. Roseberg, Dworshak National Fish Hatchery, Box 18, Ahsahka, Idaho 83544 and Mark Karnes, Ross Foundation, 1039 Henderson, Arkadelphia, AR 71923.

BOTANY

Session Chairperson: Gary Tucker

SIZE-DEPENDENT COMPETITIVE ABILITY IN PLANTS.
James B. Grace, Department of Botany and Microbiology, University of Arkansas, Fayetteville, AR 72701.

VEGETATION COMMUNITIES OF PEA RIDGE NATIONAL MILITARY PARK, BENTON COUNTY, ARKANSAS.
Edward E. Dale, Jr., Department of Botany and Microbiology, University of Arkansas, Fayetteville, AR 72701.

TALLGRASS PRAIRIES OF NORTHWESTERN ARKANSAS: QUANTITATIVE VEGETATIONAL AND SOIL DATA.
Nancy M. Eyster-Smith, Department of Botany and Microbiology, University of Arkansas, Fayetteville, AR 72701-1201.

COMPARISON OF GROUNDCOVER VEGETATION AMONG HABITATS OF THE UPPER BUFFALO RIVER.
Mark A. Paulissen, Department of Zoology, University of Oklahoma, Norman, OK 73019.

SOME STUDIES ON INTRODUCING *Castilleja coccinea,* INDIAN PAINTBRUSH, INTO PRAIRIE VEGETATION.
Robert D. Wright, Department of Biology, University of Central Arkansas, Conway, AR 72032.

Lomariopsis, THE FIFTH GENUS OF TROPICAL FERN KNOWN FROM NORTH AMERICA TO FORM INDEPENDENT, PERENNIAL GAMETOPHYTES.
James H. Peck and Carol J. Peck, Department of Biology, University of Arkansas at Little Rock, Little Rock, AR 72204 and State Plant Board, Little Rock, AR 72203.

FORESTRY IN THE REPUBLIC OF CHINA.
Timothy T. Ku, Department of Forest Resources, University of Arkansas at Monticello, Monticello, AR 71655.

PRELIMINARY STUDY ON THE POISONOUS PLANTS OF ARKANSAS.
Edward L. Richards, Department of Biological Sciences, Arkansas State University, State University, AR 72467.

CHEMISTRY I

Session Chairperson: Neil Allison

CARBON-14 ISOTOPE EFFECT STUDIES OF THE MECHANISM OF THE COPE ELIMINATION REACTION OF SUBSTITUTED 2-PHENYLETHYLDIAMETHYLAMINE OXIDES.
Daniel R. Wright, Leslie B. Sims and Arthur Fry, Department of Chemistry, University of Arkansas, Fayetteville, AR 72701.

THE SYNTHESIS OF STEROSPECIFICALLY LABELED (+) - B DEUTERODESOXYEPHYDRINE.
Richard B. Walker, Associate Professor, College of the Ozarks, 415 College Avenue, Clarksville, AR 72830.

THE EFFECT OF SOLVENT ACIDITY ON THE ISOMERIZATION OF ALPHA IONONE.
Deanna King, Paul Krause, Jerald Manion, University of Central Arkansas, Conway, AR 72032.

COMPARATIVE ISOMERIZATION RATES OF PSI AND ALPHA IONONE.
Randall Black, Paul Krause, Jerald Manion, University of Central Arkansas, Conway, AR 72032.

METALLACYCLOACYL-CARBENE COMPLEX.
Warunee Yongskulrote and Neil T. Allison, Chemistry Department, Chemistry Building, University of Arkansas, Fayetteville, AR 72701.

METALLAPHENANTHRENE.
R. Ferede, J. Hinton, N. Allison, Chemistry Department, University of Arkansas, Fayetteville, AR 72701.

SYNTHESIS AND CHARACTERIZATION OF A SILYL SUBSTITUTED IRON CARBENE.
Bruce E. Landrum and N. T. Allison, University of Arkansas, Fayetteville, AR 72701.

THE REDUCTION OF ALKYL HALIDES BY METAL HYDRIDE REAGENTS.
Ronald A. Shumate, c/o Dr. Richard Walker, College of the Ozarks, Clarksville, AR 72830.

STEREOCHEMISTRY AND HYDROGENATION OF 2-METHYL-1-METHYLENECYCLOHEXANE OVER A RHODIUM CATALYST.
W. Brian Harrod and Victoria F. Ku, Department of Natural Sciences, University of Arkansas at Monticello, Monticello, AR 71655.

DIECKMANN CONDENSATION OF A DOUBLY BOUND POLYMER-SUPPORTED ADIPATE ESTER.
Warren T. Ford, Department of Chemistry, Oklahoma State University, Stillwater, OK 74078; Paul M. Nave and Michael Wells, Department of Chemistry, Arkansas State University, State University, AR 72467.

IRRADIATION OF DIENES IN THE PRESENCE OF AMINES.
William M. Bednar and Norbert J. Pienta, Department of Chemistry, University of Arkansas, Fayetteville, AR 72701.

ALKYLATIONS USING OXYPHOSPHONIUM SALTS.
Slaton E. Fry and Norbert J. Pienta, Department of Chemistry, University of Arkansas, Fayetteville, AR 72701.

CHEMISTRY II
Session Chairperson: Robert Steinmeier

RADIATION LEVELS AROUND A NUCLEAR POWER PLANT AS MEASURED BY DIFFERENT METHODS.
Claude Epperson, Dale Swindle, Orville Cypret, Thomas Rolniak, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

BETA RADIATION ATTENUATION CHARACTERISTICS OF TYPICAL PROTECTIVE EYEWEAR USED AT A NUCLEAR POWER PLANT.
Orville Cypret, Arkansas Power & Light Company, PO Box 551, Little Rock, AR 72203 and C. E. Epperson, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, AR 72205.

APPLICATION OF DIFFUSE REFLECTANCE SPECTROSCOPY (DRS) IN HETEROGENOUS CATALYSIS.
Lawrence Orgi and G. Blyholder, University of Arkansas, Fayetteville, AR 72701.

ELECTRON MICROSCOPE STUDY OF THE SOLID-SOLID REACTION BETWEEN POTASSIUM SULFATE AND GRAPHITE.
Jamie Dudley and J. E. Bennett, Department of Chemistry, Arkansas State University, State University, AR 72467.

MASS SPECTROMETRIC STUDIES OF HIGH TEMPERATURE VAPORS FROM THE SOLID-SOLID REACTION BETWEEN SODIUM SULFATE AND GRAPHITE.
Ali Al Ghamdi and J. E. Bennett, Department of Chemistry, Arkansas State University, State University, AR 72467.

FRAGMENTATION PATTERNS IN ENERGETIC COLLISIONS OF ARGON MICROCLUSTERS.
Ralph J. Wolf, Department of Chemistry, University of Arkansas at Little Rock, Little Rock, AR 72204.

ROTATIONAL STATE RESOLVED STICKING AND DESORPTION PROBABILITIES IN DIATOM - SURFACE COLLISION PROCESS.
Ricardo C. Davis and Ralph J. Wolf, Department of Chemistry, University of Arkansas at Little Rock, Little Rock, AR 72204.

GEOLOGY
Session Chairperson: Victor Vere

DEVELOPMENT OF ALGAL-BRYOZOAN-FORAMINIFERA CARBONATE MOUNDS BLOYD FORMATION (MORROWAN-PENNSYLVANIAN), MADISON COUNTY, ARKANSAS.
Jeffrey R. Marsh, Department of Geology, University of Arkansas, Fayetteville, AR 72701.

MORROWAN (LOWER PENNSYLVANIAN) LITHOSTRATIGRAPHIC PROBLEMS, NORTH-CENTRAL ARKANSAS.
Wilson Hawkins, Arkansas Department of Pollution and Ecology Control, Little Rock, AR 72701 and Daniel Murdaugh, Tenneco Oil Company, Lafayette, LA 70501.

AMMONOID BIOSTRATIGRAPHY OF THE GENE AUTRY SHALE (MORROWAN-PENNSYLVANIAN), SOUTH-CENTRAL OKLAHOMA.
Marilyn S. Miller, Department of Geology, University of Arkansas, Fayetteville, AR 72701.

UPPERMOST MORROWAN (PENNSYLVANIAN) CONODONT BIOSTRATIGRAPHY, NORTHWESTERN ARKANSAS AND NORTHEASTERN OKLAHOMA.
Michael G. Gray and Walter L. Manger, Department of Geology, University of Arkansas, Fayetteville, AR 72701.

HYDROGEOCHEMICAL EXPLORATION FOR MISSISSIPPI VALLEY TYPE DEPOSITS, ARKANSAS.
Kenneth F. Steele, Department of Geology, University of Arkansas, Fayetteville, AR 72701 and T. F. Dilday III, Exxon Company, Houston, TX 77001.

FLUID SENSOR TECHNOLOGY - DISCOVERY.
Sedley Joseph Greer, Jr., Geologist, 2500 McCain Street, Suite 121, North Little Rock, AR 72116.

STRATIGRAPHY OF THE MIDDLE ATOKA, SOUTHERN ARKOMA BASIN, ARKANSAS.
Pamela K. Williams, Geology Department, University of Arkansas, Fayetteville, AR 72701.

IMPLICATIONS OF HYDROCARBON AND HELIUM GAS ANALYSES OF SPRINGS FROM THE OUACHITA MOUNTAINS, ARKANSAS.
Tandel T. Cox, Kenneth F. Steele, Department of Geology, University of Arkansas, Fayetteville, AR 72701.

RESISTIVITY PROFILING OF OUACHITA RIVER SAND DEPOSITS IN THE MORO BAY, ARKANSAS AREA.
Owen Carpenter and Robert A. Wells, Department of Natural Sciences, University of Arkansas at Monticello, Monticello, AR 71655.

INVERTEBRATE ZOOLOGY
Session Chairperson: Peggy Dorris

INSECT POPULATIONS ASSOCIATED WITH SUNFLOWERS IN NORTHEASTERN ARKANSAS WITH NOTES ON DAMAGE BY *Mecas inornata* (COLEOPTERA:CERAMBYCIDAE).
Julia L. Reid and H. E. Barton, Department of Biological Sciences, Arkansas State University, State University, AR 72467.

LABORATORY REARING OF *Chelysomidea guttata* (HEMIPTERA: SCUTELLERIDAE), WITH DESCRIPTIONS OF IMMATURE STAGES.
Julia L. Reid and H. E. Barton, Department of Biological Sciences, Arkansas State University, State University, AR 72467.

ANATOMY OF THE MALE AND FEMALE REPRODUCTIVE SYSTEMS OF ADULT *Menecles insertus* (SAY) (HEMIPTERA: PENTATOMIDAE).
Linda A. Lee and Harvey E. Barton, Arkansas State University, State University, AR 72467.

COLLECTING, IDENTIFYING AND PRESERVING SPIDERS.
John S. Heiss, Department of Entomology, University of Arkansas, Fayetteville, AR 72701.

A SIMPLIFIED SILVER-STRAIN TECHNIQUE FOR GRASSHOPPER CHROMOSOMES IN FIXED TISSUES.
Jeff Angel and W. L. Evans, Department of Zoology, University of Arkansas, Fayetteville, AR 72701.

INFLUENCE OF THE PAYMENT-IN-KIND (P.I.K.) PROGRAM ON *Psorophora columbiae* AND *Anopheles quadrimaculatus* MOSQUITO POPULATIONS IN A NORTHEAST ARKANSAS RICEFIELD COMMUNITY.
Larry A. Olson and Julia L. Reid, Department of Biological Sciences, Arkansas State University, State University, AR 72467.

MICROBIOLOGY AND IMMUNOLOGY
Session Chairperson: A. L. Barron

THE UTILIZATION OF *Pachysolen tannophilus* IN SIMULATED SIMULTANEOUS SACCHARIFICATION AND FERMENTATION PROCESS.
Hunsa Punnapayak and George H. Emert, Biomass Research Center, University of Arkansas, Fayetteville, AR 72701.

EVALUATION OF SUGAR CANE BAGASSE AND RICE STRAW AS PROCESS SUBSTRATES FOR THE PRODUCTION OF ETHYL ALCOHOL.
Douglas B. Rivers, Gisella Zanin, George H. Emert, Biomass Research Center, University of Arkansas, Fayetteville, AR 72701.

METABOLISM OF NITROPOLYCYCLIC AROMATIC HYDROCARBONS BY HUMAN INTESTINAL MICROFLORA.
Carl E. Cerniglia, National Center for Toxicological Research FDA, Jefferson, AR 72079.

GROUP B STREPTOCOCCAL LIPOTEICHOIC ACID ENHANCES INFECTION AND ALTERS PULMONARY SURFACTANT IN THE MOUSE.
D. E. Wennerstrom, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, AR 72205.

INFLUENZA TYPE A VIRUS-MEDIATED ADHERENCE OF TYPE Ia GROUP B STREPTOCOCCI TO MOUSE TRACHEAL TISSUE *In Vivo*.
Jay H. Menna and William T. Jones, Department of Microbiology & Immunology, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, AR 72205.

CHLAMYDIAL PNEUMONITIS INDUCED IN NEWBORN GUINEA PIGS.
Roger G. Rank, Aubrey J. Hough, Jr., Richard F. Jacobs, Cynthia Cohen, Almen L. Barron, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, AR 72205.

THE BINDING OF BACTERIA TO AVIAN LYMPHOCYTES; RESULTS OF PRELIMINARY INVESTIGATIONS.
G. T. Pharr, L. J. Paulissen, Department of Microbiology, University of Arkansas, Fayetteville, AR 72701 and J. D. Story, Campbell Soup Company, 1100 West 15th, Fayetteville, AR 72701.

EXTRACELLULAR CYCLIC AMP PHOSPHODIESTERASE FROM THE GROWTH MEDIUM OF THE MYXOMYCETE *Physarum flavicomum*.
Thomas J. Lynch and Mary E. Farrell, Biology Department, University of Arkansas at Little Rock, 33rd & University, Little Rock, AR 72204.

MODE OF SLUG MOTILITY IN *Dictyostelium discoideum*.
F. W. Spiegel, Assistant Professor, Botany & Microbiology, University of Arkansas, Fayetteville, AR 72701.

Streptococcus mutans AND PENICILLIN.
Timothy A. Kral and James W. Norys, University of Arkansas, Fayetteville, AR 72701.

INDUCED MUTATION OF *Colletotrichum gloeosporioides* F. SP. *aeschynomene* FOR TOLERANCE TO BENZIMIDAZOLE FUNGICIDES.
D. O. TeBeest, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

EFFECTS OF SIDE-CHAIN OXIDATION IN THE FUNGAL METABOLISM OF LIGNIN.
Patrick Fenn, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

RABBIT FCγ RECEPTOR - BEARING BONE MARROW CELLS SUPPRESS THE RELEASE OF A BONE MARROW GROWTH FACTOR.
Lee, S. F. Soderberg, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, AR 72205.

VERTEBRATE ZOOLOGY
Session Chairperson: John A. Sealander

ALTITUDINAL VARIATION IN THE MEXICAN VOLE *(Microtus mexicanus)*.
Meredith J. Hamilton and Gary A. Heidt, Department of Biology, Memphis State University, Memphis, Tennessee and Department of Biology, University of Arkansas at Little Rock, Little Rock, AR.

DISEASE SURVEY OF WHITE-TAILED DEER IN ARKANSAS.
Hal Harger, Chuck Harger and Gary A. Heidt, Department of Biology, University of Arkansas at Little Rock, Little Rock, AR 72204; Melody Parsley and Mike Parsley, Arkansas Livestock and Poultry Commission, Little Rock, AR 72205.

DISTRIBUTION, STATUS AND MANAGEMENT OF FOX IN ARKANSAS AS DETERMINED BY FUR HARVEST REPORTS (1941-1983) AND MAIL SURVEY OF TRAPPERS.
Gary A. Heidt and James H. Peck and Lew Johnston, Department of Biology, University of Arkansas at Little Rock, Little Rock, AR 72204.

POSTNATAL OSTEOLOGY OF THE NORTHERN GRASSHOPPER MOUSE, *Onychomys leucogaster*.
Joe W. Bailey and Gary A. Heidt, Department of Biology, University of Arkansas at Little Rock, Little Rock, AR 72204. Present address for Joe W. Bailey, Fort Roots, V.A. Medical Center, North Little Rock, AR.

THE RIVER OTTER IN ARKANSAS. III. SEASONAL FOOD HABITS IN A SOUTHWESTERN ARKANSAS BEAVER SWAMP.
Renn Tumlison and Mark Karnes, Department of Zoology, Oklahoma State University, Stillwater, OK 74078 and the Ross Foundation, P.O. Box 335, Arkadelphia, AR 71923.

CHARACTERISTICS OF OTTER LATRINES ALONG BEAVER INHABITED WATERCOURSES IN SOUTHWEST ARKANSAS.
Mark R. Karnes, The Ross Foundation, P.O. Box 335, Arkadelphia, AR 71923 and Renn Tumlison, Department of Zoology, Oklahoma State University, Stillwater, OK 74078.

HOME RANGE AND DENSITY OF THE BOBCAT, *Felix rufus*, IN ARKANSAS.
Richard A. Rucker, Memphis State University, Route #9, Box 17B, Oden, AR 71961.

PROTECTION OF ENDANGERED GRAY BAT *(Myotis grisescens)* COLONIES IN ARKANSAS.
Michael J. Harvey, Ecological Research Center, Department of Biology, Memphis State University, Memphis, TN 38152.

A SUMMARY ACCOUNT OF THE CAROLINA PARAKEET IN ARKANSAS.
Daniel McKinley, Department of Biological Sciences, State University of New York, Albany, NY 12222 and Douglas James, Department of Zoology, University of Arkansas, Fayetteville, AR 72701.

FIRST RECORDS OF THE POCKET GOPHER *(Geomys bursarius missouriensis)* FROM ARKANSAS.
V. R. McDaniel, J. D. Wilhide, C. R. Tumlison and J. D. Copenhaver, Department of Biological Sciences, Arkansas State University, State University of AR 72467.

AQUATIC AND ENVIRONMENTAL II
Session Chairperson: John K. Beadles

THE CONCENTRATION OF SELECTED IONS IN RAINWATER.
D. M. Chittenden II and J. L. Chittenden, Department of Chemistry, Arkansas State University, State University, AR 72467.

ABSORPTION OF TRACE METALS ON MANGANESE OXIDE COATED GRAVELS.
J. Nix, Department of Chemistry, Ouachita Baptist University, Arkadelphia, AR 71923.

WATER QUALITY CHARACTERISTICS OF AN AGRICULTURAL WETLAND IN EAST CENTRAL ARKANSAS.
Carl R. Stapleton, Biology Department, University of Arkansas at Little Rock, Little Rock, AR 72204.

WATER QUALITY OF GILLHAM LAKE AND ASSOCIATED TAILWATER DURING DRY AND WET PERIODS.
Stephen B. Smith and Thomas E. Moen, U.S. Fish and Wildlife Service, Multi-Outlet Reservoir Studies, Arkadelphia, AR 71923.

FACTORS LIMITING THE REVEGETATION OF BAUXITE MINESOILS IN ARKANSAS.
Jarvis Harper, Aluminum Company of America, P.O. Box 300, Bauxite, AR 72011 and A. E. Spooner, Plant Science Department, University of Arkansas, Fayetteville, AR 72701.

FLY ASH AS A FERTILIZER AND LIME SOURCE IN ARKANSAS.
Stanley L. Chapman, Cooperative Extension Service, University of Arkansas at Little Rock, Little Rock, AR 72203.

BOTANY II
Session Chairperson: James L. Wickliff

ORIGINS OF CHLOROPLASTS IN *Euglena gracilis:* A PRELIMINARY STUDY OF CHLOROPLAST SOURCES AND RESEARCH METHODS.
Clell J. Ford, Hendrix College, P.O. Box H-330, Hendrix, College, Conway, AR 72032.

MICROSCOPY OF HEAT INDUCED MORPHOLOGICAL CHANGES IN ISOLATED CHLOROPLASTS.
Roy Z. Gehring, Arkansas State University, State University, AR 72467.

EFFECT OF TENTOXIN ON THYLAKOID PROTEINS OF DEVELOPING MUNG BEAN PRIMARY LEAVES.
Judy L.-H. Jwo and J. L. Wickliff, Department of Botany & Microbiology, University of Arkansas, Fayetteville, AR 72701.

EFFECT OF LIGHT ON MICROTUBULES OF MESOCOTYL PARENCHYMA CELLS IN DEVELOPING MAIZE SEEDLINGS.
D. A. deSarcos and J. L. Wickliff, Department of Botany and Microbiology, University of Arkansas, Fayetteville, AR 72701.

GAS CHROMATOGRAPHIC ANALYSES OF POTENTIAL BIOCRUDE-PRODUCING TREES.
Roy Z. Gehring and Bob D. Johnson, Arkansas State University, P.O. Box 92, State University, AR 72467.

CHEMISTRY III
Session Chairperson: Robert Steinmeier

REAL TIME DATA AQUISITION FOR GAS ELECTRON DIFFRACTION PATERNS.
David W. Paul, John D. Ewbank, Joel Benston and Lothar Schafer, University of Arkansas, Department of Chemistry, Fayetteville, AR 72701.

MICROBALANCE ELECTROCHEMISTRY, A NEW METHOD FOR STUDYING ELECTROSORPTION AT SOLID ELECTRODES.
Linda Holm Grande and David W. Paul, University of Arkansas, Department of Chemistry, Fayetteville, AR 72701.

EFFECTS OF SPECIFIC ADSORPTION ON ELECTROCHEMICAL REACTIONS.
Doni G. Grande and David W. Paul, University of Arkansas, Department of Chemistry, Fayetteville, AR 72701.

GLC DETERMINATION OF STREPTOMYCIN IN INJECTABLE PREPARATION.
Joaquin Jessup and A. M. Hoyt, Jr., University of Central Arkansas, Conway, AR 72032.

CONSTRUCTION OF A MICROCOMPUTER CONROLLED pH TITRATOR.
Edmond W. Wilson, Jr. and James E. Mackey, Department of Physical Science, Harding University, Searcy, AR 72143.

SOFTWARE DESIGN FOR A MICROCOMPUTER CONTROLLED TITRATION APPARATUS.
Dennis K. Thompson and Edmond W. Wilson, Jr., Department of Physical Science, Harding University, Searcy, AR 72143.

THE MICROCOMPUTER AS A LABORATORY INSTRUMENT: A MICROCOMPUTER POLAROGRAPH.
Kenneth E. England and Edmond W. Wilson, Jr., Department of Physical Science, Harding University, Searcy, AR 72143.

DIFFERENCE IN ASSOCIATION OF THALLOUS ION WITH GRAMICIDIN A AND GRAMICIDIN B IN LYSOPHOSPHATIDYL CHOLINE MICELLES DETERMINED BY TL-205 NMR.
William L. Whaley, Dikoma Shungu, James F. Hinton, Roger E. Koeppe II, and Francis S. Millett, Department of Chemistry, University of Arkansas, Fayetteville, AR 72701.

THE HEMORRHAGIC COMPONENT FROM THE VENOM OF THE SOUTHERN COPPERHEAD.
Randal K. Tucker and Colis R. Geren, Department of Chemistry, University of Arkansas, Fayetteville, AR 72701.

LOXOSCELES SPIDER VENOMS.
Mary Rekow, Elizabeth Wilder and Collis R. Geren, Department of Chemistry, University of Arkansas, Fayetteville, AR 72701.

A HEMORRHAGIC COMPONENT OF EASTERN COTTON-MOUTH VENOM.
H. Raymond Allen, Patrick Dopp and Collis R. Geren, Department of Chemistry, University of Arkansas, Fayetteville, AR 72701.

PARASITOLOGY AND BIOMEDICINE
Session Chairperson: John Sorenson

HELMINTHS OF ARKANSAS *Quiscalus quiscula*.
Arthur A. Johnson, Professor of Biology, Hendrix College, Conway, AR 72032.

SURVIVAL OF *Trypanosoma cruzi* IN DEAD CHRONICALLY INFECTED MICE.
Lawrence W. Hinck and James L. Stevens, Department of Biological Sciences, Arkansas State University, State University, AR 72467 and Roger Dale Meurer, Merck Institute for Therapeutic Research, Rahway, New Jersey.

THE LIFE CYCLE OF *Aganthatrium pipistrelli* MACY 1941 (TREMATODA:LECITHODENDRIIDAE) FROM NEWTON AND JOHNSON COUNTY IN NORTHWEST ARKANSAS.
John F. Bridgman and Mark Hardgrave, Department of Biology, College of the Ozarks, Clarksville, AR 72830.

A COMPARISON OF HEART RATE RESPONSES DURING SINGLES AND DOUBLES COMPETITION IN RACQUETBALL.
Leland F. Morgans, Department of Biology, University of Arknasas at Little Rock, Little Rock, AR 72204; James A. Scovil, Kay M. Bass, Department of Medicine, Non-Invasive Cardiology Laboratory, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

IN SEARCH OF THE TRANSCRIPTION TERMINATION REGION OF THE GALACTOSE OPERON FROM *Escherichia coli*.
Joe S. Jeffers, Department of Chemistry, Ouachita Baptist University, Arkadelphia, AR 71923.

ULTRAVIOLET LIGHT REACTIVATION OF GAMMA-RAY INDUCED CHROMOSOME ABERRATIONS IN G1 PHASE *Xenopus* CELLS.
Susan Kulp, Linda Rogers and Gaston Griggs, Department of Biology, John Brown University, Siloam Springs, AR 72761.

NITROSAMINE FORMATION VIA NITRITE ESTERS.
Robert W. Allen, Assistant Professor of Chemistry, Arkansas Tech University, Russellville, AR 72801.

THE EFFECT OF *Edwardsiella ictaluri* INFECTION ON PLASMA CORTICOSTERONE LEVELS IN CHANNEL CATFISH *(Ictalurus punctatus)*.
J. L. Cooper, S. N. David and J. K. Beadles, Department of Biological Sciences, Arkansas State University, State University, AR 72467.

FACTORS CONTROLLING THE METHEMOGLOBIN REDUCTION IN THE RED CELL.
A. Mansouri, VA Medical Center and University of Arkansas for Medical Sciences, 111G, 300 E. Roosevelt, Little Rock, AR 72206.

SCIENCE EDUCATION
Session Chairperson: Neal Buffaloe

TEACHING AND TESTING IN LARGE SECTIONS OF GENERAL CHEMISTRY.
A. W. Cordes, Department of Chemistry, University of Arkansas, Fayetteville, AR 72701.

TOWARD A CONCISE DEFINITION AND PERSPECTIVE FOR BIOLOGY.
Arthur V. Brown and Kristine Basinger Brown, Department of Zoology, University of Arkansas, Fayetteville, AR 72701 and West Fork High School, West Fork, AR 72774.

JUNIOR HIGH SCIENCE AND HUMANITIES SYMPOSIUM - AN ENRICHMENT PROGRAM FOR THE GIFTED HIGH SCHOOL SCIENCE STUDENT.
Tom Palko, Arkansas Tech University, Russellville, AR 72801.

ARKANSAS BIOLOGICAL CURRICULUM DEVELOPMENT CONFERENCE - 1983.
Arthur A. Johnson, Professor of Biology, Hendrix College, Conway, AR 72032.

PHYSICS TEACHERS VISIT RUSSIA AND CHINA.
Paul C. Sharrah, University of Arkansas, Department of Physics, Fayetteville, AR 72701.

THE USE OF MICROCOMPUTERS AS LABORATORY INSTRUMENTS.
James A. Wisman, Department of Chemistry, University of Arkansas, Fayetteville, AR 72701.

SCIENCE OUT OF CONTEX: PRESCRIPTION FOR DISASTER.
Art Hobson, Department of Physics, University of Arkansas, Fayetteville, AR 72701.

THE WATER CRISIS - AN APPROACH FOR TEACHERS IN GRADES 7-12.
George L. Harp, Department of Biological Sciences, Arkansas State University, State University, AR 72467.

MICHAJLO VASILIEVIC LOMONOSOV WHO?
Billie G. Broach, Department of Chemistry, University of Arkansas at Little Rock, 33rd and University, Little Rock, AR 72204.

VERTEBRATE ZOOLOGY II
Session Chairperson: Gary Heidt

AGE STRUCTURE AND REPRODUCTIVE RECRUITMENT OF ARKANSAS RACCOONS *(Procyon lotor)* AS DETERMINED FROM HARVEST DATA OF 1981-83.
J. D. Copenhaver, J. D. Wilhide, V. R. McDaniel, J. S. Hudspeth and C. R. Tumlison, Department of Biological Sciences, Arkansas State University, State University, AR 72467.

A REGIONAL ANALYSIS OF FOOD CONSUMED BY RACCOONS *(Procyon lotor)* HARVESTED DURING THE 1981-83 ARKANSAS FUR-TRAPPING SEASONS.
J. D. Wilhide, J. S. Hudspeth, V. R. McDaniel and C. R. Tumlison, Department of Biological Sciences, Arkansas State University, State University, AR 72467.

FIRST RECORDS OF THE EASTERN HARVEST MOUSE, *Reithrodontomys humulis*, AND THE BLACK RAT, *Rattus rattus*, FROM SOUTHERN ARKANSAS.
D. R. England, Department of Biological Science, Southern Arkansas University, Magnolia, AR 71753; V. R. McDaniel, J. D. Copenhaver and C. R. Tumlison, Department of Biological Sciences, Arkansas State University, State University, AR 72467.

A COMPARATIVE STUDY OF THE DIVING PHYSIOLOGY OF REPTILES.

Robert Rountree, Dennis Baeyens and Alvan Karlin, Biology Department, University of Arkansas at Little Rock, 33rd & University, Little Rock, AR 72204.

MINE UTILIZATION BY HERPTILES IN THE OUACHITA MOUNTAINS.

Darrell Heath, Gary A. Heidt, Department of Biology, University of Arkansas at Little Rock, Little Rock, AR 72204 and David Saugey, U.S. Forest Service, Hot Springs, AR 71901.

ADDITIONAL ECO-REPRODUCTIVE OBSERVATIONS ON A POPULATION OF SLIMY SALAMANDERS, *Plethodon glutinosus glutinosus* (GREEN) FROM THE OUACHITA MOUNTAINS OF ARKANSAS.

David A. Saugey, U.S. Forest Service, Hot Springs, AR 71901.

POSTNATAL OSTEOLOGY OF THE NORTHERN GRASSHOPPER MOUSE, *ONYCHOMYS LEUCOGASTER*

JOE W. BAILEY* and GARY A. HEIDT
Department of Biology
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Little Rock, AR 72204

ABSTRACT

Two hundred forty-two specimens of *Onychomys leucogaster*, ranging in age from day of birth to the twenty-eighth day were cleared and stained using both Alizarin KOH and Alcian Blue/Alizarin-Trypsin staining methods. Thirty centers of ossification were studied. The data demonstrate the following: 1) skeletal centers of the appendicular skeleton ossify and mature earliest; 2) a new sesamoid bone lateral to the distal condyles of the femur was discovered; 3) the skeletal ossification of the baculum is present at one day of age; 4) a high degree of individual variation precludes aging of this species by skeletal maturation.

INTRODUCTION

In mammals, much of the completion of skeletal ossification occurs postnatally (Romer and Parsons, 1978); however, few studies have examined this process in detail (Nesslinger, 1956; Kirkland, 1970, 1973). Techniques have been available to clear and then stain the skeletal elements of a neonatal specimen with Alizarin Red (Nesslinger, 1956; Humason, 1979). Recently, use of Alcian Blue to counterstain cartilage has been demonstrated (Dingerkus and Uhler, 1977). Using these techniques, detailed analysis of skeletal ossification can be conducted.

This study was undertaken to determine the patterns and rates of postnatal ossification in the northern grasshopper mouse, *Onychomys leucogaster*. From these data, a better understanding of skeletal development in association with the appearance of specific locomotor activities can be elucidated. In addition, an attempt to use postnatal skeletal development as an early aging technique in this species was examined.

METHODS AND MATERIALS

Specimens used in this study were obtained from a colony of northern grasshopper mice maintained in the Basic Animal Services Unit of the University of Arkansas at Little Rock. Original members of the colony were captured in Greer and Harmon counties, Oklahoma. Mice were maintained in the laboratory on Purina Formulab #5008 and provided water *ad libitum*. Preserved specimens from the study are deposited in the UALR Vertebrate Collections.

Groups of mice (consisting of 11 individuals) were taken from day of birth to day 14 and then every other day until day 28. Of these 242 specimens, 220 were sacrificed, cleared and stained by the Alizarin-KOH method (Nesslinger, 1956; Humason, 1979). In order to examine ossification in conjunction with existing cartilage, the remaining 22 specimens were sacrificed, cleared and stained by the Alcian/Alizarin-Trypsin method (Dingerkus and Uhler, 1977). All specimens were subsequently stored in glycerol.

The specimens were examined using a standard American Optical binocular dissecting microscope with a zoom lens. Observations were done qualitatively, noting the presence, absence and degree of ossification.

RESULTS AND DISCUSSION

A total of 30 centers of ossification were examined in detail. These particular centers represented components of both the axial and appendicular skeletons and were chosen for study because they represented critical areas with the least amount of ossification at birth. The results of the study are summarized in Table 1 and Figures 1-5. Based upon the results presented in these figures and table, the 30 centers of ossification are examined in the following discussion.

Axial Skeleton:

Occipital Region

The occipital region forms the posterior surface of the skull. It fuses anteriorly with the parietal bones and laterally with the temporal region. Growth and ossification of this region was noted for dorsal fusion with the interparietals and laterally with the temporal region. Fusion with the interparietal occurred first. This fusion consisted of finger-like projections that bridged the gap between the two bones and was completed by day 8. Lateral fusion took longer as the occipital region grew to meet the temporal region. Fusion in this area was completed by day 12. Fusion in both areas appeared to be caused by the growth of the occipital as neither the interparietal nor the temporal region seemed to extend toward the occipital.

Vertebral Column

The vertebral column plays an important role in mammals, being essential for protection of the spinal cord and nerves and giving support to the body. At birth, the vertebrae are composed of one ventral and two lateral plates. The lateral plates grow dorsally and fuse to form the neural spine. The ventral plate grows dorso-laterally to meet and fuse with the two lateral plates. The vertebral column is divided into five regions: cervical, thoracic, lumbar, sacral and caudal. Each of these five regions was studied independently.

Cervical — As in most animals, there are seven cervical vertebrae. At birth, the vertebrae are complete cartilagenous rings where calcium is eventually deposited. The order of fusion and ossification in this region is: vertebra 4, 7, 3, 5, 6, 2, 1. Complete ossification of some vertebrae is first seen on day 10, but the region is not completed until day 18.

Thoracic — Ossification of the 13 thoracic centra is completed by day 14, however, only one of the neural spines (on the second vertebra), characteristic of this region, was formed by day 28. The order of final ossification in this region is: 5, 4, 3, 6, 7, 8, 9, 10, 2, 1, 11, 12, 13.

Lumbar — The six lumbar vertebrae show signs of ossification on day 8 and are completely ossified by day 18. These vertebrae ossify in descending order.

Sacral — There are four sacral vertebrae in the grasshopper mouse. While eventually fusing completely to form the dorsal surface of the pelvic girdle, fusion had only occurred in the first two sacral vertebrae by day 28. Fusion of sacral vertebrae is also in descending order.

Caudal — The number of caudal vertebrae ranges from 6-15 averaging 14. Only the first three have neural crests and these were ossified by day 8 in descending order.

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Table 1. Postnatal ossification of the northern grasshopper mouse, *Onychomys leucogaster*. Numbers indicate the number of specimens in each age group which demonstrate ossification.

Center of Ossification	Age in Days																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	18	20	22	24	26	28
Axial Skeleton																						
Occipital Region	-	-	-	-	3	6	8	9	10	10	10											
Cervical Vertebrae	-	-	-	-	-	-	-	-	2	5	3	5	8	8	9	9	10	10	10			
Thoracic Vertebrae	-	-	-	-	-	-	1	-	6	9	4	9	8	8	10	10	10					
Lumbar Vertebrae	-	-	-	-	-	-	-	-	5	7	5	7	8	8	10	10	10					
Sacral Vertebrae	-	-	-	-	4	7	10	10	10													
Caudal Vertebrae	-	-	-	-	1	8	10	10	10													
Sternum	-	-	-	-	-	-	-	-	2	1	-	9	10	10	10							
Appendicular Skeleton																						
Carpal #1	-	-	-	-	2	2	6	5	8	10	10	10										
Carpal #2	-	-	-	2	2	2	6	3	8	10	10	10										
Carpal #3	-	1	-	-	2	3	6	6	8	10	10	10										
Carpal #4-5	-	1	-	8	7	10	10	10														
Centrale	-	-	-	7	6	10	10	10														
Radius-Intermedium	-	-	-	3	2	7	6	7	9	10	10	10										
Ulnare	-	-	-	5	2	10	10	8	9	10	10	10										
Sesamoid	-	-	-	-	2	1	7	3	9	10	10	10										
Pisiform	-	-	-	1	2	5	8	7	9	10	10	10										
Olecranon	-	4	-	8	10	10	10															
Tarsal #1	-	-	-	6	4	6	-	-	7	10	10	10										
Tarsal #2	-	-	-	-	-	-	-	-	8	10	10	10										
Tarsal #3	-	-	-	-	1	1	-	3	8	10	10	10										
Tarsal #4-5	-	-	-	2	3	8	9	9	9	10	10	10										
Astragalus	-	6	8	10	10	10																
Calcaneus	7	10	9	10	10	10																
Tibiale	-	-	-	-	-	-	-	-	9	10	10	10										
Centrale	-	1	-	5	4	2	4	6	9	10	10	10										
Tibia-Fibula	-	-	-	3	5	10	10	10														
Patella	-	-	-	-	-	-	-	-	1	3	-	-	4	3	1	4	10	10	10			
Distal Femur	-	-	-	-	-	-	2	1	7	10	9	9	10	10	10							
Pelvic Girdle	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	10	10	10	10
New Sesamoid Bone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	10	10		

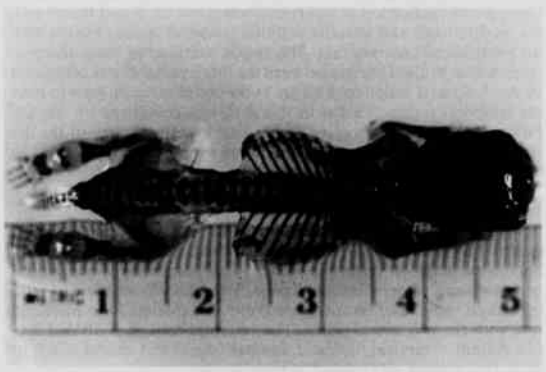

Figure 1. Dorsal view of 4 day old grasshopper mouse. Note degree of ossification of the vertebral column and pelvic region.

Figure 2. Lateral view of 9 day old grasshoper mouse. This age group represents the greatest single day completion of ossification centers (see Table 1).

Sternum

The sternum of the grasshopper mouse, like many other mammals, has six elements, all of which are present at birth. The elements, however, differ in size with the fifth sternal element being smallest. In several specimens, the fifth element at birth consisted of two or three small specks. Nesslinger (1956) found multiple specks replacing the fourth and fifth sternal elements in his work with the Virginia opossum. He reasoned that this area has multiple sites of ossification which subsequently fuse together to form the fifth sternal element as seen in the adult.

Appendicular Skeleton:

Wrist

The wrist of the grasshopper mouse is composed of nine bones: carpals 1-5 (four and five being fused), centrale, radius-intermedium, ulnare, pisiform, sesamoid. Complete ossification in this area is accomplished by day 10. The order of ossification is: centrale, carpal 4-5, ulnare, pisiform, radius-intermedium, sesamoid, carpal 3, carpal 1, carpal 2.

Olecranon Process

The olecranon is proximal to the semilunar notch of the ulna. While absent at birth, the olecranon is seen as early as day 1 and is fully ossified in all specimens by day 4. This process was expected to ossify early because of its important for muscle attachment.

Ankle

The ankle is composed of eight bones: tarsals 1-5 (four and five

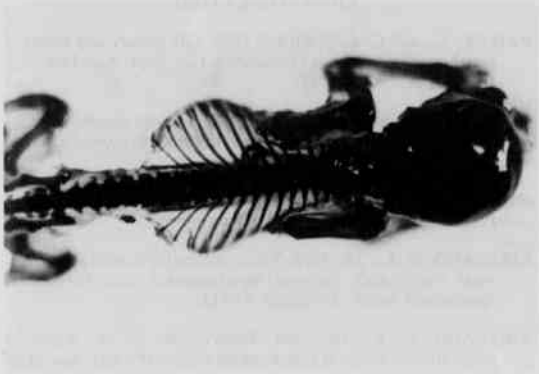

Figure 3. Dorsal view of 14 day old grasshopper mouse. Note ossification of vertebral column and pelvic region. By this age, 77% of the ossification centers studied were completed.

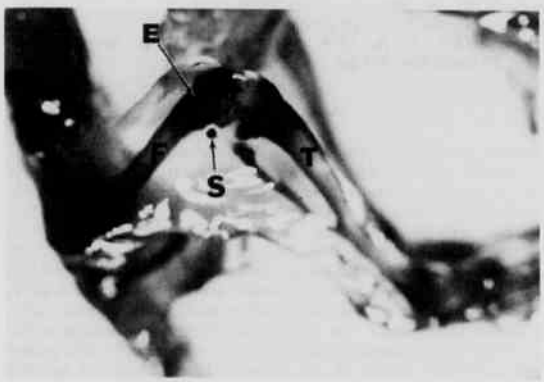

Figure 4. Hind limb of 28 day old grasshopper mouse showing new sesamoid bone (S). The femur (F) and tibia (T) are labelled for reference. Note the distinct epiphyseal plate (E) of the distal femur.

being fused), astragalus, calcaneus, tibiale, and centrale. The calcaneus is the first bone to appear, being present at birth. All components of this area are ossified in the following order by day 10: calcaneus, astragalus, tarsals 4-5, centrale, tarsals 1-2-3, and the tibiale.

Tibia-Fibula

The tibia and fibula are closely associated bones in mammals. In most rodents, this close association leads to fusion along all or part of their lengths (Romer and Parsons, 1978). This partial fusion is also present in the grasshopper mouse. The fusion of the two bones begins on day 5 as net-like strands across the notch at the proximal crossing of the bones. The fusion proceeds distally until the distal end of the fibula is met, after which the two bones cannot be distinguished along the area of fusion. The proximal ossification of the tibia and fibula differ greatly with respect to their time of appearance. Ossification of the tibia appears on day 9, while ossification does not appear until day 14 on the fibula.

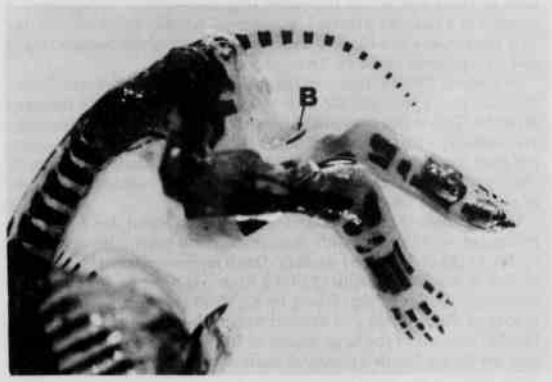

Figure 5. Posterior region of 6 day old grasshopper mouse showing the baculum (B).

Patella

The patella is a sesamoid bone that aids in the protection of the knee joint. At birth, the patella is observed as a sheet of cartilage covering the knee. Ossification is first seen as a small speck of bone on day 18 and is complete by day 26. As the patella is not as essential as other bones in the leg, the delay in its ossification is not unexpected.

Femur

The shaft of the femur in grasshopper mice, as in other mammals, is separated from the condyles by the epiphyseal cartilage. The separation of the distal condyles from the shaft makes it an ideal point for study, as growth and ossification can easily and accurately be observed. The condyles are formed in several ways (Kirkland, 1970). The mode of formation in the grasshopper mouse is via a bi-lobed or dumbbell shaped growth, first appearing as a tiny speck about day 8. The condyles subsequently grow proximally until they meet the epiphyseal cartilage at day 12.

Pelvic Girdle

At birth, the three bones of the pelvic girdle, ilium, ischium and pubis, are separate and distinct rod-like bones located on either side of the animal. Two points of fusion can be observed in this region. First, and most important, is the fusion of the three bones forming the acetabulum. The second point of fusion is that of the ischium and pubis, forming the obturator foramen. Completion of the pelvic girdle does not occur until day 22. However, Alcian stained specimens show that the region is complete at birth and that calcium is deposited later. The fact that the structure is complete, although not yet calcified, may be due to the importance of the pelvic girdle in support and locomotion. Fusion of the pelvic girdle and sacral vertebrae was not complete at day 28.

New Sesamoid Bone

A previously undescribed sesamoid bone, lateral to the distal condyles of the femur, was discovered in this study. The bone (Fig. 4) was first seen on day 20 and was thought to be a staining artifact. However, closer observation revealed a small depression on the lateral surface of the condyle which appeared to be a counterpart to the shape of the new bone. The appearance of this bone was rapid, with all specimens exhibiting it on day 20 and thereafter. The Alcian stained specimens illustrated the bone as a small spot of cartilage several days before its appearance. The function of this bone has not been determined.

Baculum

The baculum is a heterotopic bone of the penis, found in most insectivores, bats, carnivores, non-human primates and rodents (Vaughan, 1978). The baculum first appeared in one-day-old specimens and was

seen in every age group thereafter (Fig. 5). At first appearance, it appears as a rod-like structure having no other characteristics. By day 28 it appears as a rod-like structure with an enlarged and rounded distal end. No grooves could be detected.

In general, Table 1 illustrates that those elements of the appendicular skeleton ossify and mature at a faster rate than those of the axial skeleton. This is not unexpected as early neonatal locomotor patterns are necessary for nest and suckling movements. In addition, grasshopper mice leave the nest around 15-20 days of age (Bailey and Sperry, 1929) and the skeletal support systems for general locomotion must be in place at that time.

Initial complete ossification of centers began about day 3 and then proceeded very rapidly, with most ossification centers being in place by day 14 (23 of 30 centers studied). Day 9 represented the greatest completion of ossification, with 15 of the 30 centers being completed. These results are similar to that found by Kirkland (1970) who studied two species of *Peromyscus* and showed ossification completed by day 14. Finally, because of the large degree of individual variation in ossification, we do not feel that postnatal ossification in the grasshopper mouse lends itself to age determination.

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TOWARD A CONCISE DEFINITION AND PERSPECTIVE FOR BIOLOGY

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ABSTRACT

The content of introductory biology courses at the high school and college levels are currently being re-examined. Many biological educators favor revision of the high school biology courses to a format that directly addresses current social issues to "promote good citizenship". We believe it would be more meaningful to teach the fundamental principles of biology, the study of living systems. Properly defining biology, by defining a living system, should help identify important topics to include in a basic biology course. Emphasis should be placed on subjects which would contribute most to the development of a basic understanding of how all living systems function. The course content should not be restricted by historical traditions or molded by social whims, but change with the development of knowledge through biological research.

INTRODUCTION

Biology is commonly defined as the study of "life" or "living systems". However, these terms are not defined, therefore biology is left essentially undefined. Biology teachers sometimes seem to have a slight obsession with terminology; therefore it is unusual that biology itself has escaped meaningful definition. Nearly all introductory biology texts begin by stating that life is undefinable. Then, in lieu of a definition, they list and describe the basic characteristics of living organisms or the living condition. These characteristics usually include biogenesis, metabolism, growth, cellular organization, motility, irritability, adaptation, homeostasis, and perhaps others, with considerable variation among texts. This method for beginning a biology course has been popular for decades and certainly has its merits. However, the characterization method may also be the source of some problems, and is considerably out of date concerning our current knowledge of biological science, as a proper definition is no longer beyond our ability.

Students may be apprehensive about their ability to understand biology because it is a study of "life", a subject which has defied definition. A discipline that is based on the study of a subject that is undefinable may at least sumliminally seem to be shrouded in mystery. Some students and research scientists have been attracted by the mystery of biology, but teachers generally recognize a need for a concise definition. The definition should be a foundation upon which to build, and provide a proper perspective for beginning students.

THE PROPOSED DEFINITION

During the past few years we have developed the following definition for a living system:

> *A living system is a unique set of common chemicals that is capable of utilizing energy to organize matter according to its information content in a way that results in self-perpetuation.*

Life is anything that has all the characteristics of a living system. The extent to which a chemical system (such as a virus) fits the definition is the extent to which it should be considered living.

The chemical elements (matter) composing living systems are not unusual, they are found throughout the earth's crust and atmosphere, but they do comprise a unique set. They exist in rather precise relative proportions to each other and react in predictable ways. There are numerous homeostatic mechanisms built into this system to ensure that equilibria are maintained. Variations in this set of chemicals among diverse forms of life are very small compared to their overwhelming similarities. Photosynthesis and anaerobic respiration are essentially reversible reactions, excluding the light-capturing pigments and associated photosystems, because most of the enzymes which catalyze these reactions are interchangeable. Depositional chemicals that form such things as cell walls, feathers, chitin, bone and hair are characteristic of only certain taxa, but their production probably requires only minor alterations in the universal set of metabolic pathways. The fact that living things are first and foremost a unique set of chemicals points out the importance of studying molecular relationships to understand biology.

Emphasizing the similarities among life forms to students can develop the attitude that learning biology is not such a formidable task. They realize that if they learn the basics of how one living system functions, they will have a fundamental understanding of how all living organisms function. Emphasizing similarities also encourages a feeling of companionship with other life forms, which may foster a concern for environmental protection.

Matter and energy are basically the only two components of our universe and they have interacted and continue to interact in diverse ways to produce the contents of the universe. Recently Carl Sagan in the television science-drama, Cosmos, has appealingly reminded us that we are made of cosmic dust and the light of a star. We know this to be true of everything in the universe, so it is certainly no distinction. In a very broad sense "stardust" can be construed as the matter composing the universe and "starlight" the energy with which it reacts. Science is the human endeavor to explain our perceivable universe through refined techniques of careful observation.

Living systems do not defy the Second Law of Thermodynamics as was once believed (see Denbigh 1951, Wiegert 1968). They must be capable of obtaining energy either directly from the sun as phototrophs do, or indirectly by consuming organic matter produced by photosynthesis. Energy flows through them and can only be attenuated and transformed during the use process. A substantial portion is lost with each transformation. If the energy flow is stopped, the system must

become inactive or deteriorate. Many living systems are not continuously active so the phrase "is capable of" is needed in the definition. Virtually all organisms vary their metabolic rates. This is one of the tactics used by organisms to maintain a positive time and energy balance to stay alive. Some organisms become completely inactive, such as *Nostoc*, a blue-green alga which can withstand extreme dessication for extended periods. Others escape harsh environmental conditions by a combination of physiological and behavioral mechanisms which dramatically lower their metabolism, such as hibernating endotherms. Similarly many temperate zone plants become dormant during winter. Nearly all seeds and spores are capable of surviving extended periods of extremely reduced (if not zero) metabolic activity. Energy is utilized by living systems to organize matter and keep it organized by providing the driving force necessary for most of the chemical reactions in living systems. It is sometimes useful to consider energy to be that which holds matter together. Chemical bonds can be considered to be units of energy.

Perhaps the most important thing that distinguishes living from nonliving matter is that living things contain information sufficient for their maintenance and self-reproduction. This information directs all of the chemical reactions and thereby determines how the systems are organized. For individual cells, information is contained in a chemical subset and is transmitted into action primarily through manufacture and activities of enzymes. In the case of more complex metazoans there is a slight shift in this responsibility to other means of information storage such as the memory banks of the central nervous system. Hormones certainly contain and transmit information also, probably to some extent in all forms of life, e.g., edcysone informs invertebrates concerning time to molt. Quantities of other specific chemicals are occasionally used to store information, for example, certain plants store phytochromes for information concerning photoperiod.

The ultimate measure of success of a biological system obviously is whether or not it remains alive, i.e., its self-perpetuation. This is equally true for individuals and species, but in a rather different way. Individuals have a relatively inflexible, solitary information set (despite repetitiveness), especially to the extent that the individual depends upon its unalterable genetic code for information. Genetic redundancy, phenotypic plasticity, the ability to store and retrieve information by the brain, and transmit information from one individual to another provide species various amounts of flexibility of information content within individuals. A species population on the other hand essentially has all the separate information sets of each of its members. The proper measure of biological success of an individual is the number of offspring it leaves in the next generation. This measure of the reproductive success of an organisms is a meaningful way to express the fitness or suitability of its information set in a particular environment (see Pianka 1983). Each genetically-controlled trait of an organism can be correctly evaluated (whether we can actually measure it or not) in terms of the contribution it makes toward keeping the organism alive and optimizing its reproduction. Evolution is the process by which species continuously remodel their information sets to accomplish self-perpetuation in their changing environments. This is, for the most part, simply a fortuitous probability because those individuals which are most fit for the environment in which they exist are most likely to survive and reproduce. Any genetic trait that results in altruistic behavior (other than within a family) does not contribute to the survival of its possessor, by definition. (Proponents of the theory of 'reciprocal altruism' would disagree of course.) Therefore there is no mechanism for the survival of such altruistic genes.

The proposed definition can be diagrammatically represented as in Fig. 1. The central circle represents the living system with its unique set of chemicals and information subset. Energy flows through the system and, while it may be temporarily stored, it is not recycled but ultimately is dissipated to the atmosphere and then outer space. Matter is cycled between the living and non-living realms as it is assimilated, rejected, secreted, shed (e.g., exuviae), etc., or when a living system fails (i.e., dies) and decomposes. The model may be thought of as representing a living cell with the boundary of the circle as the plasma membrane and the information set consisting of DNA. It can also represent an entire multicellular organism with no change in the model and little change in perspective by the viewer. This simplest version of the

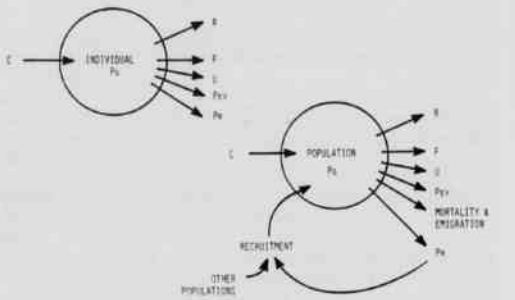

Figure 1. Diagram of living system, illustrating its interactions with nonliving matter and energy.

model shows matter and energy to have somewhat independent entries and exits. In this respect the model best represents green plants and other autotrophs. Energy enters green plants in the form of light independent of any matter and exits as heat, exuviae (leaves, etc.) and fruit. Most of the matter enters green plants as CO_2. Heterotrophs take in practically all of their matter and energy together in the form of food, of course. Mammals and birds (endotherms) release approximately 98% of this energy as metabolic heat, which is unavailable to do meaningful work, i.e., cause useful changes in matter for their living systems. Ectotherms can retain approximately one-half of their energy intake to use for growth and reproduction (e.g., see Brown and Fitzpatrick, 1978).

The First law of Thermodynamics forms the basis for energy budget equations (Wiegert, 1968) which can be used to represent the energy flow through individuals, populations or entire communities:

$$C = P + R + F + U$$

where: C = Consumption (of energy)
P = Production (storage of chemical energy)
R = Respiration (heat loss)
F = Feces (energy not absorbed by heterotrophs)
U = Urine (energy absorbed but not assimilated)

$$A = P + R$$

where: A = Assimilation of energy, or energy flow

$$P = P_g + P_r + P_{cv}$$

where: P_g = Growth
P_r = Reproduction

P_{cv} = Exuviae

Energy flow diagrams can be elaborated from the basic model of a living system and these energy budget equations as illustrated in Fig. 2. These diagrams can be quite useful when introducing the subject of

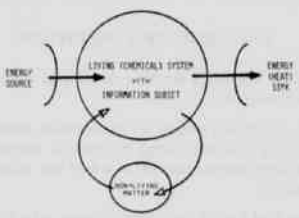

Figure 2. Energy Flow diagrams for heterotrophic individuals and populations. See text for definition of symbols.

energy flow to students. Larger hierarchial divisions of biological systems (populations, communities, ecosystems, biomes or the entire biosphere) are adequately and usefully represented by this model. However, the information content concept should shift from the individual's genotype to the gene pool at the population level and then to inter-organism and interspecific interactions at the community level (Margalef, 1958; MacMahon et al., 1981).

CONCLUSIONS AND PERSPECTIVES

The proposed definition can be used to elucidate the most fundamental concepts for an introduction to the study of biology. It could provide a cohesive reference point for use by teachers and students when assessing the contribution of each unit in an introductory biology course to the ultimate goal of achieving knowledge of how biological systems function. The definition does not have to displace listing of the characteristics of life, but it is useful when introducing biology to students. Various subdisciplines and specific topics for study in biology can be placed in proper perspective for students by using the proposed definition and model. For example, energy flow and cycling of matter are identified as two fundamental emergent properties of living systems, and as such are basic concerns of the study of biology at all levels from molecular to ecosystem studies.

This is a purely mechanistic definition as opposed to a vitalistic one. It does not include recognition of any vital force because it is in the realm of science. Science has specific limitations, and the presence of a vital force cannot be tested by scientific means. However, the proposed definition should not be misconstrued by perceiving it as being incompatible with Christianity just as it is not correct to presume that evolution and creation are alternatives, as discussed by Moyer (1981).

The Biological Sciences Curriculum Study prepared high school texts and lab manuals (especially the Blue Version) that are problem oriented and address the topics essential to developing an understanding of how living systems function. Many college-level texts have adopted a similar format or design, but too many retain the entire phylogenetic and systemic approaches as well, and as a result have become formidably large. Moyer (1982), Yager (1982), and other biology educators are concerned that many teachers confronted with encyclopedic textbooks and/or pressures stemming from social issues are retreating to the basics (so called) of phylogenetic and/or systemic approaches to teaching biology at the high school and introductory college levels. They are encouraging teachers to confront this situation by discussing pressing social issues which relate to biology (e.g., abortion, sex education, women's rights, etc.) in biology class (Bybee, 1982; Kennedy, 1982; Patrick and Remy, 1982). This would consume valuable class time that could be used to teach biology, i.e., the study of living systems and how they function. Perhaps a retreat to the basics is the best strategy, but the basics should be those topics which contribute most to understanding how all living systems function.

Historically the beginning biology course originated as a replacement for botany, zoology and human physiology courses and as a result, studies of systematics and human organ systems have often been considered to be the "basics" of the course ever since. Perhaps the beginning college biology course can eventually come into its own and transcend its traditional bifid role as a service course for the pre-medical profession students and a survey of the variety of life forms, each of which should (and usually does) constitute separate sophomore-level courses. An essential core of content should be identified that would provide a foundation from which teachers and students could meaningfully address related socially-important issues, perhaps in some other subsequent course(s). This core of information would change with the development of biology as a science through research, not in response to the vagaries of the social, political or religious environment. By defining biology the necessary unifying theme and core content for introductory biology courses become evident.

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FLY ASH AS A FERTILIZER AND LIME SOURCE IN ARKANSAS

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ABSTRACT

Percent calcium carbonate equivalent (neutralizing value) of five fly ash samples ranged from 34 to 41. Field soils at three sites were treated with fly ash at rates that ranged from 1 to 6 tons per acre. Fly ash applications had opposite effects on extractable P, B, Fe, and Cu at Sites 1 and 2. A three-fold increase in total B occurred in wheat plants taken from one field treated with fly ash. At Site 3 test results of soil samples collected three, six, nine and twelve months after treatment showed that 2 tons of agricultural limestone was equivalent to 4 to 6 tons of fly ash in raising soil pH. Most of the chemical changes occurred in the upper 2.5 cm of soil and within three months after treatment.

INTRODUCTION

Large quantities of fly ash are being produced by the burning of powdered coal by electric power generating plants at three locations in Arkansas. Three years of agronomic research with this product by Professors Spooner and Brown, associated colleagues, and graduate students at the University of Arkansas has indicated that it may be used as a liming material for acid soils (Davis, 1982; Hodgson, 1982; and Hodgson, Dyer, and Brown, 1982). At least one commercial company is marketing the by-product as an agricultural liming substitute. Questions remain as to preferred rates and longevity of fly ash applications and to the beneficial or detrimental effects of heavy metals and essential plant nutrients (Adriano et al., 1978; Bern, 1976; Martens, 1971; and Plank, Martens, and Hallock, 1975).

MATERIALS AND METHODS

Samples of fly ash were collected in August and September, 1982, from the Arkansas Power and Light electric power generating plant at Redfield, Arkansas, by Chem-Ash, Inc.

Total chemical analysis was conducted on three samples by the Arkansas State Plant Board at Little Rock. Calcium carbonate equivalent was determined on these and two additional samples from the Redfield plant by methods commonly used to evaluate the neutralizing capacity of agricultural liming materials.

Fly ash was surface applied to silt loam field soils at two locations in Jefferson County (Sites 1 and 2) and one location in Pulaski County (Site 3). Application rates ranged from 1 to 6 tons per acre. A 2-ton per acre rate of ground agricultural limestone was compared to three rates of fly ash at Site 3. Treated and untreated soils were tested before and after treatment. Soil samples were tested for extractable plant nutrients by the University of Arkansas Soil Testing Laboratories at Fayetteville and Marianna using procedures outlined in *Southern Cooperative Series Bulletin 289* (Kriz et al., 1983).

Wheat plant samples were collected at the tillering stage at one location and analyzed for total plant nutrients by the University of Arkansas Agricultural Diagnostic Lab at Fayetteville.

RESULTS AND DISCUSSION

Chemical Composition of Fly Ash

Total chemical analysis of three fly ash samples revealed calcium concentrations that range from 13 to 18 percent. Other elements ranged in concentration from around 4 percent for iron to 130 ppm for copper (Table 1). One particular concern with fly ash is the potentially phytotoxic concentration of boron at high application rates (Plank and Martens, 1974). Boron concentrations range from 820 to 970 ppm or an average of 1.72 pounds per ton of material. Most silt loam soils contain less than 10 ppm of total boron, of which only a small fraction is available to growing plants at any one time.

Table 1. Chemical Composition of Fly Ash From Redfield, AR[1]

Ingredient	Analysis of 3 Samples[2] Average	Range
	-------- % --------	
Phosphorus (as P_2O_5)	0.23	0.18-0.31
Potassium (as K_2O)	0.23	0.20-0.25
Calcium	16.17	13.15-17.85
Magnesium	2.45	1.94-2.81
Sodium	2.79	1.87-2.94
Sulfur	0.74	0.50-0.89
Iron	4.19	3.65-4.51
	-------- ppm --------	
Manganese	277	220-310
Zinc	293	220-330
Copper	152	130-163
Boron	860	820-970

[1]Samples collected in late August, 1982, by Chem-Ash, Inc.
[2]Analysis by Arkansas State Plant Board.

The average percent calcium carbonate equivalent (neutralizing value) of five fly ash samples was 38.2. The values ranged from 34.3 to 40.8, compared to 95 for good-quality agricultural limestone.

Effect of Fly Ash on Soil at Sites 1 and 2

Fly ash applied to Site 1 raised the soil pH from 5.5 to 6.2 (Table 2). Extractable iron and copper were considerably lower where fly ash

Table 2. Extractable Plant Nutrients in Treated and Untreated Soils.

Soil Test Parameter	Treatment [1] Site 1 Check	Site 1 Fly Ash	Site 2 Check	Site 2 Fly Ash
	ppm			
Phosphorus	40	81	17	10
Potassium	75	85	60	45
Calcium	150	150	350	450
Magnesium	40	25	25	25
Sulfate	37	39	34	38
Manganese	8	8	5	5
Iron	120	75	30	70
Zinc	0.7	0.65	0.65	0.3
Copper	0.65	0.4	0.3	0.45
Boron	0.14	0.28	0.26	0.22

was applied. This is to be expected since heavy metals become more difficult to extract as soils become less acid. Boron and phosphorus were twice as high in the fly ash treated soil. The other elements tested were essentially the same for both the check and the fly ash treated soil.

Fly ash applied to Site 2 raised the soil pH from 6.4 to 6.8. The effect on extractable plant nutrients was almost the opposite of that from Site 1. Extractable iron and copper were more than 50 percent higher than the check. Phosphorus and zinc were considerably less than the check. However, all of the extractable plant nutrients were relatively low in both the check and the fly ash treated soils.

Except for iron, copper, zinc, and boron, there was little difference in chemical composition between wheat plants from the check and from the fly ash treated soils (Table 3). Zinc and copper concentrations were cut in half by the fly ash treatment, while iron and boron concentrations were increased. Boron concentrations in the wheat tissue were increased three-fold. Davis (1980) observed a four- to six-fold increase in boron concentrations in alfalfa plants treated with high rates of fly ash. Boron uptake by wheat appears to be much greater than is indicated by extractable soil test levels. There were no obvious visual differences in appearance or yield of wheat from the fly ash treated plots and the remainder of the field which was not treated.

Effect of Fly Ash on Soil at Site 3

Average test results of soil samples collected three, six, nine, and twelve months after treatment with one rate of agricultural lime and three rates of fly ash showed the fly ash was effective as a liming material on Leadvale silt loam soil (Table 4). However, 2 tons of agricultural lime was equivalent to 4 to 6 tons of fly ash in raising soil pH. This is in agreement with what most researchers have reported. Agricultural lime was much more effective than even the highest rate of fly ash in increasing available calcium One advantage to fly ash was that it increased the extractable magnesium level by about 40 percent over the check and the lime treatment. There was very little difference in levels of soluble salts (E.C.) and extractable P,K, and Na between treatments. For the most part, the effects of the various treatments were manifested within three months after treatment.

Extractable sulfates and micronutrients in soil samples collected from the surface 15 cm of depth showed that boron was the only element that increased linearly with increasing rates of fly ash (Table 5). The high level of copper in the highest fly ash treatment was attributed to contamination from a previous treatment of that plot with copper sulfate.

[1]Fly ash was applied to raise the soil pH of Site 1 from 5.5 to 6.2; of Site 2 from 6.4 to 6.8.

Table 3. Chemical Composition of Wheat Plants From Fly Ash Treated and Untreated Soils.

Element	Treatment [1] Check	Fly Ash
	%	
Phosphorus	0.24	0.19
Potassium	1.7	1.6
Calcium	0.35	0.35
Magnesium	0.17	0.15
Sulfur	0.11	0.13
	ppm	
Iron	440	770
Manganese	85	83
Zinc	20	10
Copper	5.0	2.5
Boron	5.9	18.5

Table 4. Soil Test Results of Leadvale Silt Loam Topdressed With Ground Agricultural Limestone and Different Rates of Fly Ash.[1]

Soil Test Parameter	Treatment Check	Limestone (2 T/A)	Fly Ash 2 T/A	Fly Ash 4 T/A	Fly Ash 6 T/A
pH	5.2	5.8	5.5	5.7	5.9
			µmhos/cm		
E.C.	37	51	36	41	40
			ppm		
Phosphorus	37	38	39	37	45
Potassium	45	55	55	50	47
Calcium	213	338	213	238	238
Magnesium	58	54	79	81	82
Sodium	52	53	56	55	55

The greatest chemical change occurred in the upper 2.5 cm of soil (Table 6). The agricultural lime was much more effective than the same rate of fly ash in promoting chemical changes. The pH change in favor of the agricultural limte is to be expected since its neutralizing value is about 2½ times that of the fly ash. The lone exception to this was the two-fold increase of extractable boron from the fly ash treated plot. A two-fold increase in extractable calicium and sulfate occurred with the agricultural lime treatment. The extractable magnesium was decreased by the treatment with agricultural lime. This was probably due to mass action and dilution of the magnesium by excess calcium. The

[1]Fly ash applied to raise soil pH of check from 5.7 to 6.4.

[1]Fly Ash and lime applied in October, 1982. Values are averages of 3 replications and 4 sampling periods.

Table 5. Extractable Sulfates and Micronutrients in Leadvale Silt Loam 3 Months After Surface Application of Fly Ash[1].

Soil Test Parameter	Application Rate (Tons Per Acre) 2	4	6
	ppm		
Sulfates	51	54	54
Iron	118	123	123
Manganese	10	8	10
Zinc	11	10	10
Copper	0.4	0.4	2.0
Boron	0.35	0.7	0.8

Table 6. Soil pH, % Organic Matter, and Extractable Plant Nutrients in the Surface 2.5 cm of Leadvale Silt Loam 3 Months After Surface Applying Fly Ash and Agricultural Limestone[1].

Soil Test Parameter	Check	Two Tons Per Acre Treatment Fly Ash	Agri Lime
pH	5.6	5.9	6.7
		ppm	
Calcium	300	300	650
Magnesium	110	120	65
Phosphorus	64	34	41
Potassium	60	75	85
Sulfate	30	48	63
Boron	0.3	0.65	0.3
		%	
Organic Matter	1.4	2.8	2.8

levels of sulfate corresponded closely to levels of organic matter. However, the 2.8 percent organic matter in the limed and fly ash treated soils as compared to half that amount in the check soil could not be explained. One of the benefits of liming may have been the stimulation of microbial decay of organic matter, increasing available sulfates.

CONCLUSIONS

It may be concluded from this study that fly ash may be used as a liming material for silt loam soils. It has limited value as a plant nutrient source depending on the needs of a particular soil. The liming value of fly ash is about 40 percent of that of agricultural limestone. Thus, about 2½ times as much material must be applied to neutralize the same level of soil acidity. Rates of up to 6 tons per acre of fly ash should not be toxic to growing plants. Multiple application rates totaling more than 10 tons per acre may be toxic to some seedlings due to high concentrations of boron. Additional research is needed to define the conditions for most efficient use of the material.

[1]Fly ash applied in October, 1982. Values are averages of 3 replications for each treatment rate.

[1]Fly ash and agricultural limestone applied in October, 1982. Soil test values are averages of 3 replications for each treatment.

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THE EFFECT OF *EDWARDSIELLA ICTALURI* INFECTION ON PLASMA CORTICOSTERONE LEVELS IN CHANNEL CATFISH *(ICTALURUS PUNCTATUS)*

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ABSTRACT

Channel catfish *(Ictalurus punctatus)* were innoculated with a new host specific bacterium, *Edwardsiella ictaluri*, to observe the influence of bacterial infection on plasma corticosterone levels at various temperatures. The fish were innoculated intraperitoneally. The infected fish were separated from the controls. Plasma corticosterone concentrations were determined by radioimmunoassay.

The plasma corticosterone concentrations in non-innoculated catfish were about 6.15 ng/ml and nearly 5.63 ng/ml in the infected fish. The lower level of the hormone in the infected catfish was not significantly different from the control level. High temperature was a stress factor which increased plasma corticosterone levels whereas *E. ictaluri* retarded the response of corticosterone secreting cells of the fish kidneys.

INTRODUCTION

Edwardsiella ictaluri is a bacterium which causes a deadly disease called "Enteric Septicemia of Catfish." This organism was identified and named by Hawke in 1979 when the disease erupted in the channel catfish farms of Georgia and Alabama. Hawke reported that the disease produced listlessness, spinning, external petechial hemorrhages of throat and mouth regions, hypertrophies of kidneys and spleen, hemorrhagic necrosis of liver and petechia of viscera, dorsal musculature and intestine. Death of the fish followed these signs within two weeks when the disease was waterborne or within 96 hours if the bacterium was innoculated into the peritoneal cavity.

The rapid advance of the pathological changes culminating in the death of the infected fish indicated total impairment or suppression of the host's immune and inflammatory mechanisms. These defense systems are often suppressed in animals by high concentrations of corticosteroids. According to Hans Selye (1976) the adrenal cortex would secrete these hormones excessively if the animals are stressed. The inhibitions of immune responses and inflammatory responses in channel catfish could be due to this reason.

Regardless of the structural and anatomical peculiarities of the ductless glands in fish, the fish endocrine system is functionally similar to that of the tetrapods. There are no distinct adrenal glands in fish. Instead, the corticosteroids are secreted by the acidophilic cells of the interrenal tissue in these animals. One or more layers of the cells form a sheath around the posterior cardinal veins and their branches which ramify within the cranial regions of the head kidney (Hoar, 1957). Gorbman and Bern (1962) confirmed that these cells secreted cortisone, cortisol, corticosterone, and dehydrocorticosterone. Selye (1949) observed that the fish pituitary and interrenal tissue responded to environmental stress by increased production of the glucocorticoids after the same manner as the responses to obnoxious stimuli by the hypophysis and the adrenal glands of higher animals. According to Selye (1976) environmental factors such as salinity changes, temperature extremes, captivity, crowding, muscular fatigue, hypoxia, hemorrhage and trauma can influence the glucocorticoid secreting cells to increase the production of the hormones. Significant increases in the plasma levels of glucocorticoids due to stress were observed in fish by Rasquin and Rosenbloom (1954), Hoar (1957), and by Ball and Slicher (1962).

The effect of temperature on plasma corticosteroid concentrations in channel catfish was studied by Davis et al. (1979). They reported 10 ng/ml as the baseline value of total corticosteroids in catfish held in an aquarium at room temperature. In water at or below 10° C the level of plasma corticoids rose to a range of 18.7 to 40.7 ng/ml. The concentrations of corticoids in the plasmas of channel catfish decreased to a value between 4.1 to 6.1 ng/ml when the fish were transferred to water temperatures of 15 to 20° C.

In a study on *Oncorhynchus kisutch*, an increase in serum cortisol and a decrease in interrenal ascorbic acid were indicators of non-specific stress in temperature shock at 10 ± 7° C (Wedemeyer, 1969). Low temperature slows down metabolic and osmoregulation rates, central nervous system responses, swimming speed, and feeding (Umminger and Gist, 1973). Fish acclimated to a low temperature have higher total tissue oxygen consumption rate. The liver, skeletal muscle and stomach have a higher oxygen consumption rate than the brain under these conditions (Rieck et al., 1960). Compared to channel catfish maintained at 20° C under the same conditions, those maintained at 15° C and confined in a net had a longer lag phase, lower active secretion phase, and a lower maintenance phase in plasma corticosterone levels (Davis et al., 1979). Goldfish acclimated to 10° C demonstrated more symptoms of stress due to handling and sham injection than did fish acclimated to 32° C. High temperature studies revealed that females are usually more heat tolerant than males. There is no difference in heat tolerance between a spawning female and a non-spawning female, but there was diurnal rhythm in heat resistance (Johnson, 1976).

In fish the thermoregulatory center is located in the rostral brainstem. These animals change their body temperatures by heat exchange with the environment. They move from an area of higher than optimum water temperature to cool regions in an apparent effort to maintain a fairly constant body temperature (Crawshaw and Hammel, 1974) which is the "preferred temperature" in which the species functions physiologically and becomes evolutionarily adapted (Umminger and Gist, 1973; Brett, 1971). Rapid temperature changes can bee detrimental to poikilotherms including fish. The temperature which is fatal to 50% of a population is defined as the lethal temperature. The fish with the lowest thermal tolerance is the *Oncorhynchus* and the most eurythermal is the Ameriuridae (Brett, 1956).

Channel catfish *(I. punctatus)* have a narrow range of temperature tolerance. Their upper lethal limit is 33° C if acclimated gradually (Love, 1980). In *I. lacustris,* the upper lethal limit is 33.5° C and the lower lethal limit is 0° C (Brett, 1956). When the temperature reaches 23.0° C from mid-May to mid-June in Arkansas, channel catfish spawn (Brady, 1981). Channel catfish optimum digestion temperature is 26.6 to 29.4° C and they are most active at this heat level (Shrable, Tiemeier, and Deyoe, 1969).

The common bacterial diseases of channel catfish are *Pseudomonas flourescens, Aeromonas hydrophila, Flexibacter (Chondrococcus) columnaris,* and *E. tarda* (Hawke, 1979). *Aeromonas hydrophila* and *F. columnaris* make up 82.8% of all bacterial cases in fish. Bacterial diseases appear between April and September while viral diseases may

appear between June and September (Plumb, 1975). The bacterial onslaught occurs in the southern United States concurrently with the optimum temperature for *E. ictaluri* (Hawke, 1979). Environmental stress such as high temperature and poor water quality caused by overcrowding, over feeding, over fertilization, chemicals, algae die-offs, and seasonal water upheavals predispose fish to bacterial infections (Walters and Plumb, 1980).

The endotherms are known to develop fever in response to bacterial infection. The ectotherms require a higher than normal environmental temperature when they are infected by microorganisms. The desert iguana *(Dipsosaurus dorsalis)*, goldfish *(Carassius auratus)*, largemouth black bass *(Micropterus salmoides)*, and bluegill sunfish *(Lepomis macrochirus)* when injected with killed *Aeromonas hydrophila* moved to an area with a higher temperature. *D. dorsalis* regulated its body temperature by walking back and forth between a cool and a warm chamber (Vaughn et al., 1974). This movement is a behavioral adaptation to absorbe heat from the environment and to increase the body temperature until it was in equilibrium with the hypothalamic thermal set point which was raised by pyrogens (Covert and Reynolds, 1977). Endotoxin secreted by the bacteria triggers the release of endogenous pyrogens and prostaglandins which influence the hypothalamus to demand an elevated body temperature (Vaughn et al., 1974; Reynolds et al., 1976; Kluger, 1978). It has been postulated that at the high temperature, the immunological system is accelerated to synthesize immunoglobulins and enhance phagocytosis (Covert and Reynolds, 1977). Goldfish respond to bacteria by the production of precipitating agglutinins and neutralizing antibodies in the blood (Chavin, 1973). Thus, fever seems to have a protective effect on animals because fish that are allowed to thermoregulate behaviorally have no mortality when compared to those with no choice of temperature (Covert and Reynolds, 1977).

The purpose of this study was to determine the plasma corticosterone concentrations of channel catfish *(Ictalurus punctatus)* which are innoculated with *Edwardsiella ictaluri* and held in various water temperatures, and to observe if stress was a cause for the immune deficiency of these animals.

MATERIALS AND METHODS

Yearling channel catfish *(Ictalurus punctatus)* were obtained from Kueter's Lake, T 16 N, R 6 E, S 8 and 90° 27 ' W by 36° 2 ' S, Paragould, Arkansas. They were held in indoor fish tanks at a density of 15 fish per 180 liters of water. The water was aerated and filtered continuously with electric air pumps and bottom filters. Constant temperature of 21° C, 23° C, 25° C, 27° C and 29° C were maintained in separate aquaria by means of thermostatic heaters. For each of these temperatures there were two groups of fish namely the controls (n = 15) and the infected (n = 15). Prior to the start of the experiment the fish were acclimated to the assigned temperature. These animals were not fed during the experiment.

On day one at the beginning of the experiment the experimental fish were anesthetized by immersion in an 80 ppm solution of MS-222 and by means of a tuberculine syringe fitted with 25 × 5/8 needle they were innoculated intraperitoenally with *Edwardsiella ictaluri* supplied by Stoneville Research Laboratories, Stoneville, Mississippi. The dose was 0.05 ml containing approximately 1.3×10^7 organisms. The fish were allowed to recover from anesthesia before returning them to the aquaria. The control fish were anesthetized, allowed to recover and were returned to aquaria.

At 7:00 p.m. on day three and at the same hour of the following days the fish were serially sacrificed to collect blood and tissue samples. For this purpose, a fish was gently lifted from the water with a hand net and was stunned by a hard knock on the head. Quickly blood was drawn from the fish by cardiac puncture into a heparinized syringe. Minimally about 1 milliliter of blood was obtained by this method. The blood was transferred to heparinized vacutainer tubes and was centrifuged at 3000 rpm in an International Clinical Centrifuge. The plasma was pipetted into labelled vials and was stored at − 10° C until assayed. The fish was grossly examined and the observed lesions were described. Also, samples of liver, spleen, and kidney were collected for bacterial count.

The plasma was thawed and was mixed with 15 ml of methylene chloride by shaking the tubes for fifteen minutes. The mixture was filtered through #2 Whatman Filter Paper and the filtrate was collected and dried under a current of dry air. The residue was redissolved in 1 milliliter of methanol and the amount of corticosteroid in this solution was determined by radioimmunoassay using a corticosterone specific antiserum prepared by the Endocrine Sciences, Tarzana, California.

The data collected in this study were analyzed by Student t-test and by two-way analysis of covariance to determine the significance of differences (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The body weights of the control catfish and the experimental catfish were similar. There was no marked variation of the body length between the controls and the innoculated. Consequently the body weight to body length ratios of the innoculated fish were not markedly different from similar ratios of the controls (Table 1).

The infected fish had petechia of the throat and mouth skin areas. Similar lesions were observed on the internal surface of the body wall and on the external surfaces of stomach and intestine. The control fish were not affected by similar lesions. Bacterial counts were available from the livers, spleens, and kidneys of the infected fish only (Table 1).

The plasma corticosterone levels of the fish in this study are presented in Table 2. The plasmas of the controls and of the experimental fish from 21° C water, contained similar amounts of corticosterone. In the fish from 23° C environment, the plasma concentrations of corticosterone were not significantly different when these values of the innoculated fish were compared to those of the controls. At 25° C water temperature the infected fish had no markedly varied amounts of circulating compound B in comparison to the quantity of the same substance in the control fish. The 27° C controls and innoculated catfish showed no significant difference of plasma corticosterone concentrations. The catfish maintained at 29° C had high concentrations of plasma corticosterone; but these levels in the innoculated fish were not statistically different from the control levels at that temperature.

There was a significant difference which was observed when the plasma concentrations of corticosterone in the fish maintained at 21° C was compared to the similar values of the fish living in 29° C water, whether the fish were infected by *E. ictaluri* or not ($P = 0.01$).

According to the data obtained from this study the channel catfish developed "Enteric Septicemia of Catfish" when *E. ictaluri* was introduced into them under experimental conditions. The infection was fatal to these animals as reported by Hawke (1976). However, there was no increase or decrease of circulating corticosterone due to *E. ictaluri* infection in channel catfish. There was a significant increase of Compound B in the fish maintained at 29° C, when compared to the amounts of this hormone in the plasmas of fish living in 21° C environment.

The plasmas for the determination of corticosterone concentration were collected at 7:00 p.m., the hour at which the hormone reached the peak value of diurnal rhythm as observed by Boehlke, Tiemeier and Eleftheriou (1966). The amounts of corticosterone in the plasma samples were determined by radioimmunoassay because of its specificity, reproducibility and sensitivity. Using this method the fish plasmas contained about 6.15 ng/ml of plasma. This value is similar to the plasma level of corticosteroids determined by Davis et al. (1979) in fish of the same species and age maintained in water between 15-20° C. Davis et al. (1979) determined the corticosterone concentration in fish plasma by the "competitive protein binding method" which is closely related to radioimmunoassay.

The control catfish did not possess any bacteria in the kidneys, liver or spleen. In the innoculated catfish these organs were infected. Therefore, the functional efficiency of these tissues could be impaired.

The amount of corticosterone in catfish plasma as reported by Boehlke et al. (1966) was very high when compared to the values obtained by Davis et al. (1979). The fish in the experiment conducted by Boehlke et al. were older and heavier ranging from 350 to 550 g. Also, the plasma corticosterone in them was assayed by a fluorometric method which

Table 1. Body lengths, body weights, and density of *Edwardsiella ictaluri* in different tissues of channel catfish *(Ictalurus punctatus)* maintained at various temperatures.

Temperature centigrade	Group	Number	Body Length centimeter	Body Weight gram	Body Weight / Body Length	Average $\log_{10}$ of *Edwardsiella ictaluri* per gram of tissue		
						Kidney	Liver	Spleen
21	I Control	7	16.64 ± 4.74	68.09 ! 8.71	4.09	----	----	----
	II Innoc.	10	19.15 ± 0.52	90.44 ± 6.37	4.72	7.77	7.66	8.13
23	III Control	3	17.13 ± 1.43	69.38 ±21.18	4.05	----	----	----
	IV Innoc.	12	20.29 ± 0.32	111.71 ± 5.87	5.51	8.05	8.04	8.58
25	V Control	15	19.09 ± 0.46	93.17 ± 6.13	4.88	----	----	----
	VI Innoc.	12	21.31 ± 0.36	120.71 ± 5.31	5.66	7.23	8.85	9.56
27	VII Control	13	15.99 ± 0.55	55.37 ± 5.54	3.46	----	----	----
	VIII Innoc.	9	16.56 1 0.81	66.58 ±11.49	4.02	9.40	8.27	10.08
29	IX Control	14	21.81 ± 0.37	127.44 ± 5.98	5.98	----	----	----
	X Innoc.	13	21.20 ± 0.38	118.95 ± 4.91	5.61	8.79	8.84	9.44

Table 2. Effect of temperature on plasma corticosterone concentrations of channel catfish infected by *Edwardsiella ictaluri*

Temperature ° C	Number of fish (control/innoc.)	Corticosterone Concentration ng/ml of plasma Mean Standard Deviation	
		Control	Innoculated with *Edwardsiella ictaluri*
21	7/7	4.54 ± 1.21[a]	3.52 ± 1.04[c]
23	3/3	6.45 ± 1.09	4.89 ± 1.18
25	12/12	4.22 ± 1.33	5.73 ± 1.57
27	9/9	7.63 ± 2.39	5.89 ± 3.36
29	12/12	7.84 ± 2.93[b]	6.73 ± 1.70[d]

a vs b P = 0.01

c vs d P = 0.01

is not as specific as the method of competitive protein binding or radioimmunoassay.

There was no significant effect of bacterial infection on plasma corticosterone concentration in channel catfish. However, the plasma levels of compound B in the infected catfish were consistently lower than the control values for fish maintained at 21, 23, 25, 27, and 29° C. The decreased corticosteroid secretion by infected catfish can be related to the pathological changes observed in the kidneys of these animals. Whether or not the interrenal tissue in these animals had lost their function was not established by challenging the endocrine gland with exogenous ACTH.

The plasma corticosterone concentration can vary in animals from time to time according to the changes in the rate of secretion, metabolic utilization, protein-binding, and renal excretion. The change in the rate of secretion of adrenocorticoids in animals is regulated by the hypothalamo-hypophyseal adrenal axis by a negative feedback mechanism which can be stimulated by emotional, environmental, mechanical, or chemical factors (Zarrow, Yochim, and McCarthy, 1964). Generally, a significant increase in the secretion of these hormones occur when animals are stressed by one or more of these factors (Selye, 1949). According to this theory, the animals in this study did not show a significant increase in the secretory rate of plasma corticosterone due to the infection. The increase in temperature of the fish tanks did cause increased plasma corticosterone concentrations in these fish. Previously it was reported that a decrease in environmental temperature caused a rise in the level of plasma corticosterone in channel catfish (Davis et al., 1979). Therefore, cold and hot environments seem to be stressful to catfish.

CONCLUSION

The suppressions of immuno response and inflammatory reactions in channel catfish infected by *Edwardsiella ictaluri* are not the results of excessive amount of circulating corticosterone. The environmental temperatures, except 29° C, were not stressful to the fish in this experiment. Hence, the failure of the defense mechanisms during bacterial infection in channel catfish is not due to the action of stress on the hypothalamic hypophyseal adrenal axis.

ACKNOWLEDGMENT

The authors acknowledge the generosity of Mr. Tom Kueter of Kueter Lake, Paragould, Arkansas, who donated the channel catfish used in this study.

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IMPLICATIONS OF HYDROCARBON AND HELIUM GAS ANALYSES OF SPRINGS FROM THE OUACHITA MOUNTAINS, ARKANSAS

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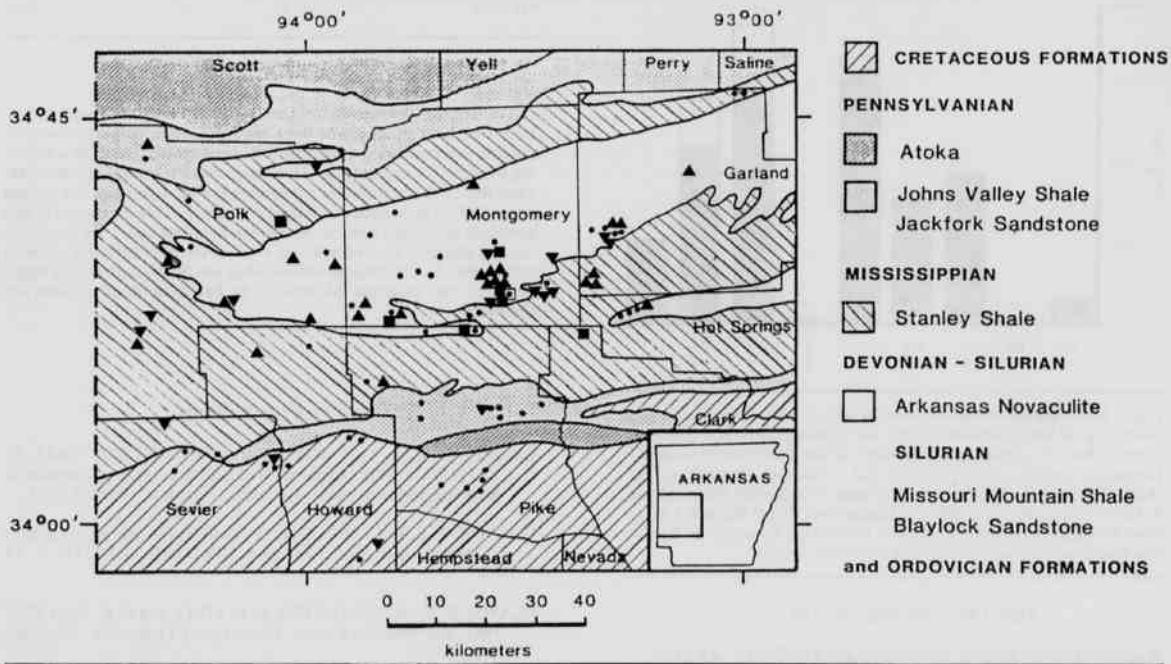
ABSTRACT

One hundred and three ground water samples (predominantly springs) were analyzed for headspace light hydrocarbon gases and helium. Four of the formations (Arkansas Novaculite, Bigfork Chert, Stanley Shale, and Womble) having the highest mean methane values are the only Ouachita Mountain facies to produce petroleum or exhibit marginally commercial production. This observation suggests that the mean methane values are useful as an indication of the relative hydrocarbon content of these formations. Anomalous helium values are generally associated with mapped faults.

INTRODUCTION

Light hydrocarbon and helium concentrations for 103 ground water samples from the western Ouachita Mountains, Arkansas were obtained by a hydrogeochemical survey conducted as part of the National Uranium Resource Evaluation. In order to obtain meaningful analyses of the metals in the ground water in this mineralized area, spring water was utilized predominantly so as to avoid plumbing contamination. Sample sites (92 springs and 9 wells) were selected to emphasize the mineralized districts, and the locations were also controlled by availability and accessibility of springs (Figure 1). The samples were analyzed for pH, conductivity, total alkalinity and concentrations of selected elements (Steele, 1982). Analysis of the headspace gas of the ground water samples for light hydrocarbon and helium was performed as a peripheral portion of the survey. It is the purpose of this paper to interpret these data and to assess usefulness of this method in evaluation of the potential hydrocarbon productivity of the area.

Figure 1. Map showing concentration ranges of ground water headspace methane value with regards to location and geologic formations. The concentration ranges are in ppm by volume as follows: (▲) > 700, (▼) 500-700, (■) 300-500, (□) 100-300, and (•) < 100.



Implications of Hydrocarbon and Helium Gas Analyses of Springs from the Ouachita Mountains, Arkansas

GEOLOGY

Paleozoic rocks ranging from Pennsylvanian to Lower Ordovician in age occupy the major parts of the study area; whereas, Lower Cretaceous rocks occur in the southernmost part (Figure 1). The Paleozoic rocks include thick successions of Carboniferous sandstone and shale flysch facies, and pre-flysch successions of shale, chert, and sandstone. Structurally, the Paleozoic strata are characterized by generally east-west oriented intense folding and associated imbricate thrust faulting. The core region of the Arkansas Ouachita Mountains (the Benton Uplift) exposes the Lower Paleozoic sequence. The Cretaceous rocks are an essentially undeformed flat-laying overlap on the southern flank of the Paleozoic rocks (Flawn et al., 1961).

METHODS OF INVESTIGATION

The parameters used for this present study include light hydrocarbon gas (through butane) and helium concentrations, surface temperature, subsurface temperature (based on silica geothermometry), location, the geologic formation from which the spring issues and whether the site is within 150 feet of a fault. Although the movement of ground water in this area is complex, it is assumed the ground water will reflect the characteristics of the formation from which it issues and also will be affected by faults. It is also assumed that biogenic methane will have minimal effect on the water sample. The ground water samples for gas analyses were collected in soft drink bottles leaving about 2cc of air space, immediately capped and stored in an inverted position (to minimize loss of gases) for shipment to the laboratory where the headspace gases were analyzed by gas chromatography and mass spectrometry. Silica was determined colorimetrically on a separate water sample that had been filtered through 0.4 micron pore size membrane in the field. See Dromgoole (1982) and Steele (1982) for more information on collection and analytical methods.

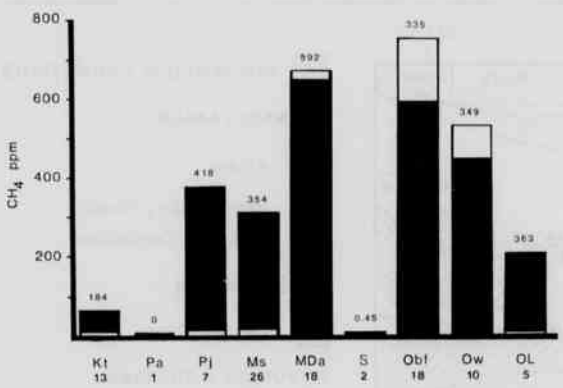


Figure 2. Histogram comparing median (top of white portion of bar), mean (top of black portion of bar) and standard deviation (number at top of bar) of methane concentrations for the individual formations. Formation symbols are as follows: (Kt) Cretaceous Formations, Pa (Atoka Formation), (Pj) Jackfork Sandstone, (Ms) Stanley Shale, (MDa) Arkansas Novaculite, (S) Silurian Formations, (Obf) Bigfork Chert, (Ow) Womble Shale and (OL) Lower Ordovician Formations. Sampling frequency is given below each formation symbol.

RESULTS AND DISCUSSION

A summary of the data for this study is given in Table 1. All of the methane, about one-third of the propane, only one (6.7 ppm) ethane, and no butane analyses were above the detection limit of approximately 1.0 ppm for these hydrocarbons. Helium concentrations are relatively uniform across the study area with a mean value of 6.0 ppm (Table 1).

There is a definite difference in the median and mean methane values for most of the formations (Figure 2), reflecting their organic contents. Of the five formations exhibiting the highest methane values for this survey (Figure 2), the Arkansas Novaculite, Bigfork Chert, Stanley Shale, and Womble Shale are the only Ouachita facies to produce oil and/or gas, or exhibit marginally commercial potentials in their western extentions in Oklahoma and Texas (Morrison, 1981). The production histories of these formations suggest that the mean methane values are an indication of the relative hydrocarbon content of these formations, i.e. those with higher mean methane contents have had greater production.

Highly mobile free helium atoms readily move up permeable zones, and therefore anomalous helium gas concentrations in ground water can be used for structural mapping (Eremeev et al., 1973). Although the helium concentrations are relatively low (6.0 ppm) and uniform across the study area, 84% of the anomalous helium concentrations are associated with mapped faults.

Table 1. Summary of data for study area. In calculations for propane values below detection were treated as zero. Concentrations are ppm based on volume.

	Methane	Propane	Helium
Mean	386	1.7	16.5
Standard Deviation	450	3.2	70.6
Median	125	<1	6.0
Maximum	2100	15	530
Minimum	1	<1	5.0

CONCLUSIONS

The fact that the highest methane values are associated with formations that have produced or have some potential for petroleum production is encouraging regarding the use of ground water as a sampling medium for exploration. This observation is especially important since there are uncertainties concerning the movement of the ground water and the importance of biogenic methane. This conclusion is also especially significant considering that the sampling design for the survey was not designed for hydrocarbon exploration in mineralized areas. It also appears that anomalous helium values can be utilized to locate major faults in the Ouachita Mountains as has been done successfully elsewhere.

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THE EFFECTS OF COMMERCIAL FISH REMOVAL, ON SPORT FISH POPULATIONS IN TWO ARKANSAS RESERVOIRS

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ABSTRACT

Commercial netting occurred October through April, 1971-1976 on Nimrod Lake and from October through April, 1973-1977 on Blue Mountain Lake in west central Arkansas. Using 7.6 cm or larger mesh gill and/or trammel nets, commercial fishermen harvested commercial fishes (buffalofishes [*Ictiobus* spp.]; common carp [*Cyprinus carpio*]; carpsuckers [*Carpiodes* spp.]; drum [*Aplodinotus grunniens*]; gars [*Lepisosteus* spp.]; suckers [*Catostomidae*]; and catfishes [*Ictalurus* spp. and *Pylodictis olivaris*]).

During the study period, cove rotenone samples were conducted on an annual basis. Fishes collected were placed into age classes and enumerated. Data were then grouped into general categories (black basses [*Micropterus* spp.], crappie [*Pomoxis* spp.], sunfishes [*Lepomis* spp.], clupeid fishes [*Dorosoma* spp], commercial fishes and catfishes) and analyzed.

Substantial reductions in standing crops of commercial fishes were noted in both reservoirs. However, in Blue Mountain Lake, reductions were temporary and commercial fish biomasses had begun to increase markedly by the end of the study period.

As catfishes were the species principally sought by commercial fishermen, it was somewhat surprising that total catfish biomasses increased in Blue Mountain Lake following the start of netting. For three of the four years prior to netting, catfish spawns were not recorded from Nimrod Lake. However, following the instigation of netting, spawns were recorded for the following four years.

Sport fish populations for the most part were unharmed by the commercial netting. In some instances, sport fish populations appeared to improve during the study period.

Visual analysis revealed that the increases in numerical standing crops of smaller sport fishes in Blue Mountain Lake appeared to correspond to decreases in commercial fish biomasses. Significant ($P \leq 0.05$) increases in sport fish young-of-the-year numbers were recorded for crappie in Blue Mountain Lake and sunfishes in Nimrod Lake and Blue Mountain Lake. Increases in numerical standing crops were also observed among intermediate black bass and crappie populations of both study lakes.

Adult black bass mean numerical standing crops were virtually unchanged in both study lakes. Adult crappie and sunfish population exhibited some variation in the study. A small increase in the adult crappie population was observed in Nimrod Lake, while a significant increase ($P \leq 0.05$) in the adult sunfish population was noted in Nimrod Lake.

In Blue Mountain Lake, an expansion of the forage base occurred which included an increase in the number of both adult and intermediate clupeid fishes present. Adult shad populations decreased in Nimrod Lake, while the numbers of young-of-the-year shad were lower in both study lakes.

It was felt that the netting program contributed significantly to the increases in sport fish populations in both lakes. However, other uncontrollable factors may have also influenced the sport fish populations in the two lakes (i.e. winter and spring water levels).

INTRODUCTION

Commercial fish removal programs have long been utilized as fisheries management tools (Moyle et al., 1950; Rose and Moen, 1952; Grice, 1958; Heard, 1959; Scidmore and Woods, 1961; Carroll et al., 1963; Starrett and Fritz, 1965; and Jester, 1972). The use of this type of management has resulted basically from four theories:

1. The removal of these fishes caused a biological void in the fishery into which forage species could expand, providing a larger forage base for game species (Simmons, 1981).
2. Since virtually all fishes go through various similar trophic levels in their development, detrimental competition between commercial (primarily rough species) fishes and sport fishes seemed plausible (Grinstead, 1975).
3. In stable bodies of water, the reproductive potential of commercial fishes was so great; through overcrowding and secretion of repressive reproductive substances; sport fish reproduction was greatly reduced (Swingle, 1956; Grinstead, 1975).
4. That commercial fishes represent an often underutilized resource which can be harvested without damaging sport fish populations (Arkansas Game and Fish Commission, 1976).

Methods which have been used in past commercial fish removal programs have varied and have included the use of explosives (Copeland, 1958), extensive shoreline seining (Rose and Moen, 1952; Hoffarth and Conder, 1967), chemical toxicants (Pintler and Johnson, 1958; Becker, 1975; Filipek, 1981), and gill and/or other types of netting (Ricker and Gottschalk, 1940; Seidensticker, 1977). Netting programs have been conducted by both investigators (Ricker and Gottschalk, 1940) and by private commercial fisherman (Heard, 1959; Carroll et al., 1963; Parrack et al., 1968; and Seidensticker, 1977).

Recently, a controversy arose concerning the use of this type of management technique with the results of a 48 state survey being instrumental in the banning of commercial netting in one southern state (Earnest Simmons, Chief, personal communication, Fisheries Division, Texas Parks and Wildlife Department, Austin). According to the survey, most states responding had used some type of commercial fish removal program, but none knew of any documentation that sport fish populations did in fact benefit from the use of this type of management.

As a fisheries management tool and as a means to use underutilized fish species, special commercial netting seasons had been conducted on two Arkansas reservoirs. It was felt that data collected from these programs might give some insight into the above controversy. The ob-

jective of this study was to retrieve data collected from past special commercial netting seasons and analyze it is an attempt to determine the impact, if any, that commercial fish removal had upon standing crops of sport fishes.

METHODS AND MATERIALS

Special commercial netting seasons occurred on two U.S. Army Corps of Engineers reservoirs in west central Arkansas. Using 7.6 cm or larger mesh gill and/or trammel nets, commercial fishermen were allowed to harvest commercial fishes and catfishes. Fishing occurred from October through April, 1971-1976 on Nimrod Lake, an 1,437 ha flood control impoundment and from October through April, 1973-1977 on Blue Mountain Lake, an 1,178 ha flood control impoundment.

As an ongoing part of the fisheries management program of the Fisheries Division of the Arkansas Game and Fish Commission, annual cove rotenone sampling was conducted on both lakes by district fisheries biologists. Rotenone sampling methodology was according to Surber (1960). Fish collected were identified to species, grouped into age class (Table 1) and enumerated.

Table 1. Size groups (cm) of fishes comprising adult, intermediate and young of year age classes.

Species	Adult	Intermediate	Young-of-Year
Black Bass	>22.9	7.6-22.9	2.5-7.6
Crappie	>17.8	7.6-17.8	2.5-7.6
Sunfishes	>12.7	7.6-12-7	2.5-7.6
Catfishes	>30.5	10.2-30.5	5.0-10.2
Gar	>61.0	10.2-61.0	5.0-10.2
Paddlefish	>61.0	10.2-61.0	5.0-10.2
Drum	>25.4	10.2-25.4	5.0-10.2
Buffalofishes	>40.6	10.2-40.6	5.0-10.2
Common Carp	>35.6	10.2-35.6	5.0-10.2
Carpsuckers	>30.5	10.2-30.5	5.0-10.2
Suckers	>30.5	10.2-30.5	5.0-10.2
Gizzard Shad	>17.8	7.6-17.8	2.5-7.6
Threadfin Shad	> 7.0	2.5-7.6	2.5-7.6

For purposes of analysis, fishes were grouped into the following general categories: black bass (*Micropterus* spp.); crappie (*Pomoxis* spp.); sunfishes (*Lepomis* spp.); clupeid fishes (*Dorosoma* spp.); catfishes (*Ictalurus* spp. and *Pylodictis olivaris)* and commercial fishes (buffalofishes [*Ictiobus* spp.]; common carp [*Cyprinus carpio]; carpsuckers [Carpiodes* spp.]; drum [*Aplodinotus grunniens*]; gars [*Lepisosteus* spp.]; and suckers [*Catostomidae*]).

Population sample data were arranged into two arbitrary groupings. To insure uniformity of the data, pre-netting data were selected to include the period four years immediately prior to the start of commercial netting. Post-netting data were selected to include a three year period following the start of netting. Data analysis in this study was by both visual analysis and by use of a students t-test for significant difference (Zar, 1974). In an attempt to determine any possible effects of outside factors on the study, historical stocking and reservoir water level data were retrieved and examined.

RESULTS

During the pre-netting period, commercial fishes comprised the bulk of the fish population of both study lakes. About 1½ years prior to the start of commercial netting, standing crops of commercial rough fishes had increased to 69.9 and 83.2 kgs/ha in Nimrod and Blue Mountain Lakes, respectively (Figure 1).

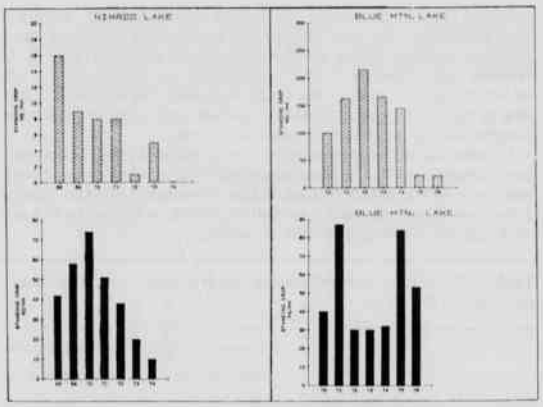

Figure 1. Commercial fish standing crops of Nimrod (1968-1974) and Blue Mountain (1970-1976) Lakes (▨ young-of-the-year fishes; ■ harvestable fishes).

Substantial reductions in adult commercial fish biomasses were observed once commercial netting began. Over a two year period, the commercial rough fish biomasses had decreased 53.8 kg/ha in Blue Mountain, while a 57.1 kg/ha decrease occurred in Nimrod Lake over a four year period. However, the reduction which occurred in Blue Mountain Lake was only temporary and by the end of the study period the commecial fish population had begun to increase markedly.

Although not as drastic, reductions in the number of young-of-the-year commercial fishes were also observed during the study (Fig. 1). Mean numerical standing crops decreased from about 144 to 53 fish/ha and from 9 to 3 fish/ha in Blue Mountain Lake and Nimrod Lake, respectively and although larger numers were present in Blue Mountain Lake, the percentage of the decrease was similar in both lakes.

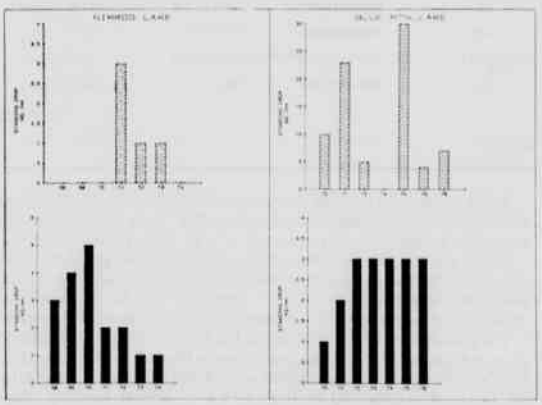

Figure 2. Catfish standing crops of Nimrod (1968-1974) and Blue Mountain (1970-1976) Lakes (▨ young-of-the-year fishes; ■ harvestable fishes).

A gradual increase in the numbers of adult catfish (Fig. 2) was observed in Blue Mountain Lake, as the mean standing crop increased about 1 kg/ha (Table 2) in the post-netting period. A decrease of similar size was also noted in Nimrod Lake (Table 3).

Catfish spawns, as indicated by the number of young-of-the-year fish

The Effects of Commercial Fish Removal, on Sport Fish Populations in Two Arkansas Reservoirs

collected (Fig. 2), fluctuated widely both before and after netting in Blue Mountain Lake, with little change in the mean numerical standing crop observed during pre and post-netting periods (Table 2). In Nimrod Lake, however, catfish spawns were not documented in any of the pre-netting years of the study. Following the initiation of netting in late 1971, young-of-the-year catfish were taken in population samples during the next four consecutive years.

Commercial netting appeared to have a minimal effect upon black bass populations (Fig. 3). Mean numerical total standing crops of black bass were 49.1 and 46.8 fish/ha in Blue Mountain Lake and Nimrod Lake, respectively during the pre-netting period, compared to 38.6 and 48.8 fish/ha following the start of netting.

Table 2. Pre-netting and post-netting standing crops from Blue Mountain Lake (1970-1976).

Species	Age Class	Pre-netting Mean Standing Crop (No. Fish/Ha)	Post-Netting Mean Standing Crop (No. Fish/Ha)
Blue Mountain Lake			
Black Bass	Adult	4.1	6.0
	Intermediate	15.7	16.71
	Young-of-Year	29.3	16.0
Crappie	Adult	20.2	9.3
	Intermediate	12.4	31.0
	Young-of-Year	182.5	427.7
Sunfish	Adult	22.7	13.7
	Intermediate	91.3	32.5
	Young-of-Year	26.1	71.09
Catfish	Adult	1.4*	2.16*
	Intermediate	0.7*	0.5*
	Young-of-Year	8.84	12.2
Forage Fish	Adult	140.7	241.1
	Intermediate	13.5	203.8
	Young-of-Year	276.0	165.7
Commercial Fish	Adult & Intermediate	44.7*	56.2*
	Young-of-Year	144.1	53.0

*kg/ha

Adult black bass populations fluctuated similarly both before and after the initiation of commercial netting (Fig. 3). A slight increase in the mean numbers/ha was observed in both lakes (Table 2 and 3).

Visual analysis revealed that intermediate black bass populations increased in both lakes during the second half of the study (Fig. 3) and although not entirely conclusive, it appeared that the increases corresponded to decreases in the commercial fish biomass which occurred.

The greatest overall variation in the black bass populations examined occurred among young-of-the-year fishes in Blue Mountain Lake (Fig. 3). Fluctuations in the population were noted both before and after the onset of commercial netting, as the numerical standing crops during the latter half of the study were significantly lower ($P \leq 0.05$). However, black bass spawns in Nimrod Lake remained relatively unchanged during the entire study. The only exception was a brief increase of approximately twofold which occurred during 1973, late in the study.

The adult crappie population of Blue Mountain Lake continued to fluctuate throughout the study period (Fig. 4). Overall mean standing crops decreased from about 20 fish/ha to 9 fish/ha. However, Nimrod Lake adult crappie populations were characterized by low, stable pre-netting populations, followed by a rapid expansion during the post-netting period (Fig. 4).

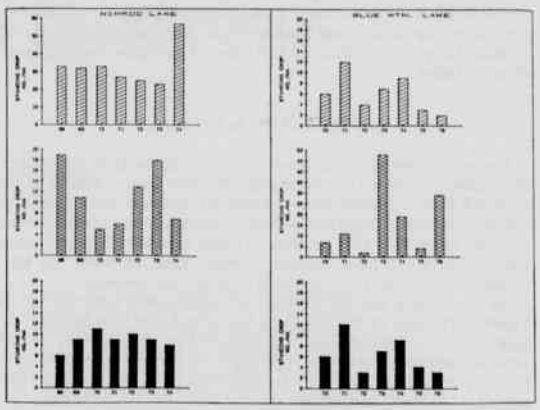

Figure 3. Black bass standing crops of Nimrod (1968-1974) and Blue Mountain (1970-1976) Lakes (▨ young-of-the-year fishes; ▩ intermediate fishes; ■ adult fishes).

Table 3. Pre-netting and post-netting standing crops from Nimrod Lake (1968-1974).

Species	Age Class	Pre-Netting Mean Standing Crop (No. Fish/Ha)	Post-Netting Mean Standing Crop (No. Fish/Ha)
Nimrod Lake			
Black Bass	Adult	7.8	8.3
	Intermediate	9.2	10.3
	Young-of-Year	29.8	30.2
Crappie	Adult	9.8	13.6
	Intermediate	16.3	28.4
	Young-of-Year	267.4	146.9
Sunfish	Adult	47.3	76.7
	Intermediate	187.9	159.8
	Young-of-Year	256.7	774.7
Catfish	Adult	1.77*	1.14*
	Intermediate	1.57*	0.3*
	Young-of-Year	0.8	0.1
Forage Fsih	Adult	598.5	236.5
	Intermediate	0.0	369.3
	Young-of-Year	453.7	184.3
Commercial Fish	Adult & Intermediate	52.5*	22.0*
	Young-of-Year	9.44	3.36

*kg/ha

Intermediate crappie populations (Fig. 4) from both lakes remained relatively low during the first half of the study period. However, during the post-netting period, significant increases ($P \leq 0.05$) occurred in both lakes.

As indicated by the number of young-of-the-year fishes collected, crappie spawns did not appear to follow any particular trend during this study, as the population increased in one lake and decreased in the other (Fig. 4). Although the increase began just prior to the start of

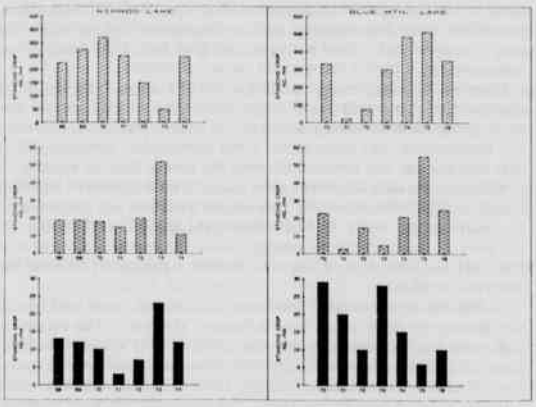

Figure 4. Crappie standing crops of Nimrod (1968-1974) and Blue Mountain (1970-1976) Lakes (▨ young-of-the-year fishes; ▩ intermediate fishes; ■ adult fishes).

netting, post-netting populations were significantly higher ($P \leq 0.05$) in Blue Mountain Lake. Crappie spawns recorded from Nimrod Lake recorded an overall decrease, until a substantial increase occurred late in the study period.

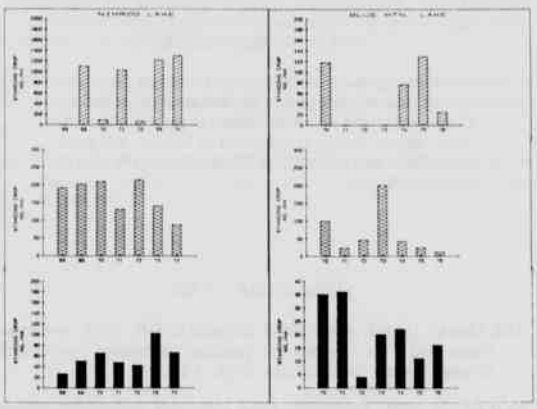

Figure 5. Sunfish standing crops of Nimrod (1968-1974) and Blue Mountain (1970-1976) Lakes (▨ young-of-the-year fishes; ▩ intermediate fishes; ■ adult fishes).

After the initiation of commercial netting, one lake exhibited a significant increase ($P \leq 0.05$), while the other exhibited a decrease in the number per hectare of adult sunfish (Fig. 5). The mean numerical standing crop increased about 30 fish/ha in Nimrod Lake, while decreasing about 9 fish/ha in Blue Mountain Lake.

Intermediate sunfish populations did not appear to follow any definite trend during the study period (Fig. 5). The Nimrod Lake population exhibited some fluctuation both before and after the onset of commercial netting, as the mean standing crop decreased about 30 fish/ha. Likewise, an even more substantial decrease occurred in Blue Mountain Lake (Table 2).

Significant increases ($P \leq 0.05$) in sunfish spawns were recorded from both lakes during the post-netting portion of the study (Fig. 5). Extreme annual fluctuations occurred early in the study in Nimrod Lake, however, during the last two years monitored, good spawns occurred for two consecutive years. Sunfish spawns were not documented for three of the four pre-netting years in Blue Mountain Lake, but young-of-year sunfishes were collected for the next three years following the beginning of commercial netting.

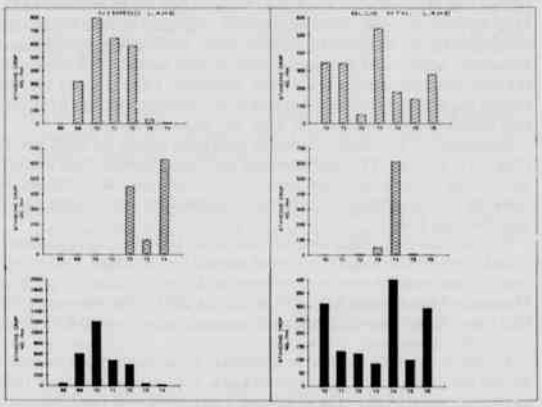

Figure 6. Clupeid standing crops of Nimrod (1968-1974) and Blue Mountain (1970-1976) Lakes (▨ young-of-the-year fishes; ▩ intermediate fishes; ■ adult fishes).

Table 4. Fish supplementally stocked into Blue Mountain Lake (1970-1976) and Nimrod Lake (1968-1974).

Lake and Date Stocked	Fish Stocked	Age Class	Total Number Stocked
Blue Mountain Lake			
January, 1971	Sunfish	Intermediate	60,000
March, 1971	Sunfish	Intermediate	111,000
May, 1971	Sunfish	Intermediate	7,200
	Catfish	Intermediate	900
August, 1971	Black Bass[1]	Young-of-Year	16,000
November, 1971	Black Bass[1]	Young-of-Year	15,000
Nimrod Lake			
August, 1972	Catfish	Intermediate	1,886
	Sunfish[2]	Intermediate	2,000
September, 1972	Catfish	Intermediate	800
October, 1972	Catfish	Intermediate	3,600

[1]Largemouth Bass

[2]Mixed Sunfish

Just prior to the start of netting, a decline in the adult clupeid population (Fig. 6) of Nimrod Lake was observed, which continued throughout the remainder of the study. The mean numerical standing crop decreased from 598.5 fish/ha to 236.5 fish/ha. The trend observed in Nimrod Lake did not repeat itself in Blue Mountain lake where an overall increase occurred in the study period (Fig. 6). Relatively stable, low pre-netting clupeid populations were followed by higher, somewhat fluctuating populations, as an increase in mean standing crop of about 100 fish/ha was observed.

In both study lakes, a significant increase ($P \leq 0.05$) in the nubmers of intermediate shad was noted during the post-netting period (Tables 2 and 3). In Nimrod Lake, there was no evidence of this size shad for the entire pre-netting period, however, a population expansion began

during the first post-netting year (Fig. 6). Likewise, no shad of this size were collected three of the four years prior to netting in Blue Mountain lake. Like the situation in Nimrod Lake, an increase in the population occurred immediately following the start of netting (Fig. 6).

In both lakes, substantial decreases in mean standing crops of young-of-the-year clupeids were recorded (Tables 2 and 3). In Nimrod Lake, after an early increase in the population, a gradual decline began that continued for the duration of the study (Fig. 6). The decrease appeared somewhat accelerated during the post-netting period. The young-of-the-year shad population from Blue Mountain Lake (Fig. 6) did not exhibit the gradual decrease, but was more variable with some fluctuation observed both before and after the onset of netting.

Supplemental stockings occurred in both lakes during the study period (Table 4). During 1971, intermediate sunfishes, intermediate catfish, and young-of-the-year black bass were stocked into Blue Mountain Lake. In 1973, intermediate catfish and intermediate sunfish were placed into Nimrod Lake.

For the most part, water levels of both study lakes fluctuated seasonally and were within ranges considered normal for each lake. One exception was an extreme winter drawdown which was conducted on Blue Mountain Lake during late 1970 and early 1971. The volume of the lake was reduced from 30.8 million m^3 at conservation pool to 6.2 million m^3 for the drawdown.

Excessive rainfalls resulted in abnormal water levels being recorded during the springs of 1970 in Nimrod Lake, 1973 in both lakes and 1975 in Blue Mountain Lake. The volume of water in Nimrod Lake increased from 35.8 million m^3 at conservation pool to 218.2 million m^3 during 1970. In 1973, volumes of 318.1 million m^3 and 456.4 million m^3 were recorded from Blue Mountain Lake and Nimrod Lake, respectively, while in 1975 the spring storage maximum recorded in Blue Mountain Lake was 147.9 million m^3.

DISCUSSION

For the most part, the special commercial netting seasons were successful in reducing harvestable commercial fish populations in both study lakes. The increase which occurred in Blue Mountain Lake, late in the study period, was attributed to lowered fishing pressure following the initial decline of higher valued commercial species such as the buffalofishes. Similar population increases resulting from lowered commercial fishing pressure were also reported by Jester (1972).

Catfish populations were affected little during the study period and since catfish usually are the species most actively sought by commercial fishermen, it was somewhat surprising that a greater impact was not observed. Another indication that catfish populations were not adversely affected were the unchanged spawns recorded from Blue Mountain Lake and the marked increase in numbers of young-of-the-year catfish collected from Nimrod Lake following the instigation of commercial netting.

It has been shown that "cropping" or partial harvest increases biomasses of catfish in the pond environment (Snow, 1976). Although entirely speculation, it might be that cropping has some application in the reservoir environment and could merit future investigation.

In determining the possible effects of commercial fish removal on the standing crops of sport fishes in the two study lakes, it was felt that any effects on fish available to the sportfishermen would be of foremost importance. Generally, this would represent adult fishes, but under the classification system used (Table 1) also encompassed some intermediate fishes.

Of the twelve harvestable sport fish populations in the study (including both adult and intermediate fishes), increases were observed in eight of the populations. With the exception of crappie and sunfish populations of Blue Mountain Lake, all adult populations expanded during the post-netting period. This might be an indication that gill and/or trammel netting with 7.6 cm or larger mesh nets does not harvest large numbers of catchable sport fishes. Similar opinions have been expressed by Heard (1959), Bailey (1971) and Seidensticker (1977).

As both increases and decreases occurred, no definite trend could be established concerning possible effects of the commercial fish removal upon sport fish spawning activities. The increased numbers of intermediate sport fishes present during the post-netting period would tend to suggest that at least in this study higher larval fish survival and subsequently, a higher recruitment potential existed.

Likewise, although mean standing crops of young-of-the-year shad decreased, higher numbers of intermediate shad suggest greater survival during the post-netting period of this study. The increase of adult and intermediate shad might reflect views expressed by Simmons (1981) that commercial fish removal allowed the forage base to expand.

Although periodic stockings were made in both bodies of water, it would be highly doubtful that they would have had any influence on the results of this study. In Blue Mountain Lake, all stockings occurred prior to the initiation of netting, while the numbers stocked into Nimrod Lake were deemed too small to have significantly affected the observed results.

As both lakes are primarily flood control structures, water level regulation during the study could have influenced the study. The extremely high water levels observed during the spring of 1973 would have inundated large areas of normally dry shoreline and would have caused a "new reservoir" environment (Keith, 1974). This undoubtedly was a primary factor in the increased sport fish spawns which were recorded during 1973.

For the most part, the sport fish population observed in this study did not appear to be harmed by commercial netting. Also, since some populations did expand during the post-netting period, the distinct possibility exists that the commercial fish removal by netting was beneficial. However, since many uncontrollable factors exist in the reservoir environment (i.e., water level regulation, fishing pressure, etc.), it would be impossible to emphatically state that the observed results of this study were directly the result of the commercial fish removal.

ACKNOWLEDGEMENTS

Sincere appreciation is expressed to present and former district fisheries personnel of the Arkansas Game and Fish Commission who were instrumental in gathering the data. Also, sincere thanks is expressed to Messrs. Bill Keith, Larry Rider and Mike Freeze for graciously reviewing the manuscript.

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TEMPERATURE PREFERENCE AND TOLERANCE OF GRASS CARP *(CTENOPHARYNGODON IDELLA)*

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ABSTRACT

Grass carp, acclimated at 24 °C, were tested for temperature preference in a laboratory, horizontal gradient tank. After a 6-day period of exploration the grass carp gravitated to a final thermal preferendum of 35 °C. In the temperature tolerance test the ultimate upper incipient lethal temperature (TL50), was estimated as 41.5 °C. The results are compared to those of a similar study with hybrid carp (female grass carp x male bighead carp).

INTRODUCTION

Knowledge of temperature tolerance and preference of a fish species is valuable in understanding interactions between fish and their environment especially regarding distribution, growth, and viability. This paper reports on the temperature preference and tolerance of grass carp, *Ctenopharyngodon idella,* under laboratory conditions.

MATERIALS AND METHODS

The temperature preferendum experiment was conducted in a horizontal gradient tank filled with 160 l of water. The gradient tank was divided into six compartments (each 112 cm long × 66 cm wide × 36 cm deep) by plexiglass partitions having openings (15.5 × 13.5 cm) that could be closed by a sliding plexiglass plate. The static thermal gradient was maintained by the placement of a variable number of 200 and 150 watt immersion heaters in the compartments and a cooling coil at one end of the tank. Thermal stratification was prevented by aerating with air stones. A relatively uniform gradient existed in each compartment except occasionally in the area in or immediately adjacent to the intercompartmental openings. Water temperatures were measured at least 4 cm away from the openings when determining the mean water temperature of each compartment.

The grass carp used in this study were obtained from Malone's Fish Hatchery, Lonoke, Arkansas in April 1982. Twenty-one juvenile fish (total length range 250-300 mm, weight range 130-250 g) were individually marked by numbered anchor tags and were acclimated to 24 °C over a one month period prior to testing in the gradient. The fish were maintained on a diet of water cress *(Nasturtium sp.).*

On the 8th of August, 1982, at the end of the acclimation period, the grass carp were released into the compartment corresponding to their acclimation temperature. They were given two hours to adjust and settle down before the intercompartmental doors were opened. At 24 h intervals at approximately 1500 CST, the openings in the intercompartmental partitions were covered and the presence of fish and water temperatures in each of the compartments were recorded. The temperature preferendum experiment was conducted for 11 days employing the gravitational method. Due to the extended length of the study period, each of the compartments was provided daily with 100 g of water cress as a maintenance ration for the fish. Water clarity was somewhat reduced due to feeding but the fish could be clearly observed throughout the study. Uneaten plant remains were removed and water added periodically to make up for evaporative loss.

After completion of the temperature preferendum experiment, six grass carp (average total length and weight, 277 mm; 185.5 g) acclimated to 34 °C, were placed in a glass tank (89 cm long × 46 cm wide × 51 cm deep) with 170 l of water and aerated with air stones, to determine temperature tolerance (upper ultimate incipient lethal temperature). The water temperature was raised by 1 °C per day increments by the addition of hot water and was maintained thermostatically. At each of the test temperatures, the fish were observed for 24 h for the occurrence of mortalities. Criteria for death were cessation of body, fin, and opercular movements. The upper ultimate incipient lethal temperature (UUILT), (Cocking 1959), was estimated by interpolation at which 50% of the test population died (TL50). During the temperature tolerance test a small amount of water cress (<75 g) was provided for approximately the first hour after raising the test temperature.

RESULTS

Temperature Preferendum

Fish were released into the gradient at 24 °C, at all subsequent observations the fish were in compartments with a minimum temperature at or above 28.8 °C. There was evidence of exploratory behavior during the first six days of the study. During this exploratory period

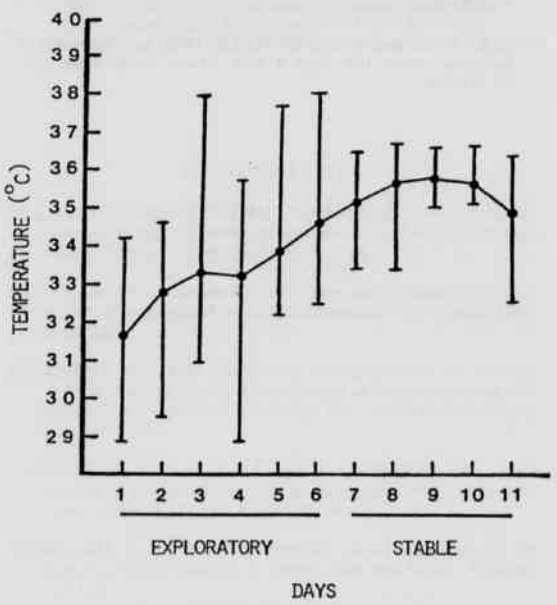

Figure. Daily mean preferred temperature and range of occurrence of grass carp in a thermal gradient.

Table. Frequency of occurrence of grass carp in the thermal gradient.

Temperature (°C)	Frequency of occurrence (%)	
	Exploratory phase	Stable phase
29	21.0	-
30	-	-
31	11.3	-
32	-	-
33	19.4	18.8
34	19.4	-
35	19.4	45.8
36	-	35.4
37	8.9	-
38	0.8	-

the fish had an average daily water temperature range of occurrence in the gradient of 6 °C (Figure), with no clear mode in their distribution (Table). On the 3rd, 5th, and 6th days of the experiment, 7, 4, and 1 fish, respectively, were observed in compartments with a mean water temperature above 37 °C.

On the 7th through 11th days the fish had an average daily range of occurrence of 2.5 °C (Figure), indicating that they had stabilized around their final thermal preferendum. The final thermal preferendum was estimated to be 35 °C based upon the occurrence of a single mode during the stable period (Table). Schooling behavior was observed throughout the temperature preferendum experiment.

Temperature Tolerance

There were no mortalities up to the 41 °C test temperature, however all the fish died at 42 °C. The UUILT was estimated as 41.5 °C. The observed normal feeding activity of entering vegetation clumps, and tearing loose and consuming plant material was observed up through 40 °C. From 34 to 40 °C schooling behavior was not affected and no apparent change in opercular movements were observed. At 41 °C feeding activity was markedly reduced, opercular movements were increased, and the fish often swam with their mouth near the air-water interface. The fish did, however, maintain at least some schooling behavior. At 42 °C the fish showed no interest in feeding. Opercular movements became very fast, the fish were nervous or excitable, moving around constantly and erratically, and surfacing frequently. At 42 °C the fish died within 2.5 h.

DISCUSSION

The thermal preference test was conducted for an extended period of time (11 days), thus each compartment was provided daily with water cress. All compartments received the same amount of food, thus availability probably had little effect on the results.

The final thermal preferendum of grass carp (35 °C), was much higher than the temperatures reported for spawning activities, 14 to 22 °C, (Kuronuma, 1958; Martino, 1974). Due to the lack of published information concerning the temperature preferendum and tolerance of Chinese carps, comparisons are made with that of a hybrid carp, female grass carp × male bighead carp *Aristichthys nobilis* (Kilambi and Galloway, 1985).

It has been reported that for most fish species of North America, one to three days in the thermal gradient is sufficient time for the final thermal preferendum to be reached, (Richards et al., 1977; Reynolds and Casterlin, 1979). In this study the exploratory phase represents that part of the test period when the fish are still gravitating to the final thermal preferendum and are exploring a relatively wide range of available temperatures. The stable phase represents that part of the test period when the fish have gravitated to the final thermal preferendum and are exploring a relatively narrow range of available temperatures. Both the grass carp and hybrid carp had extended exploratory phases, of 6 and 9 days, respectively. The ranges of temperatures being encountered during this phase were relatively greater than during the stable phase; 6 vs 2.5 °C for grass carp and 4.5 vs 1.3 °C for the hybrid carp. During the exploratory phase the grass carp had a steady upward gravitation toward the final thermal preferendum (35 °C), and only a few fish explored temperatures as high as 38 °C. The grass carp thus avoided temperatures near the UUILT (41.5 °C). In contrast, Kilambi and Galloway (1985), showed a bimodal distribution during the exploratory phase for the hybrid carp. The hybrid carp had a nine day exploratory phase. On the first day they explored temperatures 3 to 9 °C above their final thermal preferendum and near their UUILT of 39.2 °C with some occurrence of mortalities due to heat stress. Then the fish gravitated downward to temperatures near their final thermal preferendum for two days followed by a second upward gravitation to a temperature of approximately 34 °C. Subsequently, the hybrids gravitated to a final thermal preferendum of 29 °C.

In the temperature tolerance experiment, the grass carp remained behaviorly unstressed up to 40 °C. At 41 °C the fish showed signs of heat stress, however, the grass carp were still exhibiting at least some schooling behavior. After raising the temperature to 42 °C, grass carp moved as very excitable individuals, opercular movements were rapid, and all fish lost equilibrium and died within 2.5 h, thus the UUILT was determined as 41.5 °C. In contrast the hybrid carp exhibited stress as early as 35 °C, when schooling and feeding behavior were first effected (Kilambi and Galloway, 1985). Schooling behavior ceased at 37 °C with one fish surviving as high as 41 °C. The UUILT was estimated as 39.2 °C.

Studies of both the grass carp and hybrid carp indicate that extended studies may be necessary to determine the final thermal preferendum of these subtropical-temperate species. The grass carp was similar to many species of North American fish as the difference between the initial (acute) preferred temperature and final thermal preferendum was less than 5 °C, and the gravitation was steady and upward with some evidence of overshooting. The hybrid carp behavior is unique with their two large overshoots of the final thermal preferendum, including exploration of highly stressful water temperatures near their UUILT by the majority of the fish. Further elucidation of this phenomenon will await temperature preference and tolerance studies on the bighead carp.

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GAS CHROMOTOGRAPHIC ANALYSES OF BIOCRUDE-PRODUCING TREES

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ABSTRACT

Gas chromotographic procedures were used to compare commercial diesel fuel with cyclohexane, ether, and methanol extracts from various tree species. Standard n-paraffin hydrocarbons ranging from C-10 thru C-34 were used as standards. These analyses indicated that several extracts, notably those from *Juniper virginiana* (juniper) and *Pinus echinata* (pine) trees of Northeast Arkansas and the Brazilian tree *Copaifera langsdorffii* (copaiba), contain numerous hydrocarbon and selected chemical products which serve as potential renewable biocrude sources.

INTRODUCTION

Photosynthetic plants produce extractable chemicals called biocrude which can be used directly as petroleum-like chemicals (Buchanan et al., 1978a; Buchanan et al., 1978b; Calvin, 1977, 1979; Buchanan et al., 1980; Wang and Huffman, 1981; McLaughlin and Hoffmann, 1982; Campbell, 1983). Biocrude is the hydrocarbon and hydrocarbon-like chemical fraction of plants which may be extracted by organic solvents and upgraded to liquid fuels and chemical feedstocks (McLaughlin and Hoffmann, 1982). Feedstocks are mixtures of materials, derived from the source by physical and/or chemical means, whose composition is controlled to make specific primary chemicals or fuels (Lipinsky, 1981). Extractable biocrude may contain waxes, terpenoids, resins, phytosterols, latex, terpenes, fats, fatty acids, polyphenolics, phlobaphenes, oils, and tannins (McLaughlin and Hoffmann, 1982).

Since many of the plant extractives that could serve as potential liquid fuels have high carbon numbers and are not combustible at low temperatures, these fuel stocks could be subjected to catalytic cracking (Wang and Huffman, 1981). Major plant substances that could be catalytically converted are terpenoids, phenolics (flavonoids, phenols, and polyphenols) and long chain aliphatics (waxes, triglycerides, fatty acids) (Adams and McChesney, 1983). Weisz et al. (1979) studied the mechanism for the conversion of plant extracts rich in hydrocarbons and/or hydrocarbon-like compounds into low molecular weight fuels. They found that Mobil's zeolite catalyst could catalyze molecules such as latex and oils into products comparable to fuel gas. In all cases studies, there was a high degree of conversion into benzene (C-6), toluene (C-7), xylenes (C-8), and other aromatics. Although they could convert various plant materials into high grade liquid fuel, the economic feasibility of the process was a concern, that was yet to be determined.

Much of the current interest in biocrude research seems to be focused on identifying the best biocrude producing plants and determining economic feasibilities. Buchanan et al. (1980) and Adams (1982) suggested that agricultural production of hydrocarbons would be economically feasible only if the entire plant were harvested and processed. This concept would involve the development of multi-use crops (biocrude, fiber, food) with the final choice of multi-use plant species dependent upon survival, growth rate, ease of obtaining biocrude, productivity, and the quality and quantity of extractibles. Species should also be evaluated on the need for fertilizer, especially nitrogen.

Bassham (1977) and Calvin (1979) have suggested the development of biocrude farms or plantations. Calvin (1979) further suggested developing these farms in the arid Southwest. This would put to cultivation immense areas of unused land unsuitable for conventional crops. Johnson and Hinman (1980) recommended development of marginal lands for biocrude farming because they would not compete with food and fiber crops. Calvin (1979) identified members of the genus *Euphorbia* and the genus *Asclepias* as the best hydrocarbon crops for these and southwestern lands, especially *Euphorbia lathyris*. Here, entire plants would be harvested and processed.

This preliminary study utilized gas chromatographic analyses to identify trees having potential for biocrude production. Economic feasibility of biocrude production was not considered.

MATERIALS AND METHODS

Copaifera langsdorffii Desf. seeds were obtained from Brazil and grown in a greenhouse. All other experimental species were collected from their natural habitat in Northeast Arkansas. Samples consisting of young stems without leaves were collected from *Asimina triloba* L. Dunal (pawpaw), *C. langsdorffii* (copaiba), *Gleditsia tricanthos* L. (thorn), *Juniper virginiana* L. (juniper or eastern red cedar), *Pinus echinata* Mill. (short leaf pine), *Rhus copallina* var. Latifolia (dwarf sumac) and *Sassafras albidum* (Nutt.) Nees. (sassafras). The samples were oven dried and ground before weighing.

Tissue samples were extracted with ether overnight (Fig. 1A). Pigments and polar materials were removed with Darco G-60 activated charcoal. Internal standard was added before the extracts were dried under nitrogen. Separate stem tissue samples were extracted in a Soxhlet extractor for approximately 12 hours with 150 ml of cyclohexane (Fig. 1B). The cyclohexane extract was transferred to a rotary evaporator to remove excess cyclohexane. The ground stem tissue, which had been extracted with cyclohexane, was then extracted with methanol as previously described for the cyclohexane extraction (Fig. 1B). Internal standard was added to the cyclohexane and methanol extract before drying. The dried extracts obtained with ether, cyclohexane, and methanol solvents were stored dry at -10°C in vials covered with teflon tape. Each extract was redissolved in one ml of ether before a 4µl sample was injected into the chromatograph. A second chromatographic analysis was run with the methanol extract redissolved in 87.5% methanol.

Gas chromatography of extracts was performed using a Perkin-Elmer Model 3920 B chromatograph with dual flame ionization detectors (F.I.D.). The chromatograph was equipped with a 6 ft × 0.085 I.D. stainless steel column packed with 5% silicone SE 30 on 100/120 chromosorb WHP (Alltech Associates, Inc.), The chromatograph was programmed for an injection temperature of 190°C with an initial temperature of 125°C (8 min) changing at a rate of 8°C/min with a final temperature of 290°C (32 min) and a nitrogen flow rate of 8 ml/min. The chromatograph was attached to a Varian Vista 401 data system which collected, analyzed, and stored all data.

Standard n-paraffin hydrocarbons (Alltech Associates, Inc.) ranging from C-10 to C-34 were used for preliminary identification and quantification of extracts. N-triacontane (C-30) was the internal standard

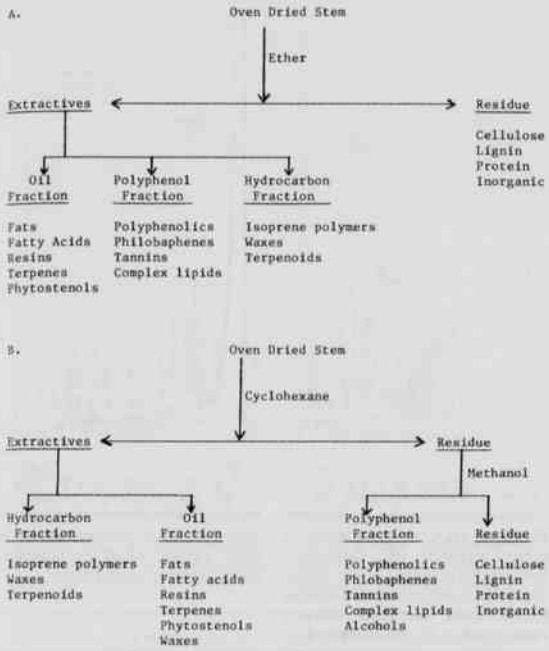

Figure 1. Scheme for extracting stem components (A. modification of the scheme presented by Buchanan et al., 1979; B. Buchanan et al., 1979).

(0.186 mg/ml) added to all extracts and was the basis for determination of correction factors for all other n-paraffins (Table 1). Final data was corrected to mg of extract detected by F.I.D. per gm dry weight of stem tissue. The chromatograph was calibrated each day to update the retention times for the n-paraffin standards. Typical retention times for the standards are shown in Fig. 2. These retention times and their corresponding n-paraffin standards were recorded as references in the chromatograms of each sample.

RESULTS

The commercial diesel fuel samples contained a wide variety of components (Fig. 3). Several components had retention times comparable to those of the small n-paraffin standards (Fig. 2). The copaiba tree ether extracts were similar to the diesel fuel in the numerous components were present. These extracts, however, had a greater proportion of larger molecules and no components were indicated with retention times less than the C-14 n-paraffin standard (Fig. 4). Ether extracts of the sumac and thorn tissues contained relatively few components, all in meager amounts (Figs. 5, 6). Additional analyses indicated that the most abundant component in the thorn extract eluted between the C-28 and C-30 n-paraffins (Fig. 7). The retention times of the components in the pawpaw and shortleaf pine ether extracts indicated the presence of medium to large molecules in relatively abundant amounts (Figs. 8, 9). In contrast, sassafras ether extracts contained small amounts of many small nonpolar molecules and conspicuously large quantities of two large components (Fig. 10). Juniper ether extracts were exceptionally abundant in a wide range of molecules as evidenced by retention times ranging from less than five min to more than 40 min (Fig. 11).

All the cyclohexane extracts of the various tree tissues were similar in that each extract contained several minor components and only one

Table 1. n-Paraffin Standards

Peak Number	Retention Time	Carbon Number	n-Paraffin	Correction Factor[a]	Amount (mg/ml)
1	2.76	*C10	Decane	2.67675	0.190
2	4.18	*C11	Hendecane	1.47443	0.230
3	6.61	*C12	Dodecane	1.45125	0.240
4	10.26	*C13	Tridecane	1.29877	0.246
5	13.02	*C14	Tetradecane	1.26864	0.254
6	15.24	*C15	Pentadecane	1.34584	0.176
7	17.01	*C16	Hexadecane	1.18011	0.192
8	18.58	*C17	Heptadecane	1.20771	0.184
9	19.97	*C18	Octadecane	1.15569	0.244
10	21.29	*C19	Nonadecane	1.04052	0.226
11	22.51	*C20	Eicosane	1.05701	0.228
12	23.65	*C21	Hereicosane	1.08329	0.384
13	24.78	*C22	Docosane	1.05617	0.204
14	25.83	*C23	Tricosane	1.08899	0.204
15	26.84	*C24	Tetracosane	1.08337	0.168
16	27.80	*C25	Pentacosane	1.04970	0.238
17	28.74	*C26	Hexacosane	0.99540	0.196
18	30.77	*C28	Octacosane	1.10684	0.250
19	33.56	*C30	Triacontane	1.00000	0.186
20	37.64	*C32	Dotriacontane	1.10721	0.268
21	43.77	*C34	Tetratriacontane	1.25787	0.202

[a]n-paraffin correction factors were calculated on the basis of C-30 which was assigned a value of 1.00.

major component. The retention time for the major component was similar to n-paraffin C-24. The shortleaf pine and sassafras extracts also contained minor components that eluted after the internal standard (Figs. 12-17).

The dried methanol extracts were gummy residues only slightly soluble in ether or methanol. However, the extracts were almost completely soluble in warm 87.5% methanol. The GLC chromatograms of extracts redissolved in ether showed internal standard (C-30) peaks whereas the chromatograms of extracts redissolved in 87.5% methanol did not. The thorn methanol extracts were low in biocrude materials (Figs. 18, 19). The methanol extracts of pawpaw, sumac, pine, sassafras, and juniper tissues contained much biocrude materials. Of these, the juniper extracts should be noted for their relatively high quantities of biocrude substances and the sassafras extracts noted for their abundance of low molecular weight biocrude components (Figs. 20-29).

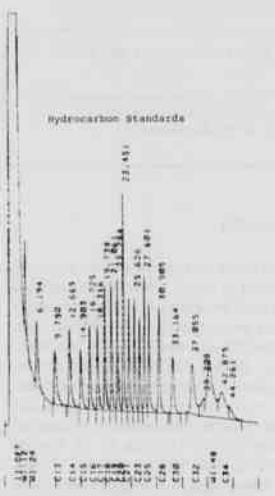

Figure 2. GLC chromatogram of n-paraffin hydrocarbon standards.

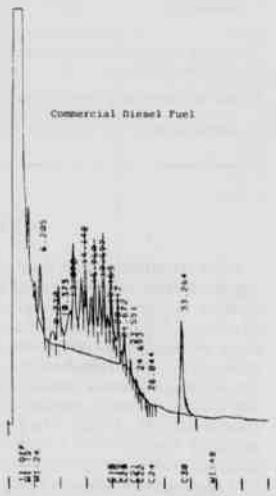

Figure 3. GLC chromatogram of commercial diesel fuel.

Solvent extraction yields for the species analyzed are given in Table 2. The highest yield by ether extraction of oven dried stem tissue was obtained from eastern red cedar. Copaiba was a distance second followed in decreasing order by pine, pawpaw, sassafras, sumac, and thorn.

Copaiba was not extracted in cyclohexane-methanol. Sassafras gave the highest yield in cyclohexane. Sumac gave unexpected high cyclohexane yields equal to red cedar followed by pawpaw. Pine, a species known to be high in resin, gave a lower cyclohexane yield than expected. Thorn, again, gave the lowest cyclohexane extraction yields.

Soxhlet extractions yielded higher extraction concentrations with methanol than cyclohexane in all species studied. This was consistent with data reported by Erdman and Erdman (1981) and McLaughlin and Hoffmann (1982). GLC chromatograms of methanol extractives redissolved in ether indicate much lower yields than when these extracts are redissolved in 87.5% methanol/water. This is probably due to the high concentration of polar compounds in this fraction with low ether solubility. Eastern red cedar (juniper) again yielded the greatest quantity of extractives followed in decreasing order by sumac, pine, pawpaw, sassafras, and thorn.

Table 2. Summary of biocrude extracts[a,b]

Species	Ether extract	Soxhlet Extraction: cyclohexane	Soxhlet Extraction: methanol: ether	Soxhlet Extraction: methanol: 87.5% methanol
Asimina triloba (pawpaw)	1.14	0.15	0.4	6.45
Copaifera langsdorffii (copaiba)	2.40	--	--	--
Gleditsia tricanthos (thorn)	0.12	0.08	0.17	0.12
Juniper virginiana (juniper, eastern red cedar)	6.33	0.18	1.25	21.42
Pinus echinata (short leaf pine	1.80	0.09	0.56	7.78
Rhus copallina (dwarf sumac)	0.31	0.18	0.42	8.25
Sassafras albidum (sassafras)	0.87	0.22	0.17	4.87

[a] Reported as mg of extract detected by F.I.D. per gram day weight of stem tissue.

[b] Ether extracts were redissolved in ether; cyclohexane soxhlet extracts were redissolved in ether; and, methanol soxhlet extracts were redissolved in ether or 87.5% methanol.

DISCUSSION

Ether extraction of oven dried stem tissue was performed by suspending dried tissue overnight (12-15 hours) at room temperature without shaking. Soxhlet extraction was not used because of the high volatility of ether. F.I.D. analyses of the ether extract gave higher values than those obtained with cyclohexane possibly due to the higher solubility parameter of ether (Buchanan et al., 1978b) which permitted extraction of the polyphenolic fraction. The cyclohexane extraction of sassafras was the only exception possibly due to the high oil content of sassafras (Buchanan et al., 1978a). The higher cyclohexane-nethanol extraction probably resulted from a high polyphenolic fraction in all species analyzed which is consistent with data reported by Adams (1982), McLaughlin and Hoffmann (1982), Adams and McChesney (1983), and others.

The total cyclohexane-methanol extract was significantly below that reported by Buchanan et al. (1978a), Erdman and Erdman (1981), McLaughlin and Hoffmann (1982) and others. Adams and McChesney (1983) reported a minimum of 20 hours of soxhlet extraction produced more than 95% extraction with both cyclohexane and methanol.

Assuming sassafras is a potential biocrude source (Buchanan et al.,

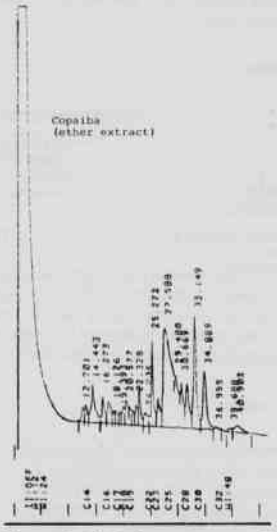

Figure 4. GLC chromatogram of ether extract of *Copaifera langsdorffii* (copaiba) stem tissue. (All extracts are redissolved in ether except those methanol extracts redissolved in 87.5% methanol.)

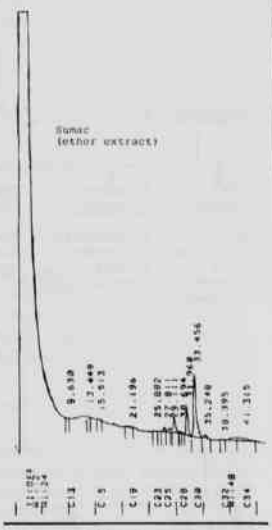

Figure 5. GLC chromatogram of ether extract of *Rhus copallina* (dwarf sumac) stem tissue.

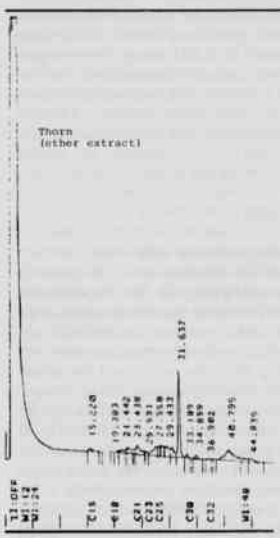

Figure 6. GLC chromatogram of ether extract of *Gleditsia tricanthos* (thorn) stem tissue.

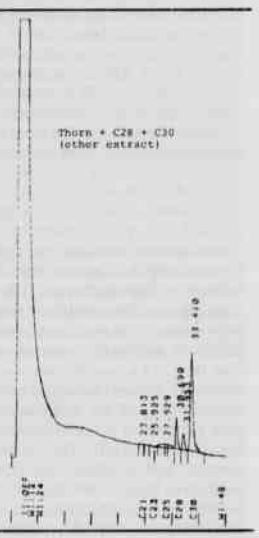

Figure 7. GLC chromatogram of ether extract of *Gleditsia tricanthos* (thorn) stem tissue plus octacosane and triacontane n-paraffin hydrocarbons.

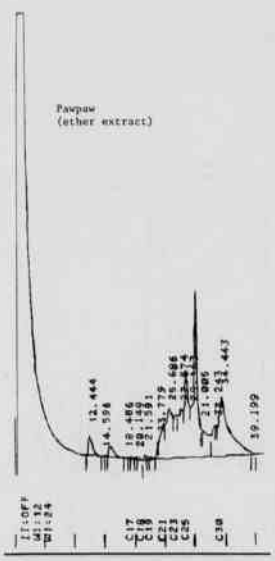

Figure 8. GLC chromatogram of ether extract of *Asimina triloba* (pawpaw) stem tissue.

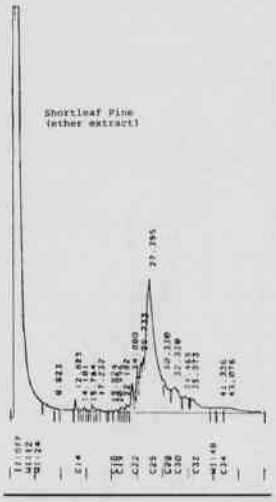

Figure 9. GLC chromatogram of ether extract of *Pinus echinata* (shortleaf pine) stem tissue.

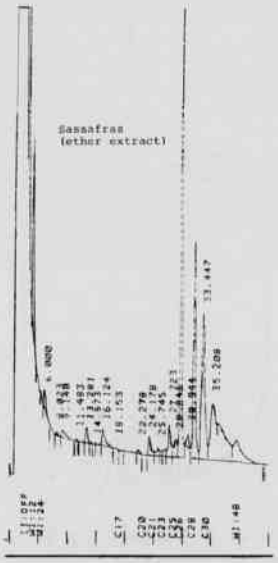

Figure 10. GLC chromatogram of ether extract of *Sassafras albidum* (sassafras) stem tissue.

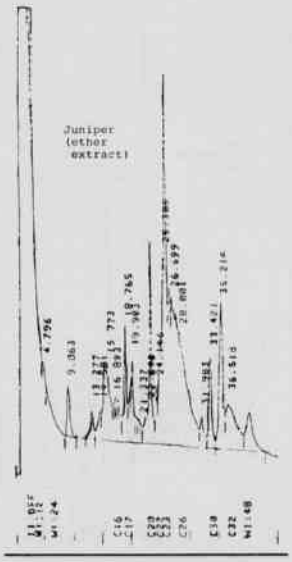

Figure 11. GLC chromatogram of ether extract of *Juniper virginiana* (eastern red cedar) stem tissue.

1978a), all species with greater total cyclohexane-methanol extract could also be considered as potential sources of biocrude (see Table 2). Pawpaw, pine, sumac, and eastern red cedar have larger total cyclohexane-methanol extract values than sassafras.

The cyclohexane extract, containing the oil and hydrocarbon fractions (Buchanan et al., 1978b), is the high energy components of plants most efficiently converted into burnable liquid fuels and feedstocks (McLaughlin and Hoffmann, 1982). Adams (1982) reported that the

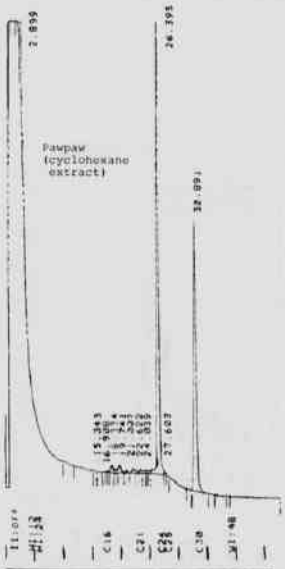

Figure 12. GLC chromatogram of cyclohexane extract of *Asimina triloba* (pawpaw) stem tissue.

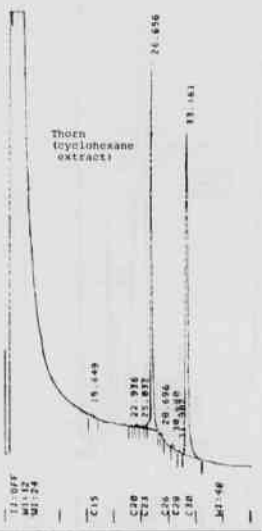

Figure 13. GLC chromatogram of cyclohexane extract of *Gleditsia tricanthos* (thorn) stem tissue.

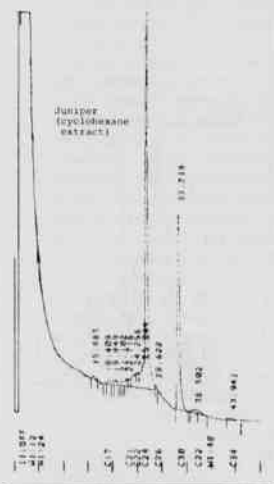

Figure 14. GLC chromatogram of cyclohexane extract of *Juniper virginiana* (eastern red cedar) stem tissue.

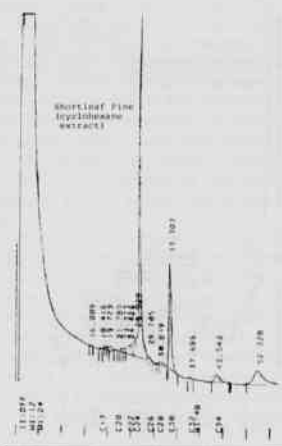

Figure 15. GLC chromatogram of cyclohexane extract of *Pinus echinata* (shortleaf pine) stem tissue.

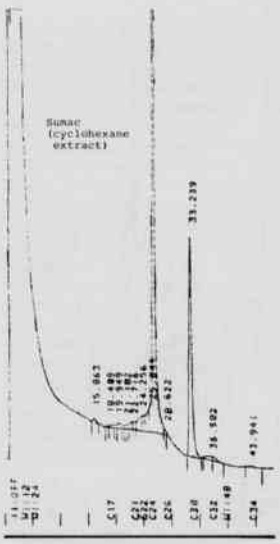

Figure 16. GLC chromatogram of cyclohexane extract of *Rhus copallina* (dwarf sumac) stem tissue.

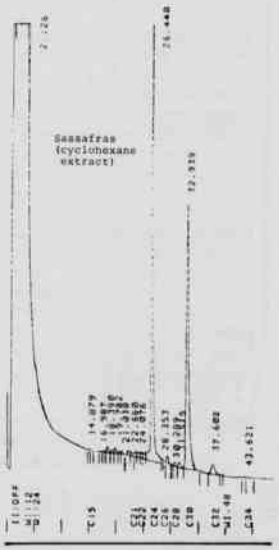

Figure 17. GLC chromatogram of cyclohexane extract of *Sassafras albidum* (sassafras) stem tissue.

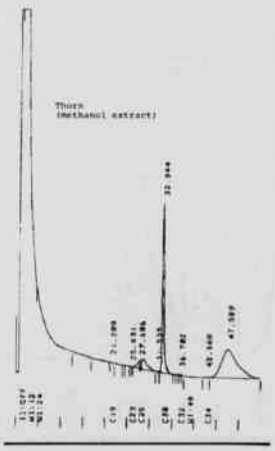

Figure 18. GLC chromatogram of methanol extract of *Gleditsia tricanthus* (thorn) stem tissue.

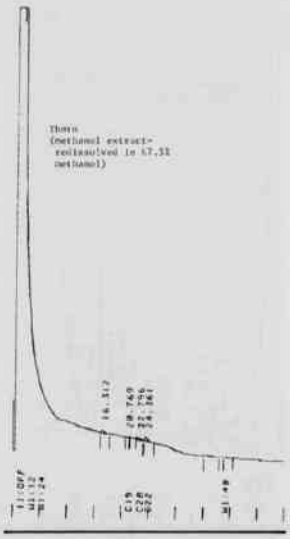

Figure 19. GLC chromatogram of methanol extract of *Gleditsia tricanthus* (thorn) stem tissue (redissolved in 87.5% methanol).

heat value of the hexane (cyclohexane) extract is comparable to crude oil. Although the cyclohexane extract has more than 2.2 times the heat value of the methanol extract (Erdman and Erdman, 1981) the high quality of methanol extract compensates for its lower heat value.

Plants producing the highest amounts of biocrude are latex and resin producers (McLaughlin and Hoffmann, 1982). Both are composed of isoprene polymers and represent promising potential fuel sources and can be readily collected by tapping. Latex has molecular weights rang-

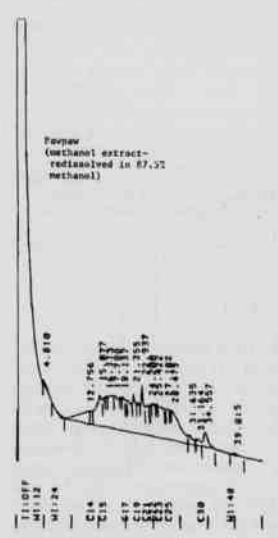

Figure 20. GLC chromatogram of methanol extract of *Asimina triloba* (pawpaw) stem tissue.

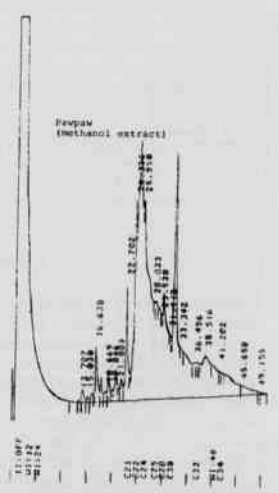

Figure 21. GLC chromatogram of methanol extract of *Asimina triloba* (pawpaw) stem tissue (redissolved in 87.5% methanol).

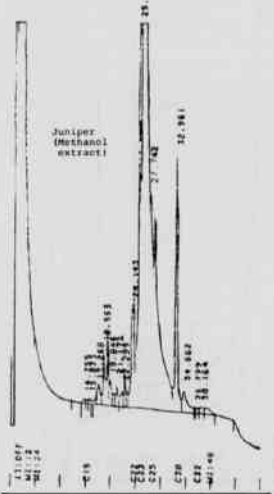

Figure 22. GLC chromatogram of methanol extract of *Juniper virginiana (eastern red cedar) stem tissue.*

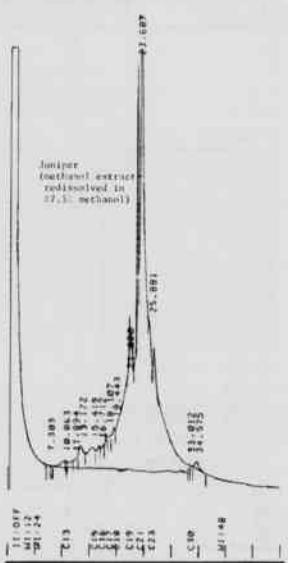

Figure 23. GLC chromatogram of methanol extract of Juniper virginiana (eastern red cedar) stem tissue (redissolved in 87.5% methanol).

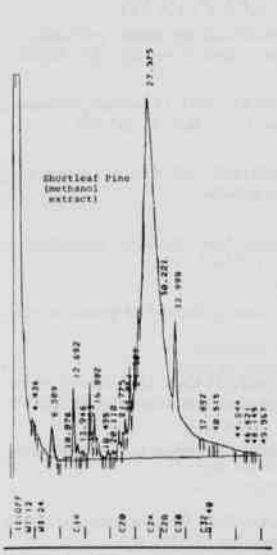

Figure 24. GLC chromatogram of methanol extract of *Pinus echinata* (shortleaf pine) stem tissue.

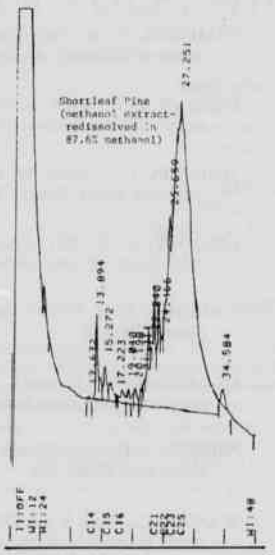

Figure 25. GLC chromatogram of methanol extract of *Pinus echinata* (shortleaf pine) stem tissue (redissolved in 87.5% methanol).

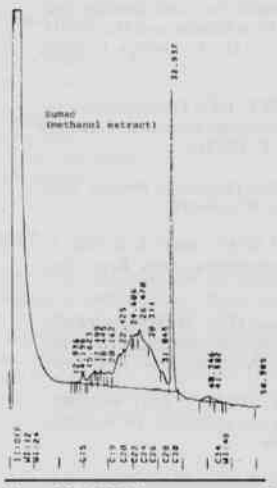

Figure 26. GLC chromatogram of methanol extract of *Rhus copallina* (dwarf sumac) stem tissue.

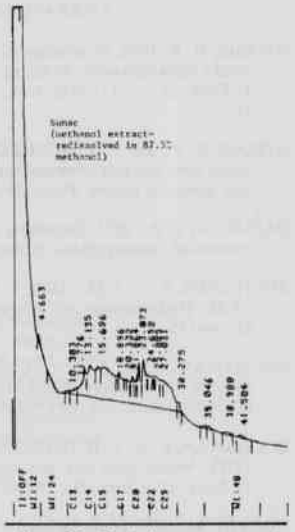

Figure 27. GLC chromatogram of methanol extract of *Rhus copallina* (dwarf sumac) stem tissue (redissolved in 87.5% methanol).

ing from 500,000-2,000,000 down to 50,000 or less. The higher molecular weight latex would be most economically used as natural rubber. The lower molecular weight range could be catalytically cracked to liquid fuel (Wang and Huffman, 1981).

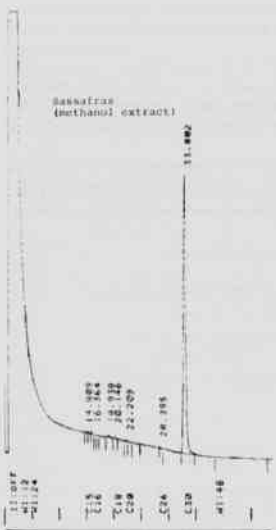

Figure 28. GLC chromatogram of methanol extract of *Sassafras albidum* (sassafras) stem tissue.

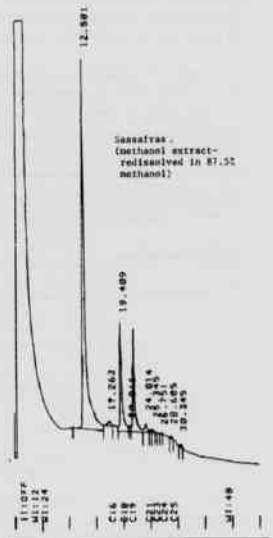

Figure 29. GLC chromatogram of methanol extract of *Sassafras albidum* (sassafras) stem tissue (redissolved in 87.5% methanol).

Pines represent a major source of resin readily collected as a volatile oil called turpentine. Turpentine is highly combustible and should be considered an important potential fuel source. Turpentine has a heat content equivalent to gasoline and butanol (40 gigajoules per metric ton) and could be used as liquid fuel or mixed with gasoline (Wang and Huffman, 1981).

Maugh (1979) identified the exudate from *Copaifera langsdorffii* Desf. trees as a source of an oil (oleoresin) sap obtained by tapping that could be used as diesel fuel. Wang and Huffman (1981) estimated that one acre of 100 *C. langsdorffii* trees could produce 25 barrels of exudate per year. This amounts to 1375 gallons (1 barrel = 55 gallons) of exudate per acre per year, enough to drive 27,500 miles per year at 20 miles per gallon. If we assign diesel fuel a cost of $1.10 per gallon, this represents a monetary value of $1,512.50 per acre per year.

This research represents a preliminary study to determine the feasibility of using trees as a source of biocrude. The development of photosynthetic plants as fuel feedstock has been hindered by the difference in production when compared with fossil fuels. However, biocrude is renewable, has flexibility afforded by crop rotation, and can be genetically altered to modify the chemical composition of extractives. These advantages may be partially offset by the high technology and market development of the petroleum industry and the seasonal dispersion of plant resources (Lipinsky, 1981).

Although current interest is focused on whole plants for biocrude (Buchanan et al., 1978a,b; Calvin, 1979; Erdman and Erdman, 1981; Adams, 1982; Campbell, 1983) we believe that biocrude-like tree exudates could be a potential alternative. Adams (1982) reported that harvesting only the exudate would result in a loss of ⅓ of the oil, ½ of the resin, and ⅓ of the latex. We believe these losses could be partially offset by the ecological benefits of trees. Farmers would see a direct economic benefit from planting trees. Trees could reduce wind erosion, improve the water table, improve wildlife habitat, provide shade for homes and livestock, and serve as a source of liquid fuel, lumber, pulp, and firewood.

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MICROGEOGRAPHIC VARIATION IN THE MEXICAN VOLE, *MICROTUS MEXICANUS*

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ABSTRACT

Altitudinal variation was assessed in 115 (62 male; 53 female) Mexican voles *(Microtus mexicanus)* from six localities in Jalisco, Mexico. Univariate and multivariate statistical techniques were employed in the data analyses. A total of 49 skeletal measurements were investigated, and of these, 17 showed significant interlocality variation. Rostral breadth, depth of braincase, rostral height, width of third molar and nasal length were found to be the most variable characters. Component I (a size factor) accounted for 36% of the total phenetic variation; components II and III accounted for 30% and 19%, respectively. Larger individuals were found to occur at lower altitudes; smaller individuals occurred at higher altitudes. Size variation was expressed in reverse to Bergmann's Ecogeographical Rule.

INTRODUCTION

Large scale geographic variation studies, those spanning several states or complete countries have increased in recent years. These studies have played major roles in debates concerning the nature of species and speciation, as well as reflecting evolutionary processes. On the other hand, spatial variation over more local areas, or microgeographic variation, has received little attention. Dubach (1975) and Price and Kennedy (1981) demonstrated genic variation in a small geographic area to exhibit levels as great as those previously reported for larger geographic areas in the deer mouse, *Peromyscus maniculatus*. Possibly, the levels of genic or morphologic variability selected to represent regions for large scale studies, may in reality reflect only the site chosen within a local area. An understanding of microgeographic variation appears to be critical in order to completely understand adaptive strategies at the macrogeographic level.

The adaptive significance of spatial variation in body size has been discussed by Bergmann (1847), Rosenzweig (1968), McNab (1971), and others. The classical interpretation of body size is reflected in Bergmann's Ecogeographic Rule. In relation to endotherms, small forms of individuals of the same species are located in southern parts of the range and large forms in northern parts of the range. The validity of Bergmann's Rule remains a central issue in discussion of modern systematics and evolutionary theory.

The Mexican vole, *Microtus mexicanus*, appears to provide an excellent model for studying the patterns of microgeographic size variation and for exploring the occurrence of Bergmann's Rule on a microgeographic scale. This boreal rodent ranges from southernmost Utah and Colorado south to Oaxaca de Juarez and occurs at higher elevations. The isolated nature of mountains in the Southwest provides for a series of small isolated populations along mountain tops

Table 1. Collection sites of *Microtus mexicanus* from Jalisco, Mexico.

Locality No.	Collection site/altitude	N Male	N Female
1	24.3 mi. W Atenquique, ca. 2770 m	6	6
2	26 mi. W Atenquique, north slope Volcan de Fuego, ca. 2835 m	20	30
3	27.3 mi. W Atenquique, Volcan de Fuego, ca. 2835 m	4	6
4	25.1 mi. W Atenquique, ca. 2850 m	6	5
5	26 mi. W Atenquique, north slope Volcan de Fuego, ca. 2896 m	13	3
6	26 mi. W Atenquique, north slope Volcan de Fuego, ca. 2990 m	5	3
	Total	62	53

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Table 2. *Microtus mexicanus* Interlocality variation in 17 skeletal characters

Character	d.f.	F-ratio[1]
Greatest skull length	5,96	2.214
Incisive foramen length	5,107	3.964
Diastema	5,108	3.883
Width of third molar	5,109	5.982
Mastoidal breadth	5,103	2.229
Pre-lambdoidal breadth	5,103	2.602
Interorbital constriction	5,106	2.169
Rostral breadth	5,109	10.367
Nasal length	5,101	5.626
Rostral height	5,105	6.218
Depth of braincase	5,104	6.662
Foramen magnum height	5,105	3.167
Tibia length	5,42	2.225
Tibia proximal width	5,99	2.568
Pelvis length	5,93	2.506
Obturator foramen length	5,104	2.153
Width of fused vertebrae	5,90	2.612

[1]Single classification analysis of variance; $p \leq .05$ for F-ratio exceeding 2.17.

Table 3. Character loadings on the first three principal components of interlocality phenetic variation in *Microtus mexicanus*.

Character	Principal Components I	II	III
Greatest skull length	.691	-.640	.032
Incisive foramen length	-.338	-.899	-.096
Diastema	-.617	-.654	.427
Width of third molar	.524	-.547	.482
Mastoidal breadth	-.746	-.417	-.283
Pre-lamboidal breadth	.619	-.383	-.587
Interorbital constriction	.540	-.459	-.697
Rostral breadth	.467	.066	-.824
Nasal length	.839	-.330	.276
Rostral height	.606	-.253	-.261
Depth of braincase	-.279	-.758	.200
Foramen magnum height	-.799	-.532	.082
Tibia length	-.474	-.798	-.167
Tibia proximal width	.569	.343	.640
Pelvis length	.487	-.244	.790
Obturator foramen length	.340	-.837	.057
Width of fused vertebrae	.838	-.254	.085

throughout its range. Gene flow across mountain tops is not likely; therefore, disjunct populations (suggesting population fragmentation) may exist.

M. mexicanus has been studied over a larger geographic area in New Mexico, Arizona, Utah and Colorado (Findley and Jones, 1962), and in Utah and New Mexico (Wilhelm, 1982). These studies involved distribution patterns, geographic variation and evolutionary relationships. Also, *M. mexicanus* has been studied karyotypically by Lee and Elder (1977).

The purpose of the present investigation was to evaluate microgeographic size variation in *M. mexicanus* using univariate and multivariate statistical techniques. This study was designed to concentrate on morphologic variability over a small geographic area, as well as provide additional insight into the validity of Bergmann's Ecogeographical Rule at the microgeographic level.

METHODS AND MATERIALS

From 31 December 1978 through 4 January 1979, 126 *M. mexicanus* were live-trapped from six localities on a volcanic mountain site in Jalisco, Mexico. Elevations of trap sites ranged from 2770 m to 2990 m above mean sea level. Specific location and corresponding sample sizes for each trap site are presented in Table 1. Specimens were prepared as standard museum study skins and skeletons and are housed in the Memphis State University Museum of Zoology. Specimens were aged according to the criteria of Choate and Williams (1978) and assigned to four age classes: old adult, adult, sub-adult and juvenile. Following their recommendations, sub-adults and juveniles were not included in the statistical analysis. The resulting sample size was 115 animals, consisting of 62 males and 53 females.

Twenty-three cranial and twenty-five post-cranial measurements were taken to the nearest 0.1 mm with the aid of dial calipers. Skeletal measurements followed Servinghaus (1976), Best (1978), Coate and Williams (1978), Kennedy and Schnell (1978) and Wilhelm (1982) with the following exceptions: *atlas width,* greatest distance across the atlas; *diagonal through the orbit,* greatest distance across the orbit on a diagonal; *foramen magnum width,* greatest distance across the foramen magnum; *foramen magnum height,* greatest height of foramen magnum; *ilium length,* distance from the anterior tip of the ilium to the nearest edge of the acetabulum.

Univariate procedures were used initially to test the character set for sexual dimorphism followed by determination of interlocality variability. A single classification of analysis of variation for character means (ANOVA) and sum-of-squares simultaneous test procedures (SS-STP) were carried out by a modified univar program developed by Powers (1969). Trends in variation of individual characters and identification of maximally non-significant subsets were obtained from the SS-STP procedures. Characters exhibiting sexual dimorphism, when tested with a critical F-ratio of 3.92 at the 0.05 significance level, were removed from the data set; and sexes were combined in the remaining statistical analysis. Interlocality variation at the 0.05 significance level was indicated by an F-ratio exceeding 2.17.

Multivariate analyses were performed using the NT-SYS programs of Rohlf et al. (1978), which computed matrices of Pearson's product-moment correlations and derived phenetic distance coefficients from standardized character values. Clusters of characters were obtained with the unweighted pair-group method using arithmetic averages (UPGMA). Correlations among characters were summarized using dendrograms generated from the correlation matrices. Principal components were calculated from a correlation matrix among characters, and projections of localities were plotted onto the first three components. A shortest minimally connected network (computed from the original matrix of distances between localities) was superimposed upon the resulting three-dimensional plot and was used to connect most similar localities. The relationship between size and elevations was assessed using Kendall's correlation routine of SPSS (Nie et al., 1975).

RESULTS

Of the 49 original measurements examined, four showed significant sexual dimorphism: obturator foramen width (F-ratio: 7.419); pelvis depth (F-ratio: 7.448); pubis length (F-ratio: 7.950); and pubis width (F-ratio: 33.442). Seventeen of the remaining 45 measurements were found to have significant interlocality variation ($P < 0.05$). These characters are presented in Table 2. The remaining characters exhibited no significant variation; these characters were removed from subsequent analysis, following Sneath and Sokal (1973). Relative to the degree of interlocality variation, rostral breadth showed the greatest interlocality variation followed by depth of braincase, rostral height, width of third molar and nasal length. Whereas, obturator foramen length, interorbital constriction, greatest skull length, tibia length and mastoidal breadth had the lowest F-values.

A dendrogram summarizing variation among characters is presented in Fig. 1. There were two major clusters each containing two sub-clusters.

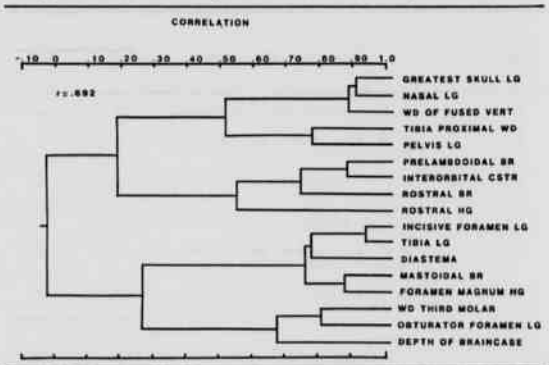

Figure 1. Dendrogram summarizing variation between characters for *Microtus mexicanus* from Jalisco, Mexico.

The sub-clusters were composed of greatest skull length through pelvis length; pre-lambdoidal breadth through rostral height; incisive foramen length through foramen magnum height; and width of third molar through depth of braincase. Incisive foramen length and tibia length were the most highly correlated characters. Additionally, greatest skull length and nasal length were highly correlated. Overall, the correlations appeared relatively low, indicating little redundency in information. Rostral height, depth of braincase, pelvis length and foramen magnum height were characters which contained independent information.

Principal components extracted to summarize character variation among localities indicated that 85% of the total phenetic variation was accounted for by the first three principal components. Therefore, reduction of the 17 character matrix to three dimensions resulted in little distortion. Character loadings indicating the correlation of characters with the first three principal components are presented in Table 3. Projections of the six localities onto the first three components are presented in Fig. 2.

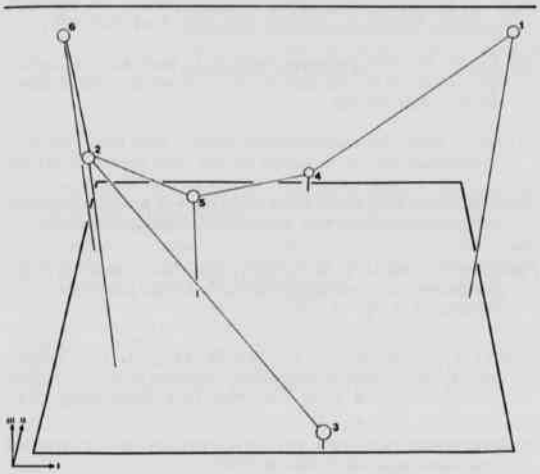

Figure 2. Three-dimensional projection of 6 localities onto the first three principal components of variation in the matrix of correlations of 17 morphological characters of *Microtus mexicanus* from Jalisco, Mexico. Numbers correspond to those given in Table 1. Shortest minimally connected network values between localities; 1-4 = 1.488; 4-5 = 1.290; 5-2 = 1.076; 2-6 = 0.932; 2-3 = 1.218.

Principal component I accounted for the majority of the total interlocality variation. This factor is taken to represent body size in the classic meaning of ecogeographical variation analysis (Niles, 1973). Nasal length, width of fused vertebrae, foramen magnum height and mastoidal breadth loaded high for this component. Variation along this axis showed a decrease in size of animals from left to right in Fig. 2 (animals from locality 1 were the largest; those from locality 6 were the smallest). This decrease, in general, corresponded with an increase in elevation. Therefore, animals obtained from locality 1 (2770 m) were relatively larger than those obtained from locality 6 (2990 m). Individuals obtained from those localities positioned in the middle of Fig. 2 were intermediate in size. The only exception to the generalization were the animals in locality 2. These were small animals but at a low elevation. Furthermore, a Kendall's correlation on principal components 1 and elevation showed that there was a statistical relationship between size and elevation, indicating elevation should be considered in examining the species over large geographic areas.

Component II had highest correlations (negative) for incisive foramen length, obturator foramen length, tibia length, depth of braincase, greatest skull length and diastema length. Component II explained 30% of the phenetic variability. Animals from localities that were situated near the front of Fig. 2 had long, deep skulls and relatively longer tibiae. Those from localities situated toward the back had short shallow skulls and short tibiae.

The third component had its highest correlations with rostral breadth, interorbital constriction, pelvis length and tibia proximal width. It accounted for 19.3% of the variability. Localities with individuals which tended to be relatively large for those highly-correlated characters are shown as being highest above the base of Fig. 2 (having tall "sticks").

DISCUSSION

Wilhelm (1982), using cranial and external measurements, found depth of skull and interorbital breadth to be sexually dimorphic. In this study, using both cranial and postcranial measurements, we found obturator foramen width, pelvis depth, pubis length and pubis width to be the only characters to be sexually dimorphic. Sexually dimorphic characters in our study corresponded well with those reported for other Microtines: *M. californicus* (Dunmire, 1955), *Clethrionomys glareus* and *M. agrestis* (Brown and Trigg, 1969) and *M. ochrogaster* (Servinghaus, 1976). The sexually dimorphic characters found in this study were those associated with the pelvic girdle; these characters would be expected to be dimorphic, considering demands placed on females during the delivery of young.

The univariate analysis indicated that there was significant morphologic interlocality variation in single characters for *M. mexicanus* within the study area. Although no overall continuous altitudinal gradients were found in the characters examined, there were some apparent trends in regard to principal component I. A decrease in the character means was found to generally correspond to an increase in elevation.

The present study indicated that specimens from about the same elevation (localities 3 and 4) which were only a few kilometers apart may be approximately the same size. However, localities 2 and 3 (occurring at about the same elevation and also only a few kilometers apart) appeared dissimilar in size. The amount of overall difference in size between individuals from localities 2 and 3 or 1 and 6, while shown at separate ends of the principal component I axis (Fig. 2), was only a few millimeters. The degree of morphologic variation at the local level may not be great enough to distort overall broad patterns. However, this study suggested that microgeographic variation should be given attention when studying variability at the macro level.

In regard to Bergmann's Rule, James (1970) reformulated the rule to predict that larger size should be found in cooler, drier locales which would correspond to areas of high elevation or latitude. In this study, overall size (which was represented by principal component I) exhibited the reverse of Bergmann's Rule. With the exception of locality 2, larger animals were found at lower elevations, and the smaller animals were found at the higher elevations. The exception probably reflected the very close proximity of localities 2, 5 and 6. There appreared to be some type of selective pressure influencing body size other than the combined effects of temperature and humidity which produced the situation described by Bergmann's Rule. Otto (1978) found significant differences in skeletal variation of male bank voles *C. glareus* along an altitudinal gradient. He described a situation wherein smaller animals were found at intermediate elevations. He attributed this distribution pattern to density factors which might put a selective premium on larger size.

Brown (1975) implied that important resources of habitat were competed for and partitioned among species on the basis of size. However, the occurrence of no other microtine species in the study area indicated that this is probably not in effect here. Another approach to account for the pattern of distribution exhibited by principal component I might be to relate a change in amount of available habitat or food over the range of elevations. Rosenzweig (1968) discussed body size in relation to primary productivity. He suggested that one way for natural selection to adjust consumers to various energy flow rates might be to modify body size in accordance with productivity. Relatively meager food supplies tended to set upper limits to body size. Habitat changes significantly across the range of elevations in this microgeographic area. This could correlate with a change in primary productivity and perhaps influence overall body size.

Principal components II and III are considered to represent shape rather than size (Niles, 1973). Principal component II indicated a pattern wherein animals from extreme elevations (localities 1 and 6) grouped as an intermediate form and animals from the intermediate elevations (localities 2, 3 and 4) grouped as relatively large or small forms. In this situation there are also selective pressures, perhaps the same as those suggested for principal component I, influencing the distribution pattern. Principal component III showed no recognizable trends.

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AN ANALYSIS OF GRAY FOX *(UROCYON CINEREOARGENTEUS)* FUR HARVESTS IN ARKANSAS

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ABSTRACT

An investigation was conducted on gray fox *(Urocyon cinereoargenteus)* fur harvest in Arkansas. Data were gathered from a mail survey of Arkansas trappers and from Arkansas Game and Fish Commission fur harvest records from 1939 to 1983. Analyses of these data demonstrated: 1) gray fox were abundant statewide with lower levels in the Delta region; 2) there was a need for fox trappers to keep better records on their trapping efforts, success and composition of catch, including sex and age data; 3) market price:harvest correlation was high ($r = 0.956$, $p < .001$); 4) over the past 10 years, the Ozark Mountain region provided the greatest contribution to annual fox harvests, the Ouachita Mountain and Gulf Coastal Plain regions were similar to each other, but lower than the Ozarks, and the Delta region contributed the least, but with a generally stable harvest.

INTRODUCTION

Arkansas falls well within the geographic range of the gray fox *(Urocyon cinereoargenteus)* (Hall, 1981). These relatively secretive carnivores have long been an important wildlife resource in the United States, both as a valuable furbearer and as a recreational sport animal. For example, in Arkansas, the gray fox is the sixth most harvested species (2% of total pelts) and is the third highest in total value (6% of total furbearer harvest value) (McArdle, 1983). In spite of this importance, our knowledge of gray fox biology is limited in the Southeastern United States (Carey, 1982; Hensley and Fisher, 1975; Nicholson, 1982; Spencer, 1982; Sullivan, 1956; Sumner and Hill, 1981; and Wood et al., 1958) and is almost non-existent in Arkansas (Fooks, 1961; King, 1981; and McArdle 1979, 1983).

The management problems presented by furbearers, in general, have increased in number, scope and intensity during the past decade in response to 1) rapidly growing demands for furbearers and their products, 2) enactment of endangered species regulations and treaties, 3) major decline in upland wildlife hunting opportunities and 4) growing antihunting and antitrapping sentiment (Hubert, 1982). Thus harvest management remains the principle focus of most furbearer management programs. The mechanics of harvest management programs, however, are not clearly understood. The success of management programs, now and in the future, requires an understanding of the variables which ultimately determine the size of furbearer populations and of subsequent expected harvests (Erickson, 1981, 1982; Hubert, 1982).

The numerous variables which influence furbearer harvests must be identified and evaluated for each furbearer species. The same factor may have a different importance value for a different species in the same state and may differ in its importance from state to state (Erickson, 1981; Hubert, 1982).

Erickson (1981) examined a number of variables (mean pelt value, population indices, harvest efforts, season lengths, weather and pelt values) of four furbearers (beaver, muskrat, raccoon and coyote) in Missouri. Of these variables, he found that mean pelt value correlated with total harvest for all four species and that exceptionally high correlations existed between mean annual pelt value and total harvest of the two carnivore species, raccoon and coyote. Market price played an important role in the harvest of otter and bobcat in Arkansas (Tumlison et al., 1981; McArdle, 1982).

Fur harvest data have traditionally been used as the primary source of data for analysis of the condition of furbearer populations and subsequent management (Erickson, 1982; Hubert, 1982). Arkansas, along with other states in the Midsouth and Midwest, has relied heavily on these data (McArdle, 1979, 1983). In an effort to determine the accuracy of fur dealer records in Arkansas, Tumlison et al. (1981) compared the number of fur buyers licensed in each county with the number of otter pelts attributed to the harvest from each county. In many counties with large harvests, there were no resident, licensed furbuyers; whereas, few otters were reported from some counties with many resident furbuyers. Since furbuyers usually listed counties other than their own as sources for pelts, the harvest data with respect to county of origin of the pelt were considered sufficiently accurate to allow for an analysis of harvest by region.

The objectives of this study were: 1) to assess trapper work effort in harvesting gray fox; 2) to assess the character of the gray fox fur harvest in Arkansas; and 3) to assess the correlation between the market price (mean pelt value) and harvest size for gray fox in Arkansas.

METHODS AND MATERIALS

A questionnaire for fur trappers was prepared in accordance with standard methods for wildlife opinion surveys (Filion, 1980). The survey examined trapping effort, trapping success, and composition of the fur harvest over the past three trapping seasons (1980-81, 1981-82 and 1982-83). Only data on gray fox were used for analysis. The questionnaires were mailed to the 1200 members of the Arkansas Trappers Association (ATA) on 14 June 1983. The members of the ATA represented 25% of an estimated population of 4800 Arkansas fur trappers (P. Dozhier, *pers. comm.*). Unforseen time limitations necessitated that all responses be returned by 30 June 1983. A total of 235 respondents replied (19.6%). A total of 230 questionnaires were used for data analysis, as five were physically damaged and unuseable. The 230 ATA respondents represented 5% of the estimated trapper population in Arkansas.

Fur harvest records used in this study were compiled since 1939 by the Arkansas Game and Fish Commission (AGFC). Data were available for mean annual pelt value, total gray fox harvested, and regional contribution of harvest for all but a few years. For purposes of analyses, years with missing data were generally omitted from consideration. In the case of missing mean annual pelt values, a value was extrapolated for Arkansas based on mean annual pelt prices in Missouri. No correction factors were applied to the data to correct for out-of-state sales of Arkansas fur. Furthermore, dollar values were uncorrected for inflation.

The data were statistically analyzed using a microcomputer statistical program (STATPAK by Northwest Analytic, Inc.) on an Epson QX-10 microcomputer. A linear regression equation relating the variable of mean annual pelt price to the number of pelts sold was formulated; a correlation coefficient and a coefficient of determination was calculated. The correlation coefficient was tested for significance with

a single tailed t test at the .001 level of significance.

RESULTS AND DISCUSSION

Trapper Survey

The 230 ATA respondents reflected a statewide distribution with residency in all but nine of 75 Arkansas counties (Boone, Chicot, Crittenden, Jackson, Lafayette, Lee, Newton, Phillips and Prairie). The accountability and comparability of the data provided by the respondents was considered to be high, as 1) 85% of the respondents signed their name to the questionnaire form, 2) 73% of the respondents indicated that they specifically trap for fox, and 3) 40% of the respondents indicated that they hunted as well as trapped fox. Altogether, the respondents indicated that they had trapped or hunted for fox in all Arkansas counties except three (Boone, Lee and Newton), and that they had searched for fox in all 75 counties.

Gray fox observations by the respondents were well distributed across the state. Positive sightings were reported in all counties except two (Lee and Lincoln). McArdle (1979, 1983) previously rated Arkansas gray fox as abundant based on an index of relative density with *rare* being defined as positive sightings being reported by fewer than 25% of observers, *occasional* being reported by 26-50%, *common* being 51-75%, and *abundant* being 76-100%. As 91% of the respondents reported positive sightings, gray fox was rated as being abundant in terms of relative density across the state.

Table 1. Mean work effort, mean trap success, and composition of the gray fox harvest by trappers in Arkansas over the past three seasons (1980-81, 1981-82, 1982-83). Calculated values were rounded to nearest whole integer.

	Trapping Season		
	1980-81	1981-82	1982-83
Mean # Traps/trapper	22	22	19
Mean # Nights Trapped	31	29	27
% Season Length Used	50	41	45
Total Trap-nights per season	690	642	506
Mean # animals harvested per trapper	68	61	65
Mean # Gray Fox sold by each respondent	7	7	7
Total % Trap success	10	10	13
Total Harvest by all respondents	15,587	14,125	14,873
# Gray Fox harvested by all respondents	1,666	1,704	1,559
% Gray Fox of Total Harvest by all respondents	11	12	10
# Gray Fox pelts sold out-of-state by all respondents	208	251	207
% Gray Fox pelts sold out-of-state by all respondents	12	15	13
% Total Arkansas Gray Fox Harvest sold by respondents	23	34	30

The respondents were asked to provide data on their trapping effort, trap success and composition of their harvest, which was summarized in Table 1. In general, the respondents (71%) indicated that economic conditions have not significantly influenced their trapping efforts, consequently they reported trapping in essentially the same localities with equal effort each year. Over the past three seasons (1980-81, 1981-82, 1982-83), the respondents on the average set 21 traps on 29 nights (averaging 45% of the length of the legal season for fur taking). The respondents averaged 619 trap-nights, catching an average of 65 animals, reflecting an 11% trap success. Altogether, the respondents harvested an average of 14,862 furbearers each season, with 11% of the season total harvest being gray fox. Altogether, the respondents trapped an average of 1643 gray fox. Although the respondents represent 5% of the Arkansas trappers, they accounted for an average of 29% of the reported state harvest of gray fox. The respondents sold, on the average, 222 pelts out-of-state each year, being approximately 13% of their harvest. If the other trappers in Arkansas market furs in a similar manner, then the reported state harvest may under estimate the number of gray fox harvested in Arkansas by approximately 13%.

The respondents indicated that weather negatively influenced the trapping of gray fox in Arkansas. Over the three seasons assessed, the respondents indicated that weather played "some" influence during the 1980-81 and 1981-82 seasons (37% and 49%, respectively), but that it played a "great deal" of influence in the 1982-83 season (68% of respondents), probably being reflected in the slight decline in number of traps and nights trapped in Table 1. Although this resulted in a reduced trap effort, the trap success actually increased (Table 1), as did the total harvest for the state. Weather probably influenced the Arkansas fur harvest, but the actual impact of weather on the harvest, apparently, can not be readily derived from trapper perceptions.

The quality and extent of suitable habitat can influence the population status of furbearers. In the case of gray fox in Arkansas, 91% of the respondents indicated that habitat in their area was staying the same or was actually improving for gray fox. Thus, the respondents did not feel that habitat quality or extent was limiting population size or harvest of Arkansas gray fox.

As to condition of the fur, the respondents noted that an average of 17% of the gray fox harvest was physically damaged before harvest, reducing the market value. The major problem reported was the presence of wounds with lead or shot under the skin (7% of the fox), scars from animal bites (5%), mange (4%) and missing limbs (1%). The incidence of mange, however, rose from 3% in 1980-81 season, to 4% in 1981-82, to 5% in 1982-83, suggesting that the extent of this disease in Arkansas gray fox needs to be monitored.

Respondents were also asked to provide age and sex data on the gray fox that they harvested over the three seasons. Based on their collective recollections, the respondents reported that from 30-51% of the last three harvests had to be classed as "unknown" with respect to sex. Similarly, with respect to age of fox, the respondents indicated that 60-64% of the three harvests had to be classed as "unknown". Consequently, it was impossible to derive any meaningful data concerning age or sex ratios. The high percentage of unknown responses probably reflected a lack of accurate recording at the time of harvest, rather than an inability by the furtaker to discern sex or age (adult/juvenile) of the fox. Considering the large number of furbearers taken by the respondents, if an organized method to record data would have been provided to them before the opening of the trapping season, valuable population data could have been readily obtained.

Fur Harvest Analysis

The character of the Arkansas gray fox harvest from the 1939-40 season through the 1982-83 season was compiled (Heidt and Peck, 1983). These fur harvest data reflect over 40 years of data gathered from furbuyers by the AGFC staff. Subsequent analyses and discussions were based upon these data (detailed data can be obtained from the authors).

The total value of gray fox harvest in Arkansas and the total number of pelts sold has varied considerably from 1939-1983 (Fig. 1). Gray fox harvest in Arkansas was stimulated from World War II through the Korean Conflict (1940's through the early 1950's). Similar trends have been seen for other furbearers such as red fox in the Midwest and Canada (Sargeant, 1982; Voight and Tinline, 1982). Mean pelt values, however, were less than $1.00/pelt from the 1946-47 season through the 1965-66 season, while values of greater than $20.00/pelt have existed since the 1975-76 season. The mean annual pelt value for the 1979-80 season was 20x the value of a pelt of the 1958-59 season. Figure 1 portrays a dramatic increase in fox harvest during the 1970's and into the 1980's. The equally dramatic increase in total value of harvest reflects the large financial impact which increases in mean annual pelt price have had on total harvest.

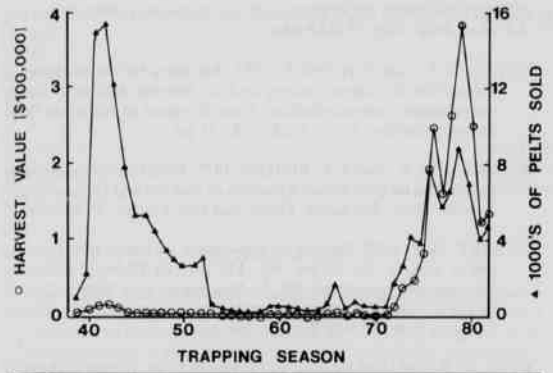

Figure 1. A comparison of the total value of Arkansas gray fox fur harvests from 1939-1982 with the total number of pelts sold at the conclusion of each season's harvest.

The magnitude of change in pelt values over the last 25 years was sufficiently large enough to influence the attitudes and efforts of furtakers, suggesting that the market price might have influenced the magnitude of the Arkansas gray fox harvest. The mean values of pelts of Arkansas gray fox were plotted against the harvest size for each season since 1954 (Fig. 2). A linear regression equation was calculated to correlate the total harvest of Arkansas gray fox and the annual mean pelt

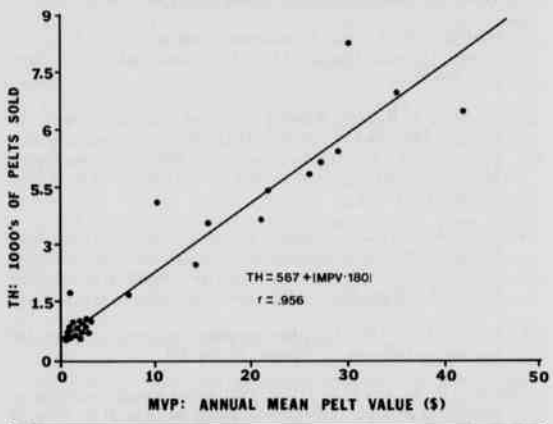

Figure 2. A scatter diagram, regression line and equation, and correlation coefficient ($P < .001$) relating annual mean pelt value in dollars (MVP) to the number of pelts sold (TH) for the 1954-1982 Arkansas harvests of gray fox.

value. The correlation coefficient ($r = 0.956$, $p < .001$) indicated a high degree of relationship between total harvest and mean pelt value. Consequently, market price accounted for 93.3% (r^2) of the variability in harvest size of gray fox in Arkansas.

The total fox harvest was also analyzed geographically, using the four major physiographic regions of the state (Ozark Mountains, Ouachita Mountains, Gulf Coastal Plain and Mississippi Delta). These regions were compared in terms of number of pelts sold (Fig. 3) and percent contribution to the total state harvest (Fig. 4). From Fig. 3, the declines from the large harvests during World War II were evident in each region,

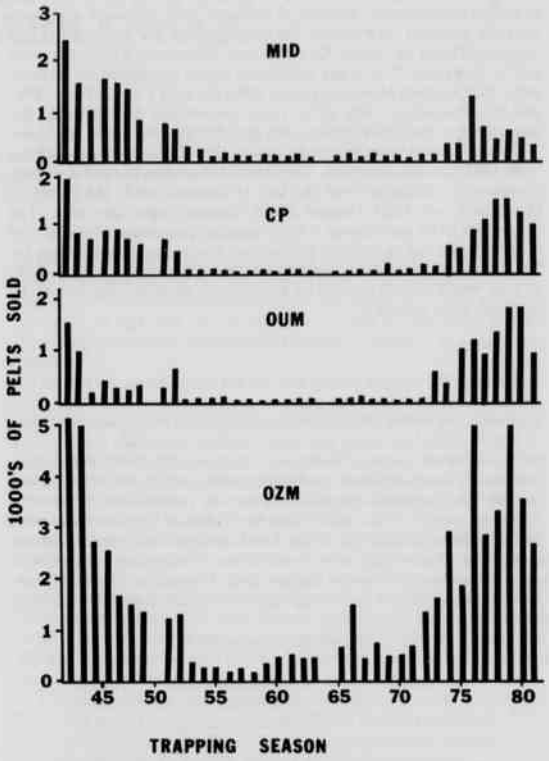

Figure 3. A comparison of the number of gray fox harvested from 1939-1982 from each of the four major physiographic regions of Arkansas: Ozark Mountains (OXM), Ouachita Mountains (OUM), Coastal Plain (CP), and Mississippi Delta (MID).

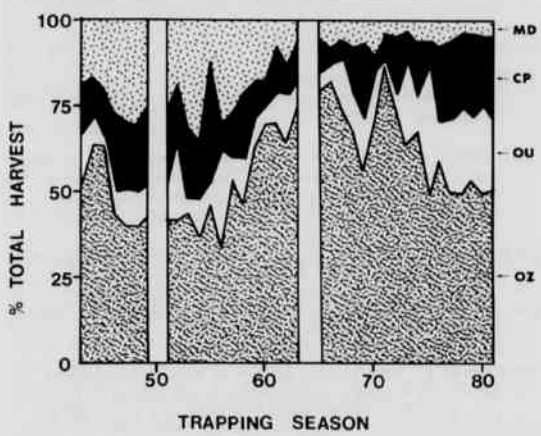

Figure 4. A comparison of the percent harvest of Arkansas gray fox from 1939-1982 from each of the four major physiographic regions of Arkansas: Ozark Mountains (OZ), Ouachita Mountains (OU), Coastal Plain (CP), and Mississippi Delta (MD).

as well as the increasing harvests of fox since 1970. Although the trends were the same for each region, the magnitude of the response in total harvest differed by region. Over the past 10 seasons, 57,355 fox were sold in Arkansas. The Ozark Mountain region accounted for 52% of sales, the Ouachita Mountain region 20%, the Gulf Coastal Plain 18%, and the Mississippi Delta 10%. These percentages demonstrated the importance of the Ozark region to the gray fox state harvest in Arkansas.

The percentage contribution by region (Fig. 4) for each season from 1940-1982 was also analyzed. The Ozark Mountains showed a slightly decreased contribution over the last 10 seasons, while the Ouachita Mountains and Gulf Coastal Plain showed slight increases. The Mississippi Delta contributed a fairly constant percentage of the harvest in spite of decreasing optimal habitat and decreasing trapper effort for fox. Furtakers in the Mississippi Delta probably expended more effort to trap water-related species (e.g., beaver, muskrat, and mink) P. Dozhier, *pers. comm.*).

CONCLUSIONS

Based on the trapper survey and fur harvest records, data from this study showed that gray fox populations are at good levels and occur statewide, but with probably fewer individuals in the Mississippi Delta. Trappers tended to utilize the same traplines and made similar work efforts from year to year. Fur harvest data indicated that economic considerations were extremely important in determining the total harvest of gray fox. Regional analysis of fur harvest records demonstrate the importance of the Ozark Mountains to the annual gray fox fur harvest and also demonstrated that while the Mississippi Delta contributes the least, it has been steady in its contribution. Finally, there is a definite need for better utilization of trapper data. Trappers should be encouraged to keep a log on their trapping efforts, success and composition of catch.

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HELMINTHS OF COMMON GRACKLES (*QUISCALUS QUISCULA-VERSICOLOR*, VIEILLOT) IN CENTRAL ARKANSAS

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INTRODUCTION

Five studies on the helminths of common grackles (*Quiscalus quiscula versicolor*, Vieillot) have been published. Welker (1962) was the first investigator to examine large numbers of the host. His sampling from the northern lake region (110 hosts), central agricultural region (263), and the southern forest-agricultural region (33) of Indiana looked at both fall (195) and spring (211) birds. He catagorized the symbionts by geographical region, season and sex of the host. Stanley and Rabalais (1971) examined 19 adult common grackles in the spring from Erie County and from South Bass Island in Lake Erie in Ohio. Cooper and Crites (1974) analyzed 13 adult and 37 juvenile birds from South Bass Island. This latter summer study (July 1 to August 15) contains the first acknowledgements of the helminths of the juvenile bird. They recovered 15 species of helminths from 48 of 50 common grackles; 10 from adult birds and 15 from juvenile birds. Three trematodes *(Ornithodendrium imanensis, Plagiorchis noblei, Prosthogonimus macrorchis)*, one cestode *(Orthoskrjabinia rostellata)*, and one nematode *(Capillaria exilis)* were reported exclusively from juvenile birds at low infection rates and in small numbers. *Conspicuum icteridorum* (77%:35%), *Choanotaenia musculosa* (23:8), and *Chandlerella quiscali* (38:3) were more prevalent in adult birds. The reverse was true for *Capillaria ovopunctatum* (31:49), *Dispharynx nasuta* (8:35), *Porrocaecum ensicaudatum* (23:54), *Syngamus trachea* (38:64), and *Plagiorhynchus formosus* (31:65). *Anonchotaenia globata* and *Hymenolepis farciminosa* were present in both age groups in small numbers and equal percentages. The authors did not separate the hosts by sex nor did they separate the intensity of the infection in juveniles and adults where the helminth species were shared. This paper also tabulated the species of helminths previously reported from common grackles. Buck et al. (1975) examined 40 adult birds from central Ohio (Columbus) for helminths. Badley and Dronen (1979) posted 21 adult common grackles from Brazos County, Texas. Collections were made during the pre-breeding period in the spring.

Banding of wintering Arkansas common grackles over the past ten years indicates that the Arkansas birds do not migrate to any of the above surveyed areas. Of the 10,000 birds banded in Conway, Arkansas, only rarely are there recoveries east of the Mississippi River. Additionally, no recoveries have been made in Texas of the central Arkansas banded birds. Further, no recoveries of Indiana, Ohio, nor Texas birds have come from the Conway trapping. The central Arkansas common grackle population thus appears to be a separate group from those previously analyzed and constitutes a population not previously examined. This is further borne out by the extensive studies of Meanley. He did not recover Arkansas winter banded birds in any of the three states. A few Indiana and Ohio breeding birds were recovered from Arkansas wintering sites. No Arkansas connection was shown with the Texas birds. Arkansas common grackles migrate north-northwest in the spring (Meanley). The Arkansas breeding population comes largely from Arkansas but also from points south. The preponderance of wintering and pre-breeding birds have northern breeding grounds. Summer birds have Arkansas breeding sites and the juveniles are Arkansas derived. The helminths of juvenile birds are thus local.

It is the purpose of this paper to 1) document the helminth community of a migratory bird throughout three seasons of the year, and in a new locality, 2) investigate the prevalence and intensity of infection as they are affected by the age and sex of the host, 3) locate the origin of the helminths geographically, 4) compare the helminths qualitatively and quantitatively with those obtained from the same host during the same time of the year in Ohio by Cooper and Crites, 5) determine the distribution of the helminths within the host. The paper means to stress the relevance of age and sex of the host, season of the year, and the geographical location when dealing with the symbionts of a migratory species.

MATERIALS AND METHODS

All common grackles were live trapped in Conway, Arkansas, using baited Glenhaven traps. Three seasonal samples of birds were examined. The winter sample from December 15 through January 31 of 1982 and 1983 consisted of 15 adult female (AF-W) and 15 adult male (AM-W) hosts. The pre-breeding group was examined from March 15-22, 1984. This sample consisted of ten adult females (AF-PRB) and ten adult males (AM-PRB). The post-breeding sample contained both adults and juveniles. This sample was taken between July 1 and August 15 of 1982. The sample contained 13 adult females (AF-POB), eight adult males (AM-POB), 15 juvenile females (JF-POB), and 15 juvenile males (JM-POB). The presence of the bursa of Fabricius was used to distinguish the juveniles from the adults.

The winter and pre-breeding birds differ basically in that there has been little opportunity to pick up additional infections since the birds are granivorous at this season of the year. Symbionts of these birds are very likely derived from some northern latitudes.

Analysis was made of the following organs and structures: gall bladder, brain, eye, trachea, digestive tube, kidney, liver, heart, body cavity and reproductive ducts. The organs in the digestive tube were isolated (esophagus, proventriculus, ventriculus, intestine) and examined separately. The intestine was divided into six equal parts and each section independently examined. Standard methods were used to extract, clean, fix, stain, clear and mount the specimens. Voucher specimens were prepared for a deposit in the United States National Museum Helminthological Collection.

It is assumed for this study that all the hosts are inhabitants of the Mississippi flyway and would have common symbionts, although the north south origins could differ.

DISCUSSION

Previous studies on the symbionts of the common grackle have opted for a tabulation of the prevalence and intensity of the infection without regard to the developmental and migration aspects of the host. Most of the studies, from northern states, are of breeding or post-breeding adults. Cooper and Crites in the Ohio sample showed the greater susceptibility of the juvenile common grackles through the greater number of incidental species found in those hosts. The differentiation and maturation of the structure and physiology of the juvenile bird apparently provides a suitable habitat for opportunistic symbionts.

All 101 common grackles examined in this study were parasitized by helminths. Table 1 lists the symbionts recovered from central Arkansas grackles and their host location. Table 2 shows the distribution of the number of species of helminths per host category. The mean is not substantially different in the eight categories. The total cases reflect the clustering of species number from three to five. The distribution ap-

Table 1. Helminths in Arkansas Common Grackles

Trematoda	
Conspicuum icteridorum Denton and Byrd, 1951	Gall bladder
Echinostoma revolutum (Froelich, 1802)	Posterior intestine
Leucocloridium macrostoma (Rudolphi, 1802) Poche, 1907	Posterior intestine
Cestoda	
Hymenolepis farciminosa Goeze, 1782	Intestine
Choanotaenia musculosa (Fuhrmann, 1896)	Intestine
Acanthocephala	
Plagiorhynchus cylindraceus (Van Cleave, 1918)	Posterior intestine
Mediorhynchus grandis (Van Cleave, 1916)	Posterior intestine
Nematoda	
Chandlerella quiscali (von Linstow, 1904)	Cerebral ventricles Under occipital meninges
Syngamus trachea (Montagu, 1811)	Anterior trachea
Acuaria quiscali Williams, 1929	Under gizzard tunic
Capillaria ovopunctatum (von Linstow, 1873)	Anterior intestine
Diplotraena sp.	Air sacs
Oxyspirura petrowi Pence, 1972	Eye
Dispharynx nasuta (Rudolphi, 1819)	Proventriculus
Eufilaria hibleri Granath, 1981	Subcutaneous tissue
Microtetrameres sp.	Proventriculus

pears to show a normal curve.

Table 3 illustrates the number of symbionts per host with numbers above 60 being unusual. The means for the host categories suggest for the adult female that there is a marked drop in the intensity of infection manifest after the breeding period and perhaps concurrent with that period. This is unexpected because the opportunities for infection are increased with the greater demand for food during the reproductive period. There is thus a suggestion of another agent at work in protecting the breeding female.

Table 2. Species of Helminths per Host

	1	2	3	4	5	6	7	$\bar{X}$
AF-W	0	2	5	5	2	0	1	3.7
AM-W	0	0	5	6	3	1	0	4.0
AF-PRB	0	2	2	4	2	0	0	3.6
AM-PRB	0	2	5	2	0	1	0	3.3
AF-POB	0	1	4	1	4	3	0	4.3
AM-POB	0	2	1	2	1	1	1	4.1
JF-POB	1	3	1	2	7	1	0	3.7
JM-POB	1	1	3	4	3	3	0	4.1
	2	13	26	26	22	10	2	

Table 3. Number of Helminths per Host

Host Category	0-20	21-40	41-60	61-80	81-101	$\bar{X}$
AF-W	5	2	4	2	2	45.9
AM-W	4	5	3	2	1	40.9
AF-PRB	3	2	1	2	2	48.6
AM-PRB	2	5	2	1	0	33.8
AF-POB	6	2	5	0	0	28.7
AM-POB	3	2	2	1	0	34.4
JF-POB	9	4	1	1	0	23.3
JM-POB	6	5	3	0	0	31.0
TOTAL	38	27	21	9	5	

Table 4 shows the percentage of infection in the eight host categories. In agreement with Cooper and Crites (1974), it is obvious that certain symbionts are incidental, present due to physiological or behavioral idiosyncrasies of the host. The following fall into that category: *Leucocloridium macrostoma, Diplotriaena sp., Oxyspirura petrowi, Eufilaria hibleri, Microtetrameres sp. Eufilaria hibleri* is a subcutaneous symbiont (Granath, 1981) and could have been missed in this study which concentrated on the viscera. Four symbionts are consistently found in high percentages and are thus the basic helminth fauna of the host in this flyway: *Conspicuum icteridorum, Chandlerella quiscali, Acuaria quiscali, Capillaria ovopunctatum.* There appears to be no age or sex difference in the infection considering that the juveniles have only had a maximum of three months to pick up the infections. Five species show a seasonal relationship to infection. *H. farciminosa* is exclusively a post breeding symbiont. The same is largely true of *C. musculosa* since the pre-breeding specimens were very small in number and size. Both acanthocephalan species are present in POB and W samples. The winter sample could be a retention of the POB infection. They are both absent in the PRB sample. This suggests that the symbionts are lost during the winter or that they are lethal to the host. *S. trachea* is distinctly a POB symbiont with some retention throughout the year.

Table 5 documents the mean intensity of infection for each symbiont for each host category. Only symbionts regarded as major symbionts are included. Juveniles appear to get a high dosage of *C. icteridorum* and the intensity decreases during the remainder of the year. *C. quiscali* has the greatest number of specimens per infected host. All adults are heavily infected. *C. ovopunctatum* is unusual in that the POB adults are so lightly infected compared to the other adults. This suggests a resistance to the infection during the breeding season with a second possible explanation being the food selection. Since the juveniles are more heavily infected, the opportunity for infection obviously exists. All of

Table 4. Prevalence (%) of Helminths in Arkansas Common Grackles

Helminth	AF-W	AM-W	AF-PRB	AM-PRB	AF-POB	AM-POB	JF-POB	JM-POB
C. icteridorum	93	100	100	100	100	100	73	87
E. revolutum	0	13	10	0	8	13	20	40
L. macrostoma	7	0	0	0	0	0	0	0
H. farciminosa	0	0	0	0	31	13	7	27
C. musculosa	0	0	20	10	8	13	7	0
P. cylindraceus	7	33	0	0	0	25	7	13
M. grandis	7	13	0	0	15	25	47	20
C. quiscali	93	80	100	90	92	100	93	47
D. tracheа	0	13	10	0	46	13	33	20
A. quiscali	67	53	40	40	39	38	40	20
C. ovopunctatum	73	93	80	60	77	75	67	53
Diplotraena sp.	20	7	0	20	0	0	0	0
O. petrowi	7	0	0	0	7	0	0	0
D. nasuta	0	7	0	0	0	0	33	47
E. hibleri	7	0	0	0	0	0	20	7
Microtetrameres sp.	0	0	0	10	0	0	0	0

Table 5. Intensity of Infection of Arkansas Common Grackles with Helminths

Helminth	AF-W	AM-W	AF-POB	AM-POB	AF-POB	AM-POB	JF-POB	JM-POB
C. icteridorum	7.6	8.9	4.8	6.6	4.9	5.3	5.8	6.0
E. revolutum	0.0	1.0	4.0	0.0	1.0	1.0	2.3	3.2
H. farciminosa	0.0	0.0	0.0	0.0	1.0	8.0	35.0	6.7
C. musculosa	0.0	0.0	1.0	1.0	2.7	1.0	2.0	8.0
P. cylindraceus	1.0	1.0	0.0	0.0	0.0	2.0	4.5	1.0
M. grandis	2.0	1.5	0.0	0.0	1.0	1.0	2.6	1.0
C. quiscali	26.6	27.7	32.7	15.5	20.3	21.5	4.5	6.5
P. trachea	0.0	2.0	2.0	0.0	2.3	6.0	3.6	2.8
A. quiscali	6.2	3.1	2.8	7.0	2.2	3.0	3.3	3.7
C. ovopunctatum	12.3	9.2	11.5	16.0	2.7	4.5	4.6	11.3
TOTAL	55.7	55.2	58.8	46.1	38.1	55.3	72.2	50.3

the other symbionts are present in small numbers, suggesting a limited carrying capacity of the host for these forms. If the adult POB males and females are compared, the females are consistently lower in intensity of infection with every symbiont.

Intestinal helminths appeared to be distributed in the organ according to species (Table 6). *Capillaria ovopunctatum* was found almost entirely in the anterior half of the intestine. The tapeworms, *H. farciminosa* and *C. musculosa*, appear to avoid the extreme segments. This latter case is somewhat biased due to the presence of 35 young tapeworms in the 4th segment of one host. *Choanotaenia* nevertheless appears to opt for a more anterior location. The two species of acanthocephala *(Plagiorhynchus cylindraceus* and *Mediorhynchus grandis)* together with the fluke, *Echinostoma revolutum*, are basically posterior half symbionts. There is a correlation between degree of invasiveness of the symbiont and posterior location. *C. ovopunctatum* are very thin nematodes which are threaded into the mucosa or are luminal. Each *H. farciminosa*

Table 6. Intestinal Helminth Distribution

Symbiont	Intestinal Section					
	1	2	3	4	5	6
C. ovopunctatum	265	256	119	13	6	1
H. farciminosa	0	0	5	45	5	1
C. musculosa	0	22	1	0	1	0
P. cylindraceus	0	0	1	5	10	2
M. grandis	0	0	0	3	26	4
E. revolutum	0	0	7	4	4	11

has 20 small hooks with which to attach to the intestinal wall. *Choanotaenia* likewise has minute but more numerous hooks. The other three species have hooks attached to a proboscis or the anterior end. Both the proboscis and the anterior end are large and are involved in the invasion.

Four of the incidental symbionts are found exclusively in the adults. *E. revolutum* has a greater incidence in the juvenile than the adult. *E. hibleri* was only found in the juveniles. *D. nasuta* appears to be an exclusive juvenile symbiont.

None of the incidentals reported by Cooper and Crites was found in the POB Arkansas birds. The comparison POB data by species concerning adult versus juvenile infection shows *C. iteridorum* (100:80), *C. musculosa* (10:4) *C. quiscali* (95:44), *C. ovopunctatum* (76:60), *D. nasuta* (0:40), *S. trachea* (33:27), *P. cylindraceus* (10:10). *P. ensicaudatum* and *A. globata* were both absent from Arkansas hosts. Ohio birds were heavily infected with the former. *E. revolutum, M. grandis, A. quiscali,* as well as the incidentals, were only found in the Arkansas birds. However, Buck, Cooper, and Crites documented *E. revolutum* in central Ohio birds. *C. icteridorum, C. quiscali, C. ovopunctatum,* and *H. farciminosa* are much more abundant in Arkansas birds than Ohio hosts. The reverse is true for *C. musculosa, S. trachea,* and *P. cylindraceus.* The observation of the heavy incidence of *D. nasuta* by Cooper and Crites in juvenile birds is borne out by the present study.

CONCLUSIONS

Sixteen species of helminths are designated as being present in Arkansas common grackles. These all constitute new locality records (Table 1). Twelve species are documented as having an Arkansas origin - present in juvenile birds.

Porrocaecum ensicaudatum, a common symbiont in Ohio birds, is absent in Arkansas grackles. *Mediorhynchus grandis* and *Acuaria quiscali* are present in Arkansas birds and not recorded in the Ohio birds. *E. revolutum, C. icteridorum, C. quiscali, C. ovopunctatum,* and *H. farciminosa* were more prevalent in Arkansas birds. The reverse is true for *C. musculosa, S. trachea,* and *P. cylindraceus. D. nasuta* is virtually an exclusive juvenile symbiont in grackles in both areas.

The major species with regard to the intensity of infection in the Arkansas birds appear to be *C. quiscali, C. ovopunctatum, A. quiscali,* and *C. icteridorum.* Other species are present in small numbers in the hosts.

The five species of intestinal helminths are partitioned along the entire intestine. Though the entire organ is used the helminths opt for certain sections by species of worm.

Juvenile birds have a higher propensity for infection with *E. revolutum.* Sex of the host is a factor when POB females and males are compared. The mean weight of all the adult females birds was 81.7 g. and of the adult male birds 106.7. Therefore, the females contain only 76% of the mass of the males. The same intensity of infection could have substantially greater consequences in the female.

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THE RIVER OTTER IN ARKANSAS. III. CHARACTERISTICS OF OTTER LATRINES AND THEIR DISTRIBUTION ALONG BEAVER-INHABITED WATERCOURSES IN SOUTHWEST ARKANSAS

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ABSTRACT

Forty-one river otter *(Lutra canadensis)* fecal deposit (latrine) sites were located during April 1983 through March 1984 along beaver *(Castor canadensis)* inhabited watercourses in Clark, Nevada, and Ouachita counties, Arkansas. Latrine sizes ranged from 64 cm^2 to 5.01 m^2 ($\bar{x} = 0.30$ m^2; S.D. = 0.88), and contained 2 to 78 scats per latrine ($\bar{x} = 9.2$; S.D. = 13.8). The most common sites for latrines included elevated leaf or moss covered banks (51.2%), beaver lodges or bank dens (17.1%), and beaver scent mounds (12.2%). Other latrine sites included felled logs over open water, bare soil along elevated banks, and exposed sandbars. Otter presence fluctuated seasonally in beaver areas, with the periods of greatest occurrence being early summer and late winter.

INTRODUCTION

Numerous studies concerning the effects of beaver pond formation on wildlife have been conducted (Beard, 1953; Rutherford, 1955; Speake, 1956; Knudsen, 1959; Arner, 1963; Reese and Hair, 1976; Allred, 1980). Practically all reports have found beaver ponds to be beneficial to the forms of life studied.

Analyzing harvest records, Tumlison et al. (1982) reported the possible existence of a commensal relationship by river otter *(Lutra canadensis)* with beaver *(Castor canadensis)*, facilitated through the development of suitable otter habitat in beaver ponds. This study was initiated to define characteristics of otter latrines and investigate the extent of utilization of beaver-inhabited watercourses by otter in southwest Arkansas.

METHODS AND MATERIALS

The study was conducted on eight beaver-inhabited watercourses during April 1983 through March 1984. Watercourse sizes ranged from small intermittent woodland branches to larger creeks and meandering sloughs in Clark, Nevada, and Ouachita counties (Fig. 1). On all watercourses beaver, through dam construction, had created ponds ranging from 0.8 ha to extensive areas of innundation 24 ha in size. Dominant vegetation of these beaver areas included bald cypress *(Taxodium distichum)*, water tupelo *(Nyssa aquatica)*, buttonbush *(Cephalanthus occidentalis)*, green ash *(Fraxinus pennsylvanica)*, smartweeds (*Polygonum* spp.), cattails (*Typha* spp.), and rushes (*Juncus* spp.).

Maximum depths in all areas sampled were greater than one meter during April through July and December through March. Due to dam removal and paucity of rainfall during the remaining months of the study, water levels were reduced to isolated pools and shallow channels in existing beaver runs.

Initially, only cursory investigations of study areas were conducted (April through June). During the remainder of the study, examinations were conducted at monthly intervals except at times when routine management activities provided more frequent examinations, or at times when, due to high water or ice cover, regular examinations were prevented.

Latrine sites were identified and size, distance from water, and elevation above waterline were recorded. When possible, estimations regarding the length of use, freshness of scats, and number of animals using a latrine were also recorded. During some sampling periods no latrine sites were discovered although tracks indicated otter were utilizing an area, or at least frequenting a particular location. To derive a more

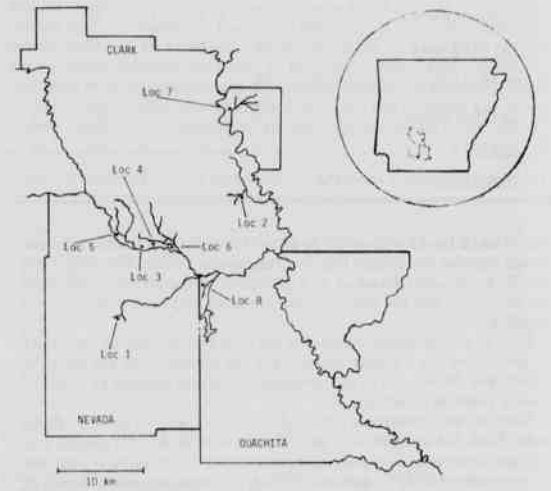

Figure 1. Locations of beaver-inhabited watercourses in southwest Arkansas.

Table 1. Numbers of latrines (track counts) recorded monthly along beaver inhabited watercourses in southwest Arkansas.

	Spring			Summer			Fall			Winter		
Location	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
#1	1	0	0	0	0	0	0	0	NI	NI	NI	0
#2	NI	0(3)	5	0	0	0	0	0	9	1(3)	4(4)	3
#3	0	0	0(1)	0	0	3	0	0	0(1)	NI	0(2)	1
#4	NI	NI	NI	0(2)	0(2)	1	0	0	0	NI	0(1)	2
#5	NI	NI	NI	0(2)	1	0	0	0	0	NI	0(1)	1
#6	NI	NI	NI	0(1)	3	0(1)	0	0	NI	NI	NI	NI
#7	0	0	0	0	1	0	0	0	0(1)	0	1	0
#8	0	0	0	0	0	0	0	0	0	0(1)	1	3

NI: Not investigated during month.

complete understanding of seasonal use of the study areas track data was recorded along with latrine data (Table). Numbers of individual otter were recorded when track characteristics indicated the presence of more than one animal.

RESULTS AND DISCUSSION

Latrine Characteristics: A total of 41 latrines was identified during the study (Table). The number of scats per latrine ranged from 2 to 78 ($\bar{x}=9.2$; S.D. = 13.8). The average latrine covered 0.30 m^2 (S.D. = 0.88), ranging from 64 cm^2 to 5.01 m^2. Distances from water and elevations above waterline ranged from 5 cm to 2.7 m ($\bar{x}=59.9$ cm; S.D. = 49.6) and 11 cm to 1.8 m ($\bar{x}=37.6$ cm; S.D. = 37.4), respectively.

Hewson (1973) reported finding spraints of European otter *(Lutra lutra)* in piles near the center of islands and beneath rhododendrons on a Scotish loch. Greer (1955) described river otter as having particular "toilets" near regular landings. Otter often defecate on large logs, rocks, logjams, sandbars, elevated banks, and any object protruding from the water (Melquist and Hornocker, 1983). The most common site for latrines found during this study were elevated debris-covered banks (21 sites or 51.2%) along main stream channels and beaver runs. Seven latrines (17.1%) were located on beaver lodges or bank dens. Interestingly, five latrines (12.2%) were found in association with beaver scent mounds. Muller-Schwarze and Heckman (1980) discussed the social role of scent marking in beaver. Along trails and dams on main travel lanes beaver scent mounds provide readily discernable signals to transient beaver that an area is currently occupied. Consequently, due to the strategic locations of these areas, otter latrines may also be deposited. Toweill and Tabor (1982) described scent marking activities among otter as including "not only deposition of excrement but also scratching together mounds of soil and debris or twisting tufts of grass together, either of which may have scent deposits or spraints deposited on top". During this study no otter scent marking consisting of mounds of soil and debris were observed, although otter latrines undoubtedly served a role in scent marking activities. Other less frequently encountered latrine sites included fell logs over water, bare soil on elevated banks, and exposed sandbars.

Many river otter latrine sites are used repeatedly (Melquist and Hornocker, 1983). Older scats at well established latrines often were difficult to classify according to age. Studying European otter, Jenkins and Burrows (1980) found 50% of spraints disappeared after two weeks and 83-94% disappeared after seven weeks. In this study exposure to rain and high water were major factors reducing the longevity of discernible scats. Scattered fragments of fish remains often indicated past use at well established latrine sites.

Fourteen (34.1%) of the recent latrine deposits were determined to represent repeated visits to an established latrine. Length of use for these latrines ranged from extended periods lasting more than two months (for two latrines situated on beaver lodges at locations 2 and 4), to repeat visits for a single latrine during a 36 hour period (location 8) after which the animal did not return. Melquist and Hornocker (1983) reported that traveling otter marked at traditional landing sites with the greatest amount of marking occurring at activity centers. When several otter congregated at activity centers, the area eventually became blanketed with scats. Liers (1951) reported that when several otter travel together each tries to be the last to leave its mark. In most instances it was impossible to ascertain the number of otter using a particular latrine, however using track counts it was determined that two latrines had been visited by more than one otter. One latrine located during August (location 5) was only minutes old when discovered and tracks along the bank indicated the presence of one large and three small otter, probably a female and young. During January, tracks in the vicinity of another latrine (location 2) indicated the presence of two otter. Melquist and Hornocker (1983) believed that frequent confrontations between unrelated otter traveling on the same stream were resolved by mutual avoidance; profuse fecal marking at activity centers served as signals to arriving otter that the site was presently occupied. Kaufmann (1983) presents a thorough discussion on the definitions and functions of dominance and territoriality. In his paper, Kaufmann summarized that there is an indivisible continuum in degrees of trespass onto territories, and functionally it is priority of access that is important rather than exclusive occupancy. This statement may describe otter dominance and territoriality. During the non-breeding season unrelated otter occupy the same general home range, move freely throughout the area, and use the same activity centers without associating with each other (Melquist and Hornocker, 1983).

Seasonal Activity Trends of Otter: Tumlison et al. (1982) compiled data indicating that trophic requirements of otter are supplied by beaver ponds on smaller streams through enhancement of all levels of the food chain. Otter were found to occur in all beaver areas throughout the course of the study (with the exceptions of October and November when all locations were virtually dry) indicating that otter make use of beaver areas so long as water levels maintain sufficient depth to provide foraging space.

Water levels probably exerted the greatest influence on seasonal habitation of beaver areas by otter. Humphrey and Zinn (1982) found seasonal changes in the distribution of aquatic habitat to be a factor involved in a seasonally declining mustelid abundance. Their observations of otter sign suggested that otter occupied permanent water bodies during the late dry season. Six of the eight locations used in this study were within 400 m of permanent creeks and rivers. This distance is easily within the expected limits of travel for a highly mobile animal such as the otter. Therefore, during the extremely dry months of October and

November otter probably returned to permanent creeks and rivers. Locations 1 and 8 were shallow intermittent branches on which beaver had constructed dams forming small ponds less than 4 ha in size. In April the dam and beaver at location 1 were removed, after which no further otter activity was observed. When the study was initiated a single beaver dam at location 8 was in disrepair, consequently no pond was present and water depth was less than 0.5 m. With the onset of rains during late November and early December, beavers reinhabited the area and repaired the existing dam. Otter presence was first recorded in January when the beaver pond had been refilled. These occurrences provide further substantiation that beaver greatly enhance otter habitat on intermittent streams.

Jenkins and Burrows (1980) concluded that changes in the numbers of spraints gave only an approximation of changes in the numbers of European otter present, although spraints could be used as indicators of the ways in which otter use habitat. Insufficient data prevents any conclusive interpretation of otter numbers, however, assuming that latrine deposition is a function of otter density (Melquist and Hornocker, 1983), and by including track counts which represent real otter numbers during a given examination period, seasonal trends of otter presence can be derived (Fig. 2).

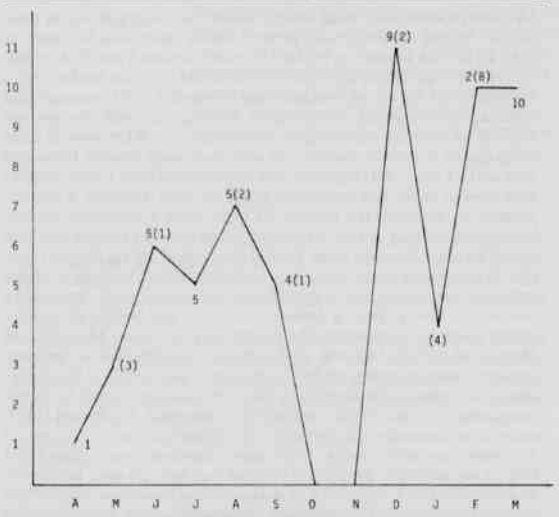

Figure 2. Numbers of otter latrines and tracks () used to derive seasonal trends of otter presence in beaver-inhabited watercourses in southwest Arkansas.

The values presented in Figure 2 probably represent conservative measurements of otter presence in beaver areas, especially during the first months of the study. The relatively low numbers of latrines and tracks recorded during April and May probably resulted from insufficient sampling of areas rather than low otter presence. The decreased values recorded during January also reflect insufficient sampling during snow and ice cover. Five of the eight locations could not be sampled during January. During the January examination period only one latrine was identified, however four individual sets of tracks were detected at two locations (Table). In February, when weather conditions permitted resumption of sampling, values increased. Also, due to elapsed time between monthly examination periods and the random arrangement of potential latrine sites it is doubtful that all latrines were identified.

Otters breed in late winter or early spring (Toweill and Tabor, 1982). The breeding season in Arkansas probably closely resembles those reported for other southeastern states (McDaniel, 1963; Lauhachinda, 1978). The period of greatest otter presence recorded during this study occurred during late winter. This period would coincide with the reported breeding season, indicating that otters may utilize beaver areas as breeding and rearing sites. Finally, this indication is strengthened by a reported preference for active and abandoned beaver bank dens and lodges as den and resting sites by otter (Melquist and Hornocker, 1983).

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EXTRACELLULAR PHOSPHODIESTERASE FROM THE GROWTH MEDIUM OF THE MYXOMYCETE *PHYSARUM FLAVICOMUM*

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ABSTRACT

The plasmodium of the myxomycete *Physarum flavicomum* secretes cyclic AMP phosphodiesterase into the medium. The extracellular enzyme had a pH optimum between 7 and 8 and a Km of about 500 μM cyclic AMP and was inhibited by theophylline, caffeine and 3-isobutyl-l-methyxanthine (MIX). A marked decrease of enzyme activity was noted in the presence of EDTA, suggesting the requirement of Mg^{++} by the enzyme. Addition of Mg^{++} and Ca^{++} stimulated the enzyme while Zn^{++}, Co^{++}, Pb^{++}, Mn^{++}, Fe^{+++}, Ni^{++}, and Cu^{++} all inhibited phosphodiesterase activity. An interesting feature of this extracellular phosphodiesterase was its ability to retain full catalytic activity after prolonged exposure to elevated temperatures.

INTRODUCTION

Cyclic AMP phosphodiesterase has been extensively studied in both prokaryotic and eukaryotic systems, and is the only known enzyme that hydrolyzes cyclic AMP. Although most phosphodiesterase is intracellular, some organisms release an extracellular phosphodiesterase which apparently acts as a signalling device. One of the best examples of an extracellular phosphodiesterase is released by the cellular slime mold *Dictyostelium discoideum*. Cyclic AMP has long been recognized as the chemotactic agent involved with aggregation in *Dictyostelium* along with an extracellular phosphodiesterase responsible in part for maintaining a gradient of cyclic AMP (Gerisch et al., 1972; Chassy, 1972; Toorchen and Henderson, 1979).

The role of cAMP in growth and differentiation of myxomycetes is not nearly as well understood. This cyclic nucleotide has been shown to be a positive attractant for the plasmodium of *Physarum polycephalum* (Kincaid and Mansour, 1979a) and the dibutyl derivative of cyclic AMP alters glucose metabolism during differentiation of the plasmodium of *P. flavicomum* into the sclerotium (Lynch and Henney, 1973). *P. polycephalum* has also been shown to release an extracellular phosphodiesterase (Murray et al., 1971; Kincaid and Mansour, 1979b) although the physiological significance of the latter is unknown.

This paper represents the first report of an extracellular enzyme from the plasmodium of *P. flavicomum*. One unusual feature of this enzyme is that it appears to be very heat stable.

MATERIALS AND METHODS

The plasmodium of *Physarum flavicomum* was grown in liquid shake cultures as previously described (Lynch and Farrell, 1984). Microplasmodia were harvested at 6-7 days by centrifugation at 2500 xg for 10 min and after decantation, medium were either frozen or used immediately.

The reaction mixture for cAMP phosphodiesterase measurements contained 40 mM Tris•HCl (pH 8.0), 5 mM $MgCl_2$, 50 μM $CaCl_2$, (^{3}H)-cAMP (.01 mM to 1.0 mM) and an appropriate amount of medium containing extracellular phosphodiesterase. The final volume of the reaction mixture was 0.1 ml unless otherwise stated. Incubation was at 30.0°C for 10-20 min.

Phosphodiesterase activity was measured by two independent techniques. One is a well established two-stage assay using an anion exchange resin to separate the substrate from product (Lynch & Cheung, 1975). In this assay (^{3}H) cAMP is converted to (^{3}H)-5'-AMP by phosphodiesterase. This first stage of the assay was terminated with .01 ml of 500 mM HCl. After two min., the solution was neutralized with .01 ml of 500 mM NaOH followed by the addition of .01 ml of 400 mM Tris•HCl pH 8.0. Snake venom (*Crotalus atrox*, 50 μg/tube) was then added as a source of 5'-nucleotidase. This quantitatively converted (^{3}H)-5'-AMP to (^{3}H)-adenosine and inorganic phosphate. An anion exchange resin (AGl-X2) is added and the tubes centrifuged. The resin bound and precipitated any remaining substract (^{3}H-cAMP). Aliquots of the supernatant, containing (^{3}H)-adenosine, were processed by liquid scintillation counting.

An alternate procedure used paper chromatography to separate substrate from product. This procedure is a one-step assay with (^{3}H)-5'-AMP as the product. Aliquots of the reaction mixture were spotted on Whatman #1 filter paper. Descending paper chromatography was done overnight using a solvent of 1M ammonium acetate and 95% ethanol (15/35, v/v). Spots corresponding to (^{3}H)-5'-AMP were visualized under ultraviolet light, cut out and the radioactivity determined by liquid scintillation counting. Cyclic AMP and 5'AMP were well separated by this solvent, with Rf values of .54 and .28 respectively.

RESULTS

Effect of Various pH Values

Extracellular phosphodiesterase from the plasmodium of *Physarum flavicomum* exhibited optimal enzyme activity in the pH range 7-8 (Table 1). All future enzyme assays were done at pH 8.0.

Table 1. The effect of pH on extracellular phosphodiesterase activity.

BUFFER	PH	nMOLE/MIN/ML	% ACTIVITY
CITRATE	4	2.5	11%
CITRATE	5	5.6	25%
PIPES	6	14.3	64%
PIPES	7	26.4	117%
TRIS	8	22.5	100%
NO ADDED BUFFER		5.5	25%

Enzyme activity was determined by the resin assay. The above indicated buffers were used to yield the desired pH. The "No Added Buf-

fer" contained no exogenous buffer other than that in the media. The pH of the media at harvest is usually between 5.0-6.0.

Kinetic Data

The extracellular enzyme followed linear kinetics with an apparent Km of about 500 μM cyclic AMP and a Vmax of about 20 nMole/min/ml of medium (Figure 1).

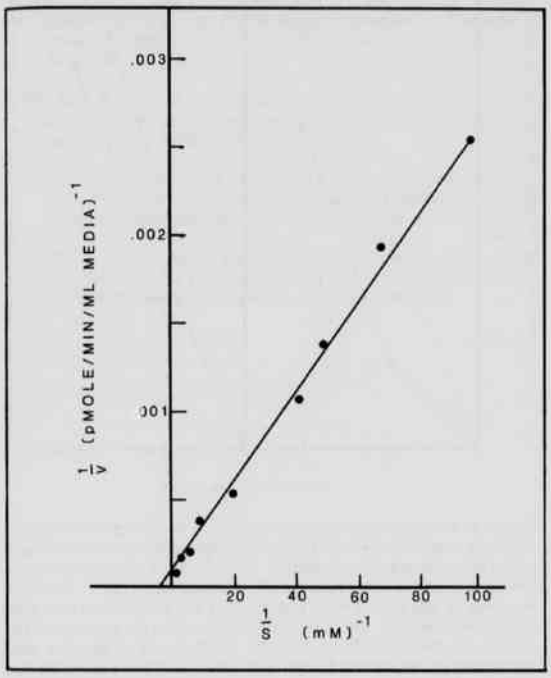

Figure 1. Lineweaver-Burk plot of the hydrolysis of (^{3}H)-cAMP by extracellular phosphodiesterase. The enzyme was assayed with (^{3}H)-cAMP concentrations from 0.01 mM to 1.0 mM for 20 min. Enzyme activity was measured by the resin procedure.

Table 2. The effect of various inhibitors on extracellular phosphodiesterase activity.

ADDITIVE	CONCENTRATION	NMOLE/MIN/ML MEDIA	%
NONE	-	18.3	100%
IMIDAZOLE	10 mM	21.6	118%
THEOPHYLLINE	10 mM	5.6	31%
CAFFEINE	10 mM	4.3	24%
MIX	2 mM	4.0	22%
EDTA	10 mM	7.4	41%
EDTA - Mg^{++}	10 mM	3.1	17%

Enzyme activity was determined by the resin assay. To determine the effect of EDTA without Mg^{++}, $MgCl_2$ was excluded from the reaction mixture.

Effect of Phosphodiesterase Inhibitors

Theophylline, caffeine, and MIX all inhibited phosphodiesterase activity with MIX showing the greatest inhibition. These compounds appeared to alter the activity of the extracellular enzyme in a manner similar to the cytoplasmic enzyme (Lynch and Farrell, 1984). EDTA also inhibited the extracellular enzyme, suggesting the requirements of magnesium for enzyme activity (Table 2).

Response to Metals

The presence of either Ca^{++} or Mg^{++} increased phosphodiesterase activity above the control and both metals together showed the highest phosphodiesterase activity (Table 3).

Table 3. The effect of metals on extracellular phosphodiesterase activity.

METAL	NMOLE/MIN/ML MEDIA	% ACTIVITY
NO ADDITION	22.6	100
Ca^{2+}	27.5	123
Mg^{2+}	24.8	110
Ca^{2+} + Mg^{2+}	29.0	129
Zn^{2+}	20.3	90
Co^{2+}	18.4	82
Pb^{2+}	16.8	75
Mn^{2+}	15.9	71
Fe^{2+}	8.2	37
Ni^{2+}	5.8	26
Cu^{2+}	4.2	19

Enzyme activity was determined by the resin assay. "No addition" indicates basal enzyme activity assayed with all metals excluded from the reaction mixture. The per cent activity in the presence of metals is based on "No addition" as 100%. With the exception of Pb^{2+} at 3 mM, the concentration of all metals is 5 mM.

Heat Stability

Throughout the course of our initial studies, several experiments suggested that the extracellular phosphodiesterase from the plasmodium was a heat stable enzyme. To investigate this possibility, samples of growth media from the plasmodium were heated in a boiling water bath for up to 20 min. and then assayed for enzymatic activity at 30 °C. The plasmodial enzyme retained full catalytic activity even after prolonged heating (Figure 2).

The enzyme activity in the above heating experiment was determined by a two-step ion-exchange resin procedure as described in Materials and Methods. To verify these results, a similar experiment was done using the resin assay and a paper chromatography procedure for determining enzyme activity. The chromatography procedure separates the substrate (^{3}H-cAMP) from the product (^{3}H-5-AMP). Two samples of media were used, one that was heated in a boiling water bath for 10 min. and one that was unheated. Both samples were then assayed for phosphodiesterase activity after 5 min. of incubation and 10 min. of incubation. Both the resin assay and the paper chromatography assay demonstrated that the enzyme was indeed heat stable (Figure 3).

DISCUSSION

The optimal pH requirement of the extracellular phosphodiesterase was in the range of 7-8. This is interesting in that the optimal pH for growth of the plasmodium was much lower. The pH of the medium

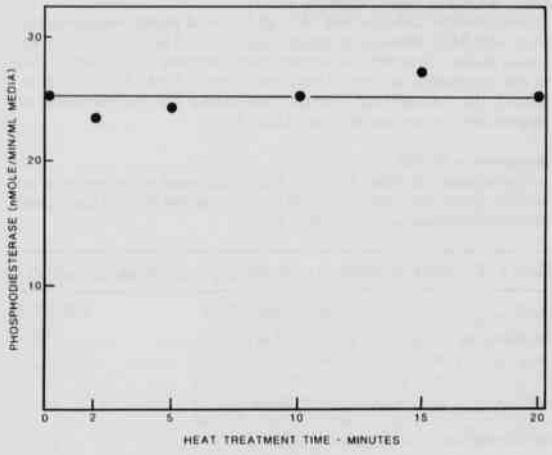

Figure 2. Heat stability of extracellular phosphodiesterase. Before each assay, aliquots of media were heated in a boiling water bath for the times indicated on the x axis. At the end of each heating, the tubes were thermal equilibrated to 30 °C and added to reaction mixture to start the enzyme assay. The enzyme was assayed for 20 min. and measured by the resin procedure.

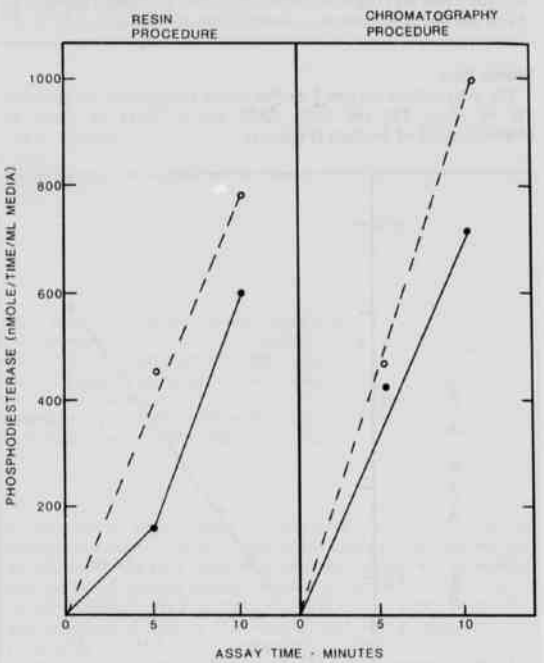

Figure 3. Comparison of enzyme activity of heat-treated (0- -0) and non-heat treated (•—•) extracellular phosphodiesterase by resin and paper chromatography procedures. Aliquots for heat treatment were placed in a boiling water bath for 10 ' and returned to 30 °C. Reaction mixtures of 300 µl each were prepared for the heat-treated and non-heat treated samples. The reaction was initiated by the addition of 100 µl each of the extracellular enzyme. Duplicate 25 µl samples were withdrawn at the indicated times and added to tubes containing 5 µl of 500 mM HCl to terminate the reaction. End product was determined using either the resin or paper chromatography procedure.

before inoculation was 4.3 and during growth the pH rose to about pH 6.0 at stationary phase. Although the enzyme did retain activity in this physiological pH range, it was far below the optimum. The role of an extracellular phosphodiesterase in plasmodial growth and differentiation is unknown. Any statements concerning the optimum growth pH versus the optimum enzyme activity pH can only be speculative at best.

The Km of the extracellular enzyme and the activity of the enzyme in the presence of various inhibitors used in Table 2 appeared to be very similar to that previously reported for the cytosolic enzyme from the same organism. Inhibition of the enzyme in the presence of EDTA suggested the requirement of magnesium by the enzyme. Phosphodiesterase from most organisms to date demonstrates increased activity in the presence of magnesium.

The effect of enzyme activity in the presence or absence of Mg^{++} (Table 3) would appear to be somewhat inconsistent with the EDTA data from Table 2. However, this is an enzyme that was released into the medium and aliquots of the medium were used for each assay. Mg^{++} was added to the medium as a trace element at a final concentration of about 0.5 mM. This endogenous Mg^{++} can be chelated by EDTA as shown in Table 2, but still maintained the enzyme close to maximum activity when no exogenous Mg^{++} was added (Table 3).

The apparent heat stability of the enzyme from the media appeared unusual. A few cases of heat stable phosphodiesterase from other organisms have been reported (Bevers et al., 1974; Sankaran et al., 1978; Shaw and Harding, 1983), but in general most phosphodiesterases were very heat labile. The cytoplasmic phosphodiesterase from *P. flavicomum* also appeared to be heat labile (data not shown). Two independent assay methods were used to verify the authenticity of the heat stability. One was a well established two-step resin assay which isolated adenosine as the end product and the other was a one-step paper chromatography procedure which separates 5'-AMP as the end product. Both techniques gave similar data and supported the concept of a heat stable enzyme.

The significance of a heat stable extracellular phosphodiesterase in this organism is unknown. The plasmodium grew best at room temperature in the laboratory and has not been known to tolerate elevated temperatures in nature. Further studies on this enzyme are in progress.

ACKNOWLEDGEMENTS

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A SUMMARY ACCOUNT OF THE CAROLINA PARAKEET IN ARKANSAS

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ABSTRACT

The extinct Carolina Parakeet *(Conuropsis carolinensis)* once was part of the Arkansas avifauna. The first two reports of the species in what is now Arkansas were made in 1673 and 1718 by early French explorers. The remaining records are from the 1800s when parakeets were found in nearly all parts of the state, often in abundance. The last literature reference for the species still definitely occurring in Arkansas pertains to birds present in the summer of 1885 along the White River at Newport.

INTRODUCTION

There are 13 species of birds that once nested in Arkansas that were extirpated in historic times as breeding birds in the state (James, 1974). At least 4 additional species have disappeared from Arkansas that formerly were present as migrants and/or wintering birds (Baerg, 1951). Of these 17, two are definitely known to be extinct species (Amer. Ornithologists' Union, 1983). These are the Passenger Pigeon *(Ectopistes migratorius)* and Carolina Parakeet *(Conuropsis carolinensis)*. This paper concerns the history of the knowledge of the latter species in Arkansas, which was the only naturally occurring temperate zone member of the parrot family found in the present North American avifauna.

EARLY REPORTS

French explorers seem to have been the first Europeans to report the Carolina Parakeet from the region of what is now the State of Arkansas. Louis Joliet, who descended the Mississippi River with Father Marquette to the mouth of the Arkansas River in the summer of 1673, had written at one point: "Perroquets fly in flocks of 10 to 12." Unhappily, it is not possible to place the reference geographically with any precision (Thwaites, 1896-1901, 58:99).

The southward advance of the Marquette party faltered somewhat at the mouth of the St. Francis River, when they were told that they were still ten days' journey from the Gulf of Mexico. Describing typical lower Mississippi valley countryside, their journalist recorded: "We thus push forward, and no longer see so many prairies, because both shores of The river are bordered with lofty trees. The cottonwood, elm, and basswood trees there are admirable for their height and thickness. The great numbers of wild cattle, which we heard bellowing, lead us to believe that the Prairies are near. We also saw Quail on the water's edge. We killed a little parroquet, one half of whose head was red, The other half and The neck yellow, and The whole body green." The party reached the mouth of the Arkansas River before giving up and beginning the return journey toward Illinois and Canada on 17 July (Thwaites, 1896-1901, 59:149,151). Again, no exact place can be determined, although an Arkansas locality is perhaps credible. It was, at any rate a great while before anyone improved upon, or even equalled, that succinct description of the parakeet.

Another early French reference is more substantial (Le Page, 1763). It is also of concern ornithologically, since the author, Le Page du Pratz, was tangentially responsible for the term Louisiana Parakeet sometimes bestowed upon western populations of the parakeet. On the basis of his remarks, Gmelin (1788:320) erected the name *Psittacus ludovicianus*, the second part of that name meaning "of Louisiana."

Le Page du Pratz, a professional engineer, lived in Louisiana (a general term that referred to the southern part of the French domain in America) for some 16 years following 1718. He traveled on the Mississippi as far north as present day Memphis, as well as elsewhere in the region. On one of his trips in what is now the state of Louisiana he ascended the "Black River" (so called because of its depth; now called the Ouachita River), possibly reaching what is now the State of Arkansas, and wrote: "The woods are like those to the East of the Mississippi, except that to the West there are more walnut and hiccory trees. These last are another species of walnut, the nuts of which are more tender, and invite to these parts a greater number of parrots" (Le Page, 1763, 1:281-282). (The "hiccory" reference may be to thin-shelled, small fruited pignut hickories, *Carya cordiformis*, or perhaps to the thin-shelled true pecan, *Carya illinoiensis;* Le Page's original French version, in fact, used the word "pacanes.")

THE NINETEENTH CENTURY

Central and Northwestern Regions

It is of interest that William Dunbar, an observant friend of the natural sciences in Mississippi, ascended the Ouachita River (by then it was called the "Washita") to the Hot Springs of Arkansas in the period 16 October 1804 to 26 January 1805. He left detailed and abundant records on game, vegetation and salt springs but said nothing at all about parakeets. We can only guess that parakeets were absent at that time of year or so common that he did not think it worth his while to notice them (Rowland, 1930).

Our confidence that Dunbar told all that he saw is shaken somewhat by the account left by George W. Featherstonhaugh (1835:72; 1844:115), a geologist and a general naturalist of undoubted competence. Thanks to Featherstonhaugh, modern readers can still saver the magnificence of Arkansas countryside as viewed in early December 1834. He was near the Ouachita at the mouth of Caddo River in northeastern Clark county: "This place is the site of an ancient village of the Caddo Indians...and a sweet sequestered situation it must have been to them, for the river contains good fish, the country abounds in game, and the sandstone, with its pines, is here exchanged for a loose soil of the greatest fertility....On sallying out, after our good cheer, we were exceedingly pleased with the scene around us; the sun was shining brilliantly, flocks of parroquets were wheeling and screaming around, the trumpet tone of the ivory-billed woodpecker was frequently heard." He recorded an extensive level cane brake and he saw "laurel" trees of large size and,

with them, holly trees up to 12 inches in diameter.

Featherstonhaugh (1844:86-87) had already seen parakeets in his overland tour through southeastern Missouri (McKinley, 1960) and north-central Arkansas. For example, in present Arkansas, he reported on about 9 November, near the Strawberry River (probably in Sharp County): "We are now on rather a flattish country with open woods, and flocks of parroqueets screaming around us."

German-born Hermann Balduin Möllhausen, artist to Lt. Amiel Weeks Whipple's exploring team, surveying the 35th parallel for a railroad route to the Pacific, left an evocative account of his ascent of the Arkansas River in early June 1853. On the way to Little Rock, he recorded that "the parrot climbs chattering from bough to bough." Later when camped in early July near the mouth of Poteau River across the Arkansas River from Fort Smith (and thus technically within what is now Oklahoma), he referred to "the chatter of the parrots on the nearest trees" (Möllhausen, 1858, 1:8,17). Before leaving the Fort Smith region on 15 July, he shot and prepared a specimen of the parakeet (Kennerly, 1859:21). (This skin is now U.S. National Museum specimen no. 3890; it is said to be from Fort Smith; it is not dated precisely and its sex was not recorded.)

A contemporary of Möllhausen, Arthur Lawrie, traveled on the Arkansas River in the winter of 1854, having progressed by steam boat from Indiana down the Ohio and Missisippi rivers and up the Arkansas River. He noted in his journal for 16 December (Lawrie, 1944:264): "Saw paroquets for the first time, on the Bayou des Rockes" (presumbly that small stream now called Rock Creek in Pulaski County, near Little Rock).

Fort Smith continued to be a stronghold of parakeets. Wrote Charles E. Bendire (1895:1,2), who had been stationed there as a member of the Medical Corps of the Fourth Cavalry: "As late...as 1860 they were still comparatively numerous through the Gulf States and the Mississippi, Arkansas, and White River valleys; and I well remember seeing large flocks...through that year in the vicinity of Fort Smith..." And again: "In the vicinity of Fort Smith...during the fall and winter of 1860-61, I frequently saw flocks of these birds in osage orange trees."

That parakeets were commonly noted in that region is further indicated by a report from just north of Fort Smith at Hermannsburg (now Dutch Mills) in present Washington County. Recalling life there Karl Friedrich Herman wrote about the brightly colored "Parroquets" (his word: he did not use the usual German term "Papageien") that moved gently in the branches of a black locust when the tree was covered with its fragrant flowers in spring (Hermann, 1900:177). Karl Hermann first arrived at Hermannsburg on 8 May 1853 and departed in 1862 (Lemke, 1965).

It is evident that parakeets held out nearly as long in northern and western Arkansas as they did anywhere (see also McKinley, 1960, 1964, 1978). For northern parts of the state, for example, Benjamin T. Gault recorded in 1888: " 'At one time Paroquets were very plentiful at Paroquet Bluff between Newport and Batesville on the White River, but none have been seen there for at least eight years.' " (Widmann, 1907:116). (This refers to Independence County.) This more or less coincides with the notice that "in 1885 Mr. W. A. Monroe reported them as summer residents at Newport" on the White River, Jackson County (Howell, 1911:44). (The Arkansas career of Gault is unknown to us; all that we can say of William A. Monroe is that he submitted migratory bird records to the U.S. Biological Survey from 1884 to 1889.)

Mississippi Valley Region

Parakeet reports from the Mississippi River valley, except for the pre-1800 French reports, are treated below. For a full picture of Mississippi River records, it is necessary to consult the accounts for Tennessee and Mississippi (McKinley, 1979, 1981).

An early nineteenth century report for the Mississippi River is that of Samuel P. Hildreth, a reliable pioneer naturalist. He left Marietta, Ohio, 21 April 1805, on a small boat bound for New Orleans. The group's trip was a speedy one (he and his companions were leaving Natchez, going southward, on 31 May); but his account hints at an uneven distribution of parakeets, even at that early day. The party evidently saw no parakeets on the Ohio River. Then, in the 140 mile stretch of wilderness (as Hildreth called it) along the Mississippi above Fork Pickering (Memphis), "As they sailed gaily along, the attention of Charles and Graham was constantly arrested by the noisy chattering of the paroquets. Their gay plumage and lively motions, as they hopped from branch to branch amongst the deep green foliage of the trees, several of which were in flower, afforded a constant theme for remark." Sandhill cranes, swans and pelicans were birds of "more staid habits" (Hildreth, 1842:131). This entry can probably safely be placed in either Mississippi County, Arkansas, or on the Tennessee side, some distance above Fort Pickering. (The uneven distribution of parakeets, even in early times, may be judged from previous analyses of historical reports found in McKinley, 1977 and 1980.)

The early botanist and ornithologist, Thomas Nuttall, found parakeets in the Mississippi River valley in the early 1800s. Thus on 7 January 1819 he wrote (Nuttall, 1821:57-58) that in the "luxuriant wilds of the Mississippi...river lands, as usual, grows platanus or buttonwood, upon the seeds of which flocks of screaming parrots were greedily feeding." At the time he was in the vicinity of the mouth of the St. Francis River, but his parakeet comments were a part of an overall commentary summarizing general conditions in the Mississippi River valley.

John James Audubon (1929) several times referred to parakeets in the valley of the Mississippi. To keep the record in one place, and because it is not possible to tell with certainty to which side of the river an entry refers, we shall cite here all his reports from the latitude of Arkansas. He floated from Cincinnati to New Orleans in 1820-1821, leaving 12 October. On 30 November, his party made 25 miles and landed in cold, wet and disagreeable weather just past the Third Chickasaw Bluff below the "Twelve Outlets": "Many birds were seen during the day;" "the Parokeets Numerous in the Woods —" (Shelby County, Tennessee, or Crittenden County, Arkansas; they passed the mouth of Wolf River the next day).

On 2 December, Audubon (1929) noted: "— the Woods literally filled with Parokeets" in rain and cold. They landed at night at "Tow Head, above Island no. 51 (below the Tennessee-Mississippi state line; now called Buck Island and currently in the state of Mississippi, opposite southern Crittenden County). The next day, they were able to float only about four miles, to the foot of the island, Audubon recording that on the island there were "Many dry Nests of Thrushes on the Willow Trees — the Tall Grass with many Sparrows — Saw 2 Flocks of Partridges, Many Parokeets." There had been only two frosts when the Audubon party landed at the mouth of White River. Parakeets were among several species of birds noted in the general area of the nearby mouth of the Arkansas River, Desha County.

On 17 December, the Audubon party landed at "Pointe Chico" (Chicot County, extreme southeastern Arkansas); they had encountered the first "Spanish Beard" (Spanish Moss, *Tillandsia usneoides*) a few miles above. They floated only a few miles on 18 December and on the 19th: "Rain and fog all day — Landed within 7 Miles of Last Nights — Killed a Carrion Crow, a Winter Wren and 16 Parokeets...Immense flocks of Parokeets and Swamp Blackbirds—." The party made only four miles on 20 December and landed on "opposite side of river," where Audubon "boiled 10 Parakeets" to try their supposed toxic effects upon his bitch, "Dash" (why boiled, we do not know, parakeet "guts" were widly alleged to be poisonous). They had by then gone to the boundary of southeastern Arkansas, or perhaps even beyond (Audubon, 1929:51,55,57,71,77,80,81,82; we thank the U.S. Board of Geographic Names for help with modern names of the Audubon localities).

The observent Paul Wilhelm, Duke of Wurttemburg (1941), did not record any parakeets on the Mississippi River between the mouth of the Yazoo River in Mississippi to near the mouth of the Ohio River on his trip up river in April 1823. This suggests either numbers of parkeets already had declined drastically or that they simply varied dramatically within seasons or years.

McKinley (1979) reported the observations of Alexis de Tocqueville and Gustave de Beaumont (Pierson, 1938:594) on the parakeet at Memphis, December 1831. Beaumont's colored sketch (now at Yale University) confirmed the identity of the parakeet. A little later in that decade, the Swedish traveler Carl David Arfwedson (1834, 2:96) steamboated up the Mississippi, having left New Orleans about 1 February 1833. His only reference to parakeets came on the fifth day, when the party was somewhat south of the mouth of the Arkansas River (therefore,

Chicot or Desha counties, or on the opposite side in the State of Mississippi). As, apparently, with pioneer travelers everywhere, they went ashore at every stop with loaded firearms: "I landed, with a few of my fellow-travelers at one of the firewood stations, with an intention of killing some of the small green parrots, which were flying in thousands about in the wood...Our sportsmen came running in every direction from the wood, carrying on their shoulders a variety of birds, among which parrots were the most conspicuous, on account of the beauty of their plumes."

Anderson (1907:126,271) mentions that "about a dozen specimens" of parakeets were in the Museum of Natural History at the University of Iowa that "were taken by D. H. Talbot's collectors at the mouth of the Arkansas River in 1882." The "Arkansas River" is apparently Anderson's interpretation of Talbot's designation "Mouth of Grand River" which has been shown to be the Grand River in present day Oklahoma where Talbot's collectors were active in 1882 (Hahn, 1963; McKinley, 1964; plus entries in the early museum ledgers, University of Iowa Museum of Natural History). Therefore, these specimens are not from Arkansas.

SOME FINAL REMARKS

The preceeding documentation shows that Carolina Parkeets once were found in most parts of Arkansas, sometimes in abundance, but were not reported after 1885. We hereby summarize a series of increasingly vague references to parakeets, all of them inferentially at least including Arkansas. Baird *et al.*, (1874:587) reported parakeets "still found in considerable numbers" in Arkansas but cited no specific localities. Oliver P. Hay (1882) saw none in his survey of birds in the lower Mississippi valley in the summer of 1881 but heard "that it had been seen in southeastern Arkansas." The second AOU Check-List (1895:152) indicated the species was present, although "only of local occurrence," in Arkansas. Robert Ridgway (1916:147), for reasons apparently known only to himself, held out the hope that "if still existing to be found only in small numbers in very restricted areas in bottom lands of southwestern Arkansas or northwestern Louisiana."

Except for the scholarly review by A. H. Howell (1911), the parakeet in Arkansas has been all but ignored in the twentieth century. H. E. Wheeler (1925:6) included the species "but considered as with meagre evidence." Baerg (1951:74) referring to Howell stated: "The most recent record for Arkansas is apparently 1885."

A final note pertains to a parakeet specimen in the U.S. National Museum. It is a mounted bird of unknown sex but immature age labeled simply "Arkansas?" and marked "rec/d prior to Sept. 1844 from Major W. B. Lewis." According to Michael J. Brodhead (letter, 28 March 1984), W. B. Lewis possibly was William Berkeley Lewis, the Nashville, Tennessee, friend of Andrew Jackson who was Jackson's quartermaster in the War of 1812 (see the entry for him in "Dictionary of American Biography"), not a major in the regular army (not named in Heitman's "Historical Register and Dictionary of the U.S. Army") but sometimes bore the honorific title of "major." Nothing, however, is known concerning the parakeet specimen he apparently possessed that eventually became specimen no. 23,700 in the U.S. National Museum.

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WATER QUALITY IN THE GILLHAM LAKE-COSSATOT RIVER SYSTEM DURING DRY AND WET PERIODS*

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ABSTRACT

Water samples were collected in the Cossatot River-Gillham Lake system during an extended dry period and after heavy rains to determine the spatial variations in certain water quality characteristics. Of particular interest was the influence of the reservoir discharge on the water quality of the tailwater compared with the effects of four tributaries entering the tailwater below the reservoir. The water quality of the Cossatot River below Gillham Lake at low-flow (dry periods) and during the first 3 days after heavy rainfall (wet period) was influenced more by the tributaries entering the tailwater than by the reservoir water release. We estimated, however, that the amount of particulate inorganic matter released to the tailwater from the reservoir after the initial 3-day wet period would be greater than the amounts entering the tailwater from the tributaries.

INTRODUCTION

Reservoirs can be regulated to benefit downstream environmental quality, however, many requirements for downstream habitats and biota are not totally understood or substantiated. Changes in various physicochemical characteristics associated with reservoir water release greatly influence the composition and abudnance of species in the tailwater communities. Methods for determining the quality, quantity, and timing of reservoir water release to maintain the tailwater ecosystem are inadequate. In 1979 the National Reservoir Research Program (U.S. Fish and Wildlife Service) and the Waterways Experiment Station (U.S. Army Corps of Engineers) began a cooperative study to evaluate environmental criteria and operational methods applicable for the maintenance of desirable tailwater aquatic habitat and associated biota (Walberg et al., 1981; Walberg et al., 1983).

Water quality measurements during the 3 year study period were taken in Gillham Lake, Arkansas, and in the Cossatot River downstream from the dam structure. Periodic collections of water samples were adequate to understand some water quality relationships during the related time periods; however, spatial variation of water quality characteristics could not be determined, particularly with regard to tributary influence on the tailwater system during extended dry periods and after heavy rainfall. We therefore began a short term study in 1981 to investigate the spatial variation of water quality in the Cossatot River-Gillham Lake system during an extended dry period and after heavy rains.

STUDY AREA

Gillham Lake (153 m above mean sea level) is a 554-ha multipurpose U.S. Army Corps of Engineers impoundment on the Cossatot River in the Little River drainage of southwest Arkansas. The reservoir was designed with multiple level water release outlets at 4.5 and 9.0 m below conservation pool to maintain lowflow (1.5 to 4.2 m^3/sec) into the tailwater, and at 19.7 m below conservation pool to discharge higher flows (4.2 to 85 m^3/sec). The lake has a drainage area of 702 km^2 and a storage ratio of 0.1. The average annual rainfall near Gillham Lake is 137.2 cm.

We selected 10 sample collection sites that would best describe the Cossatot River above and below the reservoir, the reservoir itself, and the major tributaries that might influence the water quality within the tailwater (Fig. 1).

METHODS

In situ measurements of water temperature, dissolved oxygen, pH, and specific conductance were taken with a Hydrolab model 8000**** at mid-depth at all riverine sample sites and at 1 m intervals at the two reservoir stations. We collected water samples (500 mL) at about 3-m depth intervals in the reservoir, and at mid-depth at the other sampling sites. The samples were acidified (pH less than 2) with H_2SO_4 and returned to the laboratory for analysis of total iron and manganese. An additional 10-L sample of water from each location was filtered through an 0.08-mm mesh net to retain coarse particulate matter. A 1-L subsample of the filtrate was vacuum pumped through a preweighed glass-fiber filter paper to retain fine particulate matter. The filtrate (100 mL) from the fine particulate matter was used for analysis of total dissolved solids.

Total iron and manganese were analyzed by atomic absorption spectrophotometry at the chemistry department of Ouachita Baptist University. The coarse particulate matter samples were retained on a glass-fiber filter paper for drying. The coarse and fine particulate matter filter papers were then oven dried at 60 °C for 24 hours. Inorganic portions of coarse and fine particulate matter were determined by ashing the samples at 550 °C for 20 minutes (American Public Health Association et al., 1976).

The dry period was characterized by continuous low-flow discharge (1.5 m^3/sec) from the reservoir for 41 days prior to sampling on October 1, 1981. A single rainfall (2.3 cm) occurred 17 days before sampling however, it did not raise the lake level.

A wet period (defined as occurring immediately after ≥ 5.0 cm of rain fell within a 48-hour period) did not develop in the Gillham Lake watershed until January 20, 21, and 22, 1982, when 1.65, 1.73, and 2.4 cm of rainfall occurred, respectively. The lake level during that time rose 7.2 m. During sampling on January 23, reservoir discharge was increased from 1.5 m^3/sec to 18.7 m^3/sec one-half hour before we began collecting water samples in the tailwater.

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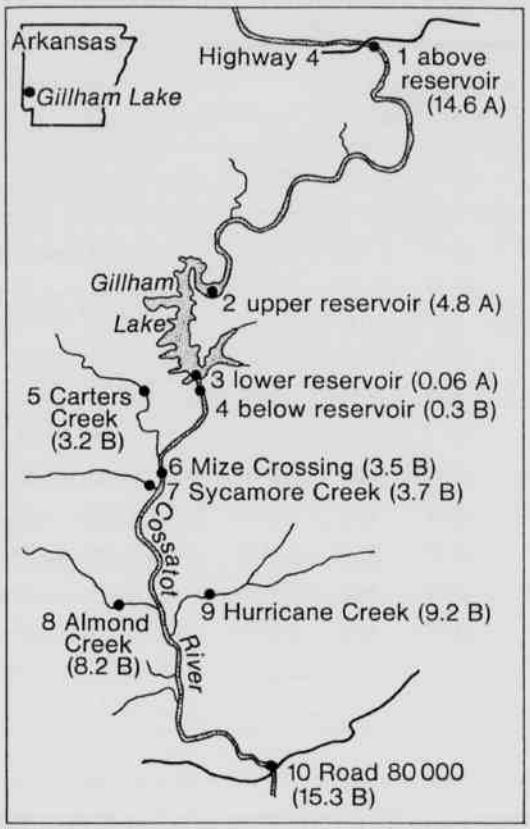

Figure 1. Water quality sampling stations in Gillham Lake watershed; numbers following station names show distance in kilometers above (A) and below (B) the dam.

Low-flow discharge into the tailwater during the dry period (1.5 m^3/sec) was only from the upper low flow gate, 4.5 m below conservation pool level. Discharge during the wet period (18.7 m^3/sec) was from all three reservoir release levels (4.5, 9.0, and 19.7 m below conservation pool).

RESULTS

Reservoir

During dry conditions, temperature variation within the water column was small at sampling sites in the upper and lower reservoir (Stations 2 and 3, respectively). However, an oxycline occurred at 5 m in the upper reservoir and at 7 m in the lower reservoir. A stable anoxic hypolimnion (dissolved oxygen, < 0.5 mg/L), in the lower reservoir led to chemically reduced conditions and higher amounts of total iron and manganese and total dissolved solids than in the upper reservoir (Table 1).

Temperature and dissolved oxygen profiles at both reservoir stations showed little variation during the wet period. However, a spate at Station 2 in the upper reservoir contained allochthonous materials from the Cossatot River above the reservoir and from the tributaries entering the reservoir above the sampling site. Total particulate organic matter (POM) was 3.5 times greater and total particulate inorganic matter (PIM) 40 times greater in the upper reservoir where samples were collected within the spate, commpared to the lower reservoir. Total iron and total dissolved solids were also greater in the freshet indicating leaching of the soils during runoff (Table 1).

River

Dry Period: Flows within the tributaries that empty into the tailwater normally ranged from 0.08 to 0.42 m^3/sec. During the dry period the surface flow within the tributaries appeared to be negligible. However, hyporheic and subterranean flow from the tributaries apparently influenced some physicochemical characteristics in the tailwater. Dissolved oxygen decreased and total iron increased at Station 6, (Mize Crossing, Fig. 1) in the tailwater below the outfall of Station 5, (Carters Creek). Water temperature downstream decreased slightly as a result of the lower water temperatures in the three tributaries entering the tailwater below Station 6. Conductivity and total dissolved solids in the tailwater showed little spatial variation, even though values in the tributaries were nearly two times greater (Table 2).

Concentrations of coarse particulate organic and inorganic matter below the dam (Station 4) were similar to those in the lower reservoir. Allochthonous material in Carters Creek resulted in increased coarse POM and coarse PIM at Station 6 in the tailwater. At Station 10 (Road 80000) the amounts of particulate matter decreased, with no apparent influence from the other tributaries (Fig. 2).

Fine POM was higher in the tailwater below the dam than in the lower reservoir. Higher values of fine POM and fine PIM were recorded at Station 6 below the outfall from Carters Creek. Both fine POM and PIM continued to increase with increasing distance downstream. The tributaries entering the tailwater (Carters, Sycamore, Almond, and Hurricane Creeks) and erosional effects of flow within the tailwater increased the amount of fine POM and PIM within the tailwater, even though surface flow from the tributaries was not evident (Fig. 2).

Wet Period: Flow in the Cossatot River above Gillham Lake (Station 1) during the wet period was about 56 m^3/sec, as a result of 5.8 cm of rainfall. The initial runoff which contained large amounts of allochthonous material had passed Station 1 when samples were collected. However, total dissolved solids and total iron concentrations in the upper reservoir (Station 2) were high as a result of the freshet which did not move from the upper reservoir during the sampling period. The reservoir water discharged into the tailwater, therefore, was not influenced by the spate. Carters Creek, which drains mainly agricultural land, contributed to the higher concentrations of total iorn, total dissolved solids, and conductivity at Station 6 in the tailwater. Additional iron from the other three tributaries (Stations 7, 8 and 9) below Station 6 resulted in additional total iron and total dissolved solids at Station 10 in the tailwater (Table 2).

Coarse POM was low at both reservoir sample sites, even though fine POM was more than twice as great in the upper reservoir. In the tailwater and tributaries course and fine POM were without a pattern (Fig. 3).

Carters Creek contained high amounts of coarse PIM, which resulted in high amounts at Station 6. However, measurements of course PIM at Station 10 were lower than at Station 6, as a result of dilution by Sycamore, Almond, and Hurricane Creeks. Fine PIM was very high in the upper reservoir as a result of the surge of flood water but was low in the lower reservoir, which had not yet received the water from the upstream freshet. Fine PIM in the tailwater increased progressively with distance downstream. Increased reservoir discharge resulted in higher flow (18.7 m^3/sec) at the tailwater dam site and at Station 6; however, the increased flow had not reached Station 10 when samples were collected. Therefore, the higher fine PIM downstream (Station 10) was contributed by Almond and Hurricane creeks (Fig. 3).

****Mention of trade names of manufacturers' does not imply U.S. Government endorsement of commercial products.

Table 1. Profiles of certain water quality characteristics during dry (October 1981) and wet (January 1982) periods in Gillham Lake, Arkansas.

Section of reservoir and depth (m)	Iron (mg/L)		Manganese (mg/L)		Particulate matter mg/L)				TDS (mg/L)		Conductance (µmhos/cm)	
					Organic		Inorganic					
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Upper												
1	0.1	0.1	0.1	0.0	2.50	2.79	1.46	50.29	44.8	57.2	53	48
4	0.1	0.5	0.1	0.0	2.84	3.87	0.15	46.68	37.8	54.7	56	48
7	0.2	0.5	0.1	0.0	1.39	4.85	2.20	79.36	38.6	53.3	58	52
10	0.2	0.9	0.1	0.1	2.59	5.81	1.93	125.53	-	66.5	65	52
13	0.7	1.9	0.5	0.1	2.61	3.27	1.48	98.01	39.6	68.5	93	53
Lower												
1	0.2	0.1	0.2	0.0	2.61	0.62	1.06	2.83	36.4	36.2	53	53
4	0.0*	0.1*	0.1	0.0	2.44	0.83	0.11	1.63	32.8	36.4	53	53
7	0.0	0.0	0.0	0.0	2.56	1.73	0.38	1.70	31.8	37.7	52	53
10	0.1	0.0*	0.3	0.0	2.16	1.16	1.42	1.70	36.5	37.1	64	53
14	2.5	0.1	0.7	0.0	1.89	1.33	1.47	1.70	47.3	37.2	79	53
17	3.9	0.0*	0.9	0.0	1.48	1.20	0.58	1.91	53.0	-	99	53

[1]TDS - Total dissolved solids
*Gate levels for reservoir water release

DISCUSSION

The changes in inorganic matter between the dry and wet periods was due to differences in the amounts of allochthomus matter entering the system during October and January. However, the changes in organic matter between the dry and wet periods were not due to differences in allochthonous matter (leaf litter) during October and January but to autochthonus differences in standing stocks of algae and zooplankton and to dilution from increased water volumes during the wet period. Other variables may have been influenced by the seasonal variation; however, discussion of these variables is limited to comparisons within the dry or wet periods.

As discharged reservoir water moves downstream, it is typically influenced by local conditions that tend to characterize each tailwater (Pfitzer, 1954). During dry conditions and within about 3 days after heavy rainfall, certain physicochemical conditions in the Gillham Lake tailwater were influenced by its tributaries, and not by reservoir water release.

During dry conditions, the tailwater tributaries (Carters, Sycamore, Almond, and Hurricane creeks) became a series of disconnected pools. Seepage between these pools by way of hyporheic and subterranean flow caused the changes in tailwater temperature, dissolved oxygen, and total iron. However, increased conductivity that typically occurs downstream at low-flow (Hynes, 1970; Smith, 1982) was not apparent in the tailwater. During the wet period in January water temperature, conductivity, total dissolved solids, and total iorn increased downstream. The tributaries, especially Carters Creek, were responsible for these changes, even though water was being released at a higher rate from the reservoir.

During dry conditions POM was 21% greater below Gillham Lake than in the water entering the lake. Sedimentation within a reservoir results in reduced passage of detritus into the tailwater; therefore, the organic matter that is important in natural streams for energy transformation is usually not as available in a tailwater stream (Walburg et al., 1981). However, autochthonous POM from within the reservoir (usually plankton) will sometimes yield substantially higher amounts of organic matter below an upper level release reservoir in comparison to a hypolimnetic release reservoir (Maciolek and Tunzil, 1968; Lind, 1971; Spence and Hynes, 1971). Plankton collections were not made during the present short-term study; however, zooplankton was sampled during the summer in 1979, 1980, and 1981 (Smith, 1983; U.S. Fish and Wildlife Service unpublished data). These collections indicated that during the periods corresonding to the dry period, zooplankton populations were usually concentrated in the epilimnion, at the same level as the release gate. During wet conditions, POM was 9% higher below Gillham Lake than above the lake. Vertical distributions of zooplankton during periods that correspond to the wet conditions were uniform throughout the unstratified water column and their densities were therefore much lower.

Farther downstream in the tailwater (Station 10), POM increased during the dry period as a result of a cumulative effect from tributary influence and allocthonous input of some leaf litter. During the wet period, tailwater POM was slightly higher at Station 6 due to the initial surge of reservoir water that had reached the collection site. Collections there (3.5 km below the dam) were made 1 hour after reservoir

Table 2. Physiocochemical characteristics at sampling locations in Gillham Lake and in the Cossatot River and its tributaries, during dry (October 1982) and wet (January 1982) periods.

Station No.	Description	pH Dry	pH Wet	Conductance (umhos/cm) Dry	Conductance (umhos/cm) Wet	Total dissolved solids (mg/L) Dry	Total dissolved solids (mg/L) Wet	Total manganese (mg/L) Dry	Total manganese (mg/L) Wet	Total iron (mg/L) Dry	Total iron (mg/L) Wet
1	Above reservoir	6.6	5.8	86	41	54	33	0.0	0.1	0.0	0.01
2	Upper reservoir (profile mean)	5.8	5.8	65	51	33	60	0.2	0.04	0.26	0.96
3	Lower reservoir (mean at release gate levels)[1/]	5.9	6.0	53	53	32	37	0.1	0.0	0.0	0.03
4	Below dam*	6.2	5.9	54	54	31	34	0.1	0.0	0.1	0.0
5	Carters Creek	6.4	6.0	159	70	136	76	0.1	0.0	0.4	0.7
6	Mize Crossing*	6.4	5.8	54	59	39	52	0.1	0.1	0.2	0.6
7	Sycamore Creek	6.2	6.0	80	55	40	51	0.1	0.1	0.1	0.5
8	Almond Creek	6.0	5.2	66	56	56	57	0.1	0.0	0.3	0.5
9	Hurricane Creek	5.1	5.8	98	58	75	66	0.2	0.0	0.6	0.5
10	Road 80000*	6.4	5.8	58	61	32	60	0.1	0.1	0.1	0.8

*Tailwater
[1]Water release was at 4.5 m below conservation pool during dry period and 4.5, 9.0, and 19.7 m below conservation pool during wet period.

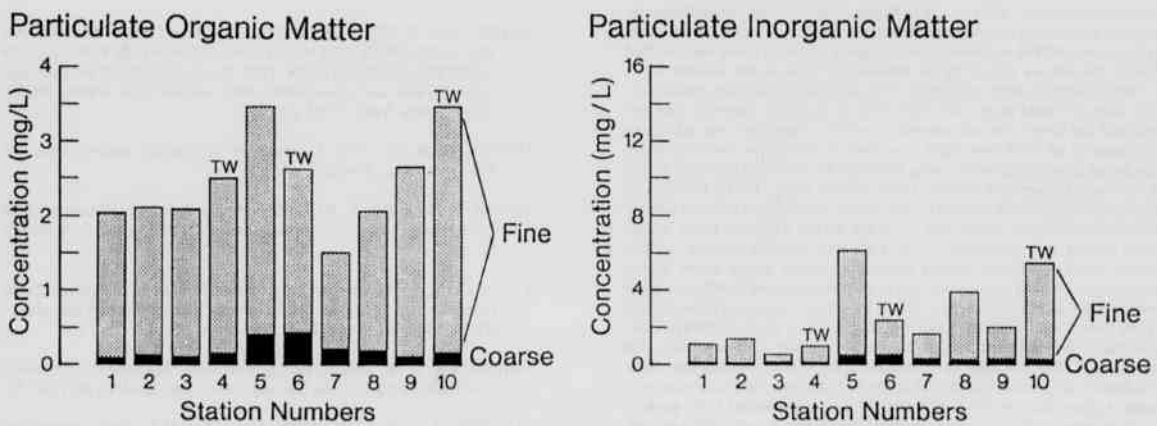

Figure 2. Fine and coarse particulate organic matter (left panel) and inorganic matter (right panel) during the dry period, October 1, 1981, in the Gillham Lake watershed. Fine particulate matter shown by the open portions of bars, and coarse particulate matter by shaded portions (TW - indicates tailwater sampling station).

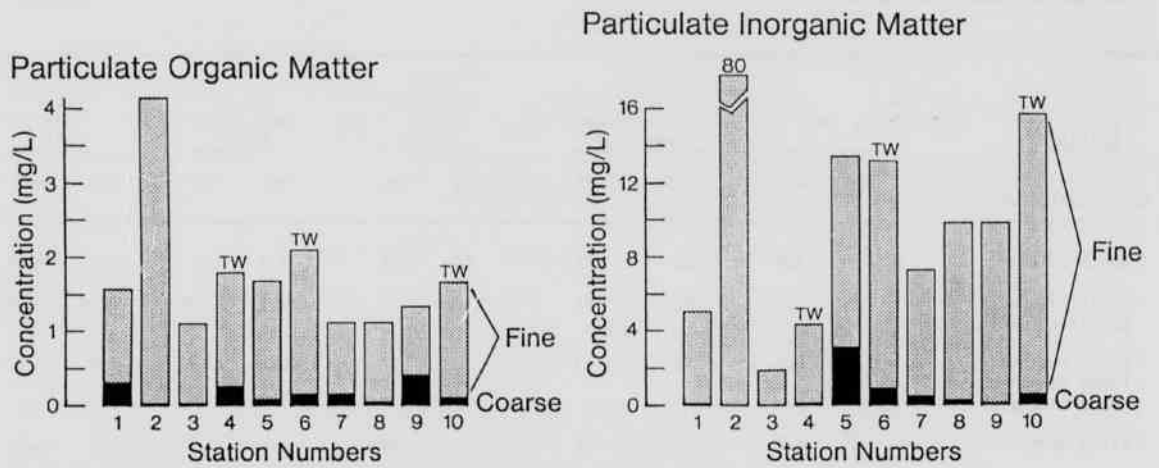

Figure 3. Fine and coarse particulate organic matter (left panel) and inorganic matter (right panel) during the wet period, January 23, 1982, in the Gillham Lake watershed. Fine particulate matter shown by the open portions of bars, and coarse particulate matter by shaded portions (TW - indicates tailwater sampling station).

discharge had been increased to 18.7 m^3/sec. Matter et al. (1983) found more POM during the initial surge of water from generation than during to pregeneration or during high flow. At Station 10, the surge of water from reservoir release had not reached the sampling station when samples were collected, resulting in lower POM there than at the upstream stations.

During dry conditions, PIM from the upper reservoir precipitated as the water moved through the reservoir. This precipitation resulted in values of PIM near the release gate in the lower reservoir of about half of the amount found in either the water entering the lake or in the upper reservoir. The PIM below the dam was higher than wighin the reservoir at the release gate level, due to resuspension of inorganic riverbed materials from the turbulence created by the reservoir water release. However, tributaries (especially Carters Creek) were the major contributors of PIM to the tailwater. The PIM in the tailwater increased as distance downstream increased. Additional input for the other tributaries and the suspension of inorganic material from the riverbed had a cumulative effect on the amount of PIM at the Station 10.

When samples were collected in Gillham Lake during wet conditions, the surge of flood water with high PIM in the upper reservoir had not reached the lower reservoir sampling stations. Therefore, we calculated the amount of PIM that might have been available for reservoir water discharge into the tailwater, using formulas for turbidity decrease within a reservoir (Jones and Rogers, 1952; Soltero et al., 1973). Particulate inorganic matter and turbidity were closely related and showed similar trends (correlation coefficient $r = 0.95$) within Gillham Lake watershed during wet conditions (U.S. Fish and Wildlife Service unpublished data). Turbidity usually decreases sharply as the water moves downstream within a reservoir, due to sedimentation of inorganic matter within a basin (Symons et al., 1964). However, increased lake volume from runoff into Gillham Lake would result in a smaller storage ratio (normally 0.1) and therefore, less sedimentation. Consequently, the amount of PIM that may have been discharged into the tailwater from Gillham Lake as a result of the spate in the upper reservoir was probably higher than the amount measured during the initial 3 day period.

In summary, the tributaries (particularly Carters Creek) had a greater effect on water quality in the tailwater during dry conditions and during the initial 3 days after heavy rains, then did the water released from the reservoir. However, estimates of PIM that may have been included in the reservoir water discharge following the initial wet period indicated that higher amounts of PIM may have entered the tailwater from water released from the reservoir than from the tributaries entering the tailwater.

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CONSTRUCTION AND SOFTWARE DESIGN FOR A MICROCOMPUTER CONTROLLED pH/ION TITRATOR

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ABSTRACT

The construction of an automated titration device is described. The major components include an Apple II + Microcomputer and 8-bit parallel interface, Fisher Accumet, Model 520 Digital pH/Ion Meter, Gilmont Micrometer Buret of 2.5 mL capacity, Sigma stepper motor, power supply and driver to operate the buret, and a constant temperature bath of ± 0.005 °C stability.

The limitations of the system are 0.001 pH/0.1 mv for the pH/ion sensing system, and 0.125 μL per step for the buret. The system as described is designed to determine equilibrium constants for metal ion-amino acid complexes. By changing the software a variety of different pH and redox titration experiments may be performed.

A computer program used to operate the stepper motor driven syringe buret and record the pH from a digital pH meter is described. The program uses both Apple BASIC and assembly language. This is a closed loop operation in which the data from the pH meter is used to control the amount of reagent delivered by the buret.

The results are displayed graphically as the titration proceeds. The variance of the pH readings are calculated using an assembly language subroutine and the calculations are done with zero round-off error.

INTRODUCTION

In the process of our research it became necessary to determine acid-base equilibrium constants for a variety of coordination compounds. Of course these values have to be accurate, precise and reproducible. Furthermore, the equilibrium constants are to be determined over the temperature interval of 5 to 40 celsius. This is to allow evaluation of the Gibbs free energy, entropy, enthalpy and heat capacity through the following relationships:

$$\Delta G^\circ = -RT\ln K$$

$$\Delta S^\circ = -(\partial \Delta G^\circ/\partial T)_p$$

$$\Delta H^\circ = -[\partial(\Delta G^\circ/T)/\partial(1/T)_p]$$

$$\Delta C_p^\circ = (\partial \Delta H^\circ/\partial T)_p$$

Because of the great amount of time and patience which must be devoted to gathering such data it was decided to construct an apparatus that could perform these experiments with a minimum of human intervention. Also, the design was to be flexible enough so that, once assembled, the apparatus could perform other related tasks by changing only the software. The apparatus to be described was built and programmed specifically to determine equilibrium constants. However, plans are being implemented to program and use it as an end-point titrator for pH or redox systems as well as a pH stat for biochemical and kinetic studies.

CONSTRUCTION DETAILS

Figure 1 is a block diagram of the system. It is a closed-loop system in that, once initiated, the microcomputer controls the course of the experiment. The major components of the system are the microcomputer, peripheral interface adapter, pH meter, BCD to binary converter, automated buret assembly, titration cell and thermostated water bath.

Microcomputer

The microcomputer system consists of an Apple II + , 64K, microcomputer, two 5½ inch mini floppy disk drives, a CRT monitor and dot matrix printer.

Peripheral Interface Adapter (PIA)

The peripheral interface adapter, PIA, is a Motorola MC-6821P, which is compatible with the Apple's 6502 microprocessor. The 6821 PIA uses TTL logic which requires a + 5 volt power supply. Figure 2 gives the wiring diagram for the interface circuit. The components of the interface are mounted on an Apple prototype board (CSI Part No. 07500-002A) which plugs into the expansion slots on the back of the Apple's main circuit board. Wire wrap staking pins and DIP sockets are secured to the board by soldering. The two 741 operational amplifiers are inserted side by side onto a single 16 pin wire wrap DIP socket. All four resistors in the circuit are 8.2 kohm, ¼ watt resistors. The circuit is assembled by wire wrapping with 30 AWG color coded insulated wire. The 18 AWG, 3 wire, cable to the stepper motor power supply is connected by soldering to staking pins. It is mechanically secured by means of a tight fitting cable hanger attached to the interface board by means of a screw, lockwasher and nut.

pH Meter

The pH meter used is the Fisher Accumet, Model 520 Digital pH/Ion Meter. The read-out is 4½ digits. The meter is advertised as having a repeatability of ±0.001 pH/ ±0.1 MV and accuracy of ±0.002 pH/ ±0.2 MV. Also, the meter has an input impedance of greater than 10^{12} ohms and a drift of less than 0.003 pH/0.18 MV drift per 24 hours. The most attractive feature of the pH meter is the digital printer output. The digital printer output information is available by means of a 30 pin edge-connector at the back of the instrument. The output is TTL logic (2.4 volt minimum impulse for digital "1" and 0.4 volt maximum for digital "0"). Each of the 4 full digits of the meter is accessed through this connector by means of binary coded decimal, or BCD, format. The ½ digit, or carry, strobe, pH, + MV, − MV indicators and common connection is also available at this connector.

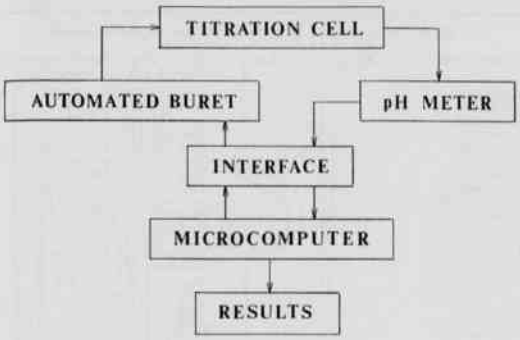


Figure 1. Block diagram of automated titration/pH stat system.

BCD to Binary Converter

The information produced by the pH meter is converted to a form usable to the computer by means of the circuit given in Figure 3. The components are mounted by means of DIP IC wire-wrap sockets glued to perf-board with super glue. The perf-board used measures 6½ x 3 x 1/16 inches with holes on 1/10 inch centers. There is a large amount of empty space in the pH meter. Therefore, the BCD to binary circuit is mounted inside the pH meter case by means of angle brackets affixed to the perf-board and back cover of the meter. Circuit connections are made by wire wrapping with 30 AWG, color-coded, insulated wires. A stranded, rainbow color coded, 28 AWG, flat ribbon cable with 24 conductors is used to connect the pH meter's 30 card edge connector to the BCD to binary circuit. The cable is 6 inches long with a 24 pin DIP male connector on one end and a 30 connection card edge female connector on the other. Heat-shrink tubing is used to insure good insulation on the card edge connector end after soldering the cable wires to the connector plug. A similar cable that is 24 inches long and with a 16 pin DIP male connector at each end is used to connect the BCD to binary circuit to the PIA circuit inside the microcomputer. Table 1 gives the cable connections for the two cables. The BCD to binary circuit is powered by the +5 volt line taken from the Apple II+ power supply via the connecting cable.

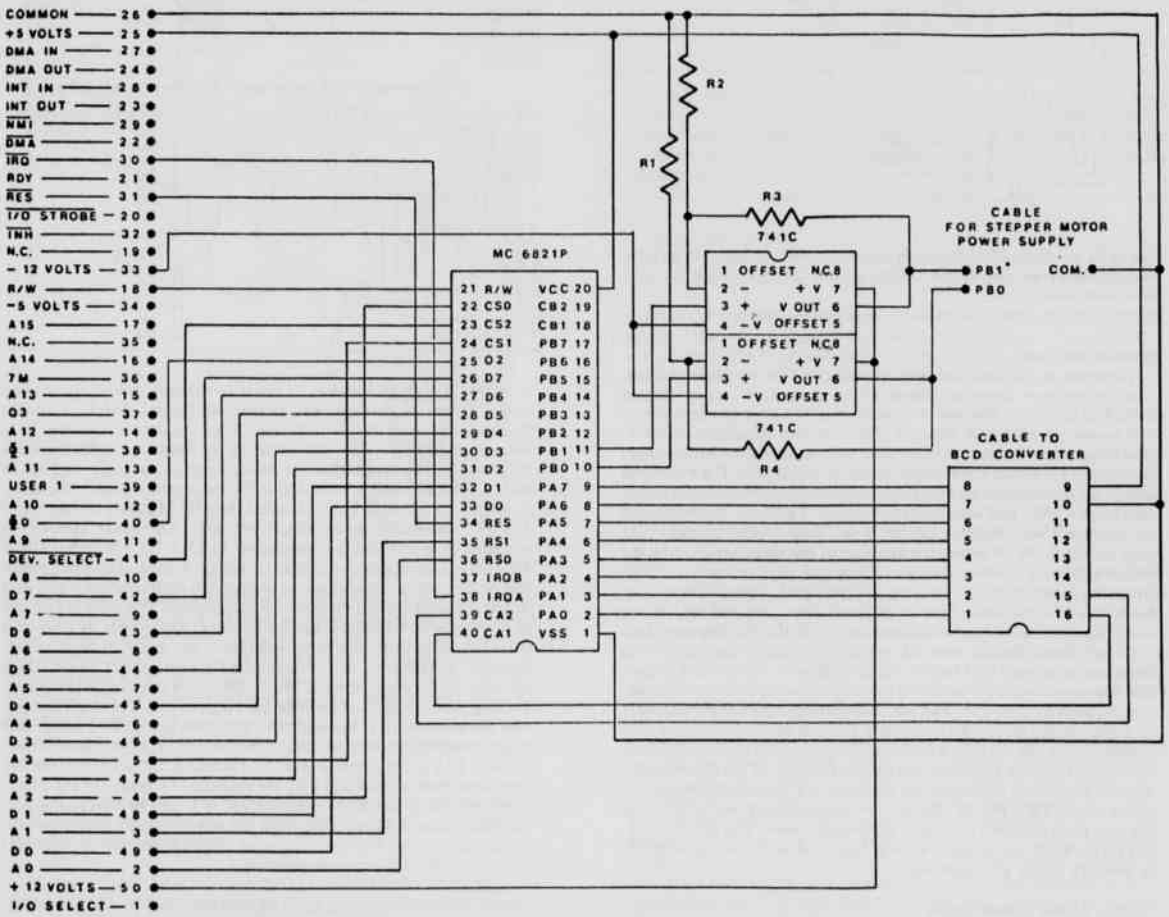


Figure 2. Peripheral interface adapter circuit utilizing the Motorola MC-6821P Peripheral Interface Adapter (PIA). All resistors are 8.2 kohm, ¼ watt.

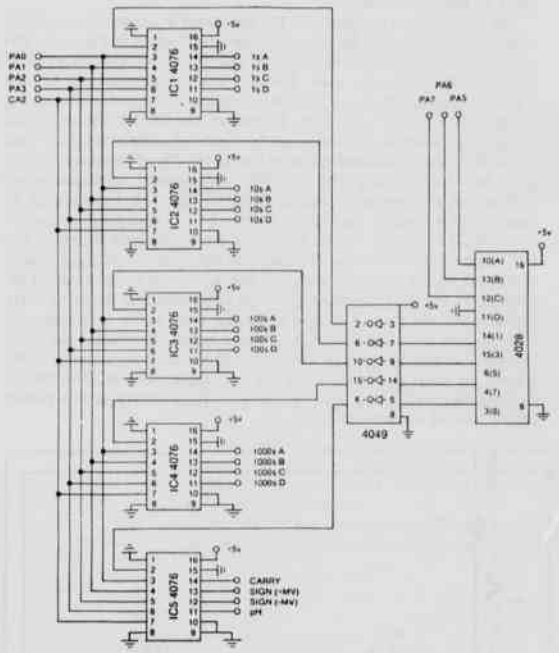


Figure 3. 4½ Digit BCD to binary converter used to read pH meter's information from its 30 pin digital printer output connector into microcomputer.

Table 1. Cable Connections

BCD to Binary Circuit to pH Meter Digital Printer Connector

pH Meter End	BCD to Binary End	BCD to Binary Board Connections from 24 pin DIP Connector
A Ones (D)	1	Ones (D) IC1, pin 11
B Ones (B)	2	Ones (B) IC1, pin 13
C Ones (C)	3	Ones (C) IC1, pin 12
D Tens (A)	4	Tens (A) IC2, pin 11
E Tens (D)	5	Tens (D) IC2, pin 13
F Tens (B)	6	Tens (B) IC2, pin 12
H Tens (C)	7	Tens (C) IC2, pin 14
J Hundreds (A)	8	Hundreds (A) IC3, pin 14
K Hundreds (D)	9	Hundreds (D) IC3, pin 11
L Hundreds (B)	10	Hundreds (B) IC3, pin 13
M Hundreds (C)	11	Hundreds (C) IC3, pin 12
N Thousands (A)	12	Thousands (A) IC4, pin 14
P Thousands (D)	15	Thousands (D) IC4, pin 11
R Thousands (B)	14	Thousands (B) IC4, pin 13
S Thousands (C)	13	Thousands (C) IC4, pin 12
1 – MV Indicator	24	– MV Indicator, IC5, pin 12
2 Ones (A)	23	Ones (A) IC1, pin 14
3 + MV Indicator	22	+ MV Indicator, IC5, pin 13
4 pH Indicator	21	pH Indicator, IC5, pin 11
5 N.C.		
6 N.C.		
7 Ten Thousands (A)	18	Ten Thousands, IC5, pin 14
8 N.C.		
9 N.C.		
10 N.C.		
11 Strobe	17	Strobe, pin 16 of cable below
12 Common	16	Common, Ground
13 Common	19	N.C.
14 Common	20	N.C.
15 Common		

BCD to Binary Circuit to Peripheral Interface Adapter Board

BCD to Binary Circuit, 16 Pin DIP Connector	PIA, 16 Pin DIP Connector
1 Pin 3, 4076	1 PA0
2 Pin 4, 4076	2 PA1
3 Pin 5, 4076	3 PA2
4 Pin 6, 4076	4 PA3
5 Common	5 PA4
6 Pin 10, 4028 (A)	6 PA5
7 Pin 13, 4028 (B)	7 PA6
8 Pin 12, 4028 (C)	8 PA7
9 N.C.	9 +5 Volts
10 N.C.	10 Common
11 N.C.	11 N.C.
12 N.C.	12 N.C.
13 N.C.	13 N.C.
14 N.C.	14 N.C.
15 Pin 7, 4076	15 CA2
16 Pin 16, Strobe	16 CA1

Automated Buret

The buret is a 2.5 mL Gilmont Micrometer Syringe Buret (S3200). The micrometer drive is connected to a stepper motor (Sigma 20-2235D200-E17). The motor is wired to give half-step operation of 0.9 degree per half-step. Figure 4 illustrates the important construction features of the buret assembly. The base plate of the buret assembly measures 13.5 x 3.25 x 0.5 inches and is of aluminum. The buret and motor are secured by 1 inch thick aluminum blocks of base 3.25 inches and height 2.625 and 4 inches, respectively. The holes for buret and motor were drilled slightly oversize. A slot in each block allows a 0.25 inch diameter bolt to reduce the diameter of the hole thus securing the motor and buret in place. A 0.25 x 2.75 inch aluminum disc is attached to the micrometer part of the buret as shown in the figure. This is done by means of a 0.25 inch wide shoulder machined on one side of the disc. Again a slot and screw arrangement locks the disc in place. Two 0.25 inch holes, slightly oversize, are drilled through the disc. These holes accept the two 0.25 inch diameter steel drive shafts which impart the rotational motion from the motor and allow the translational motion required by the buret. The two steel shafts are affixed permanently to a disc on the motor shaft that is 0.5 inches thick. The motor disc is mounted on the shaft by a 0.25 inch thick shoulder machined on the disc and a set screw tightened against the flat part of the motor shaft. The six wires from the motor are attached to a 6-terminal barrier terminal block (TRW 6-140). The barrier terminal block was attached to the base plate with two 8-32 steel, allen-head screws. The male luer fitting on the buret was mated to a 3-way Hamilton valve (Hamilton 86728) to simplify filling and emptying.

Stepper Motor Power Supply

The stepper motor requires an unregulated DC power supply of twice the motor's phase current of 2.3 amps. The power supply constructed is shown in Figures 5 and 6. In order to simplify motor operation a motor driver logic card was purchased (Sigma 29A47 Unipolar Resistance Limited Driver). The logic card required a regulated +12 volt power supply. This was achieved by means of a 7812 voltage regulator also powered from the motor supply. The logic card has to have forced air cooling to maintain its temperature below 50 celcius. A 4.5 x 4.5 x 1.5 inch fan is installed (Muffin MU2A1) on the 7 x 11 x 2 inch aluminum chassis (BUD AC-407). The entire assembly is mounted in a steel cabinet measuring 13.25 x 7.5 x 9 inches (BUD SB-2141). A six conductor cable, 18 AWG, is used to connect motor and power supply. Spade lugs are soldered to each conductor and heat shrink is placed around the soldered connections. The cable is fastened at both ends by 6-terminal barrier blocks (TRW 6-140). A resistor is placed in series with each phase winding of the motor to limit current through the motor to the recommended current of 2.3 amps. These resistors are 7.5 ohm, 50 watt, 1%. A 4066 quad bilateral switch is used to bring pins 11 and 16 of the logic card high or low. Pin 11 is the direction pin and pin 16 is the step pin. The 4066 is a CMOS device and requires amplification to 10 volts of the 5 volt TTL logic available from the 6821 PIA in the Apple (Figure 2). Therefore, the voltage of the two lines from Port B, PB0 and PB1 are amplified by means of the 74IC's to bring them up to CMOS specification. A 3-wire 18 AWG cable provides common, PB0 and PB1 from the Apple's PIA. The rest of the connections to the 4066 are connected to either ground or +12 volts to prevent the chip from overheating.

SOFTWARE DESCRIPTION

The computer software is designed with a closed loop operation in

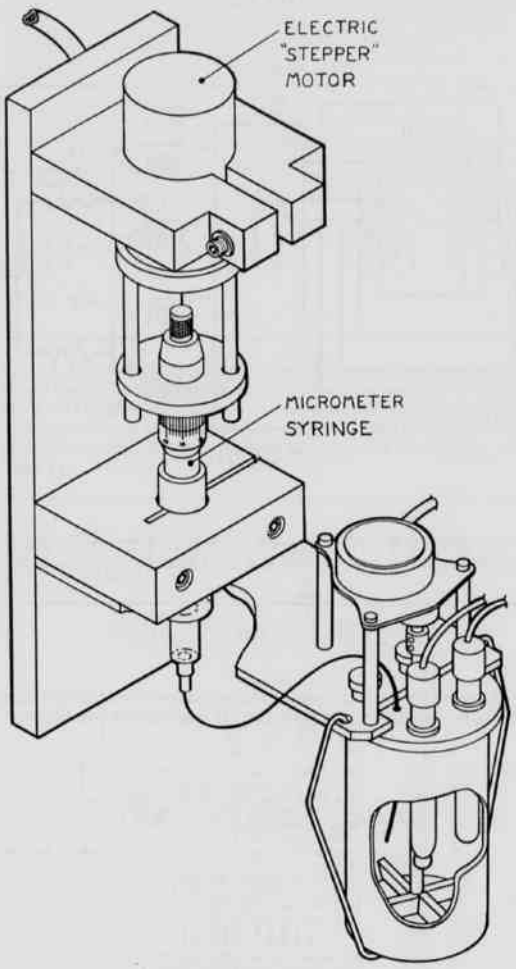


Figure 4. Isometric rendering of automated buret in its normal vertical position. The glass part of the buret and the titration cell are designed to be submerged in a thermostated bath during actual use. The titration cell has a total volume of 200 mL and is polypropylene. It is equipped with a 150 rpm glass stirrer and has provision for nitrogen gas purge. The three way Hamilton valve has been omitted from the drawing for clarity.

mind. The program controls the operation of the automated buret either at a constant increment for pH determination or variable increment for endpoint determination.

The software is designed to utilize the MC6821 peripheral interface adapter (PIA). This versatile circuit allows memory locations, internal to the computer, to be used as input or output to two ports; A and B. See Figure 7. Device A is the pH meter; device B is the stepper motor.

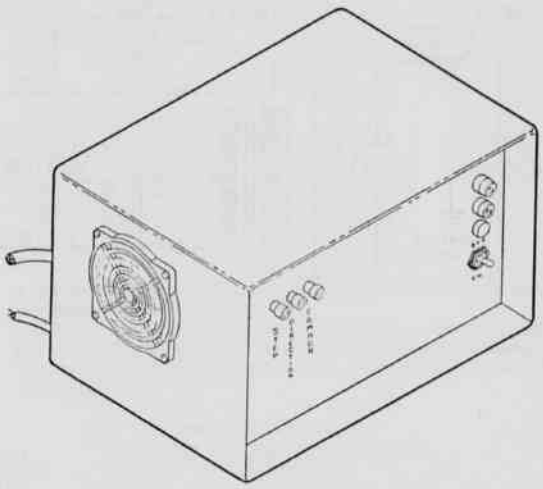


Figure 5. Sketch of stepper motor power supply and logic circuit. Two fuse holders and indicator light are on upper right hand side of panel. One of the two cables on the back is for 115 VAC power; the other is the 6-conductor cable to the stepper motor. The control cable from the computer is connected on the top left of the front panel.

Additional circuitry is needed to read the pH from the pH meter. The meter outputs the pH as binary coded decimal (BCD). BCD output is illustrated in Figure 8. A 4076 tri-state quad storage register is utilized for each of the 5 digits. Each 4076 is capable of storing 4 bits of data, the amount needed for a single decimal digit. Five 4076 IC's are utilized in the hardware configuration, one for each digit. The 4076's are accessed, one at a time, when the pH is read into memory.

The 6821 uses two memory locations for each port. One of these is the Control Register. The Control Register allows the microprocessor to control two things, the operation of the interrupt and handshaking lines (CA1, CA2, CB1, CB2) and access to the other memory location. If bit two of the Control Register is set then the other memory location is used to define which of the Port A (or Port B) lines will be input and which will be output. Since the memory location has eight bits, corresponding to eight data lines, each line can be programmed as input or output. Thus, the second memory location is called the Data Direction Register when bit 2 of the control register is set. We write a "1" in the lines of the Data Direction Register we wish to become outputs, and a "0" into those lines to be designated inputs. When bit 2 of the Control Register is not set, i.e. zero, then the second memory location becomes an output register, and the microprocessor can read the data on those lines that have been designated as input, and write data on those lines designated as output.

Since port A is connected to the pH meter, 4 lines are designated as input (required for BCD input) and 3 lines as output (for control over reading the digits). Line PA4 is not needed. It is grounded, and therefore always at logic level zero. As can be seen from Figure 3, lines PA5, PA6, and PA7 are connected to the 4028 BCD to Decimal Decoder. Only one pin of the 4028 goes high based on the conditions of lines PA5, PA6, and PA7. See Figure 9. This causes a single pin on the 4049 hex inverting buffer to go low. There are lines that go from the 4049 to pin 2 of each 4076 IC. When pin 2 of the 4076 IC goes low, the outputs (pins 3-6) can be read. These outputs are connected to PA0-PA3.

Construction and Software Design for a Microcomputer Controlled pH/Ion Titrator

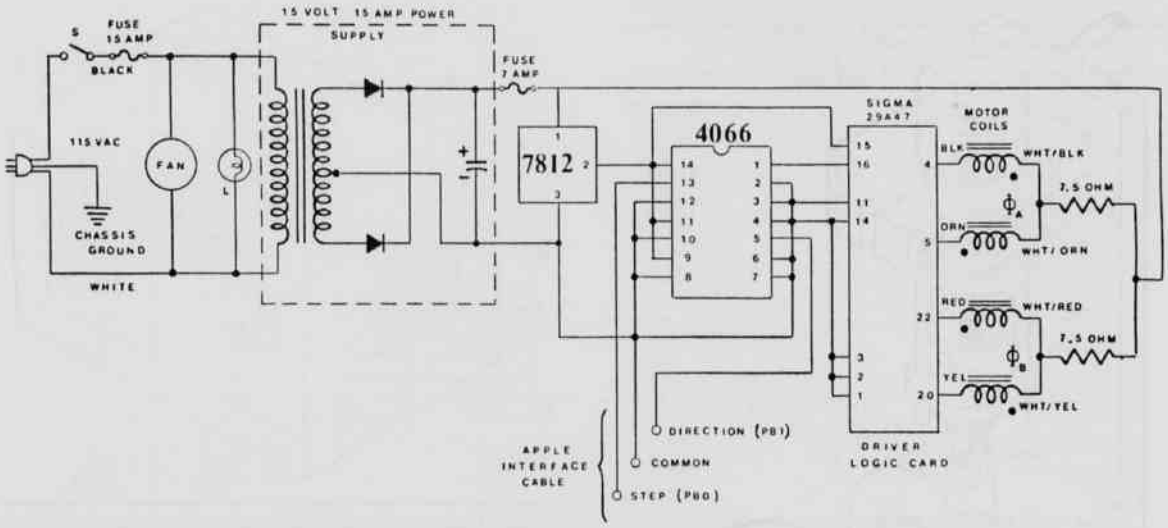


Figure 6. Schematic of stepper motor power supply and logic. The 7812 is a +12 volt regulator and the 4066 is a quad bilateral switch. L is an indicator lamp. The two 7.5 ohm resistors are 50 watt, 1%.

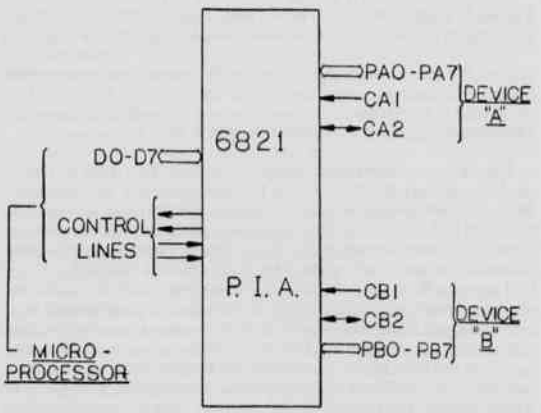


Figure 7. Simplified schematic of MC6821 Peripheral Interface Adapter.

Pin 7 of each 4076 is connected to CA2, an handshake line that is controlled via the Control Register of the PIA. This line is sent low, and then high. The 4076 IC is configured in such a manner that this procedure causes the data it "sees" on its input pins (11-14) to be latched. When pin 2 goes low, the data is placed on the data bus.

The strobe of the pH meter is connected to CA1, the interrupt line of the PIA. This line is the most significant bit (bit 7) of the Control Register. Thus, the program constantly poles the pH meter strobe that indicates a pH value is ready to be read.

The assembly language routine that sets direction of the data lines, checks for the strobe, and takes the pH reading is shown below.

```
READ    LDA   $00      Select Data Direction Register (bit 2 = 0)
        STA   $COCD    $COCD is the Control Register
        LDA   $3E0     Make lines PA5, PA6, and PA7 outputs
        STA   $COCC    $COCC is the Data Direction Register
CLEAR   LDA   $306     SELECT OUTPUT REG.; Make an active
        STA   $COCD    transition of CA1 a positive transition.
        LDA   $COCC    Reading this clears CA1 (bit 7 of the Control Register)
TEST    BIT   $COCD    Wait for a strobe
        BMI   DOIT     Is bit 7 of Control Register set?
        JMP   TEST     If not, no strobe, try again
DOIT    LDA   $334     Send CA2 low, then high
        STA   $COCD    This locks a reading in
        LDA   $33C
        STA   $COCD
        LDA   $300     Put zeroes in PA5, PA6, and PA7
        STA   $COCC    This selects the 10,000's place
        LDA   $COCC    Read the value
        AND   $301     Mask unnecessary bits
        STA   $7000    Store it
        LDA   $3E0     Put ones in PA5, PA6, and PA7
        STA   $COCC    Select 1000's place
        LDA   $COCC    Read the value
        AND   $30F     Mask unnecessary bits
        STA   $7001    Store it
        LDA   $3A0     Similarly for 100's place
        STA   $COCC
        LDA   $COCC
        AND   $30F
        STA   $7002
```

When this routine is finished, the pH value is stored in five memory locations ($7000-$7004). This BCD value is then converted to a hex number requiring two bytes for storage.

The routine that operates the stepper motor is fairly simple. Only two lines are needed to control the motor, one which specifies the direction, and one which, when pulsed, directs the motor to take a step. The motor is connected to port B of the PIA. PB0 is connected to the line that directs the motor to take a step. PB1 is connected to the line that controls the direction of the motor (high = CW; low = CCW). When PB0 is low, pin 16 of the driver card is high. The motor takes

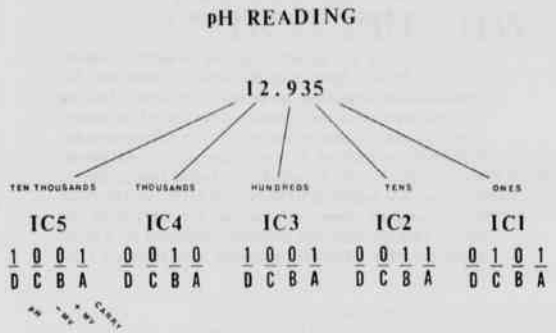
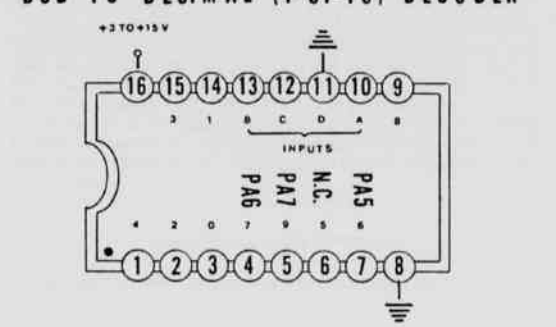


Figure 8. Diagram representing the breakdown of pH reading into five Binary Coded Decimal (BCD) numbers. Each decimal digit is latched into a 4071 quad storage register as four bits. The "A" bit is the least significant bit and is related to pins 3 (output) and 14 (input) of the 4076 IC while the "D" bit is the most significant bit and involves pins 6 and 11.



LOGIC TABLE

HEXADECIMAL NUMBER	INPUT PINS ACTIVATED A	B	C		OUTPUT PIN ACTIVATED	IC 4076 ADDRESSED
	PA5	PA6	PA7			
0	0	0	0	= 0	3	5
20	1	0	0	= 1	14	1
	0	1	0	= 2		
	0	0	1	= 4		
60	1	1	0	= 3	15	2
A0	1	0	1	= 5	6	3
	0	1	1	= 6		
E0	1	1	1	= 7	4	4

Figure 9. This device converts any 3-bit code into 1-of-8 outputs. The BCD code is input on terminals 10 through 13, with the least-significant or $2^0 = 1$ bit on "A", the $2^1 = 2$ bit on "B", the $2^2 = 4$ bit on "C", and the $2^3 = 8$ bit on "D". Positive logic with a "1" positive and a zero grounded is used.

```
LDA   $$60      Similarly for 10's place
STA   $COCC
LDA   $COCC
AND   $$0F
STA   $7003
LDA   $$20      Similarly for 1's place
STA   $COCC
LDA   $COCC
AND   $$0F
STA   $7004
```

a step when the signal on pin 16 drops below 2 volts. The signal must stay below 1 volt for 20 μsecs.

A BASIC routine determines the number of steps to be taken. Each step of the motor is 0.9 degrees. The syringe buret to which it is attached, delivers 0.05 ml for each revolution. Thus, the resolution is 0.000125 ml per step.

$$\text{BURET STEPPING RESOLUTION} = \frac{360\ \text{DEGREES}}{0.05\ \text{mL}} \times \frac{\text{STEPPER MOTOR STEP}}{0.9\ \text{DEGREES}} \times \frac{8000\ \text{STEPS}}{\text{mL}}$$

OR

0.000125 mL PER STEP

The BASIC program calls an assembly language routine via a FOR-NEXT loop for the number of steps needed. The assembly language routine is shown below.

```
BEGIN   LDA  $$00    Select Data Direction Register by making
        STA  $COCF   Bit 2 of Control Register B($COCF) a zero.
        LDA  $$FF    Make all lines, PB0-PB7 outputs
        STA  $COCE   $COCE is Data Direction Register B
        LDA  $$04    Bit 2 of Control Register B set
        STA  $COCF
        LDA  $7340   This location contains the direction (CW or CCW) given
        STA  $COCE   by the BASIC routine.
        LDA  $$20
        JSR  $FCA8   Short delay loop
        INC  $COCE   Make PB0=1; Pin 16 of driver card low; take a step
        LDA  $$10    Short delay loop; keeps pin 16 low
        JSR  $FCA8
        RTS          Return to BASIC program
```

A BASIC driver program is used to carry out a titration. At present the program inputs 60 pH values from the meter and calculates the variance (the square of the standard deviation). Sixty values are taken to allow about 30 seconds for the pH readings to stabilize. The calculation of the variance is done entirely in assembly language. Assembly language is faster than BASIC and calculations can be carried out with no round-off error. The variance is then compared to a user-set value (usually 0.001 pH). If the variance is greater than the test value, this indicates that the pH readings have not yet stabilized. In this case, another reading is taken and the first value is discarded. Then, the variance calculation is repeated on the last 60 values. When the pH has stabilized, the mean of the pH values is stored, and the program directs the stepper motor to deliver some titrant.

Table 2. Automated Buret Calibration

Apparent Vol. Delivered (mL)	True Vol. Delivered (mL)
0 to 0.5	0.5001 ± 0.0001
0 to 1.0	1.0004 ± 0.0001
0 to 1.5	1.5005 ± 0.0000
0 to 2.0	1.9999 + 0.0002
0 to 2.5	2.5001 + 0.0002

CALIBRATION OF BURET

The ability of the buret to deliver specified volumes of titrant was tested by weighing the water it dispensed at specified intervals over its range. The results are given in Table 2. As can be seen the results are excellent. The highest standard deviation in the table was 0.0002 mL which corresponds to a relative standard deviation of 0.01 percent.

CONCLUSION

We have been using this apparatus for several months now and have found it to be an accurate and reliable aid in carrying out pH titrations and in evaluating equilibrium constants.

ACKNOWLEDGEMENTS

The authors are grateful to Joe Waldrop, Senior Engineer, Phillips Petroleum, Bartlesville, Oklahoma, for some valuable suggestions on design of the sample holding device as well as for the isometric drawings of the equipment. Thanks to the instructors of the microcomputer interfacing workshop at the University of Arkansas, Fayetteville for the knowledge to tackle this problem. Also, we are indebted to Wayne Weaver of American Tool Company, Searcy, Arkansas, for fabricating the buret. This research was partially supported by a grant from the NSF-EPSCOR program and Harding University.

DISTRIBUTION AND EFFICIENCY OF HYDROCARBON-OXIDIZING BACTERIA IN A FRESHWATER RESERVOIR

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ABSTRACT

Hydrocarbon-oxidizing bacteria were identified from three stations on DeGray Reservoir, Arkansas. The organisms were primarily gram-negative rods representing 9 taxa and 37 biotypes. *Pseudomonas* spp. were the most common isolates. The largest populations were found in areas most frequently used by boaters, although seasonal fluctuations were apparent during the spring and fall. The degradation of outboard motor oil by the five most rapidly growing isolates was studied. Each species had a different decomposition profile, and substrate oxidation rates were variable. *Acinetobacter calcoaceticus* var. *anitratus* was the most efficient decomposer.

INTRODUCTION

Much research has been directed toward hydrocarbon degradation by bacteria in aquatic environments (Atlas, 1981). The marine environment has attracted the most attention and there have been relatively few studies on freshwater habitats (Cooney and Summers, 1976; Horwitz and Atlas, 1977; Atlas, 1981). The purpose of this study was to measure both the quality and quantity of hydrocarbon-oxidizing bacteria in a relatively unpolluted freshwater reservoir, and to compare their efficiencies in decomposing certain molecular weight hydrocarbons.

MATERIALS AND METHODS

The study site was DeGray Reservoir, located on the Caddo River in southwestern Arkansas. It covers approximately 5500 hectares and has been filled about 10 years. Three major compartments of the reservoir were represented by sampling stations 4, 10 and 12 (Figure 1).

Figure 1. Location of sampling sites on DeGray Reservoir.

by standard methods of membrane filtration (APHA, 1976). Cultivation was on a mineral salts medium (Aaronson, 1970) containing 1% sterile Mercury Quicksilver outboard motor oil as a carbon source and 0.01% sterile triphenyltetrazolium chloride to facilitate counting. Samples were collected at 2 m intervals from the surface to 12 m, and at 5 m intervals thereafter, from November, 1979 through October, 1980. The samples were contained in Whirl-Pak bags (NASCO) and transported on ice to the laboratory. Aliquots of 100 ml were analyzed Sterilization was by autoclaving since such treatment does not alter composition (Walker and Colwell, 1975). Purified agar (Difco) was used as the solidifying agent. Controls containing no oil were used to check for the ability of the agar to support growth, but none was observed during the study. All samples were incubated at 24 °C for 4 weeks and colonies enumerated.

After incubation, isolates were streaked on McConkey's medium (Difco) to obtain pure cultures, and identified with the API 20E system (Analytab, Inc.).

Studies to determine the rate of hydrocarbon degradation were carried out on the five most rapidly growing isolates. The strains were cultured in a liquid mineral salts medium overlaid with 1% outboard motor oil (Austin et al., 1977). After incubation, the oil fraction was extracted from replicate sets of cultures at one week intervals for 4 weeks, and from controls containing no bacteria, with 10 ml pesticide grade hexane. Extracts were concentrated to 1 ml and analyzed by injecting 3 ul into a Tracor MT 222 gas chromatograph equipped with flame ionization detectors. The instrument incorporated a 6 ' x ¼ " glass column packed with 5% SE-30 on chromosorb, W,AW,DMS,HP,80/100 mesh. Nitrogen was used as carrier gas at a flow rate of 60 cc/min and the column oven was maintained at 210 °C.

Because n-alkanes are generally considered the most readily degraded components in a petroleum mixture (Atlas, 1981), straight chain hydrocarbons (ASD/Milton Roy Co.) ranging from $C_{18} - C_{24}$ were used as markers to aid in the characterization of the motor oil fractions being oxidized.

DATA AND RESULTS

Fifty-seven isolates representing 37 biotypes were identified as hydrocarbon oxidizers (Table 1). The most common bacteria were *Pseudomonas* spp. and the unnamed group CDC Group VE-1 which

Table 1. Taxa of hydrocarbon-oxidizing bacteria isolated from all sampling stations.

Taxa	Isolates	Biotypes
Acinetobacter calcoaceticus var. anitratus	4	1
CDC Group 5E-1	4	1
CDC Group VE-1	12	9
Citrobacter freundii	2	2
Enterobacter cloacae	8	8
Enterobacter sakazakii	1	1
Klebsiella pneumoniae	1	1
Pseudomonas aeruginosa	1	1
Pseudomonas fluorescens	13	5
Pseudomonas maltophilia	4	1
Pseudomonas spp.	6	6
Serratia rubidaea	1	1

includes gram-negative fermenting rods with physiological characteristics similar to *Pseudomonas*. Approximately 95% of the total isolates were gram-negative rods.

The occurrence of larger populations in areas most frequently used by boaters was expected. In DeGray Reservoir, this area corresponds to the lower compartment (station 4). Data suggested this assumption to be generally true although heavy rainfall in March resulted in greater densities in the upstream compartments (Figure 2).

Data from all three stations were combined to determine densities for the entire reservoir during each month (Figure 3). Hydrocarbon oxidizers gradually increased throughout spring after a low density during winter. A summer decline occurred, followed by a large increase during fall.

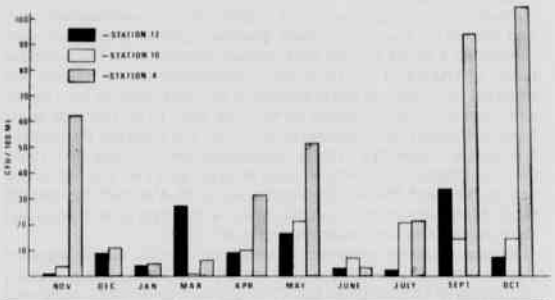


Figure 2. Comparison of populations in three compartments of DeGray Reservoir from November, 1979 through October, 1980.

Five taxa were studied to determine their abilities to degrade the major fractions of the outboard motor oil. CDC group VE-1 was found to be the least efficient (Figure 4a). It utilized only one fraction of the

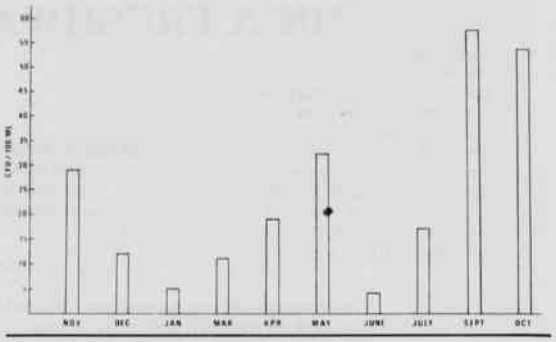


Figure 3. Mean populations for the entire reservoir from November, 1979 through October, 1980.

outboard motor oil in the $C_{22}-C_{24}$ range, reducing that fraction by approximately 72%.

Enterobacter cloacae utilized several fractions corresponding to the retention times of C_{18}, C_{18} and C_{20} standards (Figure 4b). The bacterium also decomposed the component nearest C_{22} as did CDC group VE-1.

Enterobacter sakazakii oxidized all but three of the major fractions (Figure 4c). There was a 12-100% concentration reduction in the $C_{18}-C_{24}$ range.

Most major components were decomposed by *Pseudomonas fluorescens* (Figure 4d). The organism degraded hydrocarbons in the $C_{20}-C_{24}$ range with efficiencies of 25-78%.

Acinetobacter calcoaceticus var. *anitratus* was most efficient in degrading the oil (Figure 4e). Data suggested that this bacterium was especially suited for oxidizing fractions from $C_{22}-C_{24}$. The two most fully oxidized components were diminished 81% (near C_{22}) and 93% (near C_{24}).

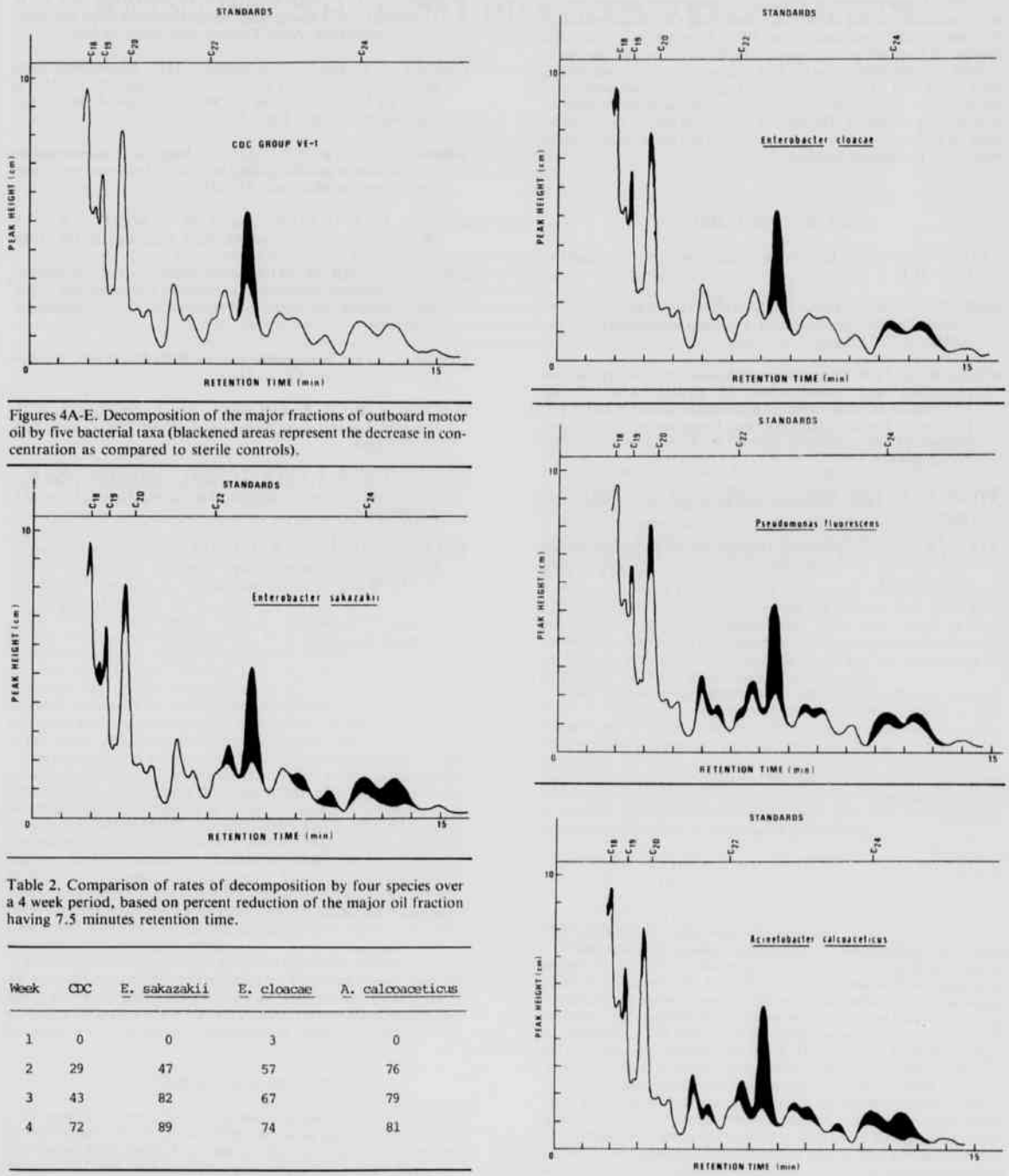
Relative oxidation rates for four species that could decompose the C_{22} (7.5 min retention time) fraction were determined (Table 2). CDC group VE-1 had an almost constant, but slow, rate during the 4 wks incubation. *E. sakazakii* and *E. cloacae* initially showed rapid rates of metabolism that began decreasing after 2 wks. *A. calcoaceticus* was most efficient, producing a 76% reduction of the oil component within 2 wks.

DISCUSSION

Many species of gram-negative bacilli are known to be capable of utilizing hydrocarbons. The species in this study have been recognized earlier in other investigations (Atlas, 1981; Austin et al., 1977; Jobson et al., 1972; Sedita, 1973). *Pseudomonas* spp. were the most comon group as reported previously (Atlas, 1980; Austin et al., 1977; Walker et al., 1975; Walker and Colwell, 1975).

Distribution of hydrocarbon oxidizers is affected by a number of variables including weathering (Atlas, 1980), nutrient composition (Austin et al., 1977; Horwitz and Atlas, 1977; Walker et al., 1975; Walker et al., 1976; Walker and Colwell, 1975) and water temperature (Atlas, 1980; Austin et al., 1977; Jobson et al., 1972). This study indicated heavy spring rains, and winter and summer temperature extremes influenced distribution. Some investigators found correlations between bacterial numbers and hydrocarbon concentrations, suggesting the possible use of these microbes as chronic pollution indicators (Austin et al., 1977; Horowitz and Atlas, 1977; Walker et al., 1975). Others have found no relationship between microbial density and hydrocarbons (Roubal and Atlas, 1977). Data presented here suggest larger populations were usually present in areas most frequently used by boaters, etc.

All species exhibited variations in decomposition rates for various fractions of the motor oil as has been reported previously (Walker et



Figures 4A-E. Decomposition of the major fractions of outboard motor oil by five bacterial taxa (blackened areas represent the decrease in concentration as compared to sterile controls).

Table 2. Comparison of rates of decomposition by four species over a 4 week period, based on percent reduction of the major oil fraction having 7.5 minutes retention time.

Week	CDC	E. sakazakii	E. cloacae	A. calcoaceticus
1	0	0	3	0
2	29	47	57	76
3	43	82	67	79
4	72	89	74	81

al., 1976). Hydrocarbons in the $C_{22} - C_{24}$ range were metabolized by all 5 organisms studied. Others have shown that $C_{20} - C_{21}$ components were most readily oxidized (Atlas, 1979; Jobson et al., 1972). The number of fractions utilized varied considerably from one biotype to another. *A. calcoaceticus* was the least selective, apparently metabolizing most of the major fractions of the oil. In general, the microbial community of the reservoir can process hydrocarbon contaminants resulting from boating recreation. The bacteria responsible are not known to depend on hydrocarbons for growth, although increased concentrations may affect population densities.

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SOME STUDIES ON INTRODUCING *CASTILLEJA COCCINEA*, INDIAN PAINTBRUSH, INTO PRAIRIE VEGETATION

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ABSTRACT

Indian paintbrush, absent from many prairie remnants in Arkansas, behaves as a biennial in certain central Arkansas prairies, growing as a small rosette one season and flowering the next. It is known to be an indiscriminate root parasite. Field sowings were made in October, March and June. Annual change in population size was monitored for one of these sowings. Laboratory studies of germination were conducted to investigate the effects of light, temperature, water potential, and host species. Haustorial connections to host roots were examined. Based on these studies, a strategy for establishing the species in prairie was developed.

INTRODUCTION

Castilleja coccinea, showy Indian paintbrush, is one of the most striking forbs of midwestern seral prairies (Steyermark, 1975). Near the southern and western limits of its range in Arkansas, this paintbrush occurs in widely scattered prairie patches, yet is totally absent from many prairies. In the Conway area there is one robust population on about 40 hectares of unplowed native hay land, but numerous similar sites have no paintbrush. As part of a prairie restoration project (Culwell and Wright, 1984), a study was designed to explore methods of introducing the paintbrush into prairie sites.

The Castillejas are among the many root parasites found in the Scrophulariaciae and Orobanchaceae. All Castillejas are designated as hemiparasites; that is, they are rooted photosynthetic plants which draw a portion of their water, minerals, and metabolites from autotrophic host plants through haustorial attachments between roots (Atsatt, 1972). *Castilleja chromosa* has been shown to maintain stable carbohydrate and water balances in comparison with its host, *Artemisia tridentata*, in the White Mountains of California (Hansen, 1979).

The hemiparasites are variously believed to have evolved as a compensating response to disease (Atsatt, 1976) or as an outcome of the tendency to form root grafts (Malcolm, 1966). Although hemiparasitic Scrophulariaceae are capable of living for some time without a host, anthesis and seed production generally depend on numerous haustorial connections (Kuijt, 1969; Atsatt and Hansen, 1978). *Castilleja coccinea* has been shown to remain in a stunted condition until haustorial connections are developed (Malcolm, 1966). As is typical for many of the herbaceous hemiparasites in the Scrophulariaceae (Werth and Riopel, 1979) *Castilleja coccinea* forms indiscriminate haustorial connections with a wide range of host species, including some that are not native (Malcolm, 1966; Heckard, 1962).

The showy Indian paintbrush generally behaves as a biennial, forming a rosette one season and flowering the following Spring. The tiny seeds, 18,000 per gram, are shed by early summer and are believed to germinate either then or the following spring. No vernalization is needed, but light is a requirement for germination (Malcolm, 1966).

MATERIALS AND METHODS

Phenological observations of the native Conway population located on the Henze prairie (Culwell, 1980) were made during the 1981 growing season. Phenology of sowings on the UCA prairie (Culwell and Wright, 1983) was observed from April, 1982 to April, 1984. These sowings consisted of broadcasting about 15 g of seed collected at the Henze prairie in a 7 x 20 m plot early in March 1981, and making duplicate sowings of 8 or 6 g of seed in 2 x 10 m plots on October 19, 1982, March 25, 1983, and June 1, 1983. Germination potential of the seed sown in 1982 and 83 was determined in November, 1982. Precipitation and temperature were monitored throughout the study.

Haustorial connections were examined in specimens from the field and from the greenhouse. Greenhouse trials were conducted to compare success of the *Castilleja* with different hosts and different densities of a given host.

Response of seed germination to temperature was determined in a lighted incubator under temperature regimes of 10 to 15 °C, 22 to 25 °C and 25 to 30 °C. Approximately 500 seeds were tested at each temperature. Seeds were maintained on filter paper saturated with distilled water in petri dishes for the duration of the test. Emergency of cotyledons was taken as evidence of germination.

Water relations of germinating seeds were investigated using a graded series of non-nutritive polyethylene glycol solutions. Carbowax 2000 (Union Carbide Corp.) was dissolved in distilled water to create solutions with water potentials ranging from −0.5 bar to −4.0 bar in increment of −0.5 bar. Five ml of solution was introduced into standard petri dishes; approximately 100 seeds of *Castilleja coccinea* were added to each dish, and the dishes sealed with tape. Plates were incubated in a lighted growth chamber at 22 °C for eight days. Emergence of cotyledons was taken as evidence of germination.

RESULTS

Phenology of the Local Native Population.

Widely scattered *Castilleja coccinea* plants in early anthesis were found March 31, 1981 on the Henze prairie. A systemtic search revealed 10 plants in ½ hectare. By April 23, the normal peak of flowering, some hundreds of *Castilleja* were visible over a 20 hectare area, still widely scattered. (By contrast, the previous season there had been many tens of thousands.) The nearest neighboring plants were from 1 cm to 15 cm distance, and included species of *Andropogon, Asclepias, Carex, Senecio, Smilax, Solanum,* and several unidentified grasses and composites. On May 28 the *Castilleja* ranged from late anthesis to mature seed, and some were growing away from any other perennial forbs or grasses. A search was made on September 23, but failed to reveal plants of *Castilleja coccinea* in any stage. (Nevertheless in Spring 1982 the density of flowering plants was much higher than in 1981.) Nineteen-eighty was a year of severe drought, with under 2 cm of precipitation recorded at the University of Central Arkansas in Conway for the July-August

period. In 1981 a total of 15.8 cm was recorded for the same period. In 1982, 9.9 cm of precipitation occurred in July and August; in 1983 the total was 11.3. June had 10 cm or more for each of the years 1980-1983.

Phenology of Sowings Made at UCA Nature Reserve.

In early March 1981, approximately 15 g of seed of *Castilleja coccinea*, collected on 1980 on the Henze prairie, was broadcast on a 7 x 20 m plot of undisturbed prairie at the UA Nature Reserve (later named the Jewel E. Moore Nature Reserve). On April 1, 1982, six paintbrush plants were observed in early anthesis. On May 18, 1982, 17 flowering individuals were counted. The most abundant plant in the plot was *Panicum linearifolium*, a small annual grass, although numerous other prairie species were represented. On May 1, 1983, 97 individuals of paintbrush were counted in the plot. On May 1, 1984, 158 paintbrush plants were located in the plot.

The three sets of duplicate field sowings made in 1982 and 1983 were from seed collected in 1982 and stored dry at 5°. Germination potential of this seed at 24° on moist filter paper in petri dishes in a lighted growth chamber was determined to be 60%. Numbers of *Castilleja coccinea* plants visible on May 1, 1984 were as follows:

October 19, 1982 sowing
plot A, 8 g seed, 5 individuals
plot B, 8 g seed, 31 individuals
March 25, 1983 sowing
plot A, 8 g seed, 4 individuals
plot B, 8 g seed, 14 individuals
June 1, 1983 sowing
plot A, 6 g seed, 0 individuals
plot B, 6 g seed, 0 individuals

The entire prairie was burned in February of 1982, 1983, and 1984. Combustion typically stopped 2 to 3 cm above the soil.

Mean temperatures at UCA for the possible germination periods were as follows: March 1981, 11.8°; April 1981, 18.5°; March 1983, 11.0°; April 1983, 13.1°; May 1983, 22.1°; June 1983, 24.8°.

On April 16, 1985, a qualitative survey indicated increased numbers in all plots, including the June 1983 sowing.

Host Dependence.

The haustorial association proved difficult to work with. Although pot trials clearly demonstrated the dramatic difference in growth between *Castilleja* plants that had and had not made haustorial connections to host broccoli plants, the hemiparasite did not respond when grown in pots of different native host *(Pentstemon alluviorim, Rudbeckia hirta, Helianthus angustifolia, Echinacea purpurea, Gnaphalium obtusifolium),* or different densities of *Rudbeckia hirta.* One certain haustorial connection to the annual *Oenothera linifolia* was found on a field specimen of a current-year seedling on May 28, 1981.

Temperature Response of Germination.

Germination at 10° to 15°C began on day 22. By 24 days it had reached 24%. No further germination had occurred by 27 days when the trial was terminated.

Germination at 22° to 25°C began on day 6. By day 11 it had reached 61%. No further germination occurred by day 14 when the trial was terminated.

Germination at 25° to 30°C began on day 7 and was complete by day 10. Total germination was 5%.

Water Relations of Germinating Seeds.

Control seeds placed on distilled water germinated at 90%. Seeds on Carbowax solutions of −0.5 bar and −1.0 bar water potential showed 50% germination. No germination occurred at −1.5 bars or lower water potential.

DISCUSSION

Since the goal of the study was to learn about introducing *Castilleja coccinea* into native prairie, results will be discussed in reference to cultural practices.

It is clear that establishment must be by field sowing, and that the numbers of seeds will be high. Natural sowing occurs in May and June from mature capsules that dehisce on the plant, allowing the very lightweight seeds to be blown or splashed out. May or June germination is possible, since the seed requires no after-ripening (Malcolm, 1966) and temperatures are favorable. Mean May and June temperatures of 19.7° to 26.8° are close to the 22° to 25° range of the highest laboratory germination. However, May-June germination is closely followed by a summer that is not only too warm for good germination but subject to periods of drought which would prevent it, as indicated by sensitivity of germination to water potential. It also seems likely that summer drought would take a heavy toll of seedlings that had not yet established haustorial connections, a process which may take some weeks (Malcolm, 1966).

Seeds that can survive until fall may germinate then or early the following Spring. Since stored seed has survived 3½ to 4½ years (Heckard, 1962) seed longevity would appear not to be a problem, but extreme sensitivity to drought as shown in the germination tests with polyethylene glycol may limit Fall germination. Thus this study appears to bear out the contention by Malcolm (1966) that early Spring is the time of field germination. The rosette discovered on May 28, 1981 was thus likely a current-season seedling, having made haustorial attachments to hosts in the previous several weeks. Early spring sowing is therefore recommended.

Field sown seed must of course reach soil and not be covered, owing to its small size and requirements of light during germination. It will parasitize plants its own age, such as the annual *Oenothera linifolia,* so presumbably could go onto lightly disturbed prairie, although the experimental sowings relied only on bare ground exposed by haying or burning. In nature as in the experimental sowings, a great many seeds are released. In the Henze and UCA prairies this study did not indicate requisite or preferred host species, but did show that establishment varies over several orders of magnitude from season to season, apparently correlated with drought during the first season of growth. It is easy to see how, without a carryover of ungerminated seed, a population could be eliminated by severe drought, and this may indeed account for the spotty distribution *Castilleja coccinea* in Central Arkansas.

It may require several seasons plus a good bit of luck for viable populations of *Castilleja coccinea* to become established. Increase of the 1981 sowing at the Jewel E. Moore Nature Reserve was several-fold from the 1982 to the 1983 season, and may have come from delayed germination of the original sowing as much as release of seed from the 1982 plants. Since from 150,000 to 300,000 seeds were sown in each plot, establishment was evidently very low. By May 1 of 1984, although sowings of the previous year had produced only a few visible seedlings, the 3-year-old plot continued to show increase in density.

Although the literature suggests that this species of hemiparasite establishes haustorial connections promiscuously, the current study does not bear this out. No connections were conclusively demonstrated in pot trials, and the low survival of field-sown seed may be partly attributable to failure to parasitize. Future field work should concentrate on a search for key host species that may correlate with field distribution in Arkansas.

In summary, introduction of *Castilleja coccinea* in central Arkansas prairie appears to be a chancy proposition. Given enough seed and time, along with good weather and the proper hosts, introduction may succeed, but it is far from certain to produce an established population.

ACKNOWLEDGMENTS

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GENERAL NOTES

ULTRAVIOLET LIGHT REACTIVATION OF GAMMA-RAY INDUCED CHROMOSOME ABERRATIONS IN G1 PHASE *XENOPUS* CELLS*

Mitigation of ionizing radiation-induced effects by appropriate administration of low UV doses, termed ultraviolet light reactivation (UVR), has been observed in a number of prokaryotic (Weigle, 1953; Rupert and Harm, 1966) and lower eukaryotic (Elkind and Sutton, 1959; Calkins and Todd, 1968; Calkins and Griggs, 1969) cells. Cross and Griggs (1978) detected a significant level of UVR of gamma ray-induced lethal damage in a number of established vertebrate cell lines, including the A8W243 *Xenopus* line. We report here an attempt to determine whether UVR extends to chromosomal aberration induction by gamma ray in G1 phase cells of a line (A8W4) which was recently cloned from the A8W243 line.

Monolayers of A8W4 cells were routinely maintained at room temperature in plastic tissue culture flasks (Falcon) in HEPES buffered F10 medium (Gibco) supplemented with 10 percent fetal calf serum (Kansas City Biological). Synchronous cultures of early G1 phase cells were obtained by a miotic harvest method similar to the one described by Griggs and Orr (1979). Techniques employed for mitotic index determination, collection of mitotic cells by colcemid, and preparation of chromosome spreads for aberrational analysis were essentially the same as described by Griggs and Bender (1972), and Wolff (1962). Gamma ray was administered at a dose rate of 50 rads/minute by a Mark IV Cesium 137 irradiator as described by Cross and Griggs (1978). UV light (254nm) was administered at a dose rate of 5 ergs/mm²/sec. in the same manner as described by Griggs and Orr (1979). All experimentation was carried out at 25 °C under red light.

In the UVR experiment the desired doses of UV and gamma ray were administered to synchrounous cultures of G1 cells and, during postirradiation incubation, many of the exposed cells progressed through S and G2 to mitosis, where samples were collected with colcemid for chromosomal aberration analysis. Nonirradiated synchronous cultures of cells normally progress through interphase to mitosis in approximately 22 hours and retain a high degree of synchrony during their progression; however, administration of variable doses of UV and gamma ray to these cultures induces complex delays in the progression of the cells through S phase, and significantly lowers synchrony. Thus, detailed mitotic index experiments (see Figure) were carried out to determine the post irradiation peaks of mitotic activity and the corresponding time ranges for collection of appropriate samples of mitotic cells for aberration analysis.

As indicated by the chromatid-type aberration data of Table 1, administration of 200 rads gamma ray plus low UV doses (in the range 0-80 ergs/mm²) fails to induce significant frequencies of chromatid aberrations. This datum is consistent with studies indicating that ionizing radiation induces few, if any, chromatid aberration in G1 phase cells (Elkind and Whitmore, 1967), and the study by Griggs and Orr (1979) indicating that UV doses in the range 0-90 ergs/mm² fail to induce significant frequencies of chromatid aberrations in G1 phase A83 *Xenopus* cells. Comparison of the chromosome-type aberration data of Table 1 reveals that the various aberration frequencies vary as a complex function of UV dose. Rings and dicentrics appear to be subject to UVR, since the numbers of both types of aberrations decrease with increasing UV dose. In contract, the number of terminal deletions significantly increases with increasing dose. Results of the brief time course of UVR study (Table 2) indicate that the UV exposure must accompany, or be given shortly after, the gamma ray exposure to effect aberration production. Results of earlier studies by Wolff and Luippold (1955) indicate that most ionizing radiation induced chromosome breaks, including those involved in production of chromosome-type rings and dicentrics, rejoin or restitute shortly after formation. Their results, coupled with the data of Table 2, suggest that UV must be administered before the gamma ray induced breaks rejoin to effect significantly aberration production.

Interpretations of these data must be highly speculative, since radiation induced aberrant processes which lead to chromosome breakage, and intracellular processes which control rejoining of broken chromosome segments, are not understood (Elkind and Whitmore, 1967). Nevertheless, the following seems reasonable. Lesions induced in G1 chromosomes by low UV doses (in the range 0-80 ergs/mm²) produce few, if any, chromosome breaks, and have relatively little influence on the chromosome breakage induced by gamma ray. However, when these low UV doses are administered shortly before or after the gamma ray exposure, UV lesions induced in or near those chromosome sites where gamma ray induced breaks occur, significantly inhibit restitution of broken ends. Thus, the UV dependent increase in frequency of terminal deletions and decrease in frequencies of rings and dicentrics observed in the experiments of Table 1 may have resulted from UV inhibition of chromosome restitution processes.

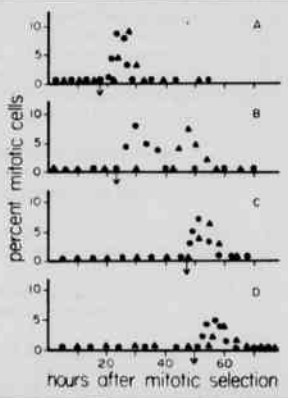

Figure. Percent mitotic cells as a function of time following irradiation of early (one hour old) G1 phase A8W4 cells with 200 rads gamma ray plus the following UV doses; 0 ergs/mm² A (circles), 10 ergs/mm² A (triangles), 20 ergs/mm² B (circles), 30 ergs/mm² B (triangles), 40 ergs/mm² C (circles), 50 ergs/mm² C (triangles), 60 ergs/mm² D (circles), 80 ergs/mm² D (triangles). Percent mitotic cells corresponding to time points to the left of downward pointing arrows were essentially zero.

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Table 1. UVR of gamma ray-induced chromosomal aberrations in G1 phase A8W4 cells as a function of UV dose. In each experiment synchronous cultures were exposed to the indicated doses of UV, one hour after mitotic selection, and 200 rads gamma ray was administered immediately after the UV exposure.

Experiment number	UV dose in ergs/mm^2	Collection time range (hours after mitotic selection)*	Number cells scored	Chromatid type aberrations		Chromosome type aberrations		
				Terminal deletions	Exchanges	Terminal deletions	Rings	Dicentrics
1	0	22-32	200	1	0	51	17	23
2	10	24-34	200	2	0	52	18	21
3	20	30-40	200	0	1	51	16	22
4	30	42-52	200	1	0	53	16	21
5	40	45-58	200	1	0	62	14	19
6	50	48-60	200	2	0	68	11	18
7	60	50-62	200	1	0	79	11	15
8	80	55-70	200	2	1	83	9	15

*Cells were collected for aberrational analysis by colcemid treatments that spanned the indicated time range.

Table 2. Time course of UVR of gamma ray-induced chromosome-type aberrations in A8W4 cells. In each experiment, a synchronous cultures of G1 phase cells was first exposed to 200 rads gamma ray (one hour after mitotic selection) and then exposed to 80 ergs/mm^2 UV, with the UV exposure beginning at the indicated time following termination of the gamma ray exposure.

Experiment number	UV dose (minutes after gamma ray exposure)	Collection time range (hours after mitotic selection)	Number cells scored	Chromosome type aberrations		
				Terminal deletions	Rings	Dicentrics
1	0	55-70	200	80	6	13
2	5	55-70	200	78	8	12
3	10	55-70	200	79	7	14
4	15	55-70	200	76	6	12
5	25	55-70	200	62	12	15
6	45	55-70	200	49	15	23
7	90	55-70	200	50	17	24

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THE WATER CRISIS — AN APPROACH FOR TEACHERS OF GRADES 7-12

The development of a water-ecology workshop has resulted from the confluence of three observations. First, several recent events attest a growing concern for the quantity and quality of Arkansas' water. In 1981 Governor Frank White appointed a committee to develop a comprehen-

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sive water code. The committee's work culminated in the presentation of House Bill 60 and Senate Bill 8 during the 1983 legislative session. During 1982 the Winthrop Rockefeller Foundation founded a public-awareness project, entitled "Arkansas Water: Why Wait for the Crisis?", which has received wide dissemination. Also during 1982 the Arkansas Department of Pollution Control and Ecology received a National Science Foundation grant to fund a scientific policy review for that agency, particularly with respect to improving stream classification, lake management, and hazardous waste disposal. Secondly, it is my experience that the older an individual becomes, the more difficult it is to modify his/her behavior, especially with regard to the environment. Finally, teachers of secondary education need inexpensive resource materials and teaching techniques that will allow them to instruct science in today's world. Accordingly, the purposes of this workshop are to provide teachers with: 1) a broad overview of the interrelationships of water and man; and 2) at least one functional freshwater-ecology unit for classroom use.

In order to serve the maximum number of teachers, the workshop is numbered to provide three semester hours of credit for either upper-level undergraduate or graduate students. Each student is required to participate in class activities, construct a teaching aid (e.g. a sampling device), and develop one water-ecology teaching unit. The teaching unit may emphasize class or field work, or a combination, depending on the constraints of that student in the school where he/she teaches. Additionally, individuals taking the workshop for graduate credit research and develop a report on a particular water use outside the workshop syllabus.

The lecture portion of this workshop is designed to acquaint teachers with the importance of water. A basic ecology unit is followed by units emphasizing water use in agricultural, industrial, recreational and municipal/domestic applications. In each of the latter units the student learns how the water is used, in what quantities, how water quality is affected by that use and what the future prospects are.

The laboratory periods are utilized for a variety of activities. Field trips are made to local streams and the sewage-treatment facility. Students become familiar with plans for constructing inexpensive water-related sampling devices and construct at least one such device. Discussion periods are held during which the students exchange information concerning environmental teaching techniques and activities with which they have had success. A resource room is utilized which has been well provisioned with water-related information. Ideas for science fair projects are developed. Ultimately, these resources are synthesized as each student develops at least one complete freshwater-ecology unit specifically designed for his/her classroom situation.

One example of a free sampling method is effective observation. It is also informative and has wide application. Observations may include the variety of aquatic life, behavior, water turbidity and substrate type. Organisms can be returned to the classroom aquarium for further observation (e.g. air bubble respiration in aquatic beetles). Measuring current speed and volume flow can also be done without expense (Phillips, R. E., Jr. 1984. A field trip to the stream. Carolina Tips. 47[2]:5-6). A thermometer, tea strainer, aquarium dip net and minnow seine bring studies of habitat requirements, species diversity and numerical standing crops within one's capabilities for little expense.

Among the information resources utilized, I have found four to be particularly u.eful in providing ideas and techniques (U.S. Environmental Protection Agency. 1974. Environmental exchange — a beginning. President's Environmental Merit Awards Program, USEPA, Washington, D.C. 21 pp.; Resh, V. H., and D. M. Rosenberg. 1979. Innovative teaching in aquatic entomology. Canadian Spec. Pub. Fish. Aq. Sci. 43. 118 pp; Miller, G. T., Jr. 1982. Living in the environment. Wadsworth Publ. Co., Belmont, CA. 671 pp; and Disinger, J. F. 1983. Learning *in* the environment. Env. Ed. Fact Sheet. No. 2. ERIC Clearinghouse for science, mathematics, and env. ed. Columbus, OH. 2 pp.). The last three contain extensive bibliographies.

Characteristically, teachers have every intention of incorporating new knowledge from their summer courses into their teaching curriculum. However, too much work and too little time often combine to thwart even the most conscientious teachers. The major strength of this workshop lies in its ensuring that each teacher takes a complete water-ecology unit, tailored to his/her specific needs, including budgetary constraints, back to the classroom. This maximizes the probability that this environmental education will be transmitted to the secondary level, where it is sorely needed.

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PROTECTION OF ENDANGERED GRAY BAT *(Myotis grisescens)* COLONIES IN ARKANSAS

The gray bat, *Myotis grisescens,* is one of three Arkansas bat taxa listed as endangered (in danger of extinction throughout all or a significant portion of their range) by both the U.S. Fish and Wildlife Service and the Arkansas Game and Fish Commission. Other Arkansas bats listed as endangered are the Indiana bat, *Myotis sodalis,* and the Ozark big-eared bat, *Plecotus townsendii ingens.*

Gray bats, unlike most bat species, are cave residents throughout the year, although different caves are usually occupied during summer than in winter; few have been found roosting outside caves (Barbour and Davis, 1969). They hibernate primarily in deep vertical caves with large rooms that act as cold air traps. They form tight clusters of up to several thousand individuals and choose hibernation sites where temperatures average 6-11 C (Barbour and Davis, 1969). During summer, female gray bats form maternity colonies of a few hundred to many thousands of individuals. Maternity colonies prefer caves that, because of their configuration, trap warm air (usually 14-25 C) or that provide restricted rooms or domed ceilings that are capable of trapping the combined body heat from clustered individuals (Tuttle, 1975; Tuttle and Stevenson, 1978). Maternity caves are rarely located more than 2 km, and usually less than 1 km, from rivers or reservoirs.

During spring and autumn transient periods, gray bats occupy a wider variety of caves. During all seasons, males and yearling females seem less restricted to specific cave and roost types. Because of their highly specific habitat requirements, fewer than 5% of available caves are suitable for occupation by gray bats (Tuttle, 1979).

Gray bats forage primarily over water along rivers or near lake shores. Most foraging occurs within 5 m of the surface. Mayflies are apparently a major item in their diet.

Approximately 250,000 gray bats hibernate in only four Arkansas caves, over 99% of these in a single Baxter County cave. During summer, ca. 150,000 gray bats occupy 40 caves scattered throughout the Arkansas Ozarks. Approximately 100,000 migrate to summer colony caves in Missouri, Oklahoma, and Kansas. Ten maternity colonies are known in Arkansas, nine of which are on private lands.

A major factor in the decline of gray bat populations has been human disturbance to gray bat colonies in caves. Hibernating gray bats, when disturbed by humans entering their hibernation caves, arouse, using up previous fat needed to survive the winter. If disturbed more than a very few times, they may starve to death before insects become available in the spring.

Maternity colonies are very intolerant of disturbance, especially when nonvolant young are present. If disturbed, baby bats may be dropped to their deaths or abandoned by panicked parents. The protection of gray bat caves from human disturbance is of foremost importance in the survival of this extremely beneficial bat species.

The following summarizes efforts made thus far to protect important Arkansas gray bat caves. Some of these efforts have been previously reported (Harvey, 1975, 1976a, 1976b, 1979, 1980; Harvey et al., 1979).

The very important gray bat hibernaculum located in the Ozark National Forest of Baxter County was gated by the U.S. Forest Service in 1975. The gate was subsequently redesigned to better fit the particular conditions at the cave and a replacement gate was installed during the summer of 1980. Five Buffalo National River caves have been fenced by the National Park Service to protect gray bat (and Indiana bat) colonies. One cave located on a private housing development in Benton County has been fenced by the developer to protect a gray bat summer colony. The U.S. Army Corps of Engineers recently constructed an artificual entrance into a gray bat cave located on Beaver Lake in Benton County, since the natural entrance is sometimes inundated during high lake levels.

In addition to the above measures, the Arkansas Game and Fish Commission, U.S. Fish and Wildlife Service, U.S. Forest Service, and National Park Service have placed warning/interpretive signs at several gray bat caves. Other agencies and organizations involved in the gray bat conservation effort include the Nature Conservancy, Arkansas Department of Parks and Tourism, Arkansas Natural Heritage Commission, National Speleological Society, Cave Research Foundation, Association for Arkansas Cave Studies, and Ozark Underground Laboratory.

Important gray bat hibernacula and summer caves will be monitored for the next several years. Additional measures will also be taken to protect other important gray bat caves. Hopefully, the conservation effort will result in removal of the gray bat from the endangered species list.

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SURVIVAL OF *TRYPANOSOMA CRUZI* IN DEAD CHRONICALLY INFECTED MICE

Trypanosoma cruzi (Chagas, 1909), the cause of Chagas' disease, is a flagellate protozoan found throughout most of Central and South America where it infects approximately 12 million people (World Health Organization, 1960). In the United States this agent has been reported in wildlife mammals in California, Arizona (Kofoid and Whitaker, 1936), Texas (Packchanian, 1941), New Mexico (Dias, 1951), Minnesota (Hedrick, 1955), Virginia (Tromba, 1951), Maryland (Walton *et al.*, 1956), Georgia, Florida (McKeever *et al.*, 1958), Alabama (Olsen *et al.*, 1964), and Louisiana (Yeager and Bacigalupo, 1960). Cited reservoir hosts include such common mammals as the raccoon *(Procyon lotor),* opossum *(Didelphis virginiana),* grey fox *(Urocyon cinereoargenteus),* and striped skunk *(Mephitis mephitis)* (McKeever *et al.*, 1958).

The normal transmission of *T. cruzi* occurs when a bug (Hemiptera:Reduviidae) takes a blood meal and deposits feces containing trypomastigotes on the skin. The parasites enter the bite wound and invade cells of many organs throughout the body as they circulate in the blood for approximately 30 days or longer. Upon entering a cell, the trypomastigote typically loses its flagellum and transforms into an amastigote which reproduces by binary fission. During the acute phase amastigotes produced intracellularly are released by cell lysis and either reinvade new cells or transform into trypomastigotes and reenter the circulation. The cells most often attacked are reticuloendothelial cells of the liver and spleen, cells of cardiac, smooth and skeletal muscle and certain cells of the nervous system. Animals surviving the acute phase typically develop chronic infections in which amastigotes form pseudocysts in the host's tissues producing only limited cell lysis. In the chronic infection, trypomastigotes in the blood are scanty and difficult to demonstrate by microscopic examination.

Epidemiologic studies of zoonoses, such as Chagas' disease, contribute to the basic understanding of the biology of the etiologic agents and provide information relating to both geographic distribution and transmission. Such information can be useful in prevention and control of these diseases which may pose a threat to man, domestic animals and wildlife. Often studies of this nature have required the capture and, in some cases, the sacrifice of potential wildlife reservoirs.

Meurer (1980), in a preliminary study, demonstrated that *T. cruzi* could be detected in dead chronically infected mice by inoculating a modified

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Tobie's diphasic medium (Tobie *et al.*, 1950) with a homogenate of heart tissue. *T. cruzi* was cultured from two of three mice that had been held for 24 hours at 25 C while three out of three mice were positive after 24 hours at 5 C. The present study was conducted to more accurately assess the relationship between holding temperature and parasite survival in dead mice that were chronically infected and to compare the efficiency of in vitro cultivation with animal inoculation for detecting *T. cruzi* in tissue homogenates of dead animals.

The Calluromys LNX strain of *T. cruzi* was used in our current study since it typically produces a less intense acute phase parasitemia followed by a chronic infection in which mice have an extended survival time. Chronic infections were achieved by injecting mice subcutaneously with approximately 6×10^4 trypomastigotes (determined by the method of Herbert and Lumsden, 1976) obtained from mice with acute infections. ASU Lilac-Wild cross mice were used for this purpose since these animals are hardy and tend to develop chronic infections consistently. Mice were considered to be in the chronic phase when microscopic examination of tail blood from previously patent animals revealed very low or negative parasitemias. In most mice this was around 50 days postinoculation.

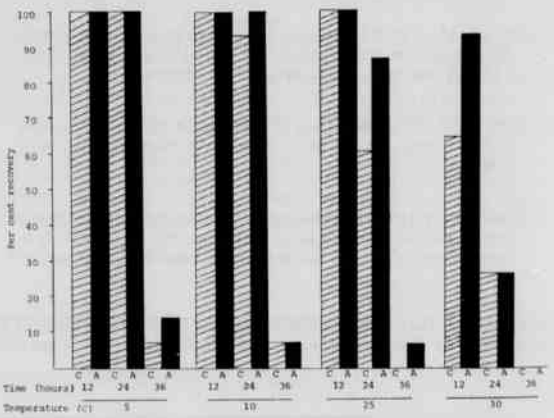
Chronic phase mice were sacrificed and held at temperatures ranging from 5 C to 35 C. Holding periods ranged from zero to 48 hours for lower temperatures while 36 hours was maximum time for higher temperatures. The heart and liver were aseptically removed from each animal and homogenized in a Ten-Broeck tissue grinder with 2.0 ml sterile Locke's solution containing 18.5 μg/ml gentamicin to prevent bacterial growth. From each homogenate, two mice (ASU Piebald) were injected subcutaneously with 0.5 ml portions and two tubes of modified Tobie's diphasic medium (with human blood) containing gentamicin were inoculated with 0.2 ml each. ASU Piebald mice were used for this phase of the study because of their susceptibility to infection. Mice were checked for parasitemias periodically for a period of 30 days after which time they were declared negative. In vitro culture tubes were incubated at 25 C for 10 days before discarding.

Results of the study indicate that survival time of *T. cruzi* in chronically infected mice is highly dependent on the holding temperature (Figure). At 5 C *T. cruzi* was detected in 100% of the mice held for as long as 24 hours when tested by in vitro cultivation or by animal inoculation. With increasing temperature, the recovery rate of *T. cruzi* from dead animals decreased. At 10 C the recovery rate for 24 hours was still 100% by animal inoculation and 93% by in vitro cultivation. The 24-hour positive fell to 60% and 87% for in vitro cultivation and animal inoculation respective at 25 C while at 30 C the rate dropped to 27% for both methods. The Table reveals that no parasites were detected in animals held at 35 C for 24 hours.

Table. Recovery of *Trypanosoma cruzi* from chronically infected mice by in vitro cultivation and mouse inoculation.

Holding time (hours)	Holding temperature (°C)	Recoveries by in vitro cultivation[a]	Recoveries by mouse inoculation[b]
0	5	15/15	15/15
6	"	15/15	15/15
12	"	15/15	15/15
24	"	15/15	15/15
36	"	1/15	2/15
48	"	0/15	0/15
0	10	15/15	15/15
6	"	15/15	15/15
12	"	14/14	14/14
24	"	14/15	15/15
36	"	1/15	1/15
48	"	0/14	0/14
0	25	15/15	15/15
6	"	15/15	15/15
12	"	15/15	15/15
24	"	9/15	13/15
36	"	0/18	1/15
48	"	0/15	0/15
0	30	15/15	14/15
6	"	14/14	14/14
12	"	10/15	14/15
24	"	4/15	4/15
36	"	0/15	0/15
0	35	15/15	15/15
6	"	14/15	15/15
12	"	2/15	3/15
24	"	0/15	0/15
36	"	0/15	0/15

[a]A modified Tobie's diphasic medium was inoculated with heart-liver homogenate and incubated at 25 C.
[b]Mice were injected subcutaneously with heart-liver homogenate.



C = Per cent recovery by inoculation of a modified Tobie's diphasic medium with heart-liver homogenate.
A = Per cent recovery by subcutaneous injection of mice with heart-liver homogenate.

Figure. Relationship of holding time and holding temperature to recovery rate of *Trypanosoma cruzi* from chronically infected mice.

The results also suggest that animal inoculation is a somewhat more sensitive method than in vitro cultivation for detecting *T. cruzi* in the tissue of dead animals. It was clear, however, from trial runs with several strains of mice that a highly susceptible line of mice is important for this purpose.

In the epidemiology of *T. cruzi* it appears likely that dead chronically infected animals might serve as a source of infection for scavenging animals for a period of at least 24 hours at moderate temperatures and possibly as long as 36 hours at cool temperatures. The results also point to in vitro or in vivo cultivation from heart-liver homogenates as an acceptable method for assessing the distribution and incidence of *T. cruzi* in wildlife. These animals could be road kills or those taken by fur trappers.

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EFFECT OF THE PAYMENT-IN-KIND (PIK) PROGRAM ON THE *PSOROPHORA COLUMBIAE* MOSQUITO POPULATION OF A NORTHEAST ARKANSAS RICEFIELD COMMUNITY

Since 1904, when rice was initially cultivated as a commercial crop within the state, Arkansas has developed into one of the five major rice-producing states in the U.S. with an annual production of ca. 6,000,000 ha (Meisch, et al., 1982). Although some rice is grown in central and southwestern counties, the majority of the crop is planted in the eastern half of the state, particularly in the "Grand Prairie" area of east-central Arkansas which includes Arkansas, Lonoke, Monroe, and Prairie counties.

It has been well established by a number of investigators in Arkansas, and in other rice-growing states, that the rice agroecosystem provides suitable breeding sites for several mosquito species including the dark ricefield mosquito, *Psorophora columbiae* (Dyar and Knab). In Arkansas, Schwardt (1939), Horsfall (1942), Whitehead (1951), Meisch and Coombes (1975), and Olson and Huggins (1983), among others, all have reported *P. columbiae* to be the dominant mosquito species wherever rice is cultivated. Whitehead (1951), in particular, noted that ricefield mosquito numbers have increased in direct proportion to the state's increased rice acreage.

In Craighead County in NE Arkansas, land devoted to rice production in 1981 and 1982 was 33,590 and 33,376 ha, respectively (Olson and Huggins, 1983). Light trap studies conducted in Jonesboro by these investigators indicated that, of 34,041 adult mosquitoes captured between 30 May and 2 October of 1981, 21,085 (61.9%) were *P. columbiae*. During the same period in 1982, *P. columbiae* numbered 24,675 (72.3%) of the 34,114 mosquitoes trapped.

In 1983, a federally funded payment-in-kind (PIK) program was implemented in an attempt to reduce national agricultural surpluses and to improve market prices by paying growers to keep land out of commercial crop production. As a result, American farmers idled some 31,000,000 ha including significant rice acreage in NE Arkansas. Under the PIK program, the amount of Craighead-County rice land was lowered by 39.0% to 20,350 ha (Fagala, Craighead Co. Extension Service, pers. comm.).

The primary aim of this study was to evaluate the effect which this acreage reduction had upon the relative abundance of *P. columbiae* in Jonesboro. To accomplish this objective, a standard New Jersey light trap was placed at each of four locations within the city limits. Sampling dates and trap locations (two peripheral and two central) were identical to those described by Olson and Huggins (1983). Light trap catches were collected daily and adult mosquitoes sorted and identified. Daily trap totals were summed for each week of the 18-week study period and compared with data obtained in 1981 and 1982.

It should be noted that, as in 1981 and 1982, all traps were in areas subjected to periodic applications of a mosquito adulticide by ground-operated ULV cold-aerosol generators. This undoubtedly lowered the total number of mosquitoes collected, particularly in the central-city area which was further from rice fields which served as the main source of *P. columbiae* reinfestation.

A total of 18,232 adult mosquitoes was captured between 30 May and 2 October of 1983, with the PIK program in effect. This represented an overall decrease in the general mosquito population of 46.5% in comparison with 1981 and 1982 pre-PIK totals reported by Olson and Huggins (1983). As anticipated, *P. columbiae,* with 13,394 trapped individuals, was the dominant species and it comprised 73.5% of the season's catch. Other genera contributing to the remainder of the total were *Anopheles* (16.0%), *Aedes* (6.5%), *Culex* (4.0%), and *Culiseta* (less than 0.1%). It was observed that the 39.0% decrease in Craighead-County rice acreage in 1983 was accompanied by a 43.4% reduction in the *P. columbiae* population trapped within the Jonesboro city limits. This was believed to have been a direct result of the lessening of available breeding sites normally

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provided by waters associated with rice cultivation.

Figure 1 clearly illustrates the relatively depressed *P. columbiae* population level throughout most of the 1983 season. It also can be observed that the 1983 ricefield-mosquito population peak occurred somewhat later than in each of the two preceding years. Rainy conditions and cool early-season temperatures which delayed rice planting in many fields, especially in 1983, were thought to have been the primary reasons for this occurrence.

Horsfall (1942) reported that although *P. columbiae* normally exhibits two periods of maximum abundance during the summer in Arkansas, these peaks may be affected by the timing of rice planting dates. A short planting interval usually results in two distinct peaks of abundance because adults emerging after the initial flood will have largely disappeared before the second peak occurs following normal mid-season drainage and reflooding. Whenever planting extends over several weeks, as in 1983, the two separate peaks of abundance tend to overlap or be somewhat obscured. This occurs because adults produced by early-planted fields, that have been reflooded following cultural drainage, will be emerging at the same time as those coming from the initial flooding of late-planted fields. In Figure 1, it can be noted that the magnitude of difference in the two 1983 population peaks was considerably less than in either 1982 or 1981. This reduction may well have been the result of the weather-related spread of the 1983 planting dates. As expected, the abrupt decline in ricefield mosquito numbers in late August of 1983 correspond closely with late-season drainage of area rice fields.

A more detailed comparison of the number of *P. columbiae* adults collected in the two peripheral and the two centrally-located traps is presented in Table 1. Despite a reduced ricefield-mosquito population in 1983, the peripheral traps, which were within 0.9 km of several nearby rice fields, accounted for over 90.0% of the total catch while the central traps, which were situated in excess of 1.2 km from the nearest rice, caught only 9.8% of the *P. columbiae* adults. This was generally consistent with 1981 and 1982 observations made by Olson and Huggins (1983) and may further substantiate the reported short flight range for this species.

In summary, our data support the conclusion that a significant decrease in cultivated rice acreage in Craighead County resulted in a corresponding reduction in the number of *P. columbiae* adults within the Jonesboro city limits. The timing of the increase and the decline of the ricefield mosquito population was closely associated with area rice-cultivation practices. The capture of more than 90.0% of all *P. columbiae* adults within 0.9 km of rice fields was in agreement with the reported short flight range for this species.

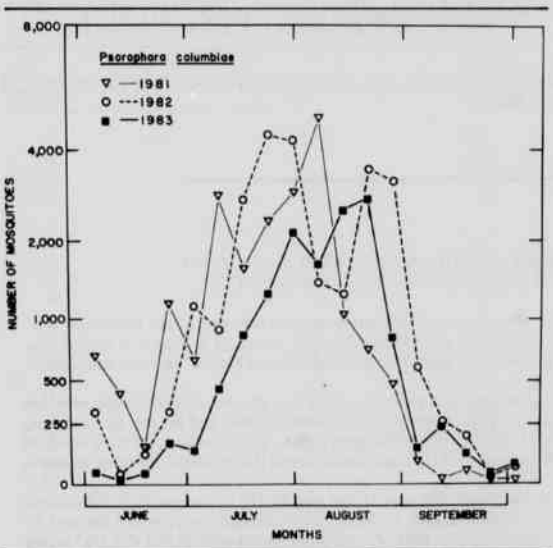

Figure 1. Comparison of weekly totals of adult *Psorophora columbiae* trapped at 4 locations in Jonesboro, Arkansas in 1981, 1982, and 1983.

Table 1. Comparison of numbers of adult *Psorophora columbiae* trapped at 2 peripheral and 2 central locations in Jonesboro, Arkansas in 1981, 1982, and 1983.

	PERIPHERAL TRAPS	N	%	CENTRAL TRAPS	N	%	ANNUAL TOTALS N	%
1981	AIRPORT	15,031	71.3	ASU	531	2.5		
	RACE ST.	5,171	24.5	CULBERHOUSE ST.	352	1.7		
		20,202	95.8		883	4.2	21,085	100.0
1982	AIRPORT	11,599	47.0	ASU	619	2.5		
	RACE ST.	11,996	48.6	CULBERHOUSE ST.	461	1.9		
		23,595	95.6		1,080	4.4	24,675	100.0
1983	AIRPORT	6,622	49.4	ASU	594	4.4		
	RACE ST.	5,461	40.8	CULBERHOUSE ST.	717	5.4		
		12,083	90.2		1,311	9.8	13,394	100.0

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EVALUATION OF SUGAR CANE BAGASSE AND RICE STRAW AS PROCESS SUBSTRATES FOR THE PRODUCTION OF ETHYL ALCOHOL

During the past ten years, a great deal of attention has been given to the production of liquid fuels, particularly ethyl alcohol, from renewable resources. A major portion of the research in this area has centered around the utilization of non food-chain resources such as waste lignocellulosics. Within this grouping municipal solid waste (MSW) has received the greatest consideration due to its availability in large, collected quantities on a daily basis. The future of ethyl alcohol production from renewable resources, however, may lie in the conversion of agricultural wastes such as sugar cane bagasse and rice straw due to their availabilities on a world-wide basis, especially in many developing countries where these are the major agricultural crops. This study reports the evaluation of both sugar cane bagasse and rice straw as potential substrates for the production of ethyl alcohol.

Microorganisms

Trichoderma reesei, QM 9414G, obtained originally from the American Type Culture Collection, Rockville, Maryland, was used to produce a full complement cellulase system consisting of endoglucanase, cellobiohydrolase, and cellobiase activities (Emert et al., 1974) for use in simultaneous saccharification fermentation (SSF) Boltkamp et al., 1978; Takagi et al., 1977). Permanent stock cultures were maintained as lyophilized spores and working stock cultures were allowed to sporulate on Difco potato dextrose agar, Difco, Detroit, Michigan, and then were maintained at 4 °C. Both seed cultures and cellulase production cultures were run according to the method of Gracheck et al. (1981).

Candida brassicae IFO 1664, obtained originally from the Institute for Fermentation, Osaka, Japan, was the yeast of choice for SSF. Permanent stock cultures were lyophilized, and working stock cultures were maintained at 4 °C following growth on Difco YM agar. Seed cultures were according to the method of Rivers (1983).

Simultaneous Saccharification Fermentation

Simultaneous saccharification fermentations were run according to the method of Rivers (1983) using F2 medium. Samples were aken at 24 and 48 hours and analyzed for ethanol and residual glucose.

Substrates

Bagasse, Sugar Cane Growers Cooperative of Florida, Bell Glade, Florida, and rice straw, University of Arkansas Agricultural Experiment Station, Stuttgart, Arkansas, were selected as prominent agricultural crop wastes.

Pretreatments

Substrates were subjected to two pretreatments, one mechanical and one thermochemical. Each substrate was ball milled in a laboratory scale ball mill using 1 inch diameter balls as the grinding medium for 4 hours. The substrates were also subjected to caustic pulping in 0.5N NaOH at 60 °C for 24 hours. Following pulping the substrate was washed in 0.005M citrate buffer to equilibrate the pH to 5.0.

Substrate Composition

Substrate components including hemicellulose, lignin, cellulose, and insoluble ash were determined by the method of Van Soest and Wine (1968).

Substrate Composition

Following pretreatment, a compositional analysis was completed for each substrate case (Table 1). Characteristically, for agricultural wates, cellulose content was found in the range of 35-45% of dry weight in the native state. Hemicellulose was also determined to be at characteristic levels for native agricultural wastes, 35-45% of dry weight. Bagasse also had typical levels of both lignin and insoluble ash; however, rice straw was found to contain lower than average lignin and higher than average insoluble ash. This variance from the agricultural waste norm may be explained by the fact that rice straw is known to contain from 15-30% silica by dry weight. Following ball milling, no compositional changes were observed; however, following pretreatment in 0.5N NaOH, changes were evident. In both substrate cases, the cellulose content was increased significantly through the semiselective extraction of primarily hemicellulose, and partially ash. The basis for this extraction lies in the type bonds found between lignin and hemicellulose. In grasses, ester bonds are the predominant linkage and are susceptible to the action of caustic whereas the ether bonds which are predominant in woody plants are not.

Table 1. Substrate Composition

Substrate	Cellulose	Lignin	Insoluble Ash
Bagasse			
Native	44.4	9.6	1.2
Ball Mill	44.4	9.6	1.2
0.5N NaOH	61.3	9.9.	1.0
Rice Straw			
Native	36.1	3.3	14.8
Ball Mill	36.1	3.3	14.8
0.5N NaOH	52.9	4.6	6.3

Table 2. Ethyl Alcohol Production

	Bagasse[1]					
	24 Hour			48 Hour		
Pretreatment	g/l	% Conversion	Gallons/Dry Ton	g/l	% Conversion	Gallons/Dry Ton
Native	0.1	0.4	0.3	0.1	0.5	0.4
Ball Mill	0.0	0.2	0.1	0.0	0.0	0.0
0.5N NaOH	10.5	61.1	63.9	11.4	67.6	70.7
	Rice Straw[2]					
	24 Hour			48 Hour		
Pretreatment	g/l	% Conversion	Gallons/Dry Ton	g/l	% Conversion	Gallons/Dry Ton
Native	7.1	21.0	12.9	7.1	21.0	12.9
Ball Mill	10.9	32.4	19.4	10.2	30.4	18.7
0.5N NaOH	21.6	64.2	57.9	24.9	74.0	66.7

1. 3% w/v cellulose
2. 6% w/v cellulose

Simultaneous Saccharification Fermentation

Following hydrolysis and ethanol production in SSF, a number of observations were made with respect to each substrate (Table 2). First, neither bagasse nor rice straw were readily hydrolyzed in the native state. In fact, bagasse was only negligibly hydrolyzable. Following mechanical pretreatment in the ball mill, conversions of bagasse were again negligible. Rice straw, on the other hand, produced a 50% increase in ethanol following the same pretreatment. Finally, following chemical pretreatment in 0.5N NaOH, both bagasse and rice straw showed dramatic increases in conversion when compared with the native state. Bagasse increased from negligible conversions in both the native and ball milled forms to greater than 60% of theoretical. At the same time rice straw increased by 350% over the native state and 200% over the ball milled case. In each case the corresponding increase in gallons of ethanol produced/dry ton of substrate is evident (Table 2) where 170.5 gallons of ethanol/dry ton of substrate is theoretical yield.

The explanation for the conversions observed are basic to lignocellulose hydrolysis. First, in the native state, the individual components of the substrate are in their most resistant form. Second, the relatively large particle size of the native substrate is prohibitive in allowing cellulase access to the β-1,4-bonds even if they were not highly unsusceptible to hydrolysis. Third, ball milling results in no alteration of the substrate com-

ponents from their native, resistant state even though particle size is reduced significantly. Finally, the caustic pretreatment, which is similar to the old caustic pulping process once used in the paper industry, resulted in dramatic increases in product yield as a result of significantly disrupting the highly resistant nature of the native substrate. As previously mentioned, this may be attributed to the fact that grassy plants contain ester linkages between the lignin and the hemicellulose. This allows the resistant nature of the substrate to be sufficiently altered in a manner which provides β-1,4-bonds which are not only accessible but also susceptible to enzymatic hydrolysis.

Agricultural wastes have great potential as process substrates for the production of ethyl alcohol and other chemicals currently produced from petroleum feedstocks. In order to effectively hydrolyze these lignocellulosic wastes, they must be pretreated in order to increase both accessibility and susceptibility to cellulases. Mechanical pretreatment in the form of ball milling is ineffective in the cases of bagasse and rice straw; however, caustic pretreatment did result in significant increases in product yields. Theoretical conversions attained were 67.6% for bagasse and 74.0% for rice straw which represent 70.7 and 66.7 gallons of ethanol produced/dry ton of the respective substrates.

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THE INFLUENCE OF DeGRAY RESERVOIR ON ZOOPLANKTON POPULATIONS IN THE CADDO AND OUACHITA RIVERS

Potamoplankton populations are usually rather limited (Hynes 1970). However, reservoirs with significant water retention time have extensive zooplankton communities and large populations can greatly influence the tailwater plankton community via releases. In 1978, at the request of the Arkansas Game and Fish Commission, MORS began sampling zooplankton populations in the DeGray Reservoir tailwater on the Caddo River (R3), in the Ouachita River above the confluence with the Caddo (R2), and in the Ouachita below the confluence (R5) (Figure).

Zooplankton samples were duplicate five minute horizontal tows with a Clarke-Bumpas sampler equipped with a No. 10 mesh (160 micron) net. Samples were taken at 4 to 6 week intervals at all three stations; all stations being sampled within a week. Sampling occurred from April through October. Sampling was not event dictated so some samples were collected during high water, some during moderate flow, and some during low flow. Cladocerans and rotifers were identified to species when possible, while copepods were identified to suborder.

Twelve cladoceran species, eleven rotifier species (Table 1), and two orders of copepods were found at the upper Ouachita station (R2). Twenty cladoceran species, twenty-one rotifer species, and two copepod suborders were found at the Caddo River station (R3). Thirteen cladoceran species, thirteen rotifer species, and two suborders of copepoda were found at the lower Ouachita (R5). Mean densities for each year were greater by two orders of magnitude at the Caddo station than at the Ouachita River stations (Table 2). Abundant cladocerans at the Caddo station (R3) were *Bosmina longirostris, Ceriodophnia lacustris, Chydorus sphaerius, Daphnia ambigua, D. galeata, D. catawba, Diaphansoma leuchtenbergianum,* and *Holopedium amazonicum.* Abundant rotifers were *Asplanchna priodonta* and *Conochilus unicornus.* Common rotifers were *Kellicottia bostoniensis, Keratella cochlearis,* and *Synchaeta stylata.* All of the preceding forms were frequently encountered in DeGray Reservoir. Littoral cladocerans such as *Latona parviremis, Macrothrix rosea, Eurycercus lamellatus, Camptocercus oklahomensis,* and *Alona sp.* were also found at the Caddo station. The only forms found at the Ouachita stations but not the Caddo station were *Scapholebris kingi, Leydigia acanthocercoides,* and *Kellicottia longispina,* which had not been found in the DeGray Reservoir. Forms found at the Caddo station but not at the Ouachita stations included *Bosminopsis deitersi, Latona parviremis, Camptocercus oklahomenses, Ceriodaphnia reticulata, Eurycercus lamellatus, Lecane luna, Platyis quadricornis,* and *Proalinopsis sp.* Some Ouachita River samples (especially during high water periods) had no zooplanktors, while many high water samples had extremely low numbers.

Cyclopoids dominated the copepod segment of the community in 96 percent of the samples at the upper Ouachita station (R2), 53 percent of the samples at the Caddo station (R3), and 85 percent of the samples at the lower Ouachita station (R5). Calanoid densities at the Ouachita River stations were always very low but were occasionally very important in the Caddo River sample.

The Caddo River station was more diverse than the Ouachita stations. This station community is composed of reservoir produced zooplankton and also those forms associated with a riverine or littoral situation. Numerically the reservoir-produced organisms dominate the population at this point but the other forms are not excluded. Hynes (1970) summarized the findings of several workers that found reservoirs and lakes greatly influence the plankton populations of their immediate tailwaters and contribute the vast majority of the constituents of the population. Edmondson (1959) states "The limnetic region of the inland lakes has a cladoceran population large in number of individuals but not rich in species." Thus this tailwater area is dominated by a relatively small group of species but not limited to just these forms. Shallow, weedy areas produce a greater variety of species (Edmondson, 1959) than any other habitat. Therefore the weedy river margins, shallow shoals areas, and the flow retarding influence of the reregulating pool account for the presence of a substantial number of littoral and/or riverine forms at the Caddo River station, even though the densities are dominated by reservoir produced limnetic zooplanktors.

The balance of the cyclopoid-calanoid ratio at station R3 is a reflection of the reservoir influence on that community. The consistent cyclopoid dominance at the Ouachita River stations leads one to speculate that they are more adapted to a limnetic and/or riverine environment than the calanoid. Hynes (1970) indicated cyclops to be a common potamoplanktor.

There is no abundant source of reservoir-produced plankton for the Ouachita River stations. Therefore, a reduced number of limnetic type zooplanktors (Table 1) are only occasional constituents of the communities there and never have high densities (1.0 organism/ Table 2). The riverine habitat is more limited at the Ouachita River stations since during much of the sampling season high flow variability did not allow the diverse vegetation to be established here that was found at the Caddo River station. Also, the Ouachita River has higher turbidity and a greater silt load that is found in the Caddo. Hynes (1970) indicated that many cladocera are eliminted from the plankton because they ingest silt or sand and then sink. Therefore, the Ouachita River stations contained fewer littoral and/or riverine species and the forms that were collected were found at lower densities than in the DeGray tailwater. The lower Ouachita River station (R5) had a slightly more diverse community (Table 1) than the upper station (R2) but the communities are similar in make up and densities.

The influx of large numbers of organisms from DeGray Reservoir into the Caddo River, which then empties into the Ouachita River, has little influence on the indigenous plankton populations in these rivers, since these limnetic organisms disappear completely by the time the Caddo empties into the Ouachita River and they do not supplant the already present littoral forms in the Caddo.

Table 1. Number of species found at the three river sampling stations.

	R2	R3	R5
Cladoceran species	12	20	13
Rotifer species	11	21	13

Table 2. Annual mean total organisms/ at the three river stations by year.

Year	R2	R3	R5	Samples
1978	.7758	3.1361	.3537	9
1979	.1166	5.5364	.2341	7
1980	.0359	4.0629	.0272	7
1981	.0031	6.6442	.0371	6
1982	.0145	3.1650	.0633	5
Average of all years	.1892	4.5089	.1431	

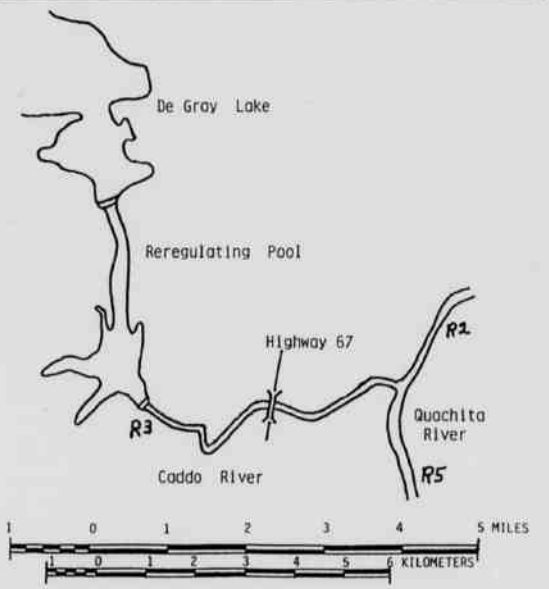

Figure. Caddo River tailwater stations.

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