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THE FORTY-SEVENTH ANNUAL SESSION

Bluefield State College

Bluefield, West Virginia

APRIL 6, 7, 8, 1972

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West Virginia Academy of Science, Inc.

The West Virginia Academy of Science was founded at Morgantown, November 28, 1924. The Academy was incorporated under the Code of West Virginia on May 9, 1959 as "a nonstock corporation, which is not organized for profit but for the advancement of learning and scientific knowledge."

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Officers

1971-72

President .... Earl L. Core, Morgantown
President-Elect .... William H. Gillespie, Charleston
Past President .... Peter Popovich, Morgantown
Secretary .... Elizabeth D. Swiger, Fairmont
Treasurer .... Joseph F. Glencoe, Buckhannon

1972-73

President .... William H. Gillespie, Charleston
President-Elect .... Elizabeth D. Swiger, Fairmont
Past President .... Earl L. Core, Morgantown
Secretary .... Elizabeth A. Bartholomew, Morgantown
Treasurer .... Joseph F. Glencoe, Buckhannon

Minutes

Minutes of the Annual Business Meeting
West Virginia Academy of Science
Bluefield State College
9:30 A.M. April 7, 1972

The meeting was called to order by President Earl L. Core. Forty-eight members were present.

President Core read a letter from President Wendell Hardway dated in August 1970, inviting the Academy to this meeting. He extended thanks for the arrangements and facilities, which were excellent as predicted.

President Hardway gave a welcoming address.

The minutes of the last business meeting were read by the secretary and approved.

The report of the treasurer was presented and approved. A copy of the report is appended.

The report of the Executive Committee was given by President Core. The problem of the Proceedings, now two years late, was explained to the membership. Two issues, those for 1970 and for 1971, are on hand. The 1970 issue is ready for the press and should be published within the next few months. No funds are yet available for the 1971 issue, although a request has been filed with the Claude Worthington Benedum Foundation and the prospects for approval look good. We hope to obtain some annual budgeted funding from all state colleges in the future.

It was suggested from the floor that the annual dues be raised to $10.00. The reaction seemed favorable but action was postponed.

Dr. Gary Larson gave the report of the nominating committee, as follows: for president, William H. Gillespie; for president-elect, Elizabeth D. Swiger; for secretary, Elizabeth A. Bartholomew; for treasurer, Joseph F. Glencoe, Jr. The report was accepted and in the absence of nominations from the floor the slate was elected by unanimous ballot.

The committee on resolutions will report at the banquet tonight.

No unfinished business was brought up. New business was then considered.

Joseph F. Glencoe, Jr., reported on the status of the Junior Academy. It has been meeting separately from the Academy since both last met together in 1970. Meetings of the Junior Academy are being held on the campus of West Virginia Wesleyan College on a year-to-year basis. The College finds it difficult to raise the required budget of $3,000.00, but Dr. David Wolfe, coordinator, wants to hold the meeting at Wesleyan again next year. Upon motion by Glencoe, seconded by Gillespie, it was decided to hold the 1973 meeting of the Junior Academy at Wesleyan.

This year the Junior Academy is meeting in conjunction with the Science and Humanities Symposium, headed by Stanley Farr. After this meeting an evaluation of the meeting place and joint meeting will be made. The report of the evaluation will be filed with officers' reports in the secretary's office.

President Core read a letter from William Bevan, Executive Secretary of the American Association for the Advancement of Science expressing regret that he could not accept our invitation to attend the Bluefield meeting.

Dr. James B. Hickman, as our AAAS representative, attended the meeting in Philadelphia in December 1971 and gave a report which will be included with the other annual reports. Dr. Hickman was appointed to represent the Academy again at the Washington meeting of the AAAS in December, 1972.

The AAAS grant fund for high school student research has accumulated $250.00. Applications should be filed with President-elect Gillespie.

Dr. James L. Hall reminded the group that as archivist he provides storage space for Academy records. Officers should send older records to him.

Dr. Peter Popovich moved that life membership dues be raised to $100.00, effective July 1, 1972. Article 4, Section 1, of the By-Laws would then read $100.00 instead of $50.00. The motion was passed.

The secretary read a letter from President Eston K. Feaster inviting the Academy to meet at Fairmont State College in April, 1973. The invitation was accepted.

Dr. W. Newman Bradshaw announced a meeting Saturday morning, April 8, of the Conference for planning a State Biological Station.

President Core thanked the group and the officers for their cooperation the past year.

The business meeting was adjourned and the Past President's address was delivered by Dr. Peter Popovich. The address was titled "Provocative Opinion: How Many Doctors?"

Respectfully submitted,
Elizabeth D. Swiger
Secretary

West Virginia Academy of Science
Financial Statement

Period, April 3, 1971 to April 6, 1972

GENERAL FUND

CASH RECEIPTS		
Balance on April 2, 1971	$ 956.93	
Dues	1,476.00	
Life Memberships	150.00	
Registrations Fees (1971 Meeting)	110.00	
Banquet Tickets (1971 Meeting)	340.25	
Science Talent Search	128.00	
Contributions	210.00	
Sale of Reprints	155.00	
Sale of Proceedings	12.50	
Total Cash Receipts		$3,538.68
CASH DISBURSEMENTS		
Banquet (1971 Meeting)	$ 319.75	
Speaker Honorarium (1971 Meeting)	100.00	
Printing	611.45	
Science Talent Search Awards	450.00	
Awards Plaques	71.94	
Research Grant	100.00	
Contributions	13.00	
Supplies	153.80	
Postage	24.95	
Total Cash Disbursements		$1,844.89
General Fund Cash Balance		$1,693.79
OTHER ACCOUNTS		
First Federal Savings and Loan Assoc. of Morgantown	$1,832.76	
First National Bank of Morgantown	488.30	
Farmers and Merchants Bank	129.25	
Total Other Accounts		$2,450.31
Total All Accounts		$4,144.10

Joseph F. Glencoe, Jr., Treasurer

Resolutions

RESOLUTIONS ADOPTED BY THE WEST VIRGINIA ACADEMY OF SCIENCE APRIL 7, 1972

WHEREAS President Hardway, his administration, faculty, students and staff at Bluefield State College have so kindly hosted and so planned the facilities for the forty-seventh annual meeting of the West Virginia Academy of Science, that the meetings were presented in an academically stimulating yet relaxed atmosphere;

THEREFORE be it resolved that we the Academy extend our most sincere thanks and gratitude to our hosts.

WHEREAS clean water is a valuable natural resource of our state of West Virginia and also serves as a very attractive recreational value to the citizens of our state and nation;

THEREFORE be it resolved that the Academy express its appreciation to the State Government for passing legislation permitting grants and loans to local governments in order to alleviate the problems of water pollution.

FURTHER, we encourage local, state, and federal governments to work with scientists on all other environmental problems.

WHEREAS the mining of coal in our state has been poorly regulated in the past and has left deep scars in our beautiful state;

Be it resolved that we the members of the Academy express our feeling that the State Government must provide regulation of the surface mining industry by adequate laws and that these regulations must be strictly enforced,

WHEREAS the National Youth Science Camp is actively promoting the ideals of science in the outstanding youth of our country and at the same time showing these youth the merits of West Virginia;

THEREFORE be it resolved that the members express our thanks to the Administration and Staff of the National Youth Science Camp and commend the State Government for adding its support to help educate the future scientists of our nation.

WHEREAS the Buffalo Creek disaster has left a deep impression on both the citizens of West Virginia and the nation.

RESOLVED that we the West Virginia Academy of Science commend 1) the prompt action taken by the State of West Virginia and the various agencies involved in aiding the people of the area, 2) the Executive Order for pumping out other such impoundments in the state and 3) for the legislation passed to study the problem to prevent a recurrence of such a disaster. We further recommend that compensation to people in the area be accelerated.

WHEREAS we as scientists are engaged in living together with our fellow man on this spacecraft called Earth. Be it resolved that we devote ourselves and our scientific knowledge to helping solve the problems that face the world.

WHEREAS the executive committee has spent much of their time and efforts on the affairs of the Academy;

THEREFORE be it resolved that we lay members of the Academy hereby express our gratitude to them for their labors.

WHEREAS the Academy has an abiding interest of long standing in the affairs of our wonderful State of West Virginia;

THEREFORE be it resolved that the Secretary of the West Virginia Academy of Science transmit these resolutions to Dr. Hardway, President of Bluefield State College, to Governor Arch A. Moore, Jr., to the President of the Senate, The Honorable E. Hans McCourt, to the Speaker of the House of Representatives, Louis M. McManus and to the Director of Natural Resources, Ira S. Latimer.

Respectfully submitted.

Anthony Winston
Dana Evans
Gary Larson, Chairman

VIRGIL GREENE LILLY

Dr. Virgil G. Lilly has been a guiding light in the West Virginia Academy of Science for as many years as a majority of you are old.

He has served as President, for years he served as editor-in-chief, he was the thorn that stung until the academy was incorporated, he insisted that a history be written, he forced young people under his guidance to participate and their careers show the effect of such wise counselling.

Dr. Lilly would merit the title of "Mr. Academy" if we but had such an award and the resolutions committee thought we should show our great regard for this man with a standing round of applause.

This tribute was presented by the Resolutions Committee and was overwhelmingly accepted by the Academy.

THE ACADEMY YEAR

Earl L. Core

The Academy Year of 1971-72 has been one of the most crucial in the history of the organization. The West Virginia Academy of Science was founded in 1924 and later incorporated as a "nonstock corporation which is not organized for profit but for the advancement of learning and scientific knowledge and the promotion of scientific work in West Virginia." In general it might be said that success has attended its efforts but there have been problems.

Perhaps the most serious, certainly the most chronic of all the problems through the years has been the matter of publication. The dissemination of knowledge through the medium of the printed page has been a major function of every scientific society and a very expensive one, in terms of financial outlay and personal effort.

This has definitely been true of the West Virginia Academy of Science. From the very beginning the Academy has required outside help in order to carry this burden. The chief publication has been the annual *Proceedings* and, starting with Volume 1, this has been published as a Bulletin of West Virginia University and the University Editor has served as Editor of the *Proceedings.* The first five volumes of the *Proceedings,* covering the first eight annual meetings, were paid for entirely by the University.

The Academy paid $42 towards the printing of Volume 6, issued in March, 1933 and by the time of the publication of Volume 9 was contributing $100 for each issue. In 1938, beginning with Volume 12, an agreement was reached whereby the University would pay two-thirds of the cost of each issue but that its share should not exceed $300 per issue.

By 1942 the Academy's share under this agreement ran to nearly $200 and the $1,000 which had been accumulated in the treasury steadily declined over the next few years. By 1946 the Academy's share, with the steady increase in the cost of printing, was approximately equal to the amount collected in dues and the balance in the treasury became dangerously low. Accordingly, the dues were raised from one to two dollars annually. This increase, together with a post-war increase in membership, enabled the Academy for a short time to operate with a surplus.

For Volume 19 (1948) the Academy contributed $400 to the printing costs and for Volume 20 it contributed $500. The inflationary developments over the next several years resulted in a rapid rise in the cost of printing and it quickly became impossible for the Academy to continue the 1938 agreement. Hence, a new agreement was reached with the University whereby the Academy was to contribute a maximum of only $500 towards the cost of each issue, regardless of the total cost.

Following the increase in dues to three dollars in 1952, the income from that source stabilized at about $750, but with other expenditures, excluding the *Proceedings*, running over $400 per year, the balance in the treasury was steadily depleted. For the next ten years the Academy was able to remain solvent only because in some instances two volumes of the *Proceedings* were combined into one issue and in some years the University paid the entire cost.

In 1961 the University again formally assumed the entire cost of printing the *Proceedings*, and the amounts raised by members' dues (now $5 annually) were used for other activities of the Academy. The cost of printing the 1969 issue (Volume 41) was about $7,000, plus editorial and other costs.

This arrangement, while resulting in financial relief to the academy, has been far from satisfactory. In the first place, the University Editor, forced to get his other bulletins out on scheduled deadlines, tended to get farther and farther behind with the *Proceedings*. The 1969 volume was published late in 1970, while the 1970 volume has only now, in the spring of 1972, gone to press. Papers from the 1971 meeting are held up, awaiting disposal of the previous year's accumulation.

This delay has had a most unfavorable effect upon potential authors of papers and upon the health of the Academy in general. Since the papers published are for the most part those presented in sectional meetings of the annual assemblies of the Academy, interest in the meetings wanes when members realize their papers may not be published for over a year; worse than that is the feeling that no particular assurance can be given *when* they will be published—or, indeed, if they will *ever* be published.

The situation was steadily becoming intolerable and it was a blessing only thinly disguised when the University in 1971 abrogated its 1961 agreement and instructed the Academy to look elsewhere for funding.

The Academy's Executive Board, on October 19, 1971, authorized the President to file with the Claude Worthington Benedum Foundation a proposal for an interim financial grant while searching for other sources of revenue. By the time of the Bluefield meeting it still could not be announced that the proposal had been approved but there were favorable indications and it was hoped that approval would come soon.

This grant from the Benedum Foundation would allow the Academy to get caught up on the publication schedule for the *Proceedings* and provide a comfortable breathing spell in which to work out permanent and more realistic programs for financing the Academy's activities.

Instructions to Authors

1. GENERAL POLICY

The Academy will only consider papers that report the results of original research or observation. The Academy will not publish papers that have been published elsewhere. Each manuscript will be reviewed by the Publications Committee and by referees designated by it. Manuscripts longer than 15 pages of double spaced typewritten copy cannot be accepted. Membership in the Academy is a requirement for publishing in the Proceedings. In the case of joint authorship, at least one author must be a member of the Academy, and the author presenting the paper must be a member of the Academy. No author, or co-author, may submit more than two papers for any volume of the Proceedings.

2. PRELIMINARY ABSTRACT

A preliminary abstract, summarizing the results of the investigation must accompany the application for a place on the program of the annual meeting. This preliminary abstract will not be considered for publication in the Proceedings.

3. ORGANIZATION OF MANUSCRIPTS

Each manuscript shall start with an abstract (not more than 250 words) which should summarize the chief results reported. The following sequence is suggested: Introduction, materials and methods, experimental results, discussion, acknowledgments, and a list of references cited. With the exception of the introduction, each division of the manuscript should be labeled. Sub-headings may be used. In general, the introductory abstract will replace a summary. The above organization may be modified when necessary. Abstracts only will not be published.

4. BY-LINE

The author's name, department, institution, city, and state should follow the title of the paper.

5. FORM

Manuscripts shall be typed double space on white bond paper. A dark undamaged ribbon should be used on typewriters in order to produce clear copy for the editors and the printer. Pages of copy should be numbered consecutively in the top right-hand corner of each page of the manuscript. Two copies, together with a set of original figures or drawings, should be given to the Section Chairman on the day of the Annual Meeting. Each table and figure should be supplied with a legend sufficiently complete to make the table or figure intelligible without reference to the text. Footnotes may be used in connection with tables and figures where they are necessary and will save space. Footnotes should be avoided where possible in the text.

6. ILLUSTRATIONS AND SPECIAL SYMBOLS

Line drawings should be carefully made with India ink on good paper for direct photo reproduction. Each figure should be numbered. Drawing instruments and lettering guides must be used. While drawings may be of any convenient size, they will be reduced to 3 x 4 inches. Letters, symbols, and figures should be not less than one mm high after reduction to printing size. In exceptional instances, a full page drawing (4½ x 6½ inches) may be used. Either original drawings or glossy photographs (mounted on illustration board with rubber cement) may be submitted. Photographic prints should be on glossy paper and have good contrast. Each drawing should be labeled on the back with the author's name and the appropriate legends.

Formulas of chemical compounds and complex mathematical equations must be drawn in India ink for direct photoproduction. Only originals or photographs will be accepted; not Xerox or other type copies.

7. LITERATURE CITED

The following style is required in all manuscripts:

A. References shall be collected at the end of the manuscript as "Literature Cited."

B. Arrange references alphabetically by author.

C. Invert name and initials of first author only.

D. The title of the papers cited and inclusive page numbers shall be given.

E. References in the text may be either by year or by number. Examples: Hall and Campbell (1957); (Hall and Campbell, 1957); or Hall and Campbell (5), or (5).

F. The journal abbreviations sanctioned by *Chemical Abstracts* shall be used by the Biology, Chemistry, Mathematics and Physics, and Geology and Mining Sections (see *Chemical Abstracts* Vol. 50, No. 23, 1956 for list). The Social Sciences Section shall use journal abbreviations sanctioned by *Psychological Abstracts*. Note: *If an author is in doubt concerning the correct abbreviation, the complete name of the journal should be given.* Example of a journal citation:
Hall J. L., and R. Campbell. 1957. Polarization of ethanol in benzene. Proc. W. Va. Acad. Sci. 29:53-57.

G. Book Citations. Use this sequence: Author, date, title, edition if other than first, publisher's name, place of publication, and number of pages. There may be added the chapter or inclusive pages to which direct reference is made. Example of a book citation:
Stacey, M., and S. A. Barker. 1960. Polysaccharides of micro-organisms. Oxford Univ. Press. London. 228 p. See Chap. 3.

8. PROOF

Galley proofs will be sent to authors for correction. Make corrections on the margins of the galley proof. Proof reader's marks may be found in dictionaries, or in the "Style Manual for Biological Journals." Changes in the text after the manuscript is in galley proof are not permitted.

9. REPRINTS

A reprint order will be sent with the galley proofs. This should be returned with the corrected proof.

The Energy Crisis*

Address by the Honorable
Jennings Randolph, United States Senate,
at the Forty-seventh Annual Meeting
of the West Virginia Academy of Science
Bluefield State College
Bluefield, West Virginia
April 7, 1972

I do not overstate the critical nature of this nation's energy future when I say that we face today and will face tomorrow, and many more tomorrows, a possible acute shortage of environmentally desirable fuels to satisfy the needs of America's industries and homes.

On July 16, 1970, I introduced legislation to establish a National Commission on Fuels and Energy. Had this Commission not been opposed by the Administration, but instead established, the energy crisis of which I speak might well have been mitigated. The study it would have carried out, however, is being conducted in-house by the Senate Committee on Interior and Insular Affairs under Senate Resolution 45 which I authored. My words of almost two years ago are valid today:

> The crisis of which I shall speak today is a real and genuine one. It is not synthetic. It is not one that has been created. It has developed with the growth of our complex society. It is a crisis that faces approximately 205 million men, women and children in the United States.

For several decades there have been massive Federal expenditures on nuclear energy research to develop long-term electrical energy supplies. The short-term—the 1970's and 1980's—was neglected. The breeder reactor holds a promise as the key to providing our electrical energy supplies in the 21st century, but, it is at least 15 years from commercial operation and 20 years from any wide-scale application as an electrical energy supply. Yet electricity amounts to less than 10 percent of our national energy demands and these requirements will remain until the next century overwhelmingly dependent on fossil fuels.

During the interim period until the year 2000, however, this country's oil reserves may be sufficiently depleted so that their use may have to be restricted to non-energy purposes. In addition, the use of non-renewable natural gas reserves, if not depleted before the year 2000, most likely will be restricted. The

*Presented as a part of the Geology and Mining Section.

critical period is from 1976 to 1990 when alternative and reliable supplies of fossil fuels, which satisfy environmental control requirements, must be developed.

There are several joint government-industry efforts to develop commercial processes for the conversion of coal into liquid and gaseous fuels. It appears that these efforts are inadequate from two standpoints:

- –first, the technologies most likely will not be available when the gap between supply and demand occurs in the mid or late 1970's; and
- –second, the amounts of synthetic fuels commercially available probably will not be sufficient to satisfy an ever-widening gap between environmentally acceptable energy supplies and demands.

If coal liquification and gasification and other advanced technologies are to contribute significantly to easing the energy crisis, increased Federal funding is needed immediately. Until recently the potential and the need for the development of these technologies has been largely ignored.

These alternatives, which rely on our abundant reserves of coal and oil shale, must be pursued more vigorously. The only alternative is an ever-increasing reliance on imports of residual and crude oil. Besides the obvious short-term implications for national security and balance of payments, there also is the possibility between now and the year 2000 that international supplies of oil and gas cannot meet international demands. The most likely reasons for this situation are insufficient transportation capacity plus environmental and political factors.

Our nation and, indeed, the world are embarked on a gigantic gamble in attempting to maintain reliable and abundant sources of crude oil, natural gas, and coal. At the same time, we hope for some substitute source of immense amounts of energy—the breeder reactor, fuel cells, or solar power—to replace non-renewable fossil fuels when they are depleted. In the meantime, should available energy supplies falter the American people and, perhaps, large portions of the earth may experience catastrophe.

Super-imposed on this picture of our energy future is a real concern for the side effects of satisfying the energy requirements which must accompany the projected growth of our economy. An "environmental ethic" has developed as a response to the realization that we are faced with an environmental and health crisis of our own making.

The time has come for our society to act to assure ample environmentally acceptable energy supplies while averting future energy crises. A comprehensive national energy policy is needed which enables us to restore the balance between man's activities and nature which is essential to quality living. Perhaps the most critical factor in re-establishing this equilibrium is man's determination to create an environment based not on economic considerations alone, but on his total physical, psychological, and spiritual needs, as well.

When the Air Quality Act was enacted in 1967, the Congress considered the potential health and environmental implications of projected energy demands. In 1970, the Congress enunciated a national policy that the protection of the health of the citizens of the United States must be assured regardless of economic cost.

The 1967 Act also included an amendment which I authored, calling for the development and demonstration of new and improved methods for the prevention and control of air pollution resulting from the combustion of fuels.

Information supplied by the Environmental Protection Agency indicates that

40 percent of the United States' population is living under conditions where sulfur oxide levels are unacceptable from the standpoint of health.

The public has expressed a desire to deal directly with the environmental costs of energy production. Therefore, utilities are being asked to take the necessary control measures to protect and enhance the quality of the human environment. The resultant expenditures are estimated to involve large sums of money for air pollution control, for cooling towers to dissipate waste heat, and for new power plants and transmission lines which are compatible with surrounding landscapes. These expenditures will be reflected in electric utility rate structures. The critical factor, however, is the availability of environmentally acceptable fuels, in particular, coal and oil.

Air pollution control regulations already enacted, for new power plants and others pending, will eliminate the use of coals containing more than 7/10's percent sulfur unless adequate means can be provided at the point of use for reducing emissions. The immediate combined effect of implementing these regulations appear to be:

- only about 18 percent (70 million tons) of the steam coal supplied in 1975 from the Appalachian and Interior regions to large Eastern steam generating capacity can be expected to meet environmental standards;
- coal cleaning is possible by mechanical or chemical means. However, it is anticipated that it will be 1985 before this technology could potentially permit all Appalachian and Interior steam coal to meet the New Source Performance Standard.

These sources of supplies, however, constitute more than 90 percent of the current total coal-fired electric utility capacity of the United States. In 1975, they will provide 70 percent of the steam coal used with the development of appropriate technologies.

Several fossil fuel sources are available as alternatives to Eastern coal. These include Western region coal, residual oil, and natural gas. There are also emission control technologies such as fuel cleaning and flue or stack gas cleaning.

We can anticipate, however, that in 1975 only 10 percent, or 40 million tons, of the total demand for new Eastern utility steam coal can be supplemented or met by Western coal sources. Furthermore, by 1975 only about 40 percent of the *total* residual oil supply required by the Eastern United States will meet the *New* Source Performance Standards.

Shortages of natural gas are expected to be on the order of 30 percent by 1975 and as much as 40 percent by 1980.

The critical question is whether the coal industry can survive the period of adjustment imposed by the new controls.

The opinion has been expressed by the Environmental Protection Agency that "it appears that the only satisfactory method for maintaining Eastern seam coal in the energy market is through conversion to synthetic gaseous or liquid fuels."

Elimination of this coal production would represent a loss of $1 billion annually in sales; a loss of a critical energy resource; and a reduction of about 30 percent in the current work force employed in the bituminous coal industry. Some 44,500 mine workers would be affected, including 15,000 in West Virginia alone.

Added to the factors I have mentioned is concern for the environmental impacts of surface mining.

In recent years, more than half of the coal mined in the United States has been produced by surface mining methods. Accompanying this increase in surface mining activity has been a heightened public awareness of the potentially adverse environmental impacts resulting from the extraction of coal by this method. In West Virginia, surface mining is perhaps the most intensely debated public issue. In 1967, the West Virginia State Legislature enacted new controls over surface mining. Since then, additional proposals have been made and there have been recommendations that all surface mining be banned in West Virginia.

On February 24, 1972, I introduced legislation providing a Federal program of strict controls of surface mining operations and requires reclamation of the highest quality. It would provide for neither abolition of surface mining nor its conduct without careful attention to its consequences.

The Air Quality Act of 1967 authorized a major government-industry program for the development and demonstration of methods for the control of air pollution from the combustion of high sulfur fuels. While there has been progress under this program, accomplishments have not met with Congressionally estimated needs. The effort has, as a whole, been totally inadequate, and both government and industry are to blame.

Section 104 of the 1967 Act, which I sponsored, was amended in 1970, at my suggestion, to provide increased emphasis on research and development activities into new and improved methods for the prevention and control of air pollution resulting from the combustion of fuels.

Congress appropriated $45 million for these developments during fiscal year 1970. However, only $36 million were obligated to this effort and expenditures were only $14 million. Obviously, Federal program efforts and funding have been unresponsive to anticipated requirements. This lack of foresight has contributed significantly to the potential coal supply problems.

In 1967 the then National Air Pollution Control Administration projected Federal expenditures for a 5-year period research and development plan for the control of sulfur oxide emissions from stationary sources was $394 million. The Congress authorized $370 million for the 5-year period 1968 and 1972 for the total research and development program. Appropriated funds fell short of these estimates and $133.5 million were appropriated for this period. Although the full funds have been obligated, expenditures have been only $85.6 million, leaving $38.9 million of appropriated funds unspent.

Of these amounts, expenditures for stationary source pollution control research and demonstration were $50.5 million. Yet expenditures for the whole technology development program have been $85.6 million—for sulfur oxide, nitrogen oxides, particulates, special industry problems, and incineration control problems.

Without the development of economically and technically feasible methods to control sulfur oxide and other emissions, the Nation's commitment to effective air pollution control cannot be honored.

Although progress can be made in developing stack gas clean-up methods for sulfur oxides and nitrogen oxides, the long-range program should be aimed at eliminating these pollutants more efficiently.

On the basis of a hearing which I chaired before the Senate Committee on Interior and Insular Affairs on February 8, it is apparent that the emphasis of the Environmental Protection Agency's long-term sulfur oxide control program must continue to be on advanced power cycles for the generation of electricity. Such methods employ coal gasification without going to the lengths necessary to produce coal gas of pipeline quality. Development of this technology is needed to assure the future use of the ample coal reserves of the Midwest and Appalachia. These processes not only offer pollution-free energy, but an efficiency considerably higher than that of conventional power plants, thus decreasing thermal pollution and prolonging the life of our coal reserves.

The Environmental Protection Agency has estimated that the total cost is approximately $75 million for development of a commercial advanced power cycle for the generation of electricity from coal. In a joint government-industry venture, industry contractor teams have indicated they are prepared to fund two-thirds of this cost. Several unsolicited proposals have been received by the Environmental Protection Agency.

Recently, however, the Administration fragmented this program, admittedly, without any assurance that the advanced power cycle will be developed to alleviate the emerging gap between coal demands and environmentally acceptable means of converting coal into electricity.

The Environmental Protection Agency requested $25 million for fiscal year 1972 to implement this program. However, the Department of the Interior, with a recognized expertise on synthetic high BTU coal gas, was alloted $3 million to pursue low BTU gas development. The Environmental Protection Agency is to pursue the development of fuel and stack gas cleaning methods and phase-out its low-BTU coal gas program.

I question the desirability of the transfer of these Environmental Protection Agency's activities on coal gasification to the Department of Interior at this time. This program has been in existence since 1969 and to fracture it now could easily result in a 3-year delay in demonstration of advanced power cycles. There also is no assurance that the advanced power cycle would even be developed under such arrangements.

Next week, the Subcommittee on Air and Water Pollution of the Committee on Public Works will conduct hearings to review the research programs of the Environmental Protection Agency that relate to non-polluting energy production. The issues I have discussed today will be explored in depth.

The resolution of questions surrounding the ability of this Nation to continue producing large quantities of energy is essential if it is to maintain the kind of society and quality of life we now enjoy. But the challenge of today is more than just energy production. The challenge is one of accepting our larger responsibilities to conduct our affairs in a way that is not counter-productive to our ambitions or, in fact, does not imperil environmental quality and life on this planet.

Meeting this challenge is not an easy task. It is, however, one to which I am sure we can rise.

Attractive Potentials for Scientific Academicians*

Address by Dr. Edward F. Turner, Jr.
President of the Virginia Academy of Science,
Chairman, Physics Department,
Washington and Lee University
Lexington, Virginia

President Core, Ladies and Gentlemen:

Before I begin, I think it is only fair to enter a disclaimer. Last fall I received a generous invitation from President Core to all members of the VAS to participate in your annual meeting. It was my pleasant privilege to transmit that kind invitation, issued on your behalf, to the members of the Virginia Academy, calling their attention to the happy prospect of hearing a distinguished speaker of international reputation, whom President Core had arranged for this occasion. I won't attempt to explain to you how it happened that I am now standing at this lectern, because I really don't quite understand it myself, but I want to make it clear to any of my colleagues from Virginia, who may be present, that this was not a devious act of deception; and to assure all present that I am, perhaps, the one with the keenest sense of disappointment and chagrin that the original arrangements made by President Core were not consummated.

Let me also use this occasion to take reciprocal action in the spirit of President Core. The VAS will hold its 50th annual meeting May 4th and 5th in Lexington, Virginia. It would be a distinct pleasure to have you come and participate in our activities. We will do our best to show you that Virginians are as hospitable in the East as they are in the West. I seriously considered retaliatory action, in the form of asking President Core to speak on that occasion, but I dismissed that notion out of fear that my precarious reputation among my own colleagues would suffer by inviting comparison of our respective performances. Perhaps, one day, when memories of my efforts here have dimmed, I will invite Virginians the opportunity to hear President Core. At any rate, I can offer you one inducement—I do *not* plan to speak at our meeting in May. East Virginians know better than to subject themselves to that particular form of entertainment.

As a representative of one State Academy of Science talking to those of you in another, it seemed an appropriate occasion to examine the institutions with which we are associated, and to assess their relationship with, and the impact they make upon the societies in which they operate. The title of my remarks not

*Presented at the banquet, April 7, 1972.

only divulges that general theme; it also discloses the conclusion I will come to at the end. Potentials exist when there are stresses of one kind or another in various force fields. The existence of stresses in our society are, I think, self-evident. I believe they give rise to unusual opportunities for Academies of Science to wield an influence for good in our society and culture, and I will attempt to identify some of the "attractive potentials" for scientific academicians.

In addition to a title, I suppose I betray my Baptist upbringing by having selected something equivalent to a "scripture lesson," so to speak. I have taken my text from several fairly recent articles in the *American Scientist* and the *Science Journal,* the feeling being that here was a source of literature with which we were all familiar and which, therefore, would serve to curtail extensive development on my part.

During the last few years there has been a spate of articles in these journals, and others, centering upon the strained relationship between science and society. For example, there has been a plethora of articles about the current disaffection of our "lay" brethren with science and scientists. In the July 2, 1971 issue of *Science,* Arnold Thackray offers some "Reflections on the Decline of Science and on Some of its Causes." In the 1 October issue of the same year Harvey Brooks asks: "Can Science Survive in the Modern Age." If one includes within the general rubric all the special concerns about declining federal support, unemployment, disruption of scientific meetings, the efforts to revolutionize the college science curriculum, and the debates about technological assessment and control, I suspect that at least half of the issues of the last several year's output of *Science* could be used to provide source material.

As you might surmise, there is a fairly broad spectrum of opinion about the seriousness of the problem—ranging from the notion that it is a passing fad to the feeling that it represents a crisis of monumental proportions. For my own purpose here, I am content to accept James Carroll's estimate of the situation as he analyzed it in the February 19, 1971 issue of *Science:*

> There is considerable speculative and observational testimony, and some empirical evidence that the scope and complexities of science and technology are contributing to the development of social alienation in contemporary society.

I wish to emphasize, however, how mild this assessment is by inviting its comparison with Thomas Blackburn's opening paragraph in his article in June 4, 1971 issue of *Science:*

> We live in a technological culture, and that culture is in trouble. Recent essays that explored the relationship between modern science and the history and psychology of technical men, have generally concluded that the scientist's quantifying, value-free orientation has left him helpless to avoid (and often a willing partner in) the use of science for exploitation and destructive ends.

Blackburn goes on to describe the rapid growth of a counter-technological culture with its epistemology of direct sensuous experience, subjectivity, and respect for intuition, and then asserts categorically:

> I believe that Science as a creative endeavor cannot survive the loss of these people, nor, without them, can science contribute to the staggering social and ecological problems that we face. More fundamentally, much of

the criticism directed at the current scientific model of nature is quite valid. If society is to begin to enjoy the promise of the "scientific revolution" or even to survive in a tolerable form, science must change.

The Nobel-Laureate, Murray Gell-Mann, in the May 1971 issue of *Physics Today* buttresses the general agreement about the declining influence of science with an assessment that is even more severe than Blackburn's:

> Some of our most successful institutions are in trouble, under attack, and even despised, sometimes by intellectuals and frequently by educated young people. . . . In our country, in particular, science is in ill repute, together with such gigantic and impressive feats of engineering as the manned flight to the moon. . . .
>
> And that is not all. We are seeing among educated people a resurgence of superstition, extraordinary interest in astrology, palmistry and Velikovsky; there is a surge of rejection of rationality, going far beyond natural science and engineering. In my opinion, some of the adverse reaction to science and engineering and even rationality is understandable.
>
> There are the unfortunate effects of carelessly deployed or carelessly diffused technology. . . . These effects are interpreted, and quite correctly, as being connected with a kind of narrow rationality, that takes into account in decision-making only things that are very easy to quantify, and sets equal to zero things that are hard to quantify. . . . We see facts and figures marshalled in huge arrays that have failed somehow to include inputs from common sense or from human values. . . . Youngsters tired of the tyranny of badly programmed computers, and of people who act like badly programmed computers, are turning to tarot cards and charlatans.

I find a remarkable convergence of opinion among these and other authors, not only regarding the increasing alienation of science and society, but with respect to a number of other themes, some of which are only hinted at in these quotations but which are more fully developed in the complete articles. I wish to call attention to a few of these as being particularly fertile areas in need of tilling. It seems to me that many of them are saying that science is experiencing, perhaps for the first time, the trauma of a rejected suitor. The brilliant technological achievements of the last six decades are as powerless to hold the jaded affections of milady as last month's sparkling jewel. The disaffection manifests itself in two diametrically opposing forms. On the one hand, we are all familiar with the growing clamor that science now wave its magic wand at the pressing problems of the environment, the population explosion, the threat of nuclear war, the rising crime rate, and the uneven distribution of justice. In one quarter the belief is widespread that if we would just divert our attention from our immediate concerns and start manipulating the social parameters into our equations, that shortly the answers will come, and they impatiently demand that we get on with it. This implicit faith in our prowess is touching, if not disconcerting.

In a different quarter, we have lost their faith entirely. To them, we are out of touch with reality. Science is irrelevant, has lost its social consciousness, and so a pox upon its house. In its most virulent form this has led to the surge of rejection of rationality that troubled Gell-Mann.

Clearly the lines of communication to both of these groups have short-circuited somewhere. All the writers, whether they represent the alienated "counter-culture" or are part of the establishment, agree on this. Finally, there is a consistent consensus that science must assume part of responsibility for this condition, and that a concerted effort is needed to reverse the tide of disaffection.

In view of the conflicting attitudes of the laity, it is not surprising that scientists are ambivalent about their roles in society, both individually and institutionally. An editorial by Warren Weaver entitled "Science in a Troubled Culture: A Prescription" appearing in the most recent AAAS bulletin attests to the institutional schizophrenia. Wherever one looks, the dichotomy is there—the unstinting effort to determine truth, together with the self-imposed restriction to do it with one eye closed; the humility in the face of fact, coupled with the arrogant assertion of a superior technique for dealing with facts; the search for order in nature, commingled with the seemingly irresistible impulse to impose an order upon it.

This description conjures up the caricature of a scientist as a modern day Diogenes who seeks to dispel the darkness by holding up such a smoky lantern that its light rays cannot pierce its own pollutants.

Scientists found it impossible to speak with a common voice on the issues of Space Exploration, the ABM, the Supersonic transport, the use of pesticides, and they may be found on both sides of the questions about the population growth and the ecology. On a more mundane level, scientists seem to be no better able than the poet, the plumber, or the palm-reader at choosing the most efficacious toothpaste, or in deciding on which brand of TV he will watch Walter Cronkite.

I have heard the scientist described as a simple-minded fellow who can deal with only one parameter at a time. It is true that such issues as I have just enumerated are much more complex than the kinds of problems scientists like to tackle. But therein lies one of our most attractive opportunities—that of demonstrating that the scientific approach to problems have a more general applicability than has hitherto been demonstrated.

Our ambivalence is not confined to technological, social or political matters. It touches our philosophy as well. Gerald Holton commented on the increasingly heuristic approach of contemporary science in the first issue of the new *Journal of College Science Teaching.* He writes about the current scientific debate concerning the existence of quarks.

> There are many doubts whether or not the hypothetical quark, for example, does exist. But by assuming it, one can explain well some puzzling results; and so Weisskopf, skeptical but in a relaxed mood, is quoted as having told the story of Niels Bohr who visited a friend's house, noticed a horseshoe nailed over the door, and asked what it meant. His friend told him, "That brings luck." Bohr was astonished and said, "Do you really believe in this?" To which his friend replied, "Oh I don't really believe in it. But I am told it works even if you don't believe in it.

"Perhaps a hair divides the false from true," wrote the Persian poet, Khayam almost 1000 years ago. Could it be that the distinction between physics and numerology is no clearer—or that the only difference between astronomy and astrology is in the spelling? A lot of people think so, and scientists seem too little concerned about helping to eliminate the confusion in the public mind. The workings of a television set is just as much of a mystery, just as "magical" as the crystal ball and, I might add parenthetically, the two sometimes seem to be equally productive.

Science, true science, is being *under*utilized. I reject Professor Roszak's assertion in his book "The Making of a Counter Culture":

> that the alleged objective consciousness of the scientist is not only a myth, but a vicious one, behind which men may perpetrate monstrous crimes against nature without acknowledging personal involvement and, therefore, guilt.

For one thing, as Blackburn pointed out, "the very amorality of science makes it not wrong, but incomplete." "As Sir Eric Ashby has observed," wrote Caryl Haskins in the January-February issue of the *American Scientist,*

> the natural sciences, inescapably and at a very deep level, share with the humanities the vital task of mastering the dialectic between orthodoxy and dissent in the evaluation of our civilization.

"If Not Reason, What?" asks Kingman Brewster in the succeeding issue. He goes on to say:

> Cynical disparagement of objectivity as a "myth" seems to be both naive and irresponsible. Its irresponsibility lies in the suggestion that since the ideal is unattainable it should not be held up as a standard to both practitioners and critics.

On this point, we must not lose our nerve. It is one of the privileges of an association of practicing scientists to hold up the mace of objectivity and rationality. We must recognize, however, that these are not the only tools on our workbench. Imagination, intuition and inspiration have all played important roles in the great discoveries of the past, both theoretical and experimental. In our separate pedagogies we have too often failed to convey this secret. To my way of thinking, it is one of the more attractive opportunities for scientists, individually and collectively, to find the way to bring about a rapprochement with our alienated cousins who are turned off by the depersonalization that they associate with science.

To whom else should we look for a clear and unambigous assertion that scientists are concerned about the human condition and the perplexing problems of our time? The pattern of specialization is so permanently dyed into the fabric of our culture that I seriously question if it can be bleached out. But the time has come to widen those narrow corridors of specialization that have channeled us into such divergent paths that physicists deign speak only to chemists, chemists to biologists, and mathematicians only with God. At the risk of being impudent, I wonder if both of our respective academies do not encourage this cleavage in the format of meetings such as this where, having assembled biologists, chemists, geologists, physicists, mathematicians, engineers and a similar variety of the social scientists, we send them off, each to their respective chambers to hear each other, each in a jargon peculiarly his own, describe the addition he has made to the scientific edifice, without any reference to the overall architectural design.

True, to pause from the daily rush to publish can be misinterpreted as a waning of productivity. "There are those," as Blackburn put it,

> who are chafing to get on with the extension of mankinds' intellectual hegemony to the understanding and complete control of our natural environment, our societies, our heredity, and our fellow men.

Clearly, such people are adherents of the "Golden Age" view of man as portrayed by Bentley Glass in the January 8 issue of *Science.*

The assumption is that nature is uniform, and a finite number of natural laws are generally applicable. The properties of matter are totally explicable in terms of 3 or 30 or 100 "fundamental" particles; or the genetic code, once decoded, will lead ultimately to full understanding of the manifold, but finite, variety of life forms. It seems to me that our experience teaches us otherwise. There is, I am told, an infinity of differential equations that defy analysis. As a trivial case, we can never complete the list of irrational numbers, or find the last decimal of π. I believe every researcher finds that his research generates more questions than were answered. Indeed, when you think about it, how can we ever know that our knowledge is complete? One of Mozart's contemporaries is supposed to have declared that all the musical themes had been exhausted, and I have been told by one of his students that Professor Robert W. Wood, author of the classic text, "Physical Optics," once began his lecture with words to this effect: "Gentlemen, today we begin our study of light. Now light is the one subject about which we know all there is to know." That was at the turn of the century, before the discovery of the photoelectric effect, before the birth of quantum electrodynamics, and long before the development of the laser. It must have been reflections of this sort that prompted Vannevar Bush to write his book "Endless Horizons," setting forth the alternative view of the future of scientific knowledge as one of infinite extension, and limitless expansion.

As a practical matter, it is really of little consequence which view one accepts. The disciples of either philosophy will agree, I think, that at present we have found only a few of the bright pebbles on the shores of a vast ocean. And yet there is a sense of urgency to make the unknown knowable that is truly puzzling.

Bentley Glass refers to this phenomenon obliquely in the article already referred to. He states:

> (Scientists) are concerned about the quantitative measurement of all sorts of phenomenon except that of the growth, stasis, or decline of their own collective activity, finding "it sufficient to harbor a personal liking for scientific exploration and discovery, without much worry about the magnitude of the edifice they are constructing, the depth of the mine they are excavating, or the extent of the continent they are invading."

This of course is the basic tenet of Tofler's provocative book "Future Shock." It suggests a view of man so inebriated with the heady wine of discovery that, lemming-like, he may toss himself into the ocean of the unknown, flailing the waves in a desperate attempt to reach an uncharted and distant shore.

Even if we have illuminated only a few crevices of the vast abyss of knowledge, the present volume of information is so great that no one organization has succeeded in organizing it for effective use. The rate of increase is already so rapid that no one individual can hope to read even the small portion of new information pertinent to his field. In the course of some research on the implications of technology for libraries a few years ago, I came across a statement by J. L. Licklider, who dramatizes the information deluge this way:

> The body of recorded scientific and technical information now has a volume of about 10 trillion alphanumeric characters (i.e., letters, numerals, and punctuation marks) and is increasing along (what for lack of precise

data is usually assumed to be) an exponential curve characterized by a doubling time in the range of ten to fifteen years.

To simplify back-of-the-envelope calculations, let us take the figures, 10 trillion characters and (say) twelve years, at face value; let us assume that one thousandth of all science and technology constitutes a field of specialization; and let us consider the plight of a scientist who reads 3,000 characters a minute, which is a rate more appropriate for novels than for journal articles. Suppose that he gathers together the literature of his field of specialization (10 billion characters) and begins now to read it. He reads thirteen hours a day, 365 days a year. At the end of the twelve years, he sets down the last volume with a great sigh of relief—only to discover that in the interim another 10 billion characters were published in his field. He is deterred from undertaking twelve more years of reading by the realization that not only the volume but the rate of publication has doubled.

Sixty years ago, according to our simplifying assumptions, the 3,000-character-per-minute reader needed only twenty-five minutes a day to keep up with everything in his field. Eleven years hence, he will have to read continuously, every hour of every day.

There is, I suggest enough known and unmanageable information at our disposal to provide us some measure of repite from the frantic pace of accretion. Furthermore, as one of my professors was found of saying: "A little contemplation can save a lot of computation." I believe our effectiveness will be immeasurably increased by a period of reflection in which we sort out the pebbles we have already collected. Again, I ask, who should assume the task of creating a new taxonomy of scientific knowledge if it is not just such institutions as this where such knowledge as we have is artificially distributed among a dozen archaic fields of specialization?

As you can see, I have not dwelt at all upon the specific challenges of the day—the ecological crisis, the population explosion, and the divers other issues of the moment. These problems must be solved of course if there is to be any science or any humanity in the future. They represent our immediate concerns, but I suggest that we have been presented with a series of challenges of another sort that are too important to ignore, and too pressing to postpone. Furthermore, they are precisely of a kind that falls squarely within the province of science academies, such as those with which we are associated. For what better purpose are we constituted than to provide a convenient mechanism for serving the common good of all in whatever ways we can bring our peculiar expertise to bear. The constitution of the Virginia Academy of Science declares as an objective of the organization, "the fostering of interest in science, the provision of a forum for presentation of scientific ideas, and facilitites for their publication and generally, in doing these things, to benefit not only its own members, but to promote the civic, agricultural, academic, industrial and commercial welfare of the people of Virginia." And I note on the front cover of your program that the West Virginia Academy is incorporated as a "nonstock corporation which is not organized for profit but for the advancement of learning and scientific knowledge and the promotion of scientific work in West Virginia." The ideal of service to our respective regions is implicit in the avowed purposes of both these institutions.

If I understand aright the implications of the assorted articles that I have used as my "text" it seems to me there are a number of attractive potentials for scientists and their academies. To recapitulate these, in closing, there is first of all the opportunity to reopen and extend the lives of communication, and to use these lines to bring about a credibility in the meaning and importance of the scientific enterprise. To the scientist the phrase "life of the mind" is as meaningful and appropriate as the "life of the laboratory."

Second, there is the challenge to maintain excellence and to be one of its producers. In meeting this challenge we must not relax our objectivity and rationality—they are proven assets. It may be, as some wag remarked that "if you can keep your head when everyone else is losing theirs, you just don't understand the situation," but it is a basic tenet of science, that understanding is not improved by irrationality. Rational thought is the moral responsibility of those who are capable of it.

Third, we are being invited to give more attention to the social *significance* of our discoveries. That is not the same thing as *relevance,* and I think the distinction is important—it is this distinction in fact that President Brewster makes with particular prescience, emphasizing that "the crisis of purpose transcends topical problems."

Fourth, we are being encouraged to expand our rationality, to recognize the insufficiency of a rationality restricted to quantifiable phenomena. The matrices are not all there are, and it ill behooves us to brand as heretics those who recognize this important truth. Even if it is heretical, science is particularly well placed, it seems to me to accept Thomas Jefferson's wise admonition to "tolerate any error so long as reason is left free to combat it."

Finally, the enclaves of science, the academies, are being offered an unusual opportunity to give direction and impetus to these assorted activities. With tongue in cheek, Thackray encapsulated the past history of science associations into three prominent periods—The first, the age of the Elegant Amateur was superseded by the era of the Poor Professional, to give way at present to what he terms the epoch of "Dominant Dukes." It is for us to determine that the epithet that describes the period into which we now are entering has a kindlier ring.

Biology Section

Primary Productivity in Relation to Chemical Parameters in Cheat Lake, West Virginia

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Abstract

Primary productivity and chemical parameters were investigated in Cheat Lake and two of its backwater areas during the summer and autumn of 1971. The main lake showed high acidity and conductivity, and low pH, alkalinity, and inorganic carbon, indicating the presence of acid mine wastes. The backwater areas have a pH near neutrality with low acidity and higher levels of bicarbonate alkalinity and inorganic carbon.

Rates of carbon assimilation were found not to be significantly different between the Rubles backwater and the lake proper, even though the two sites are vastly different with regard to water chemistry. Regression analyses showed productivity at the two sites to be under the influence of different factors. At the dam station the acid content of the water exerts considerable influence on the rate of carbon assimilation. In contrast, inorganic carbon was the most important variable controlling productivity in the Rubles backwater. Subsequent investigation is needed before precise relationships can be defined.

Introduction

Cheat Lake was created in 1926 by impoundment of the Cheat River by the West Penn Power Co. The lake extends behind the dam some 13 miles to the south. It is ¼ to ½ mile wide, and has a surface area of 1730 acres (Basnett, 1954). Water is drawn off for the generation of electric power during the weekday morning and afternoon peak load periods, with large scale drawdowns occurring during the spring and fall in anticipation of flooding.

As a result of water seepage through the numerous coal mines in the Cheat watershed the lake receives a heavy load of acid from several tributaries (Stilson, 1969). This influx of acid has been shown to have a detrimental effect on the water quality of the lake (Basnett, 1954; Gorman, 1957).

There are three backwater areas associated with Cheat Lake. Since they have no coal mines in their watersheds and are partially isolated from the main basin, it was suspected that they are relatively free of mineral acid. Preliminary investigation confirmed this.

In a biological survey of Cheat Lake, Rohr (1954) found phytoplankton to be quite scarce. Nannoplanktonic forms were most abundant. Although examination of the plankton assemblage on an enumerative basis can yield valuable information, often a more comprehensive picture can be obtained from estimates of phytoplankton primary productivity. Primary productivity estimates have been proven to be useful in studies of both marine and fresh waters, especially with regard to establishing the trophic state of a body of water.

Several investigations have been made on primary production in acid waters. Campbell, et al. (1965) and Lind and Campbell (1970) found little difference between the gross photosynthetic rates of acid and neutral strip-mine lakes. The high rate in acid lakes was attributed to the development of large populations of acid tolerant algae.

Johnson, et al. (1970) investigated primary productivity in a Canadian Shield lake contaminated with sulfuric acid wastes from the milling of uranium ore. Both primary productivity and species diversity were very low. They concluded that high acidity limited the availability of inorganic carbon, resulting in a reduction of both species diversity and primary productivity of phytoplankton.

This investigation was undertaken to determine to what extent high acidity (and its associated factors) influences phytoplankton primary productivity. Cheat Lake serves as an ideal study area for two reasons. The lake is contaminated with little else besides acid mine wastes, and therefore it is possible to avoid the complex situation resulting from the interaction of several pollutants. Secondly, the backwaters, by virtue of the absence of acid pollution serve as a natural control area. This then makes it possible to obtain productivity estimates in the absence of acid.

Currently we know little of acid waters beyond their physical and chemical characteristics. Before we can reach an understanding of energy flow we need to know more about the effects of acid on the biota of these waters. Since conversion of solar energy to potential chemical energy by phytoplankton is the initial link in energy dynamics of a lake it is a logical starting point.

Methods

The investigation was initiated in June, 1971 and continued to October, 1971. One sampling site was selected in the lake proper near the dam (referred to hereafter as the dam station), and one station in each of two associated backwater areas, Rubles Run and Morgan Run. Most of the work done in these backwaters was concentrated in Rubles Run.

Water temperature was recorded from the surface to bottom, at one meter intervals, with a thermistor thermometer. Light transmission (as percent of surface radiation) was measured from 0.1 m to the depth of 1% transmission using an underwater photometer with deck cell. Incident solar radiation was monitored with an Eppley pyranometer located at Cooper's Rock State Forest, some five miles from the study area. Since the instrument was seldom in operation on the same days that sampling was undertaken, the energy flux values reported are averages of values from several days in 1970 and 1971 having equivalent cloud cover.

Water samples for chemical analysis were taken at selected depths with a 2 liter PVC Kemmerer sampler and transferred to polyethylene plastic bottles. Analyses were performed in the laboratory with a minimum of delay. Methods given for the determination of chemical parameters are those found in Golterman (1969).

Hydrogen-ion concentration was measured with a Sargent-Welch pH meter. Specific conductance was determined with a conductivity meter, and reported as specific conductance at 25° C. Alkalinity and total inorganic carbon concentrations were determined by titration with 0.01N CO_2-free sulfuric acid. Total hot acidity was determined by titration with 0.02N sodium hydroxide.

Samples for measurement of primary productivity were taken with an opaque PVC Kemmerer sampler at the approximate depths of 50, 25, 10, 5, and 1% light transmission. Water was discharged into 125 ml light or darkened bottles with ground glass stoppers, taking care to avoid contact with bright sunlight. One ml of $NaHC^{14}O_3$ having an activity of 1 uCi/ml was added to each bottle with a tuberculin syringe from sealed glass ampules.

The bottles were then lowered on a suspension rig (Schindler, personal communication) to their respective depths. One light bottle was placed at each depth, and one dark bottle was included with each vertical series. The samples were incubated for a period of 3, 4, or 6 hours, beginning at 10 or 11 AM. Following incubation the bottles were raised and put in a light-tight box for transport to the lab. Immediately upon return to the laboratory the samples were filtered. After thorough shaking a 50 or 100 ml aliquot from each bottle was filtered through a 0.45 u membrane filter under a vacuum of no more than 300 mm Hg. The filters were then inverted over fuming hydrochloric acid for 3 minutes to remove any traces of carbonate containing C^{14} (Wetzel, 1966). Radioactive carbon on the filters was assayed on a Beckman liquid scintillation counter. The filters were dissolved in 15 ml dioxane base cocktail containing naphthalene, PPO, and POPOP (Schindler, 1966). Each vial was counted for one minute or longer, and the counts obtained were corrected to 100% efficiency. Background counts were then subtracted. Carbon assimilation rates were calculated after Vollenweider (1969).

Daily rates were derived by multiplying by the ratio I_o/I_e; where I_o is the full day solar radiation, and I_e is the solar energy received during the incubation period (Schindler and Nighswander, 1970).

Results

Cheat Lake and its backwaters showed a similar pattern of thermal stratification. The metalimnion attained a maximum depth of only a few meters and showed a progressive downward movement during the study period. Disruption of stratification occurred in the Rubles backwater in early August, and in the main lake basin in early September. Representative temperature profiles for the dam station, Rubles backwater, and Morgan backwater are shown in Table 1.

The dam station shows considerably greater transparency than do the backwaters. The mean 1 percent level was 13m at the dam and 7m in the backwaters. Thus the lake has a fairly deep euphotic zone, while that of the backwaters is shallower.

The most striking differences between the lake and its backwaters were found in the chemical parameters associated with acid mine drainage (Table 2). The presence of mine acid in the main lake basin is reflected in the high acidity and conductivity, and low pH values recorded at the dam station. The linear regression equations given below show the influence of acid content on the pH and specific conductance at the dam station.

$pH = 4.39 - 0.35\ ACID(meq/l);\ R^2 = 0.77,\ p<0.0001$

$SP\ COND(umhos/cm) = 52.4 + 381\ ACID(meq/l);\ R^2 = 0.92,\ p<0.0001$

As is the case with acid waters alkalinity was extremely low. The presence of bicarbonate alkalinity was detected infrequently, indicating that inorganic carbon in the water is in the form of free CO_2.

These chemical conditions correspond to those of strip-mine lakes in the early recovery stage as proposed by Campbell, et al. (1965).

Table 1. Representative Temperature Profiles at the Dam Station of Cheat Lake, Rubles Backwater, and Morgan Backwater.

Depth (meters)	*Temperature (°C)* Dam. Sta. 8.VII.71	Rubles 8.VII.71	Morgan 26.VI.71
surface	27.6	26.8	26.1
1	27.4	26.8	26.1
2	27.1	26.8	26.1
3	27.1	26.8	26.0
4	27.0	26.8	25.8
5	27.0	26.8	24.6
6	26.8	26.7	22.0
7	26.4	25.8	20.8
8	25.5	24.6	20.2
9	25.2	23.5	19.7
10	23.9	22.4	19.5
11	22.8	21.3	19.2
12	21.8	20.8	19.0
13	21.0	20.4	18.9
14	20.4	19.8	
15	19.4	19.5	
16	18.6	19.4	
17	18.0	19.1	
18	17.1	18.5	
19	16.2	18.1	
20	15.7		
21	14.0		
22	13.5		

In contrast, the Rubles backwater has a pH near 7, very little acidity, and at times large quantities of bicarbonate alkalinity with corresponding levels of inorganic carbon. These conditions are not surprising when one considers that this water is partially isolated from the main lake, and that Rubles Run has a small limestone quarry near its headwaters. In the Morgan backwater pH, alkalinity, and inorganic carbon levels were slightly lower, and acidity levels slightly higher than in Rubles. Conditions did not however approach those found in the lake proper.

Estimates of primary productivity were found not to be significantly different between the lake station and the Rubles backwater, even though these two areas are vastly different with respect to water chemistry. The mean rates of carbon uptake over the study period were 55.2 and 67.7 mg C/m^3 day^{-1} for the lake and Rubles Run, respectively. Even more alike are the mean rates of maximum productivity, being 94 mg C/m^3 day^{-1} at the dam station and 96 mg C/m^3 day^{-1} in the Rubles backwater. Estimates from several dates are not included here due to the anomalous dark bottle uptake recorded. The mean and range of assimilation rates on the dates included are shown in Table 3.

In the lake the rate of carbon assimilation increased steadily to a maximum of 180.8 mg C/m^3 day^{-1} on August 7. Following this there was a sharp decline in September-October. The Rubles backwater showed a similar trend, productivity

Table 2. Summary of Chemical Parameters at the Dam Station of Cheat Lake, Rubles Backwater, and Morgan Backwater.

	LAKE		*RUBLES*		*MORGAN*	
	Mean	*Range*	*Mean*	*Range*	*Mean*	*Range*
pH	4.14	2.3-5.55	6.42	4.2-7.7	6.08	5.15-6.55
Acidity (meq/l)	0.79	0.15-7.6	0.03	0-0.14	0.07	0.04-0.13
Sp Cond (umhos/cm @25°C)	355	76-2750	146	68-200	136	104-314
Alkalinity (meq/l)	0.002	0-0.04	1.26	0-8.69	0.19	0.03-0.47
Total inorganic Carbon (mg/l)	3.30	1.56-9.28	5.59	1.49-10.4	4.36	1.32-7.94

Table 3. Mean and Range of Carbon Assimilation (mg C/m^3 day^{-1}) at the Dam Station of Cheat Lake and the Rubles Backwater.

LAKE			*RUBLES*		
Date	*Mean*	*Range*	*Date*	*Mean*	*Range*
26.VI.71	58.7	20.0-109.4	19.VI.71	70.0	37.7-102.9
22.VII.71	73.1	30.2-123.1	24.VI.71	56.2	23.2-101.9
7.VIII.71	91.0	0-180.8	8.VII.71	14.8	0-24.3
5.IX.71	23.6	13.4-32.4	7.VIII.71	118.4	60.0-188.8
3.X.71	12.2	7.0-16.2	3.X.71	28.3	0-42.5

reaching a maximum of 188.8 mg C/m^3 day^{-1} on August 7 and declining in the autumn. In exception to this are the low productivity values recorded in Rubles Run on July 8. It is interesting to note that the lowest pH values recorded in the Rubles backwater also occurred on this date; the mean pH in the euphotic zone on that date was 4.77. This suggests that it is possible for considerable quantities of water to enter the backwater from the main lake basin. It is not known however, how, or with what frequency this may occur.

The relation of primary productivity to physical and chemical factors was analyzed using multiple linear regression analysis (Table 4). At the dam station temperature was the most important variable in the regression equation. The negative slope encountered in the relation of carbon assimilation with energy flux is somewhat unusual. During the time of the year that this investigation took place light intensity is high, and inhibition of photosynthesis near the surface is common in temperate lakes. There is reasonable evidence that this phenomenon occurs in Cheat Lake, since the lake is particularly transparent and maximum assimilation rates were usually encountered several meters below the surface. This frequent occurrence of surface inhibition could account for the negative relationship between energy flux and productivity shown in the regression equation.

An anomalous situation is presented by pH and acidity in this equation, both having a negative slope. Covariation between the two variables may obscure the possible relation between one of these and carbon assimilation. In any event, both pH and acidity do contribute significantly to the regression, indicating that the acid content of the water does exert considerable influence on phytoplankton productivity. Because of the complexity of the water chemistry and the interactions taking place it is difficult to define a precise relationship with the data available.

Results of the regression analysis on carbon assimilation in the Rubles backwater show inorganic carbon to be the most important variable influencing productivity (Table 4). Concentrations of inorganic carbon in this backwater were generally low to moderate, although higher levels were encountered. Carbon dioxide is a vital requirement for photosynthesis to take place, and its rate of uptake is influenced by its availability, especially at low concentrations.

In this analysis the log of energy flux proved to be more significant than the untransformed variable. Surface inhibition of photosynthesis was much less common here, and as a result the relation of primary productivity to energy flux has a positive slope.

Areal production estimates were higher at the dam station in comparison to the Rubles backwater. Mean production in the lake proper on an areal basis was 695 mg C/m^2 day^{-1}, reaching a maximum of 1514 mg C/m^2 day^{-1}. In the Rubles backwater the mean was 437 mg C/m^2 day^{-1} with a maximum of 747 mg C/m^2 day^{-1}. The greater total production at the dam station is a consequence of the deeper euphotic zone found there.

Discussion

Carbon assimilation rates in Cheat Lake are considerably higher than those reported by Johnson, et al. (1970) for two acid contaminated lakes of the Canadian Shield. They found a maximum rate of less than 24 mg C/m^2 day^{-1} (12 hour day), as opposed to a maximum rate of 36 mg C/m^2 day^{-1} in a neighboring uncontaminated lake. Most of the lakes in this region are extremely oligotrophic even in the absence of any pollution, as indicated by the low

Table 4. Results of Multiple Linear Regression Analyses Relating Phytoplankton Productivity to Environmental Factors.

Dam Station

PP(mg C/m^3 day^{-1}) = 43.78 + 15.4 TEMP(°C)*** - 450 ENERGY(ly/min)** - 35.3 ACID(meq/l)** - 66.9 pH*

$R^2 = 0.46$ ($F = 3.92$; df = 4,22; $p < 0.02$)

*** $p < 0.002$

** $p < 0.02$

* $p < 0.05$

Rubles backwater

PP(mg C/m^3 day^{-1}) = 29.76 + 16.1 INORG C(mg/l)** + 13.5 ln ENERGY(ly/min)*

$R^2 = 0.59$ ($F = 9.51$; df = 2,15; $p < 0.005$)

** $p < 0.001$

* $p < 0.08$

production recorded in the uncontaminated lake. As is the case with Cheat Lake primary productivity was slightly lower in the acid lake.

Lind and Campbell (1970) reported the periodic development of large populations of certain algal species in an acid strip-mine lake, and a corresponding maximum gross photosynthetic rate of 7.9 g O_2/m^2 day^{-1}, or about 2440 mg C/m^2 day^{-1}. Any comparison of the data is of limited value since carbon assimilation as measured by the C^{14} method is generally agreed to be somewhere between gross and net production.

Bible (personal communication) found net phytoplankton populations to be very low during the same time period that this study was undertaken. It may be then that nannoplankton are responsible for the greater part of primary production in Cheat Lake. This is the case in most lakes. We cannot however rule out the possibility of chemoautotrophic bacteria, such as *Thiobacillus ferrooxidans*, contributing significantly to carbon assimilation at the dam station. These bacteria are common in acid mine waters and derive energy for the assimilation of carbon from the oxidation of ferrous iron and other ions (McGoran, et al., 1969; Nielsen and Beck, 1972). We have no information regarding the abundance of these bacteria in Cheat Lake at the present time, although they undoubtedly exist there since they are always abundant in association with acid mine drainage.

Results of the regression analyses show inorganic carbon to be an important nutrient in controlling productivity in the Rubles backwater. This is quite reasonable in view of the levels of carbon found there. Sakamoto (1971) reported that in the presence of adequate levels of other nutrients, inorganic carbon concentrations of 5-7 mg/l were limiting to phytoplankton carbon assimilation in several lakes of the Experimental Lakes Area of the Fisheries Research Board of Canada. Concentrations of inorganic carbon in the Rubles backwater generally fell within this range.

As indicated by the regression equation for productivity at the dam station we find the presence of mineral acid exerts considerable influence on carbon assimilation. Although the rates of carbon assimilation at the dam station and the Rubles backwater are not significantly different, they are influenced by different factors.

Considerable study is necessary before we fully understand the relations of acidity to primary productivity in Cheat Lake. Estimates of carbon assimilation rates over a period of a full year would yield information concerning seasonal trends in phytoplankton production. Determination of the distribution and abundance of chemoautotrophic bacteria in the lake is needed before we can assess the fraction of carbon assimilation due to these organisms. Investigation of carbon assimilation in relation to phytoplankton biomass would give an insight into production efficiencies in the lake, and would aid in explaining variation in productivity rates.

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Two Systemic Fungicidal Benzimidazoles as Control Agents for Dutch Elm Disease

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Abstract

Dutch elm disease (DED) destroys 400,000 trees annually, a loss of $100 million in the United States. The recent federal ban from public use of the vector insecticide, DDT (dichlorodiphenyltrichloroethane), the most effective control agent for DED, has stimulated

the search for alternate methods of disease control. One of the most promising of them is the systemic fungicidal approach reported herein.

Two fungicidal benzimidazole derivatives, benomyl [methyl-l-(butylcarbamoyl)-2-benzimidazolecarbamate] and thiabendazole [2-(4-thiazolyl)-benzimidazole], were assayed for prophylactic suppression of disease symptoms. Suspensions containing 124.8 gm of each compound were forcibly injected into the rhizosphere of outplanted American elms (*Ulmus americana*), using a completely randomized experimental design. The causal fungus, *Ceratocystis ulmi*, was later artificially introduced by inoculating all trees with a spore suspension (approximately 4.8 x 10^6 spores/tree). Observations of disease control, survival of the fungus, effects on tree growth and translocation of the fungicides were made after inoculation.

Average visually-estimated foliar symptoms in benomyl- and thiabendazole-treated and control (untreated, fungus-inoculated) trees 444 days after inoculation were 1.0, 52.0, and 68.0%, respectively. The survival of *C. ulmi* was greatly reduced in benomyl-treated trees which exhibited good growth as determined by fresh weight determinations. Bioassays of residual fungitoxicants in tree tissues by the standard solvent extraction-paper disc method revealed the presence of both compounds or derivatives thereof in leaves, bark and wood; this indicated systemicity of the fungitoxicants. Extracts from older leaves were more active than those from younger ones. Fungicidal residues in treated soil were present 17 months after application.

These results illustrate a method by which DED can be controlled prophylactically (preventively), especially with benomyl. The implementation of its use on landscape specimens awaits further investigation and evaluation.

Endothia gyrosa, a Fungus Associated With a Destructive Canker of Pin Oak

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Abstract

Fruiting bodies of *Endothia gyrosa* were observed on cankers that have been destroying pin oaks (*Quercus palustris*) in tidewater Virginia. Cankers were discrete, sunken and elongate, and the lesions commonly bordered by calloused folds. The fungus produced numerous bright orange to dark brown pulvinate stromata, and these contained labyrinthiform pycnidial chambers that opened by irregular pores at the stromal surface. The chambers were lined by cylindric, slightly tapered conidiophores that produced masses of bacilloid, hyaline conidia (3.0-5.0 x 1.5-2.0 μm) commonly exuded in yellowish droplets. The morphological features and cultural characteristics of the imperfect stage were identical to those of *Endothiella*.

Regular observations of developing stromata were made periodically from September, 1970 until early summer, 1971. Only the *Endothiella* stage was seen at first, but in June, 1971, fertile, flask-shaped perithecia were observed below the pycnidial cavities. Access to the stromal surface was through long, slender perithecial necks that protruded slightly at maturity. Immature perithecia had translucent walls that darkened with age. Asci contained

eight ascospores that were thin-walled, aseptate, cylindric, and measured 7.0-9.0 x 2.0 μm, within the range of that reported for *Endothia gyrosa* (7.0-11.0 x 2.0-3.0 μm). Several stromata contained both perithecia as well as pycnidia. Our collections were similar morphologically to United States Department of Agriculture herbarium specimens and were similar in culture to authentic reference isolates of *Endothia gyrosa.* A specific diagnostic feature of this species that we found was the emission of a guaiacol-like odor not detected in cultures of *Endothia parasitica, Endothia havanensis, Endothia viridostroma* and *Endothia fluens.*

Although *Endothia gyrosa* is related to and visually resembles *Endothia parasitica,* the causal organism of the devastating chestnut blight, it has never been reported to be an aggressive pathogen. Furthermore, the presence of this destructive disease of pin oak to our knowledge is limited to the tidewater region of Virginia, and it poses a threat to the successful culture of this favorite landscape species. Studies have been initiated to evaluate the pathogenic potential of this organism.

A Comparative Study of Plankton Respiration in an Acid Polluted Lake and its Acid Free Embayments

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Abstract

Plankton community respiration rates were measured in both the acid polluted impoundment and acid free backwaters of Cheat Lake, West Virginia. Six hour dark bottle respiration experiments were employed and the following mean planktonic community respiration rates were determined as mg O_2/l/day: 0.90 near the dam (pH 4.1), 1.47 in Ruble's Run (pH 6.2), and 2.42 in Morgan's Run (pH 6.2). All rates were significantly different at the 0.01 confidence level.

The chemical parameters tested, specific conductivity, pH, total iron, and hot acidity were all significantly different ($p < 0.01$) when comparing both backwaters to the Dam Station. All parameters, except specific conductivity, were found not significantly different ($p > 0.05$) when comparing the two backwaters. At the Dam Station it appears that acid pollution limits plankton abundance, thus producing lower community respiration rates. However, no acid pollution is evident in either backwater. Therefore, the primary difference in plankton community respiration between Ruble's Run and Morgan's Run is probably due to increased planktonic abundance in the Morgan's Run backwater. This planktonic abundance difference may be the result of a nutrient limiting factor, as suggested by the specific conductivity differences between these two backwaters.

Introduction

Extensive studies have been performed analyzing the biological impact of acid mine drainage upon aquatic communities. Decreased species diversity accom-

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panying increased acidity was reported by several investigators (Lackey, 1939; Johnson et al.; and Lind and Campbell, 1970). They also report that while species diversity decreased, the populations of surviving species in acid polluted waters are often quite high (Joseph, 1953; and Robach and Richardson, 1969). This implies that surviving organisms have the capabilities to adapt to physiological stresses encountered within this acid environment.

This paper describes a 30 day study of community respiration in Cheat Lake, West Virginia. The acid polluted lake proper (pH 4.12), and two acid free backwaters (pH 6.24 and pH 6.21) which flow into the lake, were investigated.

Cheat Lake is thirteen miles long and varies in width from one quarter to one half mile. Early physical-chemical surveys indicated Cheat Lake pH values varied from 5.8 to 7.5 (Carpenter and Herndon, 1929). Gorman (1957) reported a pH range of 3.2 to 7.6 with total iron levels of 0.02-16 mg/l. A 1969 survey by Stilson produced pH values of 3.1 to 5.5 with accompanying total iron of 0.09 to 59.6 mg/l. However, Stilson also found that streams entering Cheat Lake from the east are relatively free from mineral acidity, as indicated by pH values over 7.0 and iron values of about 0.18 mg/l.

Studies of the aquatic communities in the lake have all reported impoverished conditions, presumably due to high acidity. Rohr (1954) indicated that plankton are represented by few species and Menendez (1965) reported that plankton and aquatic insects are virtually non-existent.

Cheat Lake apparently contains plankton communities inhabiting two distinct environments; the acid polluted lake proper and the relatively acid free backwaters flowing into it from the east. This investigation examined the hypothesis that plankton community respiration varies significantly ($p<0.05$) between the acid polluted lake and its acid free embayments. Further, this variation will manifest itself in a lower community respiration rate in the acid polluted environment than in the acid free backwaters.

Methods

Respiration measurements were obtained by suspending three 125 ml dark bottles at sample depths determined by pH and relative light penetration to those depths. Water samples were obtained via a 2 liter non-metallic Kemmerer sampler.

Following a six hour incubation the oxygen content of the samples was measured (Winkler Azide Modification) and compared to the initial oxygen value obtained at the start of the trial. This difference was then adjusted to a 24 hour plankton community respiration measurement.

Bottles were suspended from an oceanographic cable attached to a five gallon styrofoam filled metal drum. They were attached to plexiglass plates with slots cut into them enabling the three bottles to be horizontally accomodated by sliding their necks into these slots (Schindler, pers. comm.).

Three sampling sites were chosen: Morgan's Run (pH 6 to 7), Ruble's Run (pH 6 to 7.2), and the water immediately above the dam (pH 3.5 to 5.8). The first two sites are backwaters and flow into the eastern side of the lake about 900 meters above the dam. Sampling dates were 2 to 24 July, 1971.

The following chemical analyses were performed on surface through bottom samples collected generally at one and two meter intervals: temperature, conductivity, total iron, dissolved oxygen, methyl orange and hot acidities. The latter four analyses were all performed according to *Standard Methods for the Examination of Water and Waste Water* (A.P.H.A., 1971).

The unpaired t-Test ($p<0.05$), utilizing the West Virginia University I.B.M. 360-75 computer system, was employed to compare all sampling site data in this study.

Results

Six sampling dates were employed at the Dam Station, 3 dates corresponding to samples taken simultaneously at Ruble's Run and 3 dates identical to those of Morgan's Run. T-Test analyses comparing these two 3 date sets of Dam Station data revealed all chemical parameters non-significant ($p>0.05$, Table 1), thus permitting valid statistical evaluations to be accomplished on data comparing the Dam Station and the two backwaters.

Eighteen six hour plankton community respiration measurements were taken at both Ruble's Run and the Dam Station. Table 2 shows significant differences for all parameters at the 0.01 confidence level. No methyl orange acidity was found in the backwater. Numerous backwater sample depths lacked iron, whereas all Dam Station samples contained at least 0.01 mg/l iron.

Twenty-one six hour community respiration measurements were obtained from the Dam Station, and nineteen measurements from the Morgan's Run backwaters. For all chemical parameters significant differences were found at the 0.01 confidence level, while the mean community respiration rates were significant at $\lambda = 0.001$ (Table 3). No methyl orange acidity was found in Morgan's Run. Iron was present in all Dam Station samples, whereas several samples from Morgan's Run were iron free; especially those samples obtained from surface through five meters.

Plankton community respiration was measured utilizing nineteen six hour dark bottle incubations in Morgan's Run and eighteen in Ruble's Run. Table 4 shows that while plankton community respiration and K_{20} values were significantly different; hot acidity, total iron, and pH were not. Community respiration mean in Morgan's Run was 0.95 mg O_2/l/day higher than Ruble's Run community respiration. However, Ruble's K_{20} mean value was almost 22 umhos/cm^2 higher than the mean conductivity values in Morgan's Run.

Discussion

From the data presented it appears that the plankton in the lake proper and those in the backwaters are living in two significantly different environments with respect to the chemical parameters analyzed. The hydrogen ion concentration of the dam samples indicated the pH of these waters to be more acidic than the backwater pH's by a factor of about 100. In addition, the significantly higher phenolphthalein and methyl orange acidities found at the dam present an extremely acid environment to organisms. Conversely, plankton in Morgan's and Ruble's Run dwell in waters with little or no acidity.

As indicated previously, several authors (Lackey, 1939; Johnson et al.) have found that increased acidities will decrease species diversity. However, it was also pointed out that surviving organisms' populations increase so that community photosynthesis and respiration values are not always adversely affected by the acid environment (Lind and Campbell, 1970). Thus to imply that the high acidity in the lake proper directly inhibits plankton respiration may be misleading.

Community respiration does not consider the concentrations of the plankton community within the three sampling sites. A respiration measurement as mg O_2/individual plankton/day would permit direct and more conclusive compari-

Table 1. T-Test Analysis of Chemical Parameters for the Cheat Lake Dam Station.

Parameter		*Dam Station (2, 18, and 24 July)*	*Dam Station (8, 15, and 22 July)*	*t*	*P*
K_{20}	$\bar{x}$	160.33	177.21	1.83	>0.05, d.f. 67
(umhos/cm^2)	s^2	15.56	38.12		
pH	$\bar{x}$	4.13	4.11	0.29	>0.05, d.f. 61
	s^2	0.01	0.05		
Methyl Orange	$\bar{x}$	0.51	0.47	1.15	>0.05, d.f. 67
(mg/l $CaCO_3$)	s^2	0.36	0.18		
Hot Acidity	$\bar{x}$	23.97	20.58	2.64	>0.05, d.f. 69
(mg/l $CaCO_3$)	s^2	0.52	1.12		
Total Iron	$\bar{x}$	0.46	0.54	0.64	>0.005, d.f. 62
(mg/l Fe)	s^2	0.01	0.01		

Table 2. T-Test Analysis of Chemical Parameters from Ruble's Run and the Dam Station, 8, 15, and 22 July, 1971.

Parameter		*Ruble's Run*	*Dam Station*	*t*	*P*
K_{20} (umhos/cm^2)	$\bar{x}$	141.41	177.21	5.54	<0.01, d.f. 44
	s^2	3.55	38.12		
pH	$\bar{x}$	6.24	4.11	12.06	<0.01, d.f. 40
	s^2	0.02	0.05		
Hot Acidity (mg/l $CaCO_3$)	$\bar{x}$	3.63	20.58	14.05	<0.01, d.f. 41
	s^2	0.20	1.12		
Total Iron (mg/l Fe)	$\bar{x}$	0.08	0.54	6.03	<0.01, d.f. 34
	s^2	0.00	0.01		
Plankton Comm. Resp. (mg O_2/l/day)	$\bar{x}$	1.47	0.75	2.91	<0.01, d.f. 23
	s^2	0.05	0.01		

Table 3. T-Test Analysis of Chemical Parameters from Morgan's Run and the Dam Station, 2, 18, and 24 July, 1971.

Parameter		*Morgan's Run*	*Dam Station*	*t*	*P*
K_{20} (umhos/cm^2)	$\bar{x}$	119.08	160.33	5.60	<0.01, d.f. 55
	s^2	37.71	15.56		
pH	$\bar{x}$	6.21	4.13	22.00	<0.01, d.f. 39
	s^2	0.01	0.01		
Hot Acidity (mg/l $CaCO_3$)	$\bar{x}$	4.02	23.97	13.40	<0.01, d.f. 44
	s^2	0.04	0.52		
Total Iron (mg/l Fe)	$\bar{x}$	0.11	0.46	5.00	<0.01, d.f. 39
	s^2	0.01	0.01		
Plankton Comm. Resp. (mg O_2/l/day)	$\bar{x}$	2.42	1.05	5.90	<0.001, d.f. 21
	s^2	0.05	0.01		

Table 4. T-Test Analysis of Chemical Parameters from Morgan's Run and Ruble's Run Backwater Stations of Cheat Lake.

Parameter		*Ruble's Run*	*Morgan's Run*	*t*	*P*
K_{20} (umhos/cm^2)	$\bar{x}$	141.41	119.08	3.46	<0.001, d.f. 37
	s^2	3.55	37.71		
pH	$\bar{x}$	6.24	6.21	0.17	>0.05, d.f. 55
	s^2	0.02	0.01		
Hot Acidity (mg/l $CaCo_3$)	$\bar{x}$	3.63	4.02	0.77	>0.05, d.f. 45
	s^2	0.20	0.04		
Total Iron (mg/l Fe)	$\bar{x}$	0.08	0.11	0.94	>0.05, d.f. 52
	s^2	0.00	0.01		
Plankton Comm. Resp. (mg O_2/l/day)	$\bar{x}$	1.47	2.42	3.04	<0.01, d.f. 35
	s^2	0.05	0.05		

sons of the backwater and Dam Station plankton. Preliminary Cheat Lake plankton surveys have indicated that plankton/m^3 are most concentrated in Morgan's Run (Bible, pers. comm.). Ruble's Run and the dam possess successively lower plankton concentrations with *Dinobryon* being the dominant genus at all three sites. The higher plankton density of Morgan's Run correlates to the higher community respiration rates exhibited in this backwater. Therefore, these respiration data may actually reflect a decreasing planktonic abundance gradient from Morgan's Run to Ruble's Run to the Dam Station.

Data analyses indicate the acid polluted waters of the dam either inhibits respiration of existing species or reduces planktonic densities, producing similar results. Since Bible (pers. comm.) reports much less plankton at the Dam Station, the data further suggest that the significantly lower community respiration at the dam is due to a lower plankton abundance resulting from the higher acidities present.

This decreased concentration of surviving species in an acid environment is contrary to results reported by Joseph (1953) and Robach and Richardson (1969). The Missouri acid mine lake studies of Lind and Campbell (1970) revealed that species diversity decreased in acid Missouri lakes with concurrent significantly increased populations of surviving species. These investigators, contrary to the findings in this study, found that community respiration values from the acid Missouri lakes positively correlated to non-acid natural Missouri waters.

If the acid environment were the primary factor causing decreased community respiration, both backwaters should possess more similar community rates. Since the pH and phenolphthalein acidities are not significantly different ($p>0.05$) it appears the acid environment, while it may affect species survival (Yount, 1956), may not be the sole inhibiting factor in this study and cannot satisfactorily explain the significantly different respiration rates found between the backwaters ($p<0.01$). The only chemical parameter examined that was significantly different between these stations was specific conductivity, yet Morgan's Run community respiration greatly surpassed that of Ruble's Run. Specific conductivity is a conservative measurement of salts available to planktonic organisms, and Welch (1952) reports that conductivity values may be reduced by algal removal of nutrient electrolytes vital for their growth. This algal removal may partially account for the lower K_{20} values found in Morgan's Run, for Bible (pers. comm.) found six genera of plankton in both backwaters, but planktonic abundance was greater in Morgan's Run from surface through 16 meters. Consequently, it appears that the respiration rate differences found between Morgan's Run and Ruble's Run are attributable to increased planktonic abundance in the Morgan's Run backwater. This abundance difference could be due partially to the availability of specific nutrients within these two backwaters. An appropriate subsequent investigation to evaluate fully this possibility would involve a nutrient analysis of both Ruble's Run and Morgan's Run backwaters.

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An Analysis of the Zooplankton Community in an Acid Polluted Reservoir

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Abstract

Since its construction in 1929, Cheat Lake has been polluted by acid mine drainage. The reservoir's three backwaters are partially isolated from the main reservoir and do not receive acid except occasionally from the main reservoir. The present study examined stations in the main reservoir and two of its backwaters to determine the extent of acid mine pollution and its possible effect on the zooplankton community.

Chemical analyses indicate water in the main reservoir has high acidity and low pH, hardness and alkalinity. However, the backwaters have little or no acidity, higher pH, hardness and alkalinity than the main reservoir.

Community coefficients indicate zooplankton communities in the backwaters are similar to each other but different from the zooplankton in the main reservoir. In addition, zooplankton biomass m^{-3} was much greater in the backwaters, as was biomass m^{-2}. The effects of reduced water quality and zooplankton abundance in the main reservoir compared with conditions in the backwaters provide a basis for further study.

Introduction

Cheat Lake, a reservoir located in Monongalia County, West Virginia, was formed by construction of a hydro-electric power dam across the Cheat River in 1929. The Cheat River drainage basin in Monongalia and Preston counties and several local tributaries create a reservoir 13 miles long, up to a half mile wide, with a surface area of about 1,730 acres and a total capacity of 73,000 acre feet (Basnett, 1953). At least half the eleven tributaries, predominantly on the western side of the lake, and the Cheat River, are known to carry acid mine water into the lake (Stilson, 1968).

Three backwaters are formed a half mile above the dam by an abandoned railway which partially isolates Rubles Run, a 56 acre cove, Morgan Run, a 37 acre cove, and Cove No. 1, with an area of 11.7 acres, from the main reservoir. Stilson (1968), classified the tributaries entering the backwaters in "Group 1," a group with an average pH of 7.32. In contrast, the tributaries draining coal mine areas on the western side of the lake were classified in "group 2," with an average pH of 2.66.

Acid mine pollution results from weathering of sulfuritic material left as debris in coal mining areas (Brezina *et al.*, 1970). Parsons (1964) compared a series of strip-mine lakes and found stages of chemical aging with concomitant physical characteristics. Campbell *et al.*, (1965), found decreasing water quality and low quantities of aquatic organisms with increasing acid mine pollution while succession to less acidic conditions produced increasing variety of aquatic life. Johnson *et al.*, (1970), also found lower species diversity of phytoplankton in acid mine polluted waters.

Measurements in the backwaters and main reservoir were made in this study to determine if the stations differed significantly on the basis of chemical parameters associated with acid mine pollution. In addition, study of the zooplankton community at each station was conducted to determine if differences existed between the acid-receiving reservoir and the isolated backwaters. Comparisons of chemical and biological parameters were made in an effort to characterize conditions in Cheat Lake and to establish a basis for further studies of acid mine pollution in aquatic ecosystems.

Methods

Two sampling sites were chosen in the reservoir, and two in the backwaters. Stations were located: 1) opposite the coal pile, five miles above the dam, 2) one quarter mile above the dam, 3) the middle of Rubles Run and 4) the middle of Morgan Run.

Temperature at each station was measured in degrees Celsius with a standardized Whitney thermister. Maximum depth was found using a depth sounder and water samples were taken every other meter with a two liter PVC Kemmerer sampler. Samples were stored in either 250 ml. B.O.D. bottles for dissolved oxygen determination or in polyethylene bottles for conductivity, pH, alkalinity, hardness, methyl orange and total acidity determinations. All analyses were performed immediately upon return to the laboratory.

Conductivity, measured with a Beckman Conductivity Bridge, was expressed as micromhos cm^{-1} at 20° C. A Beckman Expandomatic pH Meter, standardized before each use and periodically throughout the analysis, measured pH. Dissolved oxygen, alkalinity, E.D.T.A. hardness, methyl orange and hot acidity, were determined titrimetrically by methods outlined in Standard Methods (APHA, 1971).

A #25 mesh Wisconsin plankton net was used for vertical tows and for filtering plankton samples obtained with a PVC Kemmerer sampler. The samples were fixed with 10% formalin.

Zooplankton counts were taken from the entire sample and expressed as number per cubic meter. Plankton biomass m^{-3} was obtained by multiplying number of organisms of each species by a conversion factor (Hall *et al.*, 1970; Schindler and Noven, 1971). Total biomass m^{-2}, derived by interpolating biomass m^{-3} values for unsampled depths and totalling all depths at each station, was expressed in milligrams m^{-2}.

Results

Chemical parameters measured at each station are seen in Table 1. The reservoir stations were very similar to each other with low pH and alkalinity and high methyl orange acidity, total acidity and conductivity. In contrast, the backwaters were nonacid with relatively high pH and alkalinity and low methyl orange acidity, total acidity and conductivity. In addition, higher carbonate hardness was recorded at Rubles Run than at the other stations, presumably due to a limestone quarry in its drainage basin.

Table 1. Chemical Parameters Expressed as Mean and (variance) at Each Station of Cheat Lake, Summer 1971.

	RESERVOIR		*BACKWATERS*	
	Coal Pile	*Dam*	*Morgan Run*	*Rubles Run*
pH	4.41	4.44	6.11	6.98
	(.41)	(.21)	(.56)	(.27)
M.O. Acidity	9.20	3.08	0.02	0.00
mg $CaCO_3$/l	(52)	(3)	(.12)	(0)
Total Acidity	26.78	17.74	6.26	1.97
mg $CaCO_3$/l	(2882)	(49)	(16)	(8)
Conductivity				
micromhos/cm	199	190	127	172
at 20° C	(2867)	(6092)	(471)	(683)
Alkalinity	0.00	0.08	3.26	18.42
mg $CaCO_3$/l	(0)	(.18)	(9)	(135)
Hardness	38.91	42.18	37.03	62.14
mg $CaCO_3$/l	(19)	(139)	(298)	(380)
Dissolved Oxygen	7.83	7.40	6.26	7.41
mg/l	(9)	(9)	(16)	(9)

Table 2 lists zooplankton means and variances for all the stations. *Brachionus* cf. *urceolaris,* which is correlated with high conductivity ($r = .18$, d.f. = 284, $P < .0027$) and low pH ($r = -.24$, d.f. = 319, $P < .0001$), was most abundant in the reservoir. *Diaptomus sp.* and nauplii larvae were the only other zooplankters occurring regularly in the reservoir, and these were most commonly found at the Coal Pile station.

Table 2. Mean and (variance) for Zooplankton m^{-3} at Each Station.

	RESERVOIR		*BACKWATERS*	
	Coal Pile	*Dam*	*Morgan Run*	*Rubles Run*
Brachionus cf.	33.22	15.17	2.54	0.78
urceolaris	(5775)	(3378)	(80)	(9)
Diaptomus sp.	4.67	0.75	0.00	0.48
	(194)	(6)	(0)	(4)
Nauplius	17.27	3.46	2.39	3.35
	(2651)	(826)	(66)	(70)
Mesocyclops edax	1.51	0.10	0.96	2.98
	(54)	(1)	(7)	(74)
Daphnia parvula	0.31	0.08	0.40	1.22
	(3)	(.6)	(8)	(12)
Bosmina	1.03	0.10	1.05	0.30
longirostris	(17)	(1)	(8)	(2)
Ceriodaphnia sp.	0.00	0.00	0.16	0.99
	(0)	(0)	(1)	(17)
Polyarthra sp.	0.82	0.00	0.00	1.83
	(49)	(0)	(0)	(51)
Keratella	0.48	0.13	0.09	1.05
cochlearis	(13)	(9)	(.5)	(14)

The zooplankton community at Rubles Run was dominated by *Mesocyclops edax,* which is found in most North American lakes (Hutchinson, 1967). Cladocerans were more abundant in the backwaters than in the reservoir with *Daphnia parvula* and *Ceriodaphnia sp.* in Rubles Run and *Bosmina longirostris* most numerous in Morgan Run.

Qualitative comparisons of zooplankton communities are presented in Table 3 as community coefficients which are based on the formula: $CC = \frac{100 \cdot c}{(a+b)-c}$, where: a = number of organisms at station 1, b = number of organisms at station 2, and c = number of organisms common to both stations. When the communities are identical, the coefficient equals 100. On this basis, Rubles Run is most similar to Morgan Run and the Dam and Coal Pile stations are most similar to each other. While Morgan Run is most like the other backwater station, it is also similar to the reservoir stations with similar community coefficients. However, comparisons of Rubles Run with the reservoir stations produced the lowest coefficients in the study, indicating Rubles Run is least like the reservoir stations. These qualitative similarities and differences between the stations may be expected from chemical conditions in the backwaters and in the reservoir.

Table 3. Community Coefficients for Zooplankton Populations at Cheat Lake, Summer 1971.

STATION	*COAL PILE*	*RUBLES RUN*	*MORGAN RUN*
Dam	67	50	66
Coal Pile		55	64
Rubles Run			72

Quantitative measures of zooplankton at each station are seen in Table 4. The reservoir had the greatest numbers of organisms m^{-3} due to high occurrence of *Brachionus*, which is associated with acid waters. However, the backwaters had the greater biomass m^{-3} due to abundance of cyclopoids and cladocerans which are heavier than *Brachionus*. Differences between the stations are most apparent when biomass in micrograms per square meter is compared. Morgan Run and the Coal Pile station, which are similar in depth, have similar amounts of biomass/m^2. However, the Dam station, which is almost twice as deep as the Coal Pile station, has an areal biomass equal to half that found at the other reservoir station. In contrast, Rubles Run consistently recorded the greatest biomass/m^2 due to greater alkalinity and hardness of the water, which, according to Ruttner (1967), supports quantitatively more plankton than soft water.

Table 4. Mean and (variance) for Zooplankton Expressed as Total Organisms m^{-3}, Biomass in Micrograms m^{-3} and Biomass in Micrograms m^{-2}. All Values Should Be Multiplied by 10^3.

	RESERVOIR		*BACKWATERS*	
	Coal Pile	*Dam*	*Morgan Run*	*Rubles Run*
Total organisms m^{-3}	38.43 (6460)	17.73 (3455)	7.30 (188)	12.12 (481)
Biomass m^{-3}	16.16 (3041)	4.89 (1085)	15.30 (3525)	22.19 (3686)
Biomass m^{-2}	238.45 (508256)	135.08 (99178)	205.30 (387638)	607.56 (2132428)

Chemical and biological differences among the four stations were determined using a series of multi-variate discriminant analyses. As seen in Table 5, all the stations were significantly different at the .0005 level for all comparisons. The differences were greatest when chemical parameters and biomass were compared. The data indicate the major factors accounting for these statistical differences are related to the acid pollution occurring at the stations in Cheat Lake. The main reservoir differed from the backwaters due to higher acidity. Differences between the backwaters may be due to evidence of mine acid entering the backwaters and to greater alkalinity in Rubles Run.

Table 5. D^2 Values and Significance Levels for Multivariate Discriminant Analyses of All Stations.

VARIABLE SET	D^2	*SIGNIFICANCE*
Conductivity, pH, Total Acidity, Alkalinity, Hardness	3238.05	P<.0005
Conductivity, pH, M.O. Acidity, Total Acidity, Hardness, Alkalinity, *Brachionus*	1013.78	P<.0005
Conductivity, pH, M.O. Acidity, Total Acidity, Hardness, Alkalinity, Total Organisms	432.38	P<.0005
Conductivity, pH, M.O. Acidity, Total Acidity, Hardness, Alkalinity, Biomass/m^3	3247.58	P<.0005
Conductivity, pH, Total Acidity, Hardness, Alkalinity, Biomass/m^2	1028.54	P<.0005
Mesocyclops, Diaptomus, Nauplius, Daphnia, Bosmina, Brachionus, Polyarthra, Keratella	86.18	P<.0005
Mesocyclops, Diaptomus, Nauplius, Daphnia, Bosmina, Brachionus, Polyarthra, Keratella, Biomass/m^2	101.50	P<.0005

Discussion

Chemically, Cheat Lake is intermediate between strip mine lakes in the early and late recovery stages from acid pollution, as classified by Campbell *et al.* (1965). Between these two stages, acidity decreased from 683 to 0 ppm, pH increased from 2.8 to 7.9, and alkalinity increased from 0 to 184 ppm. Unlike the strip mine lakes, Cheat Lake is not a recovery stage; comparisons of previous studies on the lake show a steady increase in the acidity of the water (Menendez, 1966).

Differences in bicarbonate ion concentration and the resulting distribution of zooplankton, enabled Borecky (1956), to differentiate trophically different basins in the same reservoir. Similarly, differing acid concentrations in Cheat Lake seem to have produced differences in zooplankton distribution. The backwaters, which were similar in chemistry, were dominated by zooplankters found in an unpolluted lake by Tash and Armitage (1965), while the reservoir stations were more acidic with limited zooplankton abundance, similar to that found in strip mine lakes by Campbell *et al.* (1965).

Studies of zooplankton communities usually involve numerous species existing in the same lake. Tappa (1965) studied six species of *Daphnia* in a Maine lake and Patalas (1971) found as many as five major plankters in the same lake. Acidic water conditions in Cheat Lake seem to have limited zooplankton variety.

Only eight zooplankters were observed more than once; of these, only one was dominant at each station. The variety of species was concomitantly restricted with one cyclopoid, one calanoid, three cladocerans, and three rotifers.

Schindler and Noven (1971), list oligotrophic lakes with biomasses of 24-51 mg m^{-3} and eutrophic lakes with biomasses as great as 540 mg m^{-3}. Reed and Olive (1956) found mean biomass in a mesotrophic Colorado reservoir equal to 262 mg m^{-3}. Maximum zooplankton biomass of 113 mg m^{-3} was recorded in the present study at Rubles Run. Mean biomass for the entire study was only 13.5 mg m^{-3}. This is very low compared to other lakes and may be a result of increased acidity in Cheat Lake. Further study is needed to understand the ability of acid water to maintain zooplankton populations of particular abundance and composition. This is an important step to understanding the dynamics of an acid polluted aquatic ecosystem.

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The Culture of *Nicotiana glauca* Tissue

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Abstract

Nicotiana glauca L. stem tissue cultures were obtained by routine methods and were maintained on Miller's 1966 medium. This medium contains (mg/l): KH_2PO_4, 300; KNO_3, 1000; NH_4NO_3, 1000; Ca $(NO_3)_2 \cdot 4H_2O$, 500; MgSO4 $\cdot 7H_2O$, 71.5; KCL, 65; MnSO4 $\cdot$ $4H_2O$, 14.0; H_3BO_3, 1.6; Cu $(NO_3)_2 \cdot 8H_2O$, o.35; $(NH_4)_6$ $Mo_7O_{24} \cdot 4H_2O$, o.10; meso-inositol, 100; nicotinic acid, 0.5; pyridoxine · HCL, 0.1; thiamine · HCL, 0.1; a-napthaleneacetic acid, 2.0; kinetin, 0.5; sucrose, 30,000; Bacto-agar, 10,000.

Nicotiana glauca grew satisfactorily on this medium. The next step was to determine which components were required for growth. This was studied by systematically omitting each of the organic compounds. Results indicate that a cytokinin is not required, however, an auxin (a-naphthalene acetic acid) is required for growth. Cytokinin will not replace the auxin. The other organic compounds aside from sucrose, stimulated growth but were not absolutely required.

The Effects of Acid Mine Water on Growth (Number and Size) of *Chlorella vulgaris*[1]

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Abstract

Growth in *Chlorella vulgaris* was examined under axenic conditions in a variety of Acid Mine Water (AMW) concentrations, and inorganic nutrient media, to ascertain the relationship between cell number and cell size. It was found that high concentrations of AMW (above 3/16) decreased the average cell size. Further, at concentrations of 3/16 AMW or more, cell number was greatly decreased. Finally, the normal relationship that exists between cell size and cell number during growth was greatly modified by AMW concentrations greater than 3/16.

Introduction

The great increase in population and the rapid development of agriculture and industry have caused a phenomenal increase in the use of water and have brought about many difficult problems in the procurement of clean water (usable for a variety of purposes). One major contributor to this water problem is drainage from active and inactive mines and is commonly referred to as acid mine water (AMW).

Algae are one of the major sources of energy for aquatic animals; therefore, if the algal populations are destroyed or modified by the presence of AMW, this could eventually result in a direct effect on man by limiting the available aquatic food sources and modifying existing aquatic ecosystems so that "natural" water renovation is inhibited. It has been shown by Bennett (1969), Lacky (1939) and Prescott (1961) that in water containing AMW, the number of different kinds of algal species decreases and changes in morphological structure often take place.

Methods

The organism, *Chlorella vulgaris*, Pratt strain, (Indiana University Algae Culture Collection, #398) was grown in various concentrations of AMW in combination with modified Beijerinck's medium (W. J. Shoupp, 1972). This medium contains essential macro and micronutrients only and no sugar.

The modified Beijerinck's medium was filtered through a millipore filter (type HA, 0.45μ). The samples of AMW were obtained directly from the drainage pump of a local mine and were not filtered before use.

[1]This project was supported, in part, by the Office of Water Resources Research, Department of Interior grant No. WVA B001, and the Biology Department, W.V.U.

Two separate groups of replicated experiments using different concentrations of AMW were completed as follows:

The treatments of the first experimental series (2 replicates for each treatment) were:

AMW (parts)	+	Beijerinck's medium (parts)
0		100
1/128		127/128
1/64		63/64
1/32		31/32
1/16		15/16
1/8		7/8
100		0

The treatments of the second experimental series (4 replicates for each treatment) were:

AMW (parts)	+	Beijerinck's medium (parts)
0		100
1/16		15/16
3/16		13/16
5/16		11/16
7/16		9/16
9/16		7/16
11/16		5/16
13/16		3/16
15/16		1/16
16/16		0/16

The test system used in the first experiment was an aluminum block with 88 culture tube positions bored in it (described in detail by Shoupp, 1972). For the second experiment a test tube rack was suspended over a constant light source provided by fluorescent "plant-gro" tubes of about 500 f.c. Each method allowed approximately the same amount of light to impinge upon the culture tubes. Both experiments were carried out at room temperature which fluctuated between 23° C and 25° C during the experiments. The organisms were grown in 25mm x 125mm culture modules, as described by Shoupp, 1972.

The entire culture apparatus with tubes, air tubing and culture modules was autoclaved prior to use and the culture modules (plugged with cotton) were dried to prevent inhibition of air flow due to wet cotton. Sterile humidified air was used for bubbling as described by Shoupp (1972). A sample of 0.5 ml of inoculum per tube (total volume 15.5 ml) per culture module was used to inoculate the axenic cultures.

The data were collected as follows: both the cell counts and number of cells were determined at regular intervals depending upon the rate of growth observed in the culture modules and from data previously gathered. The size of the cells was determined by using a 1000X magnification of a Nikon model SU microscope with an ocular micrometer. The ocular micrometer was calibrated to microns using a stage micrometer. To determine average cell size the first 20 Chlorella cells in a wet mount were measured, and an average determined for each treatment. Cell number was counted using a Model B Coulter Counter as described by Shoupp, 1972.

Results and Discussion

Four sample plots representing some of the results of the experiments are shown in Figures 1 through 4. Figure 1 shows the results of the treatment of 0/16 parts AMW and 16/16 parts Beijerinck's medium (control), and Figure 2 shows the results of the treatment of 1/16 parts AMW to 15/16 parts Beijerink's medium. Figure 3 shows the results of 3/16 parts AMW to 13/16 parts Beijerinck's medium, and Figure 4 shows the results of growing Chlorella in 16/16 parts AMW to 0/16 parts of Beijerinck's medium. These sample graphs show the general trend that occurred in both experiments, viz; when the con-

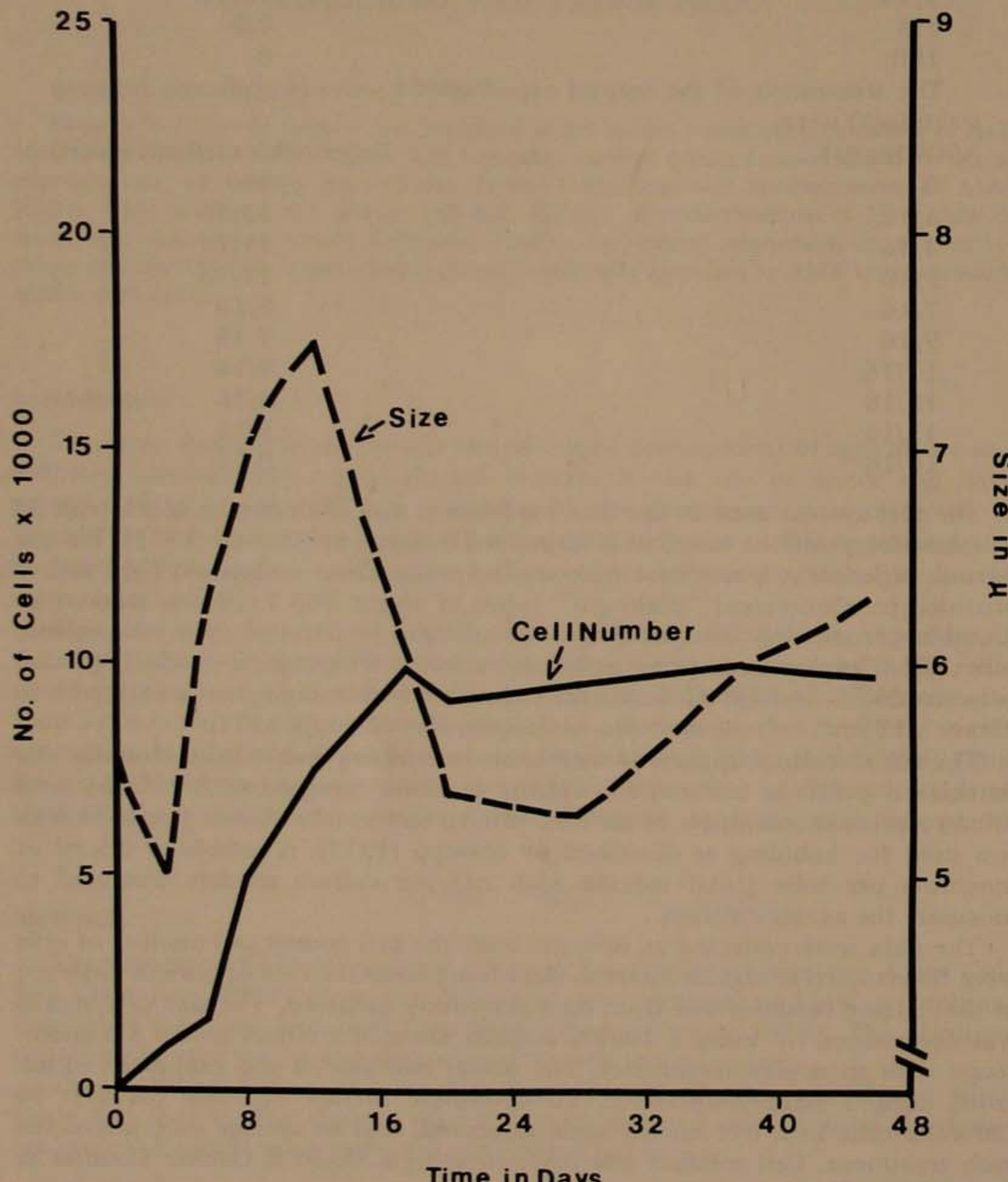

FIGURE 1. The Relationship Between Cell Size and Time and Cell Number and Time Over the Experimental Period for the 100 Percent Beijerinck's Medium.

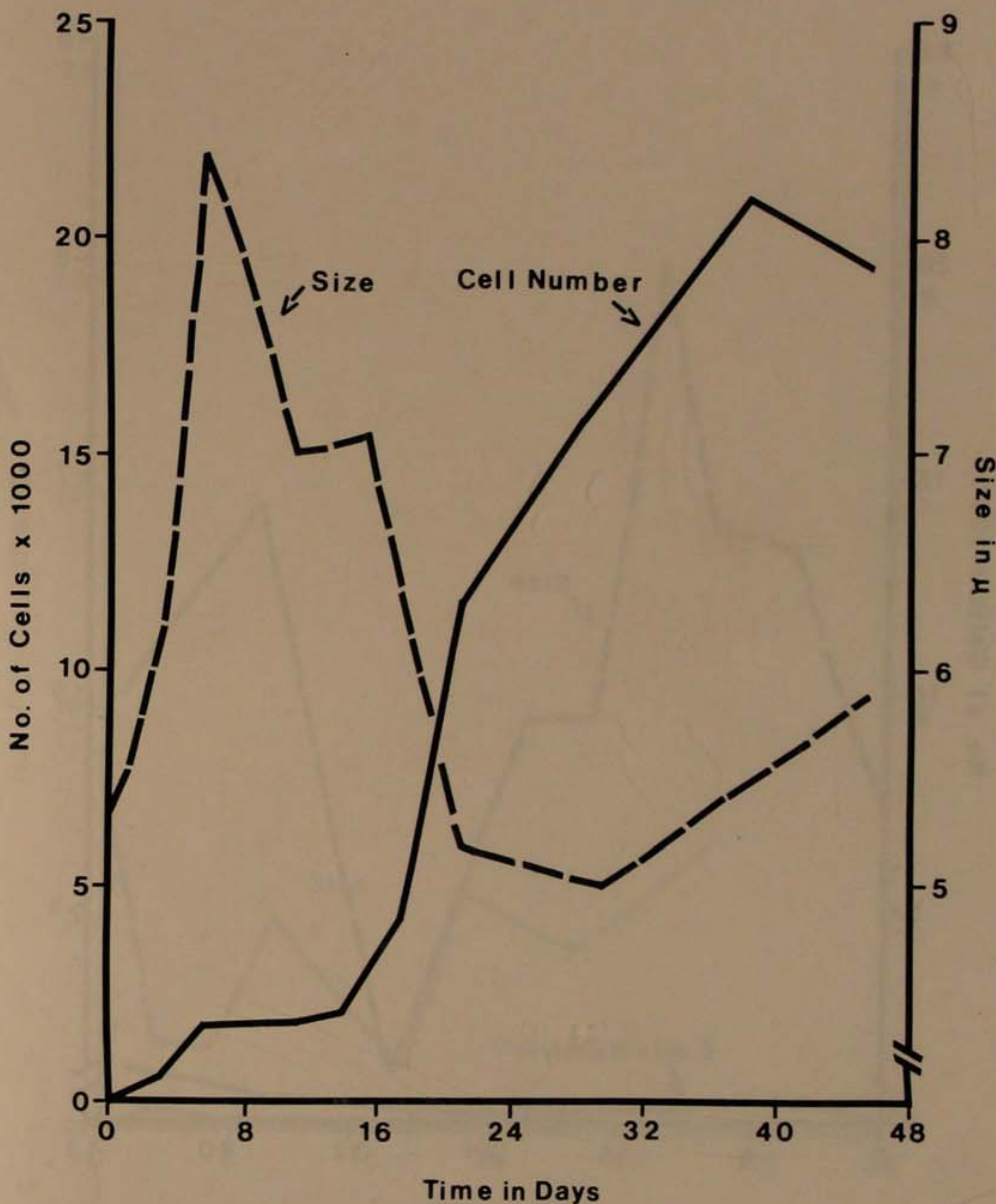

FIGURE 2. The Relationship Between Cell Size and Time and Cell Number and Time Over the Experimental Period for the 15/16 Proportion Beijerinck's Medium and 1/16 Proportion Acid Mine Water.

centrations of AMW were less then 3/16, there was a characteristic sigmoid growth curve for cell number and a corresponding curve for cell size (which at first increased then decreased, and after a period of time slightly increased again). For these lower concentrations of AMW there was a great increase in cell size during the early phases of growth followed by a decrease in average size per cell with a simultaneous increase in cell number. At concentrations of 3/16 AMW and higher, this relationship did not remain consistent. Cell number tapered off rapidly, and the patterns of change in cell size became inconsistent (Figures 3 and 4).

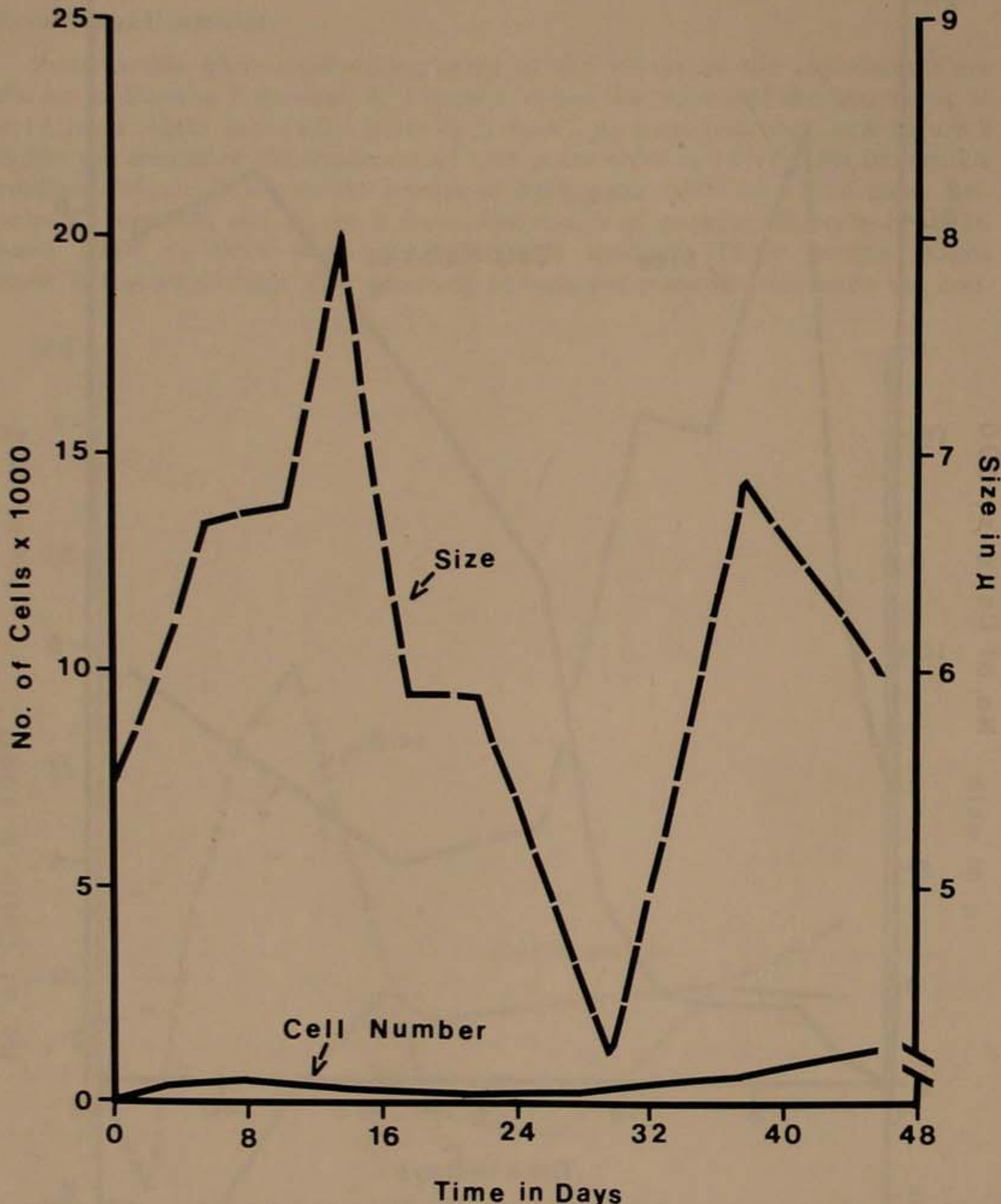

FIGURE 3. The Relationship Between Cell Size and Time and Cell Number and Time Over the Experimental Period for the 13/16 Proportion Beijerinck's Medium and 3/16 Proportion Acid Mine Water.

The relationship between cell size and cell number of Chlorella grown in 100% Beijerinck's medium (control) is presented in Figure 5, and the growth relationships of the 11/16 parts AMW to 5/16 parts Beijerinck's medium is given in Figure 6. The general relationship, as shown in the control (0% AMW to 100% Beijerinck's medium), indicated an initial sharp increase in cell size and cell number. This increase (in log phase) was followed by a sharp decrease in cell size with only a moderate increase in cell number. There was apparently another growth cycle beginning (note the increase in cell size at the uppermost point of

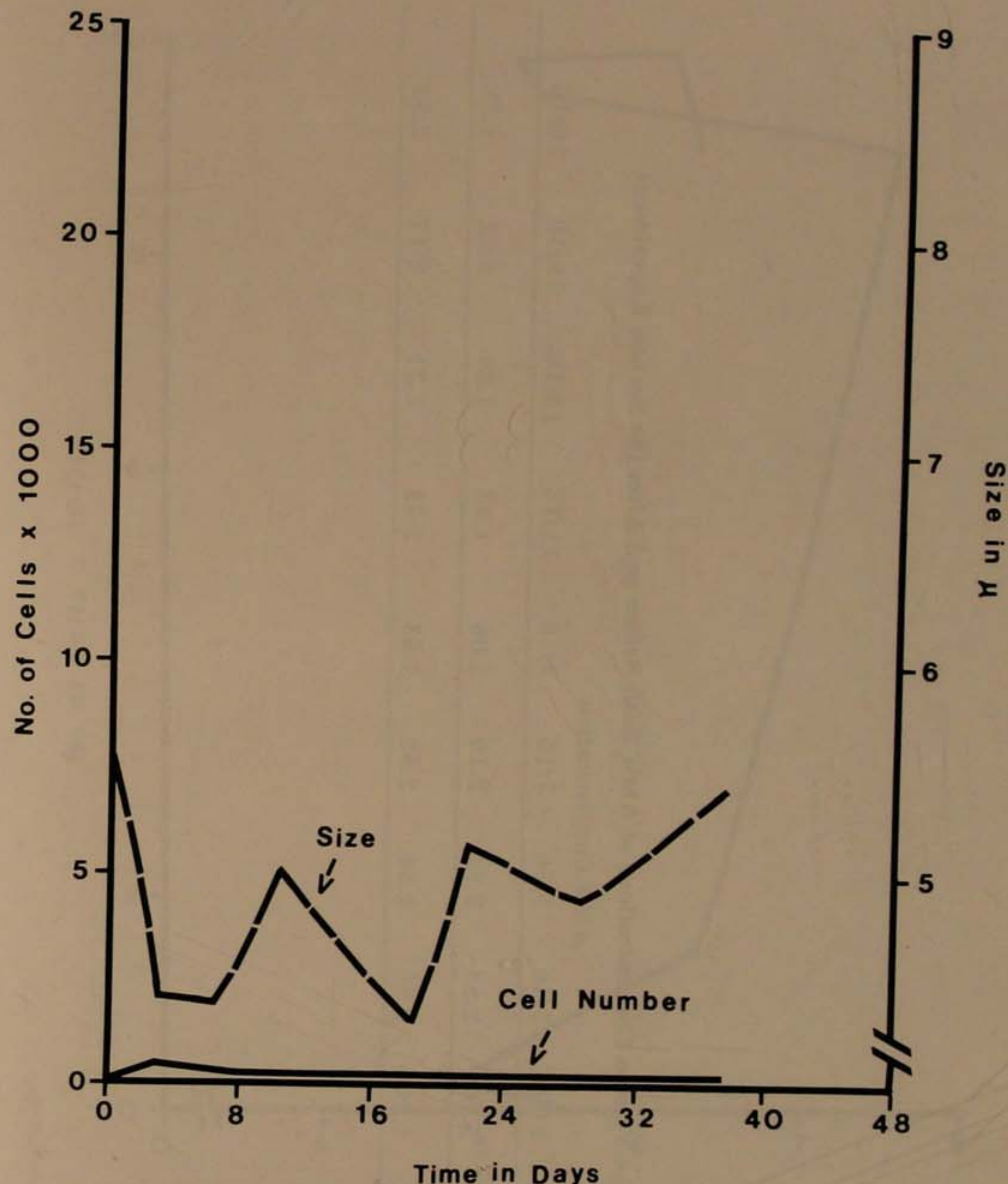

FIGURE 4. The Relationship Between Cell Size and Time and Cell Number and Time Over the Experimental Period for the 100 Percent Acid Mine Water.

the graph) but due to decreased nutrient levels, the cell number also decreased. Above 3/16 parts AMW, this general relationship disappeared as shown in Figure 6, wherein there are seemingly random fluctuations of both parameters. This disrupted relationship is apparently due to the higher AMW concentrations, or possibly the availability of iron, or pH levels *per se*.

Cell numbers may also be examined as a function of pH. At AMW concentrations above 3/16, when the pH decreased sharply, growth also sharply decreased, Table 1. High pH may be related to plasmolysis in Chlorella cells, thus resulting in a self-inhibiting system (Rice, 1954).

Table 1. pH Values of the Various Concentrations of AMW Both Before and After the Second Experiment

	AMW Concentration									
Experimental Stage	0/16	1/16	3/16	5/16	7/16	9/16	11/16	13/16	15/16	16/16
pH at the time of inoculation	6.81	5.70	2.53	2.21	2.10	2.06	1.93	1.89	1.82	1.78
pH at the end of the experiment	6.40	6.20	2.96	2.56	2.53	2.63	2.73	2.21	2.17	2.32

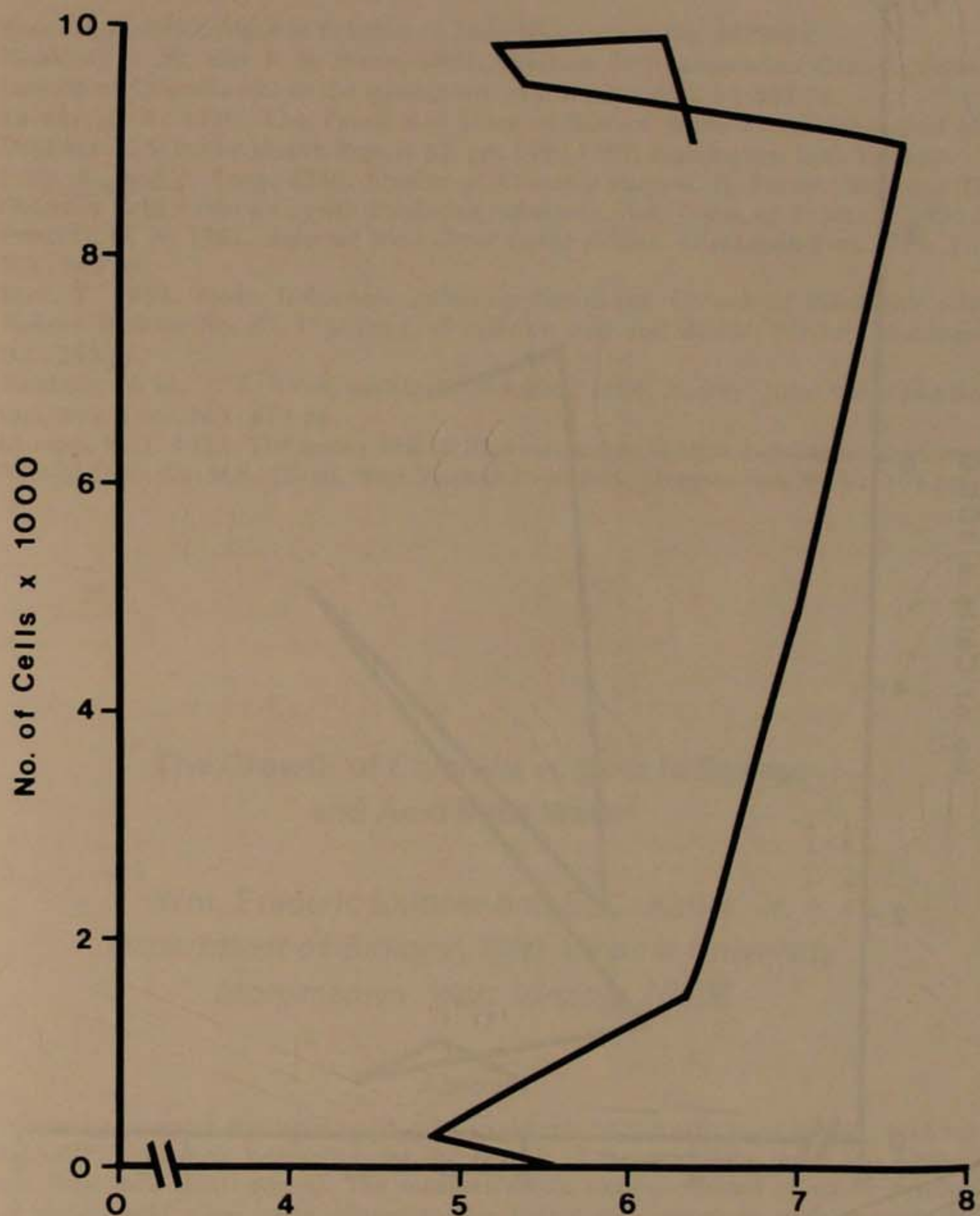

FIGURE 5. The Relationship Between Cell Number and Cell Size for Chlorella Grown in 100 Percent Beijerinck's Medium.

The initial increase in cell size during log phase of growth is probably due to the method by which Chlorella cells reproduce. The initiation of log phase generally corresponded to the mother cells releasing their progeny. This agrees with earlier work by Pratt and Fong (1940). At the higher concentrations of AMW (3/16 AMW and above) this generalized relationship becomes disrupted.

Conclusion

(1) Cell size is decreased at high AMW concentrations (above 3/16 AMW).

(2) Cell numbers plotted against time at concentrations up to, but not includ-

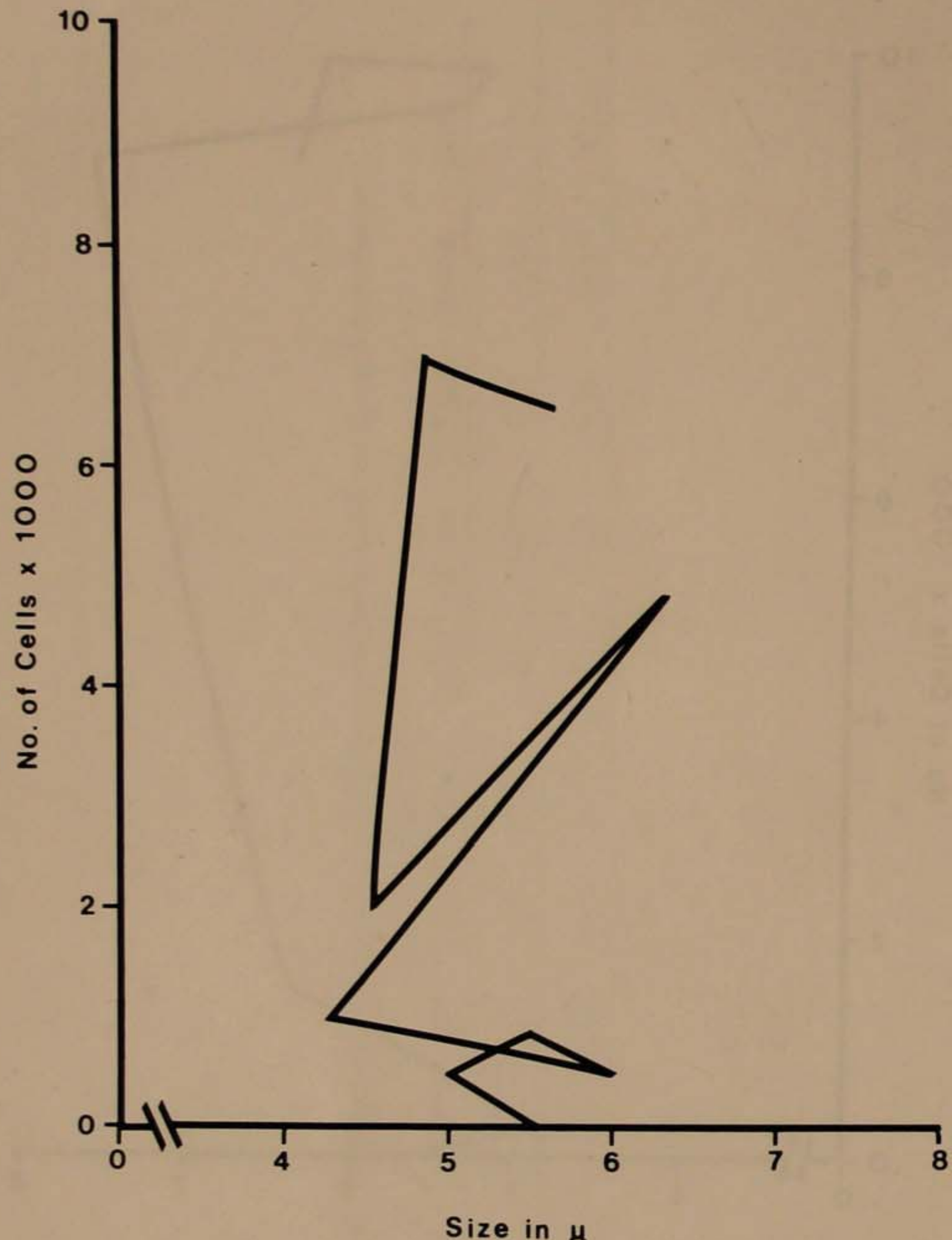

FIGURE 6. The Relationship Between Cell Number and Cell Size for Chlorella Grown in 5/16 Proportion Beijerinck's Medium and 11/16 Proportion Acid Mine Water.

ing 3/16 AMW resembled a normal sigmoid growth curve. At concentrations of 3/16 AMW or above, cell number decreased drastically, possibly due to toxic pH effects.

(3) At concentrations up to 3/16 of AMW, a distinct relationship between cell size and cell number exists, (i.e., an increase in cell size at early phases of cell number growth followed by a decrease in cell size as the log phase of growth begins) but at concentrations of 3/16 AMW and above, the growth curve no longer remains sigmoid, thus disrupting the normal relationship.

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The Growth of *Chlorella vulgaris* in Sewage and Acid Mine Water[1]

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Abstract

Preliminary research has been conducted to determine the effects of sewage, acid mine water (AMW), and their interaction on the growth of the unicellular green alga *Chlorella vulgaris* Beijerinck (Pratt strain). The nutrient media were composed of sterile synthetic sewage and/or acid mine water diluted to the desired concentrations with a standard inorganic culture medium (modified Beijerinck's medium). Six concentrations of sewage (0/0, 1/32, 1/16, 1/8, 1/4, 1/2), four concentrations of AMW (0/0, 1/31, 1/16, 1/8), alone and in all possible combinations were evaluated. The cultures were grown at 25° C in 24x150 mm glass culture tubes, aerated, and agitated with sterile humidified air, and illuminated from below by approximately 500 f.c. light intensity by "Gro-lux" fluorescent tubes. The maximum growth was obtained with the 1/2 sewage treatment, the least amount of growth occurred in the 1/8 AMW + 1/2 sewage treatment. *Chlorella vulgaris* was apparently able to utilize some of the components in the sewage since better growth occurred in all treatments containing only sewage than in the controls. However, high concentrations of sewage in combination with high concentrations of AMW inhibited algal growth.

[1]This project was supported, in part, by the Office of Water Resources Research, Department of the Interior grant No. WVA-B001, and the Biology Department, W.V.U.

Little information was available on the bacteria-free culture of *Chlorella* on sewage. Pipes and Gotaas (1960) investigated the occurrence of organic compounds in sewage which could be metabolized by *Chlorella pyrenoidosa* Chick and the growth characteristics of *C. pyrenoidosa,* when cultured in sewage, were studied by Oswald, Gotaas, Ludwig, and Lynch (1953). Wiedeman (1970) examined axenic isolates of eleven genera of green algae, including six isolates of Chlorella, for their ability to utilize several carbon sources under a variety of environmental conditions. Wiedeman concluded "certain algae are capable of assimilating preformed carbon sources under all conditions."

Shoupp (1972) investigated the growth of *Chlorella vulgaris* in four concentrations of AMW and eleven temperature levels over a temperature range of 14.6°C to 39.6°C. There were apparently some growth factors, nutrients, or increased nutrient levels in the AMW which were absent in the inorganic culture medium (control) since the alga grown in media containing 1/32 or 1/16 AMW reached a higher maximum number of cells than those cultures in the inorganic control medium (zero AMW). In order to gain some information concerning the effects of sewage and AMW and their interaction on the growth of *Chlorella vulgaris,* this preliminary experiment was run at one temperature.

Materials and Methods

Acid mine water (AMW), having a pH of 2.68, was collected from a mine pump near Osage, W. Va. Concentrations of AMW were those used in previous experiments (Shoupp, 1972) to span the approximate AMW range of tolerance of *Chlorella vulgaris;* i.e., AMW/total volume ratios of 0/0, 1/32, 1/16, and 1/8.

Synthetic sewage was formulated according to Rogers and Wilson (1966). Synthetic sewage was used instead of natural raw sewage to assure a solution of uniform predictable composition, consistency of known components, high stability upon autoclaving, and that would be relatively particle-free (to allow optimal counting accuracy on a Coulter Counter). An initial experiment was done to determine the approximate concentration of synthetic sewage that would yield a BOD level of about 200 ppm, the average for Morgantown raw sewage (Soccorsi, personal communication). Five-day BOD tests (25°C) of various concentrations of a stock solution of synthetic sewage (10x concentration of the formula of Rogers and Wilson, 1966) were conducted using a Hach manometric BOD apparatus in a constant 25°C temperature room. A 2.5 percent dilution of the stock solution gave a five-day BOD of 215 ppm. This value approximates the Morgantown average and was used in this experiment. The proportion of sewage to total volume ratios evaluated were: 0/0, 1/32, 1/16, 1/8, 1/4, 1/2. A modified Beijerinck's medium was used for control cultures as well as for the diluent of AMW/sewage combinations.

Cultures were grown in culture tube assemblies each consisting of a 24x150 mm glass culture tube fitted with a modified Morton closure through which a length of pyrex tubing extended to the bottom of the culture tube. The top end of the glass tubing was partially plugged with a cotton wad filter, the bottom end was flared and notched to allow for even distribution of sterile humidified air. This sterile humidified air was pumped through a Tygon tubing manifold into which 18 gauge hypodermic needles were inserted. To each of these needles was attached a length of amber latex surgical tubing which extended to the culture tube assembly completing the circuit from air pump to algal culture.

The culture tube assemblies were placed in two tandem aluminum thermal gradient blocks (Shoupp, 1972). These thermal gradient blocks were constructed of aluminum tool and jig plate, 4"x15"x25". A matrix of 8x11 26 mm holes was bored through the 4" thickness giving an 88 hole experimental matrix in each block. Channels were also drilled for the circulation of cooling and heating fluids through the opposite ends of each block, as well as holes for monitoring the temperature of each of the eleven rows by thermocouples or thermometers. Since this study concerns only the growth of algae at a static temperature, the temperature regulation and operation of the thermal gradient block systems will be published elsewhere (a detailed description is given by Shoupp, 1972).

A bank of 20 watt Gro-lux (Westinghouse Corp.) fluorescent tubes under each block provided a light intensity of approximately 500 f.c. impinging on the base of the gradients. An exhaust fan system above the lights removed most of the lamp generated heat so that the gradient blocks operated between 23-25°C during the experiment. Duplicate cultures of each AMW concentration, each sewage concentration, and each combination were inoculated with 1.0 ml of a 4-day old suspension of *Chlorella vulgaris* Beijerinck's (Pratt strain) (Indiana Algae Culture Collection, #398) giving 48 (24 treatments x 2) cultures of 16 ml each. The cultures were placed randomly in the blocks with one replicate per block. Sterile, humidified air was circulated through the cultures for 20 seconds in every 20 minutes for adequate aeration and agitation to facilitate gas transfer and to prevent flocculation of algae at the bottom of the culture modules.

Beginning with the first day (t=0) the cultures were counted every other day on a Model B Coulter Counter for a period of 18 days. A 0.1 ml sample was aseptically withdrawn from a culture using a 1.0 ml sterile disposable pipet. The sample was placed in a counting vessel and diluted with 10 ml of Isoton (Coulter Electronics, Hialeah, Florida) diluent. Five counts were determined on 0.05 ml sub-samples of the sample using the Coulter Counter. The highest and lowest counts were discarded and the middle three counts were utilized to give an average cell count per 0.05 ml. The highest and lowest cell counts were discarded to eliminate statistical and operational outriders. The maximum number of cells per 0.05 ml (average of 2 replicates) attained during the 18 days for each of the treatments was plotted with respect to AMW or sewage concentration (Figures I and II).

Results

Figure I shows the effect of AMW on the maximum cell number (plateau growth) of *C. vulgaris* attained in different concentrations of sewage. The differential effect of AMW was most pronounced with a concentration of 1/2 sewage. At 1/4 sewage the three lower levels of AMW yielded nearly the same maximum cell number, but for 1/8 AMW no increased growth was noted as compared to the controls. A general increasing trend of maximum cell number was indicated for the three lower AMW levels as the sewage levels increased from 0/0 to 1/4. A large non-linear interacting effect of AMW and sewage concentrations was noted on the maximal cell number attained.

The effect of sewage on the maximum growth of *C. vulgaris* in different concentrations of AMW is presented in Figure II. There appeared to be little effect of 1/32 of 1/16 sewage concentration level on maximal cell number as compared to the controls (0/0). One eighth dilution of sewage, however, showed a more pronounced increase in maximum growth with the addition of 1/32 AMW. Higher levels of AMW might have inhibited growth somewhat. At one fourth sewage concentration an increase in algal growth was noted with 1/16 concentration of AMW, followed by a rapid drop to essentially no growth in 1/8

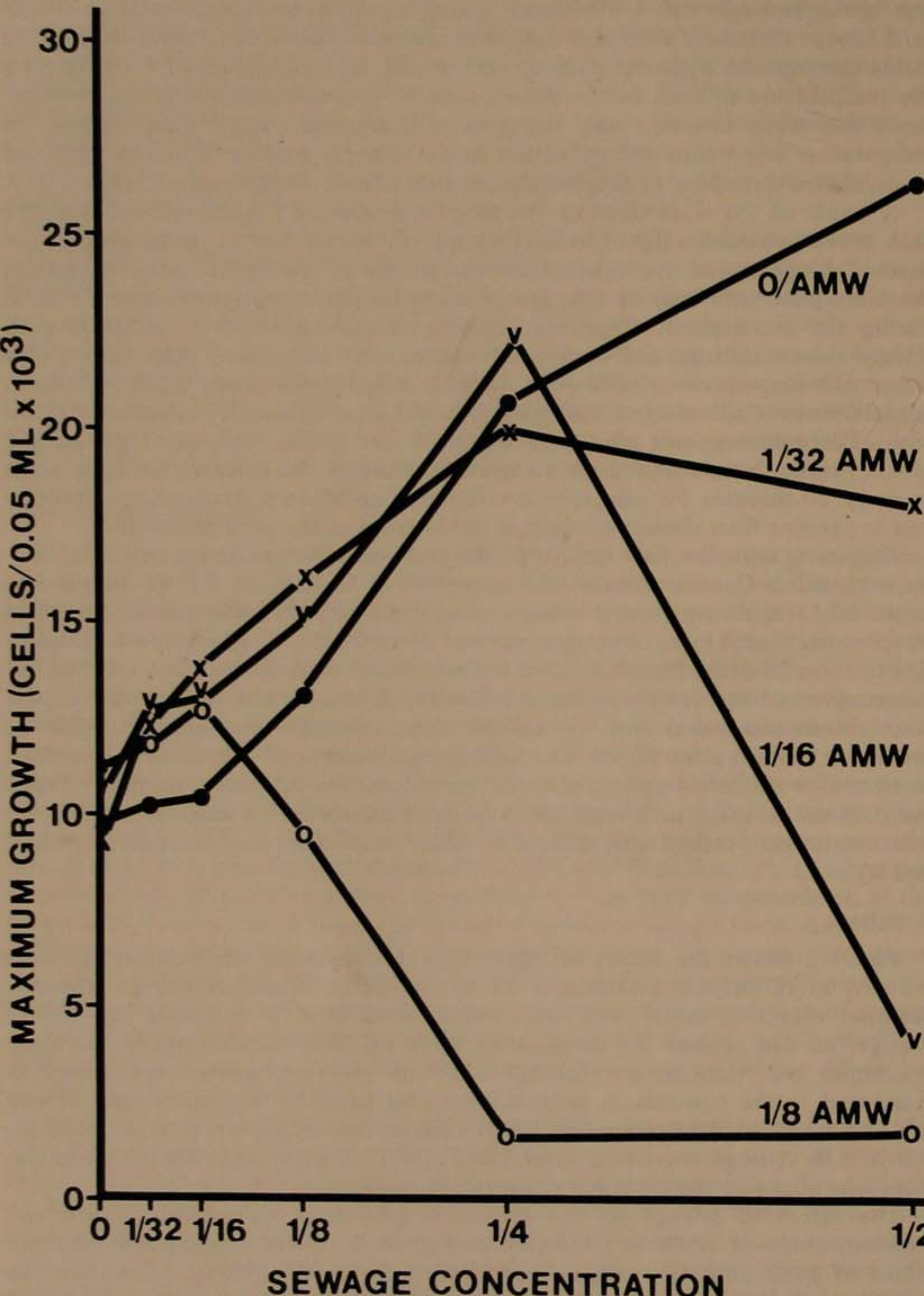

FIGURE 1. Maximum growth attained by *Chlorella vulgaris* in four concentrations of Acid Mine Water (AMW) plotted as a function of Sewage Concentration (light intensity and temperature held constant).

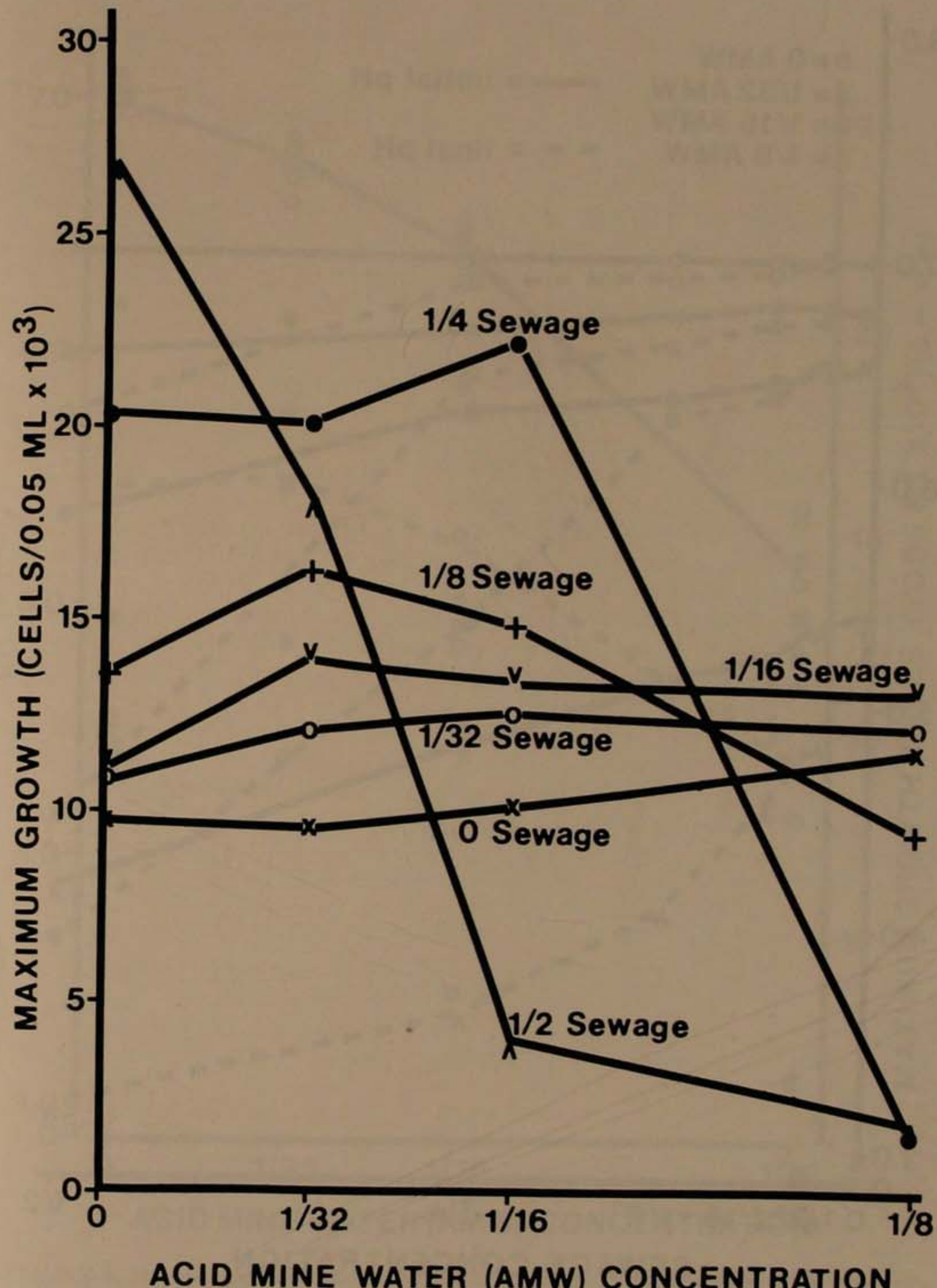

FIGURE 2. Maximum growth attained by *Chlorella vulgaris* in six concentrations of synthetic sewage plotted as a function of Acid Mine Water (AMW) Concentration (light intensity and temperature held constant).

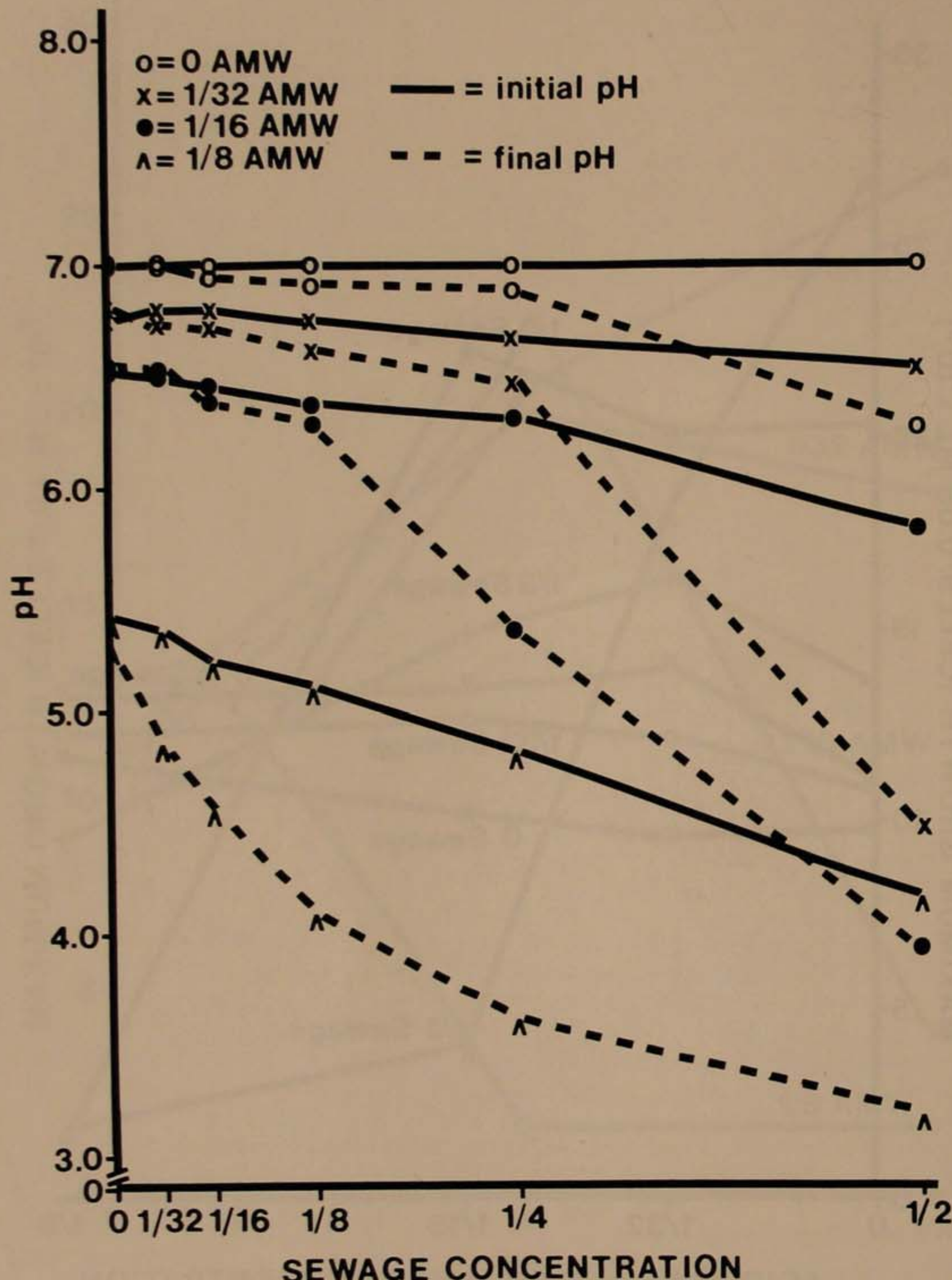

FIGURE 3. Initial and final pH of *Chlorella vulgaris* cultures plotted by Acid Mine Water (AMW) Concentration as a function of Sewage Concentration. Each point represents average of two replicates.

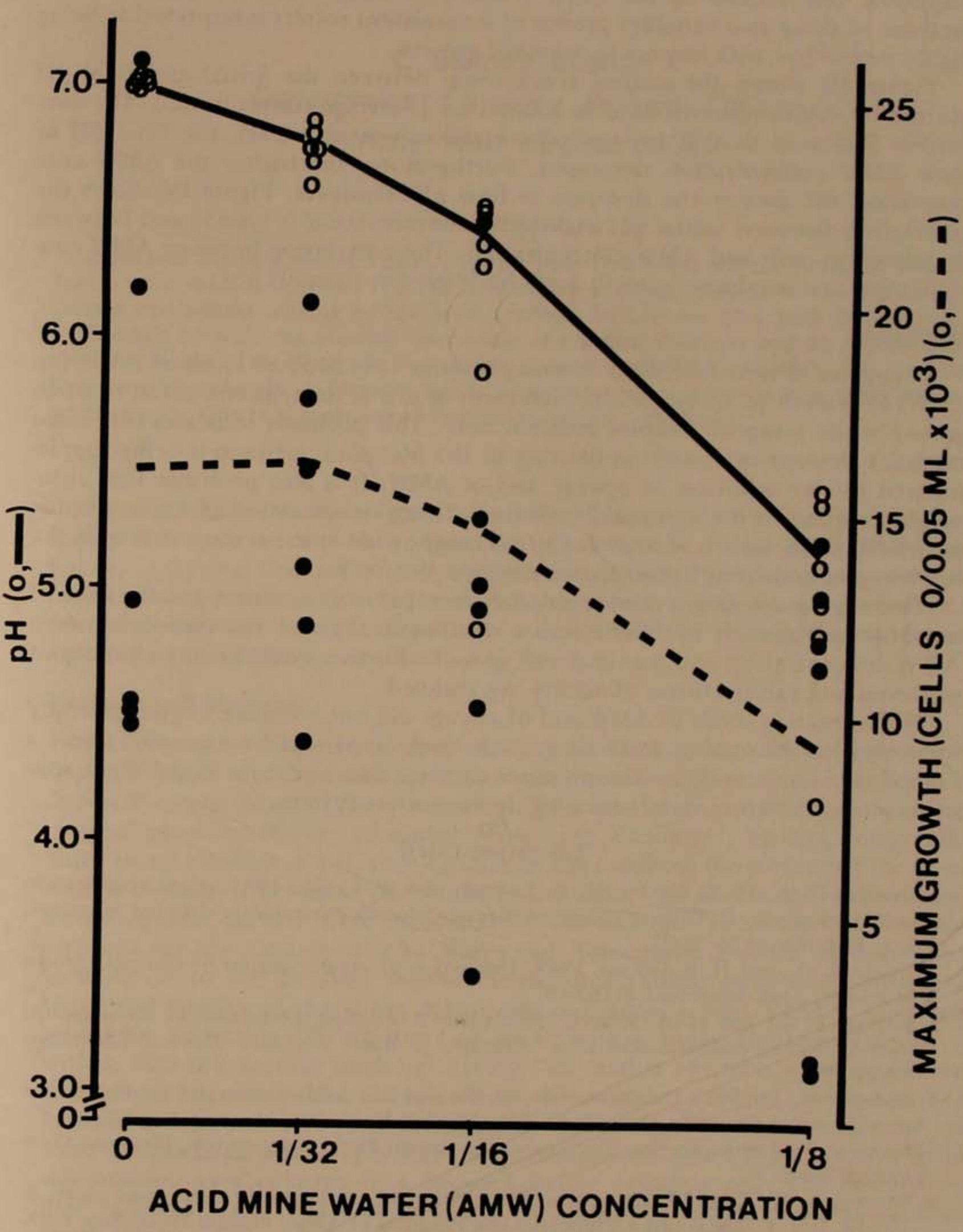

FIGURE 4. Maximum growth and initial pH of *Chlorella vulgaris* cultures in six concentrations of sewage plotted as a function of Acid Mine Water (AMW) concentration (r for initial pH vs. AMW concentration = −.063; r for AMW Concentration vs. maximum growth = −.52, significant P greater than .01).

AMW concentration. One half sewage concentration apparently produced the greatest growth in the absence of AMW, exhibiting a precipitous decrease in maximum cell number as the AMW levels were increased. Again, the additive increase of these two variables produced inconsistent results interpreted as being highly interactive with respect to maximal growth.

Figure III shows the general relationship between the initial and final pH plotted by AMW concentration as a function of sewage concentration. The data further indicated that as sewage concentration was increased, the final pH of each AMW concentration decreased. Furthermore, the higher the AMW concentration the greater the decrease in final pH. However, Figure IV shows the correlation between initial pH and AMW concentration (r=-.063) and between maximum growth and AMW concentration. The correlation between AMW concentration and maximum growth is r=-.52 (P greater than .01).

Discussion

It appears that at low levels of sewage (up to 1/4) and low levels of AMW (up to 1/16) growth of *C. vulgaris* is increased, in this system, as compared to when grown in the inorganic culture medium only. This probably indicates that some essential element or condition limiting in the inorganic medium is being supplemented by the addition of sewage and/or AMW. It is also probable that autotrophic growth in the inorganic medium is being accompanied by heterotrophic growth in the presence of organic carbon compounds in the sewage and/or in the AMW thereby yielding higher maximum growth of cells.

There is no consistent relationship between pH of the culture and the level of maximal cell growth but there was a significant negative correlation between AMW concentration and maximal cell growth. Further studies using a variety of pH levels and various forms of acidity are planned.

The increasing levels of AMW and of sewage did not combine to give generally increasing or decreasing maximal growth levels as would be expected under a completely linear system. The presence of these two variables in different concentrations, therefore, produces a highly interactive system.

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Effects of Various Cations and Anions on Several Species of Cladocerans Determined by Factorial Experiments on Survival*

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Abstract

Clones of *Daphnia magna* Straus, *Daphnia pulex* (de Geer) and *Ceriodaphnia reticulata* Jurine were studied in laboratory and field experiments with respect to the effects of the cations and anions making up the total alkalinity component of a fresh water pond. A relationship between the seasonal fluctuation of the total alkalinity and the populations of these organisms in the pond was found. LD_{50} values for $KHCO_3$, $NaHCO_3$, KCl, and NaCl have been determined. A factorial design experiment was used to analyze these values to determine the effects of these anions and cations on the organisms.

Field studies were conducted from July 1967 to July 1969 at two adjacent spring fed fresh water ponds, Pond 18 (Cascade Lake) and Pond 20 (Geyser Lake), in Spring Grove Cemetery, Cincinnati, Ohio. The average depth of the ponds was approximately twelve to fifteen inches ranging from four inches to four and one half feet. Total surface area of Pond 18 was approximately one acre and of Pond 20 approximately four acres.

Methods and Materials

The organisms studied belonged to the order Cladocera; in Pond 18 they were *Daphnia pulex* de Geer and *Ceriodaphnia reticulata* (Jurine) and in Pond 20 *Daphnia magna* Straus. The parameters measured in the ponds were dissolved oxygen, phenolphthalein alkalinity (carbonate alkalinity), methyl orange alkalinity (total alkalinity), pH, foot candles of light striking the surface of the pond, carbon dioxide (free) and continuous recordings of air and water temperatures. These aquatic factors were measured by the methods prescribed in *Standard Methods for the Examination of Water and Wastewater*, Twelfth Edition, 1965. In addition to the physical measurements, observations were made on the activity and density of the above mentioned microcrustaceans. Density was determined by collecting 10.065 liters of water with a Juday Plankton Trap fitted with a #20 silk bolting cloth net having 173 meshes per inch with an aperture size of 0.076 mm (Welch 1948). From this sample, a 30 ml. vial was filled, labeled and returned to the laboratory for counting. The organisms in the vial were anesthetized with 1 percent chloretone (Anderson 1933) and five 1 ml. sub-samples were counted in a Sedwick-Rafter counting cell. The density per liter of pond water was determined by applying the average number obtained from the counting to the following formula modified from Hazelwood and Parker (1961):

*This study was conducted at the University of Cincinnati, Department of Biological Sciences and supported in part by Public Health Service Grant 1-P10-ES00159.

N = NUMBER OF ORGANISMS PER LITER OF POND WATER

$$N = \frac{\text{(AVERAGE NO. OF ORGANISMS IN FIVE ONE ML COUNTS) X 1000}}{\text{RATIO OF 30 ML SAMPLE TO VOLUME OF WATER SAMPLED i.e. "DILUTION FACTOR"}}$$

The laboratory investigation was to determine whether variations in the composition of the pond water have an effect upon the density of any group of microcrustacea. Water samples from collecting stations in Ponds 18 and 20 were analyzed for 25 inorganic ions by the laboratory of the Water Quality Office of the Environmental Protection Agency in Cincinnati, Ohio. Three subsequent samples were analyzed at three month intervals to check for consistency. Seasonal variations of the populations and environmental factors were tested to see whether there were any changes in organism density that might coincide with changes in concentration of one or more of the elements. A correlation was found between the fluctuation of total alkalinity and carbonate alkalinity and the members of the ponds being studied. As a result of this correlation, tests were made to determine the sensitivity of the organisms to the component anions making up the total and carbonate alkalinity of the ponds. Bicarbonate, hydroxide and carbonate were the anions tested as sodium and potassium compounds. As an analytical example, I will deal only with the effects of the bicarbonate ion on the organisms as compared with the effects of the chloride ion standard. A logarithmic dilution series of a 1 M. stock solution was used to determine the lethal dose concentration threshold for 50 percent of the organisms (LD_{50}). Eight logarithmically spaced concentrations were tested twice. Each test was comprised of 10 organisms per concentration and 80 control organisms. The organisms were tested in 400 ml beakers at 50F for 48 hours. Six clones of *Daphnia magna*, two clones of *Daphnia pulex* and one clone of *Ceriodaphnia reticulata* were treated. Using the Spearman-Karber method, two LD_{50}'s were determined for each clone tested in the four compounds (KCl, NaCl, $NaHCO_3$ and $KHCO_3$). Once the clonal LD_{50}'s were obtained, they were tested in a factorial block design (Table 1) to determine whether there was a cation, anion or interaction effect.

Table 1. Experimental Factorial Design

TOTAL	a	b	A	B	AB
Yt1	0	0	-1	-1	1
Yt2	1	0	1	-1	-1
Yt3	0	1	-1	1	-1
Yt4	1	1	1	1	1

	Cl	HCO_3
Na	a_0b_0 Yt1	a_1b_0 Yt2
K	a_0b_1 Yt3	a_1b_1 Yt4

a_0 = Cl; a_1 = HCO_3
b_0 = Na; b_1 = K

Derivation of the Effects of Anion (A), Cation (B) and Interaction (AB).

$$\text{Effect of A} = \frac{(Yt2 = Yt4) - (Yt1 = Yt3)}{2n}$$

$$\text{Effect of B} = \frac{(Yt3 = Yt4) - (Yt1 + Yt2)}{2n}$$

$$\text{Effect of AB} = \frac{(Yt1 + Yt4) - (Yt2 + Yt3)}{2n}$$

The different combinations of the Yt's are derived from the arrangement of the 1's and -1's under the appropriate columns in the upper left table.

Experimental Results

A variance estimate was calculated for all sets of clonal tests to determine if the between clone variance was homogeneous or equal. By using Cochran's Test for Homogeneity of Variance, the clonal variance estimates were equal, enabling the use of a pooled variance estimate which is a better estimator of the true variance with an increased number of degrees of freedom. An Analysis of Variance table (ANOVA) was then computed to determine the presence or absence of three relationships, namely, a treatment effect for the entire data, clonal

Table 2. Analysis of Variance for Bicarbonate Series

Source	*SSQ*	*df*	*MSQ*	*F*
Between Treatments	21.758429	3	7.252809	405.86**
Between Clones	1.343981	8	0.167997	9.40**
Interaction	1.255907	24	0.052329	2.92**
Error	0.643328	36	0.017870	
Total	25.001645	71		

**F value significant at 0.01 level.

Table 3. Relative Potency Estimates, 95 Percent Confidence Limits and Main Effects for Bicarbonate Series.

	EFFECT OF ANION			EFFECT OF CATION		
Clone Number	*Potency Estimate*	*Confidence Limits lower limit upper limit*	*Effect**	*Potency Estimate*	*Confidence Limits lower limit upper limit*	*Effect**
9	0.85119	0.45568 1.58997	NE	0.96557	0.03510 0.12248	15x
12	0.53683	0.28739 1.00278	NE	0.04832	0.02587 0.09026	21x
18	1.02178	0.54701 1.90866	NE	0.09984	0.05344 0.18649	10x
600	1.09964	0.58869 2.05410	NE	0.10770	0.05766 0.20119	9x
A_4	1.09660	0.58706 2.04839	NE	0.09604	0.05141 0.17940	10x
129	0.95041	0.50879 1.77532	NE	0.09764	0.05227 0.18239	10x
T_1	0.67828	0.36311 1.26699	NE	0.06695	0.03584 0.12506	15x
700	0.60195	0.32225 1.12442	NE	0.11142	0.05965 0.20814	NE
Cerio 1	0.32957	0.17643 0.61563	3x	0.06444	0.03449 0.12037	15x

NE = No effect.
*Numbers in Effect columns represent the proportion of Cl to HCO_3 and Na to K to achieve the same result of death in the organisms.

Table 4. Relative Potency Estimates, 95 Percent Confidence Limits and Interaction Effects for Bicarbonate Series.

Clone Number	*Potency Estimate*	*INTERACTION EFFECT* *Confidence Limits* *lower limit* *upper limit*	*Effect 1**	*Effect 2**
9	0.73324	0.39253 1.36966	NE	NE
12	0.58907	0.31535 1.10036	NE	NE
18	1.28336	0.68704 2.39726	NE	NE
600	1.60959	0.86169 3.00664	NE	NE
A_4	1.05546	0.56603 1.97156	NE	NE
129	1.21112	0.64837 2.26231	NE	NE
T_1	1.40906	0.75433 2.63207	NE	NE
700	2.28292	1.22745 4.28292	4x	2x
Cerio 1	0.96286	0.51546 1.79856	NE	NE

NE = No Effect.
*Effect 1 compares Na and K as HCO_3 compounds and Effect 2 compares Na and K as Cl compounds.

differences due to the treatments and clonal interaction due to the treatments. Table 2 shows that all F tests for the above entries are significant at the 1 percent level. The ANOVA table shows the effects on the over all level, however, to ascertain the effect on the clonal level, the clones must be analyzed by the factorial block design (Table 1). Tables 3 and 4 illustrate the results of this analysis. These numbers represent relative potencies of the anions, cations and interactions. The significance of the relative potency estimates is based on a relationship that exists when it takes equal amounts of the ions being compared to achieve the same effect. When the number one is between the confidence limits and $p = 0.95$ there is no difference between the ions in terms of effect and the potency estimate is judged non-significant (Finney 1971). In the interpretation of these relative potencies the dilution assumption is made i.e. one cation is acting as a dilution of the other to achieve the same end result of death of the organisms.

Discussion

In this series, all cation effects were significant. There was a significant anion effect for the *Ceriodaphnia reticulata* clone and a significant interaction effect for Clone 700 (*Daphnia magna*). Tables 3 and 4 show the main effects and interaction effects with respect to ion combinations and clones. There does not appear to be any rearing temperature effect on the resistance or susceptibility of the clones to Na or K. That is, the clones reared at 15C do not appear to be either more susceptible or resistant to the ions than do the clones reared at 21C. Since *Ceriodaphnia reticulata* is the only clone that showed an anion effect and Clone 700 (*Daphnia magna*) is the only clone that exhibited an interaction effect, there is a possibility of a species specific susceptibility or resistance to ion concentrations. The *C. reticulata* relationship to the anion effect may in fact be true. This statement may be interpreted in two ways. For example, *C. reticulata*, in almost every testing sequence, proved to be in the most susceptible group for any specific ion potency. Anderson (1946) found the $NaHCO_3$ was innocuous to *Daphnia sp.* except when the concentration was high enough to exert an unfavorable osmotic pressure (greater than 2350 ppm threshold value.) However, he mentioned nothing about effects of bicarbonate concentration on other Cladocera. While $NaHCO_3$ may not affect *Daphnia*, it may be toxic to *C. reticulata*. On the other hand, since the confidence limits for the relative potency values have a $p = 0.95$, there is a possibility of having a positive effect by pure chance.

The interaction effect of *Daphnia magna* (Clone 700) may be true, but with reference to the work of Anderson (1946, 1950) this seems unlikely. This positive effect measured only the interaction between the cations in this testing series and Anderson (1950) found that it took approximately eleven times more sodium as a chloride compound than potassium as a chloride compound to achieve the same effect; a result much higher than that found in Tables 3 and 4. However, there is a possibility that this interaction also is positive by pure chance.

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Fatty Acid Production by Nine Bacterial Isolates From Household Refuse[1]

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Abstract

Five media, three classed as natural and two as synthetic, were inoculated with nine bacterial isolates from household refuse. After incubation periods ranging from 5 to 30 days the fatty acid production was determined. All isolates produced acetic acid in varying amounts in all media. Only acetic acid was produced in the soil extract medium but valeric and/or butyric acids were produced in refuse extract medium, soil-refuse extract medium, and glucose medium. In addition, iso-valeric and iso-butyric acids were produced in the Tryptone Medium. Except acetic acid, all organisms did not produce all the acids, in all the media. The amounts also varied.

Anyone who has ever experienced the odors of fatty acids and also has ever obtained samples from the refuse of a sanitary landfill knows that such acids are at least one of the products of decomposition.

In a simulated sanitary landfill, Lin (1) found that acetic acid was the only fatty acid formed during the first few days of decomposition. In addition its concentration remained rather constant. Soon, however, butyric acid appeared and became the predominate fatty acid during the first two months. Other acids occurring in varying amounts over a period of time were propionic, isobutyric, valeric, isovaleric, and caproic.

Thompson (3) observed that anaerobic conditions and a moisture content of at least 60 per cent were necessary for the production of all seven of the short chain fatty acids during refuse decomposition. Moistures below 30 per cent and an incubation temperature of 55 C inhibited synthesis of all seven of these short chain acids.

In a laboratory study Songonuga (2) also obtained the same acids reported by Lin during the anaerobic decomposition of fats, carbohydrates and proteins at 45, 60, and 80 per cent moisture and incubated at 25 C, 35 C and 45 C.

This study was undertaken to determine, if in pure culture, selected bacteria isolated from sanitary landfill materials would produce any of the C_2-C_6 fatty acids.

Materials and Methods

Five media, as follows, were used in the study. Three of the media considered natural media were: soil extract medium; refuse extract medium; and, soil-refuse extract medium.

Fifty grams of each substance were added to 450 ml of 0.025 M phosphate buffer and steamed for 1 hour. After filtration through Whatman #42 filter

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paper the filtrate was readjusted to pH 6.5. Each liter of filtrate was supplemented with 0.5 g sodium thioglycollate, 0.5 g agar, and 0.002 g methylene blue. The media were dispensed in 24.9 ml portions into screw cap test tubes and autoclaved at 121 C for 15 minutes.

Glucose or tryptone, equivalent to 5 g C/1, was the carbon source in the synthetic media. Other nutrients were: K_2HPO_4, 1.00; $CaCO_3$, 10.00; $MgSO_4 \cdot 7 H_2O$ plus the following: $MnSO_4$ H_2O (=0.1 mg Mn) 0.30 mg; $ZnSO_4 \cdot 7 H_2O$ (=0.2 mg Zn) 0.80 mg; $NaMoO_4 \cdot 2 H_2O$(=0.1 mg Mo) 0.25 mg; $Fe(NO_3)_3 \cdot 9 H_2O$ (=0.5 mg Fe) 3.40 mg; Nicotinic acid 0.30 mg; Ca-pantothenate 0.02 mg; riboflavin 0.05 mg; thiamine chloride 0.005 mg; biotin 0.002 mg; p-aminobenzoic acid 0.005 mg; pyridoxine 0.007 mg; sodium thioglycollate 0.50 g; agar 0.50 g; methylene blue 0.002 g; and distilled water to 1000 ml.

The nine bacterial isolates from refuse used were: *Bacillus cereus, Bacillus circulans, Bacillus laterosporus, Bacillus polymyxa, Clostridium perfringens, Clostridium tetani, Aerobacter aerogenes, Proteus vulgaris,* and *Serratia marcescens.*

Each isolate was used to inoculate Brewer Thioglycollate Medium*, incubated for 24 hours at 25 C, centrifuged at 4 C at 8000 G and the pellet resuspended in 20 ml of 0.025 M phosphate buffer. Several tubes of each medium were inoculated with 0.1 ml of each isolate suspension. Incubation was at 25 C. Tubes were removed at 5-day intervals for a total of 6 harvests. After incubation a 10 ml sample of medium was adjusted to pH 2.5 with 5 per cent H_3PO_4 and then neutralized with 0.1 N NaOH and evaporated to dryness. To the residue 99 ml acetone and 1 ml of 60 per cent H_3PO_4 were added. The tubes were then capped and stored for at least 24 hours in a refrigerator. This procedure released the fatty acids while unwanted materials were precipitated. The solution was then filtered through a Whatman #42 filter paper and the filtrate stored at -20 C until analyzed. A 2.5-microliter portion was injected into the gas chromatograph.

The gas chromatograph was a Barber-Colman Series 5000 equipped with hydrogen ionization flame detector. The column was a 6 ft. x 5 mm U-shaped glass tubing packed with 16 per cent Carbowax 20M plus 2 per cent H_3PO_4 coated on 60/80 mesh HMDS** treated Chromosorb W. The sequence of fatty acid elution was: acetic, propionic, isobutyric, butyric, isovaleric, valeric and caproic. The peak areas were determined by a Model 205 Disc Chart Integrator.

Results

Size of Inocula

Because of the different growth rates of the bacteria in Brewer Thioglycollate Broth the number of cells in 0.1 ml, the amount used as inoculum into the various media, varied considerably as shown: *B. cereus*, 2.04×10^6; *B. circulans*, 1.36×10^6; *B. laterosporus*, 1.2×10^6; *B. polymyxa*, 0.1×10^6; *Cl. perfringens*, 1.44×10^6; *Cl. tetani*, 0.016×10^6; *A. aerogenes*, 21.8×10^6; *P. vulgaris*, 1.12×10^6; and *S. marcescens*, 40×10^6.

Soil Extract Medium

In this medium acetic was the only acid produced by the bacteria. Three bacteria, *Cl. tetani, B. cereus,* and *P. vulgaris* reached a peak on the third harvest

*Bacto-Difco, Detroit, Michigan.
**Hexamethyldisilazane.

and *B. cereus* on the 5th harvest. The acetic acid production of the other bacteria fluctuated (Table 1).

Refuse Extract Medium

A general increase in acetic acid production followed by a leveling off or decline was observed for *Cl. perfringens, Cl. tetani,* and *A. aerogenes* during the 30 days of incubation. Except for *B. circulans,* whose acetic acid production continued to increase, the acetic acid production of the other organisms showed fluctuations from harvest to harvest (Table 2). *Cl. perfringens, B. circulans* and *P. vulgaris* produced trace to small amounts of valeric acid beginning with the second harvest, and *B. cereus* produced trace amounts of butyric acid throughout the 30 days of incubation.

Soil-Refuse Extract Medium

Acetic acid production by all of the organisms fluctuated from harvest to harvest in this medium. The largest quantity of acetic acid was produced by *B. laterosporus.* In fact, this was the largest quantity of acetic acid produced by any of the bacteria in any media. *B. circulans* and *B. laterosporus* also produced traces of butyric acid at the first harvest but at no other harvest. On the other hand, *Cl. perfringens* and *P. vulgaris* produced butyric acid at later harvests (Table 3).

Glucose Medium

All of the bacteria showed fluctuations in acetic acid production during the 30-day incubation period. *B. circulans, Cl. perfringens, Cl. tetani* and *P. vulgaris* produced butyric acid in addition to acetic acid. Except for *P. vulgaris,* at some harvests the butyric acid exceeded the quantity of acetic acid. *B. cereus, P. polymyxa, B. laterosporus, A. aerogenes,* and *S. marcescens* produced only acetic acid (Table 4).

Tryptone Medium

Cl. perfringens, Cl. tetani, and *P. vulgaris* were the only bacteria to produce acetic acid by the first harvest, but all had produced this acid by the second harvest. *B. cereus* produced increasing amounts of acetic acid throughout the 30 days but the amounts produced by the other bacteria fluctuated. *Cl. tetani* and *B. polymyxa* produced isobutyric and isovaleric acids. *Cl. tetani, Cl. perfringens* and *B. cereus* also produced butyric acid (Table 5).

Discussion

Acetic acid was produced in all media by each of the bacteria although it was not detected until the second harvest in Tryptone Medium for six of the bacteria.

In the soil extract medium no acid, except acetic, was detected. Butyric acid was detected in small quantities in the refuse medium inoculated with *B. cereus.* In this medium valeric acid was detected using *B. circulans, Cl. perfringens,* and *P. vulgaris* as the inocula.

Butyric acid was produced in soil-refuse extract medium by *B. circulans, B. laterosporus, Cl. perfringens* and *P. vulgaris.*

In addition to acetic acid, butyric acid was produced in the glucose medium by *B. circulans, Cl. perfringens, Cl. tetani* and *P. vulgaris.*

Table 1. Production of Acetic Acid (mg/l) in Soil Extract Medium

Organisms	Days of Harvest					
	5 days	*10 days*	*15 days*	*20 days*	*25 days*	*30 days*
Bacillus cereus	110	192	352	361	401	389
B. circulans	215	350	267	465	450	411
B. laterosporus	221	332	295	469	480	460
B. polymyxa	93	350	305	340	280	317
Clostridium perfringens	62	216	333	276	280	306
Cl. tetani	116	316	305	237	221	215
Aerobacter aerogenes	135	428	344	453	421	436
Proteus vulgaris	50	279	295	243	215	189
Serratia marcescens	65	264	259	701	610	539

Table 2. Production of Acetic Acid (mg/l) in Refuse Extract Medium.

Organisms	Days of Harvest					
	5 days	*10 days*	*15 days*	*20 days*	*25 days*	*30 days*
Bacillus cereus	244'	252'	243'	123'	130'	118'
B. circulans	20	36*	45*	272*	290*	313*
B. laterosporus	477	58	231	194	173	201
B. polymyxa	244	117	202	215	225	240
Clostridium perfringens	12	97*	136*	201*	231*	202*
Cl. tetani	106	113	202	201	221	216
Aerobacter aerogenes	46	56	178	237	150	167
Proteus vulgaris	22	55*	219*	311*	289*	249*
Serratia marcescens	55	178	85	260	103	173

'Traces of butyric acid (less than 10 mg/l) also present.
*Trace to small amounts (10-30 mg/l) of valeric acid also present.

Table 3. Production of Acetic Acid (mg/l) in Soil-Refuse Extract Medium.

Organisms	*Days of Harvest*					
	5 days	*10 days*	*15 days*	*20 days*	*25 days*	*30 days*
Bacillus cereus	466	109	178	356	317	290
B. circulans	537*	81	200	856	960	908
B. laterosporus	461*	84	231	2,687	3,100	2,973
B. polymyxa	103	79	243	1,013	2,053	1,751
Clostridium perfringens	235	152*	142*	283*	277*	291*
Cl. tetani	113	102	154	256	267	259
Aerobacter aerogenes	51	40	405	540	605	681
Proteus vulgaris	319	70	163*	599*	401*	436*
Serratia marcescens	761	131	202	416	826	506

*Traces of butyric acid (less than 10 mg/l) also present.

Table 4. Production of Fatty Acids (mg/l) in Glucose Medium.

Organisms	Days of Harvest					
	5 days	*10 days*	*15 days*	*20 days*	*25 days*	*30 days*
Bacillus cereus						
Acetic acid	18	75	240	171	215	308
B. circulans						
Acetic acid	30	561	242	129	132	117
Butyric acid	14	30	90	156	130	100
B. laterosporus						
Acetic acid	36	283	182	130	75	319
B. polymyxa						
Acetic acid	26	608	400	166	310	350
Clostridium perfringens						
Acetic acid	103	600	364	165	184	101
Butyric acid	2	10	80	100	101	116
Cl. tetani						
Acetic acid	54	607	152	162	210	127
Butyric acid	8	20	60	150	160	168
Aerobacter aerogenes						
Acetic acid	34	20	166	164	651	474
Proteus vulgaris						
Acetic acid	36	374	179	162	362	232
Butyric acid	2	10	40	95	101	124
Serratia marcescens						
Acetic acid	16	370	170	200	475	232

Table 5. Production of Fatty Acids (mg/l) in Bacto-Tryptone Medium.

Organisms	Days of Harvest					
	5 days	*10 days*	*15 days*	*20 days*	*25 days*	*30 days*
Bacillus cereus						
Acetic acid	—*	51	109	381	484	460
Butyric acid	—	—	—	36	40	60
B. circulans						
Acetic acid	—	173	473	140	420	275
B. laterosporus						
Acetic acid	—	352	190	174	516	617
B. polymyxa						
Acetic acid	—	516	121	80	443	224
Isobutyric acid	—	—	9	17	8	31
Isovaleric acid	—	107	150	146	96	100
Clostridium perfringens						
Acetic acid	32	191	101	223	141	444
Butyric acid	—	—	30	57	75	87
Cl. tetani						
Acetic acid	13	186	404	182	177	315
Isobutyric acid	—	—	—	4	5	10
Isovaleric acid	—	44	92	100	91	85
Butyric acid	—	—	—	20	24	36
Aerobacter aerogenes						
Acetic acid	—	158	73	123	279	301
Proteus vulgaris						
Acetic acid	52	320	73	578	412	316
Serratia marcescens						
Acetic acid	—	324	89	297	555	615

*Acid not detected.

B. cereus, Cl. perfringens and *Cl. tetani* produced butyric acid in the Tryptone Medium. Isobutyric acid and isovaleric acid were also produced in the Tryptone Medium by *B. polymyxa* and *Cl. tetani.*

The largest quantities of acid were produced by *B. laterosporus* and *B. polymyxa* in the soil-refuse extract medium; 3100 mg/l and 2053 mg/l respectively, at the 5th harvest. No explanation is available for these increases.

Based upon total acid production at 30 days by all nine bacteria, the soil-refuse extract medium is by far the best medium; though closely followed by the Tryptone Medium. Based upon the 15-day production the soil extract medium is the best. The highest acid yields by individual organisms in media are as follows: *B. cereus, Cl. perfringens, Cl. tetani,* and *S. marcescens* produced more acid in Tryptone Medium than in any other media, whereas *B. circulans, B. laterosporus, B. polymyxa, A. aerogenes,* and *P. vulgaris* had higher yields of acid in the soil-refuse extract medium.

Microorganisms utilize carbohydrates, lipids, and proteins for energy production and/or reducing power for growth. Numerous pathways for anaerobic catabolism of these compounds exist with the organisms used.

Even and odd carbon chain acids, C_2-C_5, but no isomers, were detected with carbohydrate as the carbon source. Acetic acid was always the first acid detected and usually its quantities declined as longer chain acids appeared. Exceptions were with *B. laterosporus* and *B. polymyxa* which produced relatively small quantities of the longer chain acids. Acetic acid decline may result from reactions, i.e., head-to-tail condensation to form butyrate, and condensation with lactate, such as occurs with many clostridia species. Propionate may arise by clostridial fermentation of carbohydrates. Numerous other end-products, for example, ethanol or lactate, may be further modified to yield short chain fatty acid.

Proteins, specifically amino acids, could give rise to C_2-C_5 acids, including the isomers isobutyric and isovaleric from leucine and isoleucine, respectively. Reductive deamination of amino acids, such as glycine to acetate or valine to valeric acids, produces numerous acids of the C_2-C_5 type. The Strickland reaction, primarily by clostridia, also yields numerous C_2-C_5 acids by oxidation-reduction reactions of amino acids.

Acetate may arise from long chain fatty acids, as hydrolytic products of lipids. Propionate may also arise from catabolism of a long odd carbon chain fatty acid or butyrate by condensation of two acetates. Propionate arising from carbohydrate and acetate from glycine to yield valerate also demonstrates the association of pathways in media such as refuse extract, which results in fatty acids.

These results contribute to our knowledge of the metabolic processes and products of microorganisms in sanitary landfills.

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Weed Control in *Taxus cuspidata* *

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Abstract

The following herbicides gave excellent weed control from July 5 to October 30 without injury to Japanese yew (*Taxus cuspidata* Sieb. and Zucc.) liners: *S*-propyl dipropylthiocarbamate (vernolate) 10 lb/A + 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine) 2 lb/A; *N,N*-dimethyl-2,2-diphenylacetamide (diphenamid) 8 lb/A + simazine 2 lb/A; *a,a,a*-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine (trifluralin) 2 lb/A + simazine 2 lb/A; 6-*t*-butyl-2-chloro-*o*-acetotoluidide (CP 31675) 4 lb/A; 3,4-dichlorobenzyl methylcarbamate (dichlormate) 12 lb/A; and 2-chloro-*N*-isopropylacetanilide (propachlor) 8 lb/A. Trifluralin 4 lb/A gave satisfactory weed control with the exception of common ragweed (*Ambrosia artemisiifolia* L.), goosegrass (*Eleusine indica* (L.) Gaertn.), and smooth crabgrass (*Digitaria ischaemum* (Schreb.) Muhl.). Two other triazines, 2-methylthio-4-isopropylamino-6-methoxypropylamino-*s*-triazine (G 36393) 2 lb/A, and 2-(*tert*-butylamino)-4-(ethylamino)-6-(methylthio)-*s*-triazine (terbutryn) 1 lb/A gave good control of annual broadleaf weeds but were weak in controlling goosegrass and smooth crabgrass. Differences in growth between yews given root dips of activated carbon and those without dips were not significant.

More information is needed regarding seasonal weed control in newly-set nursery liners. Many herbicides can be used safely around well-established woody plants without apparent damage but some of these may be too phytotoxic around small plants which have been recently transplanted. Herbicides that have been used frequently on *Taxus* without appreciable phytotoxicity are: simazine (1, 3, 4, 5, 6, 7, 9, 11, 14, 15, 16, 19, 20); *S*-ethyl dipropylthiocarbamate (EPTC) (1, 7, 8, 12, 14, 18); vernolate (12, 15, 20); trifluralin (4, 9, 12, 15, 20); dimethyl tetrachloroterephthalate (DCPA) (2, 10, 13, 15, 20); diphenamid (2, 9, 13, 16, 20); and 2,6-dichlorobenzonitrile (dichlobenil) (2, 6, 9, 12, 13, 15, 16, 19, 20).

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Although *Taxus* is generally tolerant of many herbicides, slight injury has been reported under some conditions. Ahrens (2) observed that the *capitata* variety of *T. cuspidata* is less tolerant of simazine than other *Taxus* species while Pridham (17) reported some injury when simazine was applied at 10 lb/A. EPTC 5 and 10 lb/A as soil incorporated treatments did not injure *T. media hicksii* planted within a week of application but bare root plants were injured by EPTC 15 lb/A where it was sprayed on roots or mixed with soil used to fill the planting holes (18). Dichlobenil at 4 or 6 lb/A injured *Taxus* transplants (2).

The objectives of the experiment were to evaluate several herbicides for weed control in *T. cuspidata* and to determine if root dips of activated carbon would decrease phytotoxicity, if any, from the herbicides.

Materials and Methods

The soil (sandy loam) was tilled with a rotary cultivator and smoothed with a rake. On July 1, 1964, two *Taxus* liners (10″-12″ in height) were set into each plot (4′ x 4′) after the root system of one plant for each plot was dipped into activated carbon (Aqua Nuchar A). The activated carbon was applied to determine whether the carbon would adsorb enough of a potentially phytotoxic herbicide to protect the plant. The plots were given about an inch of water with an oscillating sprinkler on July 2.

The herbicides, formulated as liquids or wettable powders, were applied with a two-gallon compressed air sprayer using a TeeJet 8004 flat fan nozzle. The amount of herbicide needed to spray a single plot was added to the tank with enough water to spray each plot. This procedure simplified the application of the predetermined rate. The granular herbicides were applied individually to each plot with a small crank-type duster. The herbicide applications, whether sprays or granules, were applied to entire plots without regard to the *Taxus* plants. This method simulated a broadcast, topical application without any effort to avoid herbicide contact with the *Taxus* liners.

The following herbicides were applied July 5, 1964 at random with four replications of each treatment: vernolate 10 lb/A + simazine 2 lb/A; diphenamid 8 lb/A + simazine 2 lb/A; trifluralin 2 lb/A + simazine 2 lb/A; CP 31675 4 lb/A; dichlormate 12 lb/A; propachlor 8 lb/A; trifluralin 4 lb/A; G 36393 (Geigy Agricultural Chemicals) 2 lb/A; and terbutryn 1 lb/A. The following herbicides were applied July 5, 1964 as single plot treatments: trifluralin 1 lb/A + diphenamid 4 lb/A; *N*-(3,4-dichlorophenyl)-2-methylpentanamide (Karsil) 4 lb/A + simazine 2 lb/A + vernolate 10 lb/A; simazine 2 lb/A; 2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine (prometryne) 2 lb/A; vernolate 10 lb/A; 2-(ethylamino)-4-(isopropylamino)-6-(methylthio-*s*-triazine (ametryne) 2 lb/A; 2-cyano-4-ethylamino-6-isopropylamino-*s*-triazine (G 10293) 2 lb/A; *a,a*-dimethylvaleryl acid-4-chloranilide (G 17269) 8 lb/A; DCPA 12 lb/A, and Karsil 4 lb/A.

Results and Discussion

The *Taxus* plants were examined in late July, August, and September for possible herbicide injury to the leaves. No injury was observed in the replicated trials. Karsil-treated plants in the non-replicated trials had some chlorotic and necrotic leaves which abscised during the middle of the summer. No evidence of foliage injury remained by September.

On October 30, 1964, the weeds were pulled, sorted into two groups (annual grass weeds and annual broadleaf weeds) and weighed. The average weights of weeds for replicated plots are given in Table 1 and for non-replicated plots in Table 2. These weights are approximate air-dried weights since most of the weeds had been killed by frost and they were allowed to dry in the sun about eight hours before weighing.

The most prevalent grass weeds found in the check plots were: barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), annual bluegrass (*Poa annua* L.), goosegrass, yellow foxtail (*Setaria lutescens* (Weigel) Hubb.), and smooth crabgrass. Broadleaf species present were: carpetweed (*Mollugo verticillata* L.), hairy galinsoga (*Galinsoga ciliata* (Raf.) Blake), ladysthumb (*Polygonum persicaria* L.), common lambsquarters (*Chenopodium album* L.), redroot pigweed (*Amaranthus retroflexus* L.), common ragweed, devils beggarticks (*Bidens frondosa* L.), common purslane (*Portulaca oleracea* L.), and common chickweed (*Stellaria media* (L.) Cyrillo).

Growth evaluations of *Taxus* plants with and without activated carbon root dips were not significantly different. Apparently the *Taxus* liners either were sufficiently tolerant to the herbicides used or the herbicides did not reach the roots in sufficient amounts to be phytotoxic.

Replicated Plots

All herbicide treatments significantly reduced the weights of grass weeds as compared to the check. The combination of vernolate + simazine was particularly outstanding; however, diphenamid + simazine, trifluralin + simazine, CP 31675, dichlormate, propachlor and trifluralin were also excellent, giving grass control of 93 per cent or better. G 36393 and terbutryn with weed control percentages of about 81 per cent were judged satisfactory on the basis of accepted standards for commercial weed control of 70 per cent or higher.

All herbicide treatments, except trifluralin and G 36393, significantly reduced weights of broadleaf weeds as compared to check. Vernolate + simazine, diphenamid + simazine, trifluralin + simazine, and dichlormate were excellent for control of broadleaf weeds. Propachlor and terbutryn gave very good control while CP 31675 would be considered satisfactory. G 36393 was not effective. Trifluralin failed because it did not control common ragweed which grew vigorously since most other broadleaf weeds were controlled.

Overall visual ratings of weed control (both grass and broadleaf weeds) made in August and again in October indicated that all treatments were satisfactory although G 36393 and terbutryn were weak on grass control, and trifluralin did not control ragweed. Since the initiation of this experiment other investigators (7, 13, 20) have reported the combination of diphenamid and simazine to give excellent weed control.

Non-Replicated Plots

Two combination treatments—trifluralin + diphenamid and Karsil + simazine + vernolate—appeared promising. Three "triazine" herbicides (simazine, prometryne and ametryne) gave satisfactory control of both grass and broadleaf weeds. Vernolate gave good grass control but poor control of broadleaf weeds, especially purslane. G 10293 and G 17269 apparently were satisfactory for controlling the broadleaf weeds present but not the grass weeds. DCPA gave borderline control of grass but failed to control broadleaf weeds, especially hairy galinsoga. Karsil generally failed to control either grass or broadleaf weeds. Crabgrass was the predominant grass weed in the "triazine" herbicide plots (simazine, G 10293, prometryne, and ametryne).

Table 1. Average[1] Weight of Weeds Pulled From *Taxus cuspidata* Plots Given Various Herbicide Treatments.

Treatment		*Annual Grass Weeds*		*Annual Broadleaf Weeds*	
Herbicide and Formulation	*Rate (lb/A)*	*(Grams/Plot)*	*% Control Based on Check*	*(Grams/Plot)*	*% Control Based on Check*
Vernolate[3], 10G + simazine, 80 wp	10 + 2	8 a[2]	99+	0 a	100
Diphenamid, 80 wp + simazine, 80 wp	8 + 2	38 b	99	0 a	100
Trifluralin[3], 4 ec + simazine, 80 wp	2 + 2	85 bc	98	0 a	100
CP 31675, 75 wp	4	46 ab	9	46 a	74
Dichlormate, 5G	12	143 bc	97	1 a	99+
Propachlor, 65 wp	8	190 bc	96	29 a	83
Trifluralin[3], 4 ec	4	363 c	93	290 c	-67
G 36393, 25 wp	2	912 d	82	78 ab	55
Terbutryn, 50 wp	1	970 d	81	34 a	80
Check	—	5089 e	—	174 b	—

[1] Average of four replicates each 4′ by 4′ weighed October 30, 1964 when weeds were nearly air dry.

[2] Treatment averages followed by a common letter are not significantly different from each other at the 5% level according to Duncan's Multiple Range Test.

[3] These herbicides were incorporated within 3 minutes after application by raking in both directions with a garden rake.

Table 2. Weight of Weeds[1] Pulled from Non-replicated *Taxus cuspidata* Plots Given Various Herbicide Treatments.

Treatment		*Annual Grass Weeds*	*Annual Broadleaf Weeds*
Herbicide and Formulation	*Rate (lb/A)*	*(Grams/Plot)*	*(Grams/Plot)*
Trifluralin, 10 wp + diphenamid, 40 wp	1 + 4	0	15
Karsil, 2 ec + simazine, 80 wp + vernolate[2], 10G	4 + 2 + 10	40	0
Simazine, 80 wp	2	84	0
Prometryne, 80 wp	2	110	0
Vernolate[2], 10G	10	76	120
Ametryne, 80 wp	2	260	0
G 10293, 50 wp	2	1020	67
DCPA, 75 wp	12	780	645
G 17269, 50 wp	8	2439	8
Karsil, 2 ec	4	3723	90
Check[3]	—	5089	174

[1]Weighed October 30, 1964, when weeds were nearly air dry. Plot size 4′ by 4′.
[2]Vernolate was incorporated within 3 minutes after application by raking in both directions with a garden rake.
[3]Check plot weights taken from replicated plot data.

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Chemical Weed Control in *Liriope (Liliaceae)* *

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Abstract

Thirty one herbicide treatments were evaluated for weed control and phytotoxicity on newly planted liriope (*Liriope muscari* cv. 'Moore's Majestic', *L muscari* cv. 'Variegata', and *L. spicata*). The five best treatments for weed control from July 14 to October 1 were: 3-*tert*-butyl-5-chloro-6-methyluracil (terbacil) 1.6 lb/A; 2,6-dichlorobenzonitrile (dichlobenil) 6 lb/A; 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (linuron) 4 lb/A; isopropyl *m*-chlorocarbanilate (chlorpropham) 8 lb/A; and the combination of 3-[*p*-*p*-chlorophenoxy)-phenyl]-1,1-dimethylurea (chloroxuron) 4 lb/A + *N,N*-dimethyl-2,2-diphenylacetamide (diphenamid) 6 lb/A. The following treatments were acceptable: 3,4-dichlorobenzyl methylcarbamate (dichlormate) 12 lb/A; *a,a,a*-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine (trifluralin) 1 lb/A; *S*-ethyl dipropylthiocarbamate (EPTC) 5 lb/A; 3-amino-2,5-dichlorobenzoic acid (as the methyl ester of chloramben) 4 lb/A; *S*-propyl dipropylthiocarbamate (vernolate) 6 lb/A and the combination of dichlormate 12 lb/A + diphenamid 6 lb/A. Dimethyl tetrachloroterephthalate (DCPA) 14 lb/A was satisfactory but not at 9 lb/A. Diphenamid 6 lb/A was satisfactory in one experiment but failed in the other. One herbicide, 3-amino-*s*-triazole (amitrole) 4 lb/A injured the liriope severely while two other herbicides, dichlobenil 6 lb/A and DCPA 14 lb/A seemed to reduce *Liriope* vigor appreciably.

Liriope, a member of the lily family (1), often is used as a ground cover in warm regions of the south (2,5) and in sheltered areas as far north as New York City (8). *Liriope spicata* referred to as Creeping lily-turf by Taylor (5) is a finer textured evergreen that resembles grass more than the taller, coarser *L. muscari* (8) which is called Blue lily-turf (5). It is tolerant of many soil types and is especially valuable on slopes and in shady areas where grass may be difficult to establish and maintain (2), but it is reported to be crowded out by severe weed competition especially grass from seed blown in from nearby lawn areas (6). *L. spicata* may be smothered by grass in three or four years so that it must be replaced (6,7) therefore control measures are necessary. Smith (3,4) suggested the use of diphenamid 4 lb/A + 2-chloro-4,6-bis(ethylamino)-*s*-triazine (sima-

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zine) 1 lb/A for *Liriope* established for one entire growing season. He also noted that DCPA 10.5 lb/A and diphenamid 6 lb/A can be used on most established ground covers so it may be assumed that *Liriope* is included. For many newly planted ground cover plantings trifluralin 1 lb/A and possibly DCPA 10.5 lb/A are recommended (3,4). Diphenamid 6 lb/A was recommended for use on new plantings (4).

The objectives of these experiments were to evaluate the effectiveness of selected herbicides in controlling grass and broadleaf weeds and to note the effects of the herbicide treatments on the vigor of newly planted *Liriope* plants.

Materials and Methods

A preliminary greenhouse experiment was conducted during 1967 and 1968 in which fourteen herbicide treatments were applied to two cultivars of *L. muscari* ('Moore's Majestic' and 'Variegata') and to *L. spicata* grown in No. 10 cans. The treatments were: chloroxuron 4 lb/A; DCPA 9 lb/A; dichlormate 12 lb/A; diphenamid 6 lb/A; 1-butyl-3-(3,4-dichlorophenyl)-1-methylurea (neburon) 4 lb/A; 4-(methylsulfonyl)-2,6-dinitro-*N,N*-dipropylaniline (nitralin) 4 lb/A; 2-chloro-*N*-isopropylacetanilide (propachlor) 6 lb/A; 2-(2,4-dichlorophenoxy)-ethyl sodium sulfate (sesone) 4 lb/A; simazine 2 lb/A; chloroxuron 4 lb/A + diphenamid 6 lb/A; chloroxuron 4 lb/A + nitralin 4 lb/A; DCPA 9 lb/A + sesone 4 lb/A; dichlormate 12 lb/A + diphenamid 6 lb/A; and diphenamid 6 lb/A + nitralin 4 lb/A. The applications were made May 11-18, 1967 and again August 16, 1968. All herbicides, except dichlormate, formulated as wettable powders (wp) were applied in water at 30 psi at the rate of 100 gpa with a single TeeJet 8006E nozzle. Pressure was supplied by an air compressor mounted on a small garden tractor. Dichlormate was applied by hand as a granule to each can. The treatments were replicated six times on each *Liriope* cultivar or species.

Three field experiments were conducted during 1969. In the first experiment the same herbicide treatments used in the greenhouse were applied to *L. spicata* plants set in the field. In the second field experiment the following treatments were applied to *L. muscari* 'Moore's Majestic': amitrole 4 lb/A; chlorpropham as "Furloe" 8 lb/A; DCPA 14 lb/A; dichlobenil 6 lb/A; diphenamid 6 lb/A; EPTC 5 lb/A; simazine 4 lb/A; terbacil 1.6 lb/A; and trifluralin 1 lb/A. Treatments applied to *L. muscari* 'Variegata' in the third experiment were: 2-chloro-2′6′-diethyl-*N*-(methoxymethyl) acetanilide (alachlor) 2 lb/A; 0,0-diisopropyl phosphorodithioate *S*-ester with *N*-(2-mercaptoethyl)benzenesulfonamide (bensulide) 4 lb/A; chloramben as methyl ester 4 lb/A; 2-chloro-4-cyclopropylamino-6-isopropylamino-1,3,5-triazine (cyprazine) 1 lb/A; linuron 4 lb/A; *S*-propyl butylethylthiocarbamate (pebulate) 6 lb/A; vernolate 6 lb/A; diphenamid 6 lb/A + simazine 1 lb/A; and EPTC 5 lb/A + simazine 1 lb/A.

The field was plowed and disced about the middle of June, 1969 but rainy weather prevented setting of *Liriope*. The field was then cultivated with a rotary tiller on July 6 to kill the weeds that had germinated. Five *Liriope* plants were set about one foot apart in the center of plots 6 ft by 12 ft. The planting was rototilled again prior to the application of herbicides on July 14. The herbicides were applied with a small 4-wheel garden tractor equipped with a boom containing TeeJet 8004 tips. A constant spraying pressure of 31 psi was maintained with a carbon dioxide tank and regulator. The herbicides in granular form were broadcast by hand. The herbicides requiring incorporation were worked into the soil with a single pass of the rotary tiller within five minutes after application. The treatments within each of the seven replications were assigned at random.

Between August 13 and October 1, 1969 the experimental plots were evaluated at weekly intervals as to overall weed control, individual weed control, and *Liriope* vigor. Eight evaluations were made. Data were analyzed by analysis of variance and compared by Duncan's Multiple Range Test.

Results and Discussion

Preliminary Experiment.

The repeat applications (May 11-18, 1967 and August 16, 1968) of 14 herbicide treatments on established *Liriope* plants growing in cans of pasteurized greenhouse soil mix were not phytotoxic to *L. spicata, L. muscarvi* cv. 'Moore's Majestic' and 'Variegata'. These treatments were the same as those in the field experiment on *L. spicata* and are listed in the first two columns of Table 1.

Field Experiment Using L. spicata

The herbicide treatments used in the preliminary experiment were applied to *L. spicata* in the field. No effects were noted on the vigor of the liriope between July 14 and October 1, 1969.

The most common weeds in check plots of all field experiments are listed in approximate order of their abundance (from high to low): barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.); redroot pigweed (*Amaranthus retroflexus* L.); common purslane (*Portulaca oleracea* L.); wild mustard (*Brassica kaber* (DC.) L.C. Wheeler); common lambsquarters (*Chenopodium album* L.); field bindweed (*Convolvulus arvensis* L.); common ragweed (*Ambrosia artemisiifolia* L.); Virginia copperleaf (*Acalypha virginica* L.); hairy galinsoga (*Galinsoga ciliata* (Raf.) Blake); yellow nutsedge (*Cyperus esculentus* L.); and yellow woodsorrel (*Oxalis stricta* L.).

Three herbicide treatments (dichlormate 12 lb/A; chloroxuron 4 lb/A + diphenamid 4 lb/A; and dichlormate 12 lb/A + diphenamid 6 lb/A) were satisfactory in controlling weeds from July 14 to October 1. The other treatments were considered inadequate (less than 70 per cent control of weeds). Nine herbicide treatments decreased the amounts of barnyardgrass compared to check; however, the following five treatments did not (chloroxuron 4 lb/A; DCPA 9 lb/A; nitralin 4 lb/A; propachlor 6 lb/A; and simazine 2 lb/A). Five treatments which reduced common purslane were: chloroxuron 4 lb/A; dichlormate 12 lb/A; neburon 4 lb/A; chloroxuron 4 lb/A + diphenamid 6 lb/A; and DCPA 9 lb/A + sesone 4 lb/A. All treatments except DCPA 9 lb/A and simazine 2 lb/A reduced redroot pigweed compared to check.

Field Experiment Using L. muscari ('Moore's Majestic')

Amitrole 4 lb/A injured the 'Moore's Majestic' severely as evidenced by chlorotic leaves with marginal necrosis while dichlobenil 6 lb/A and DCPA 14 lb/A seemed to have reduced the vigor (Table 2).

Although terbacil 1.6 lb/A in this experiment provided best overall weed control, six other herbicides were satisfactory. They were: chlorpropham 8 lb/A; DCPA 14 lb/A; dichlobenil 6 lb/A; diphenamid 6 lb/A; EPTC 6 lb/A; and trifluralin 1 lb/A. The EPTC and trifluralin treatments were soil incorporated within five minutes of application with a rotary tiller. Amitrole 4 lb/A and simazine 4 lb/A were unsatisfactory in overall weed control from July 14 to October 1.

Four herbicides (dichlobenil 6 lb/A, EPTC 5 lb/A, terbacil 1.6 lb/A, and

Table 1. Evaluation of Several Herbicides for Herbicide Injury to *Liriope spicata* and for Weed Control from July 14 to October 1.

Treatment		*Control[1] of Three Common Weeds*			*Overall[2] Weed Control*	*Liriope Vigor[3]*
Herbicide and Formulation	*Rate (lb/A)*	*Barnyard-grass*	*Common Purslane*	*Redroot Pigweed*		
Chloroxuron, 50 wp	4	64 defg[4]	93 ab	99 a	60 cde	80 a
DCPA, 75 wp	9	61 efg	84 bcd	74 bcd	57 de	81 a
Dichlormate, 5 G	12	81 ab	100 a	91 a	74 ab	84 a
Diphenamid, 50 wp	6	79 abc	87 abc	94 a	61 cde	76 a
Neburon, 50 wp	4	74 abcde	94 ab	90 a	60 cde	79 a
Nitralin, 75 wp	4	67 cdefg	87 abc	96 a	64 bcde	71 a
Propachlor, 65 wp	6	64 defg	84 bcd	84 abc	54 e	77 a
Sesone, 90 wp	4	76 abcd	89 abc	87 ab	61 cde	81 a
Simazine, 80 wp	2	59 fg	73 d	70 cd	41 f	64 a
Chloroxuron + Diphenamid	4 + 6	84 a	97 ab	100 a	80 a	77 a
Chloroxuron + Nitralin	4 + 4	70 bcdef	89 abc	96 a	60 cde	76 a
DCPA + Sesone	9 + 4	70 bcdef	91 ab	84 abc	54 e	77 a
Dichlormate + Diphenamid	12 + 6	79 abc	89 abc	94 a	70 abc	66 a
Diphenamid + Nitralin	6 + 4	84 a	84 bcd	89 ab	67 bcd	83 a
Check	—	56 g	77 cd	69 d	41 f	74 a

[1] Weed control rated as follows: 100 = complete control; 70 = acceptable control; 0 = no control.
[2] This evaluation includes all weeds found in plots. Weed control rating same as for individual species.
[3] Vigor of *Liriope* rated as follows: 100 = good vigor; 0 = plants dead.
[4] Averages, in the same column, followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test at the 5 per cent level. Experiment replicated seven times.

Table 2. **Evaluation of Several Herbicides for Herbicide Injury to *Liriope muscari* 'Moore's Majestic' and for Weed Control from July 14 to October 1.**

Treatment		*Control[1] of Three Common Weeds*			*Overall[2] Weed Control*	*Liriope[3] Vigor*
Herbicide and Formulation	*Rate (lb/A)*	*Barnyard-grass*	*Common Purslane*	*Redroot Pigweed*		
Amitrole, 90 ws	4	64 d[5]	79 bcd	71 de	56 c	39 b
Chlorpropham, 2 ec	8	76 bcd	100 a	91 abc	76 b	67 a
DCPA, 75 wp	14	79 bc	97 a	83 bcde	71 b	56 ab
Dichlobenil, 4 TP	6	87 ab	96 a	94 ab	83 ab	53 ab
Diphenamid, 50 wp	6	79 bc	83 bc	91 abc	70 b	64 a
EPTC,[4] 6 ec	5	86 ab	90 ab	76 cde	70 b	71 a
Simazine, 80 wp	4	73 cd	70 d	84 abcde	51 c	64 a
Terbacil, 80 wp	1.6	94 a	100 a	100 a	93 a	63 a
Trifluralin,[4] 4 ec	1	86 ab	87 ab	87 abcd	73 b	63 a
Check	—	71 cd	71 cd	70 e	44 c	69 a

[1]Weed control rated as follows: 100 = complete control; 70 = acceptable control; 0 = no control.
[2]This evaluation includes all weeds found in plots. Weed control rating same as for individual species.
[3]Vigor of *Liriope* rated as follows: 100 = good vigor; 0 = plants dead.
[4]Herbicide incorporated within five minutes after application with a single pass of a rotary tiller.
[5]Averages, in the same column, followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test at 5 per cent level. Experiment replicated seven times.

Table 3. Evaluation of Several Herbicides for Herbicide Injury to *Liriope muscari* 'Variegata' and for Weed Control from July 14 to October 1.

Treatment		*Control*[1] *of Three Common Weeds*			*Overall*[2] *Weed Control*	*Liriope Vigor*[3]
Herbicide and Formulation	*Rate (lb/A)*	*Barnyard-grass*	*Wild Mustard*	*Redroot Pigweed*		
Alachlor, 4 wd	2	73 cde[5]	91 ab	86 abcd	61 b	74 a
Bensulide,[4] 4 ec	4	71 de	79 c	84 bcd	59 b	64 ab
Chloramben, 2 ec	4	77 abcd	100 a	94 ab	69 ab	69 ab
Cyprazine, 1 ec	1	66 ef	91 ab	89 abcd	61 b	71 ab
Linuron,[6] 50 wp	4	83 abc	100 a	100 a	79 a	63 b
Pebulate,[4] 6 ec	6	74 bcde	89 bc	83 bcd	64 b	66 ab
Vernolate,[4] 6 ec	6	86 a	96 ab	80 bcd	69 ab	71 ab
Diphenamid, 50 wp + simazine, 80 wp	6 + 1	74 bcde	80 c	93 abc	63 b	71 ab
EPTC,[4] 6 ec + simazine, 80 wp	5 + 1	84 ab	93 ab	79 cd	64 b	63 b
Check	—	60 f	87 bc	74 d	49 c	67 ab

[1]Weed control rated as follows: 100 = complete control; 70 = acceptable control; 0 = no control.
[2]This evaluation includes all weeds found in plots. Weed control rating same as for individual species.
[3]Vigor of *Liriope* rated as follows: 100 = good vigor; 0 = plants dead.
[4]Herbicide incorporated within five minutes after application with a single pass of a rotary tiller.
[5]Averages, in the same column, followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test at 5 per cent level. Experiment replicated seven times.
[6]Surfactant WK was added to linuron.

trifluralin 1 lb/A) decreased the amount of barnyardgrass compared to the control whereas amitrole 4 lb/A, chlorpropham 8 lb/A, DCPA 14 lb/A, diphenamid 6 lb/A, and simazine 4 lb/A did not. Improved control of common purslane was provided by all herbicides used on 'Moore's Majestic' except amitrole 4 lb/A, diphenamid 6 lb/A, and simazine 4 lb/A. The five herbicides that improved control of redroot pigweed over check were chlorpropham 8 lb/A, dichlobenil 6 lb/A, diphenamid 6 lb/A, terbacil 1.6 lb/A, and trifluralin 1 lb/A.

Field Experiment with L. muscari ('Variegata')

None of the herbicides used in this experiment affected vigor. Linuron 4 lb/A did cause a temporary chlorosis which lasted about three weeks.

The only herbicide that was satisfactory in overall weed control was linuron 4 lb/A. Chloramben 5 lb/A and vernolate 6 lb/A were probably adequate in overall weed control.

All herbicides except cyprazine 1 lb/A improved control of barnyardgrass over check. Only chloramben 8 lb/A and linuron 4 lb/A provided better control than check of wild mustard. Chloramben 8 lb/A, linuron 4 lb/A, and the combination of diphenamid 6 lb/A + simazine 1 lb/A improved control of redroot pigweed over check.

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Low pH Tolerance of the Larvae of the Alderfly, *Sialis aequalis* Banks, Under Controlled Conditions

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Abstract

The larval population of the alderfly, *Sialis aequalis* Banks, was experimentally tested in the laboratory to determine its tolerance to low pH. The straight-line graphical interpolation method was employed to determine the pH value at which 50 per cent of the alderflies survived after 96 hours. The $TL_m{}^{96}$ pH value was 2.1.

Coal mining and related industries are West Virginia's principle economic base. Since accurate mining records have been kept, West Virginia coal fields have supplied greater than 7.5 billion tons of bituminous coal (Minerals Yearbook, 1969). A serious consequence of the coal mining activities has been the enormous amount of pollutants supplied to streams draining active and abandoned mining operations. The acid drainage effluent stemming from bituminous coal mining is generally recognized as the most widespread pollution problem in the Ohio River basin. It has been conservatively estimated that damages from acid mine drainage pollution in the Upper Ohio River basin exceeds 10 million dollars annually (USDI, 1968).

A perusal of the literature indicated that few species of benthic organisms, if any, are found in low pH streams. Lackey (1938) conducted a brief biological survey of the acid streams and seepages in the region about Fairmont, West Virginia, and of the lakes of southern Indiana. He reported mosquito and caddisfly larvae, mayfly nymphs, and beetles in a quiet West Virginia pool, pH 2.4. An extensive study of the water quality and the benthic fauna in the Kanawha River basin (partly in West Virginia) was conducted in 1964 (USDI, 1967). Clear Fork, an acid mine drainage tributary of the Coal River in the Kanawha River basin, above Whitesville, West Virginia, was devoid of benthic organisms. Parsons (1968), Roback and Richardson (1969), Nichols (1971), and Woodrum (1971) reported the alderfly *Sialis* in an area of continuous pollution by acid mine drainage. A few laboratory studies have been reported on the tolerance of benthic organisms of a low pH, although no study dealt with the alderfly. Bell and Nebeker (1969) conducted a study on ten species of mature larvae of caddisflies and nymphs of stoneflies, dragonflies, and mayflies, to determine their relative tolerance to low pH. They recorded the lethal pH at which 50 per cent of the organisms died after 96 hours ($TL_m{}^{96}$). The $TL_m{}^{96}$ pH ranged from 1.5 to 4.65, the caddisfly *Brachycentrus americanus* (Banks) and the mayfly *Ephemerella subvaria* McDunnough, respectively. Bell (1971) studied the effect of low pH on the survival and emergence of mature larvae and nymphs of 9 species of aquatic insects (caddisflies, dragonflies, mayflies, and stoneflies) in the laboratory. The TL_{50} values at 30 days (chronic test) ranged from pH 2.45 to 5.38, *Brachycentrus americanus* and *Ephemerella subvaria*, respectively. All species tested were more sensitive to low pH during the period of emergence. A similar test on the effects of pH was conducted by Bell (1970) on the life cycle of the midge *Tanytarsus dissimilis* Joh. At pH value of 4.0 and below mortality was 100 per cent. Pupae formed at pH 5.0 but adults failed to emerge.

This study was designed to determine the effects of low pH on the larvae of the alderfly, *S. aequalis*, in the laboratory. The detailed life history and ecology of this alderfly in an acid mine stream has been reported by Woodrum (1971).

Materials and Methods

Fifty healthy, mature alderfly larvae of comparable sizes were collected from the acid mine waters of Camp Creek of Twelvepole Creek, Wayne County, West Virginia. They were returned to the laboratory in acid creek water for observation and acclimation over a 24-hour period. Ten larvae were carefully placed in each of 5 aquaria (2 gallon) which contained the following pH values; 7.5, 6.0, 4.5, 3.0, and 1.5. Each aquarium contained approximately 1 gallon of water, natural substrate, air stone, and leaf detritus and chironomid larvae for food. A Model 5 Corning Scientific pH meter was employed to establish the various pH values using dilutions of sulfuric acid. The pH readings were periodically checked and adjusted, although the readings did not significantly change during the test period. Acidity and alkalinity were measured with a Hach chemical kit, Model AL-36-WR. Acidity ranged from 29 to 342^+ ppm at pH 3.0 and 1.5, respectively, while alkalinity ranged from 43 to 77 ppm at pH 3.0 and 7.5, respectively. Water temperature was measured with a maximum-minimum thermometer placed below the surface of the water; the range of temperatures was from 13 to 18° C. Dissolved oxygen concentrations were always kept at a high level of saturation (80-100 per cent) during the test period.

The 96-hour TL_m (median tolerance limit) test (Standard Methods, 1965) was employed as the measure of acute toxicity to low pH. The straight-line graphical interpolation method was used to determine the pH value at which 50 per cent of the alderflies survived after 96 hours.

Results and Discussion

The alderfly larvae of *S. aequalis* had a 96-hour TL_m pH value of 2.1 (Figure 1). All of the larvae survived in decreasing pH values until 3.0, while none of them survived a pH of 1.5 (Table 1 and Figure 1). Woodrum (1971) reported that the Camp Creek alderfly larval population, the same one used in this experimental study, lived in acid mine pollution drainage with a yearly pH range of 4.3 to 6.4; the average was 5.0. Therefore, this study indicated that the population could possible exist at a lower pH if further adverse acid condition occurred in the stream. Of course, various ecological factors would be important in this consideration.

Sialis sp. was reported in an area of continuous pollution (pH 2.8-4.0) by acid strip mine drainage in central Missouri (Parsons, 1968). Nichols (1971) noted that *Sialis* and *Chironomus* were the predominate benthic indicator organisms in the polluted zone (pH 4.6) of the East Fork Obey River, Tennessee. Not all *Sialis* species are associated with acid mine pollution. Minshall (1965) reported *S. joppa* Ross from Morgan's Creek, Meade County, Kentucky, an extensive karst topographic area, where the pH ranged from 7.7 to 8.3. The genus *Sialis* was part of the benthic fauna sampled by Olson (1971) in Beech Fork of Twelvepole Creek, Wayne County, West Virginia; the pH was 7.1. *Sialis* has been collected in Bowen Creek, Bryon Creek, and Mud River of Cabell County, West

Table 1. Experimental Data Obtained from Laboratory Tests of Various pH Values on *S. aequalis* Larvae.

Tank No.	*pH*	*No. of Larvae*	*No. of Larvae Surviving after 96 hours*
1	7.5 (control)	10	10
2	6.0	10	10
3	4.5	10	10
4	3.0	10	10
5	1.5	10	0

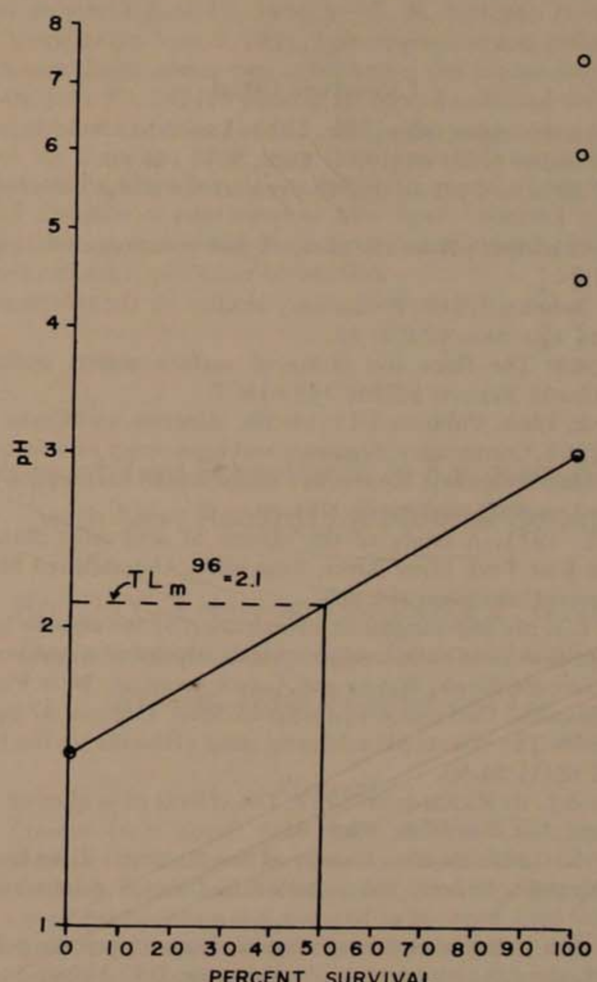

FIGURE 1. Estimation of the median tolerance limit of pH on *S. aequalis* larvae by straight-line graphical interpolation.

Virginia, with pH values of 8.0, 7.5, and 7.0, respectively (Carl Olson, personal communication).

The alderfly larvae of the genus *Sialis* have a wide tolerance range for pH (2.8-8.3). Some species (e.g. *S. aequalis*) have a low pH tolerance and are good benthic indicator organisms of acid mine drainage, but other species (e.g. *S. joppa*) are only found in good quality water. Therefore, only certain species of the genus *Sialis* are benthic indicator organisms of acid mine pollution. Future studies will be made to determine if more than one species has adapted to acid mine drainage.

Acknowledgments

The authors are sincerely grateful to Dr. Herbert H. Ross, Department of Entomology, University of Georgia, for identification of the alderfly. Special thanks to the following people, directly or indirectly, for their assistance in this study: Clara Judy, Dr. Howard L. Mills, Carl Olson, Judy Tarter and Gene Thomas.

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Calopteryx angustipenne Selys in West Virginia

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Abstract

Calopteryx angustipenne Selys is a rare dragonfly that inhabits clear, swiftly flowing streams from Pennsylvania to northern Georgia and westward to Tennessee, Kentucky and Ohio. No published record of its occurrence in West Virginia has been found. James G. Needham stated in an unpublished manuscript that one nymph had been taken from the South Fork of the Potomac River below the Smoke Hole and another nymph from the Lost River near Wardensville. Both these specimens are very small and cannot be determined specifically because nymphs of *Calopteryx angustipenne* have not been connected certainly with the adults. A single male specimen of this species is in the Carnegie Museum of Pittsburgh. It was collected June 15, 1936 by G. M. Kutchka from Patterson Creek near Burlington, West Virginia. On June 9, 1971, I photographed and collected *Calopteryx angustipenne* over Patterson Creek about two miles below the bridge that carries U.S. Route 50 over the stream. On June 10, 1971, I photographed and collected the species over the North Fork River near Ice Mountain. On June 11, 1971, the species was thinly represented over the South Fork of the Potomac River from the Smoke Hole northward into Grant County. On July 22, 1971, a single, worn female was collected over the Lost River near Wardensville. To date, adults of *Calopteryx angustipenne* have been collected in five counties of West Virginia. We hope that nymphs will be collected and reared until adults are obtained. Possibly Needham's earliest records can be verified.

Herpetology of Two Farm Ponds in Southern West Virginia, with New Records for Monroe County

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Abstract

Two Monroe County farm ponds near Marie, Summers County, West Virginia, were studied March-November, 1971, for utilization by amphibians and reptiles. A permanent pond near an oak-hickory forest and bordered by trees, stumps, and logs harbored more species than did a semi-permanent pond situated in an open field and lacking a variety of refugia along its edges. The diversity of species and numbers of individuals using the ponds indicate that the farm pond habitat is an important one for amphibians and reptiles in southern West Virginia.

New herpetological records for Monroe County resulting from the study include *Ambystoma maculatum* Shaw, *Hemidactylium scutatum* Schlegel, *Chrysemys p. picta* Schneider, *Natrix s. sipedon* Linnaeus, and *Coluber c. constrictor* Linnaeus.

There are more than 15,000 man-made farm ponds in West Virginia (Core, 1966). These ponds represent a widespread, lentic habitat type of recent origin. The extent to which this habitat is exploited by amphibians and reptiles has not been determined, nor have the factors affecting utilization of farm ponds by these animals. This study represents an initial attempt at securing ecological data which eventually will lead to an understanding of the role played by farm ponds in amphibian and reptile ecology in West Virginia.

Materials and Methods

The two farm ponds studied are approximately 400m apart in Monroe County, one mile east of Marie, Summers County, West Virginia, on a plateau at 700m elevation. The smaller pond (hereafter designated Pond A) is surrounded by open fields and has a maximum depth of 1-1.5m and surface area of 650m^2 in early Spring. The larger pond (Pond B) lies in a pasture and has a maximum depth of 2m and surface area of 1700m^2 in early Spring. The pond is bordered by large trees, stumps, and logs and lies adjacent to an oak-hickory forest along its NE margin. During the study period (26 Feb.-11 Nov. 1971), Pond B was the water source for 6-40 cattle. The fields surrounding Pond A were not used for crops or pasture during the study. Pond A occasionally dries up during the summer; Pond B is a permanent pond. Rainfall, runoff, and ground water are the only water sources for both ponds.

On each visit to the study area, a standard procedure was followed. Each pond was surveyed with binoculars to discover basking reptiles. Next, the edges of the pond were examined by wading along the margins, turning over rocks and logs, and netting or grabbing any amphibians or reptiles encountered. Water temperatures near the margins and water pH readings were taken several times during this work. Photographs of the ponds were taken from the outflow seepage areas. Rainfall since the last visit was recorded from a station near Pond A.

All amphibians and reptiles seen were recorded, whether caught or not, as long as positive identification could be made. Some of the specimens actually captured were preserved to confirm species identifications, but most animals were released immediately after capture and identification.

Results

Nineteen visits to the study area were made. Physical parameter measurements obtained on these trips are presented in Table 1. Both ponds contained water throughout the study period. The ponds were full from February through mid-June, decreased in size through September, and filled again by early November.

Two hundred fifty-three observations of individual amphibians and reptiles were recorded. Pond B yielded approximately 70% of the observations. Seasonal distribution of the observations (Table 1) indicates that herpetological activity at the ponds was greatest March-June and late September-October, with lessened activity during the summer.

Fifteen amphibian and reptile species were encountered. Eight species (*Ambystoma opacum* Gravenhorst, *Hemidactylium scutatum* Schlegel, *Eurycea l. longicauda* Green, *Bufo a. americanus* Holbrook, *Terrapene c. carolina* Linnaeus, *Sceloporus undulatus hyacinthinus* Green, *Natrix s. sipedon* Linnaeus, *Coluber c. constrictor* Linnaeus) were recorded from Pond B only. The remaining seven species (*Ambystoma maculatum* Shaw, *Diemictylus v. viridescens* Rafinesque,

Table 1. Physical Parameters at Two Farm Ponds in Monroe County, West Virginia, and Total Numbers of Individual Amphibians and Reptiles Observed.

Date (1971)	Water temp. (°C) Pond A	Water temp. (°C) Pond B	Water pH Pond A	Water pH Pond B	Precipitation (mm) since last visit	# Individuals observed
26 Feb.	6.7	6.1	5.0	3.0	–	0
6 Mar.	5.6	6.7	5.0	3.5	35.6	13
14 Mar.	14.4	13.3	–	–	5.1	8
31 Mar.	16.7	14.4	5.0	3.5	12.7	18
12 Apr.	16.7	18.9	5.0	4.5	22.9	15
28 Apr.	–	–	–	–	22.9	11
7 May	17.2	15.6	4.5	3.0	40.6	16
19 May	23.9	24.4	5.5	4.5	35.6	3
4 June	24.4	22.2	4.5	3.5	70.0	67
18 June	23.9	22.8	4.5	3.5	50.8	10
5 July	26.7	26.1	5.0	3.5	15.2	2
15 July	25.6	24.4	5.0	3.5	12.7	8
5 Aug.	22.2	20.0	5.0	3.5	78.7	7
30 Aug.	23.3	23.9	5.0	3.5	15.2	3
16 Sept.	22.2	23.3	5.0	–	40.6	9
30 Sept.	–	22.2	–	3.0	22.9	26
14 Oct.	16.7	17.8	5.0	3.0	22.9	21
28 Oct.	16.7	15.6	5.0	4.0	55.9	11
11 Nov.	10.0	8.3	5.0	3.5	15.2	5

Hyla c. crucifer Wied, *Rana clamitans melanota* Rafinesque, *Rana catesbeiana* Shaw, *Chelydra serpentina* Linnaeus, *Chrysemys p. picta* Schneider) were found at both ponds.

Ambystoma maculatum, *Hemidactylium scutatum*, *Chrysemys p. picta*, *Natrix sipedon*, and *Coluber constrictor* are new records for Monroe County. *Bufo americanus* and *Sceloporus undulatus* are new West Virginia Biological Survey records.

Discussion

Observations on phenology and habitat made during the study are in agreement with published information (Bishop, 1943; Brown, 1932; Carr, 1952; Conant, 1958; Green, 1965; Wright and Wright, 1949) and are not included here. The difference in numbers of observations at each pond probably is due to the larger size of Pond B (Pond B is 2.6 times as large as Pond A and yielded 2.4 times as many observations). However, finding eight species at Pond B only, seven at both ponds, and none at Pond A only cannot be so easily explained. Differences between Ponds A and B which might have affected this distribution of species include the impermanent nature of Pond A, the greater number and diversity of shoreline hiding places (logs, stumps, etc.) at Pond B, the proximity of Pond B to woodland, and perhaps the greater acidity of Pond B.

Five of the species found at Pond B only (*Bufo americanus*, *Terrapene carolina*, *Sceloporus undulatus*, *Natrix sipedon*, *Coluber constrictor*) probably also visit Pond A occasionally. All except *N. sipedon* are primarily terrestrial, and none of the species are confined to woodland habitats. The more varied shoreline environment at Pond B may have contributed most to recording these species at Pond B and not at Pond A. However, *Ambystoma opacum*, *Hemidactylium scutatum*, and *Eurycea longicauda* all are associated with woodlands. Whether they would have utilized Pond B for breeding, had Pond A not been present and closer to the forest, is unknown. In the case of *H. scutatum*, habitat requirements include both woodland and a bog or pond with sphagnum moss (Green, 1965; Neill, 1963). Sphagnum was found at Pond B and not at Pond A.

The species diversity and large numbers of amphibians and reptiles using these farm ponds indicates that the farm pond plays an important ecological role for aquatic and semi-aquatic amphibians and reptiles in southern West Virginia. This role is especially significant because natural ponds and marshes are unusual in this elevated, well-drained region.

Conclusions

The farm pond habitat is important in the ecology of amphibians and reptiles in southern West Virginia. Factors other than geographic proximity of the ponds determine utilization by particular species. These factors probably include surrounding habitat types, availability of shoreline refugia, and the frequency with which the ponds dry completely or are drained. Further investigations will be needed to identify other influencing factors and to reveal the impact of particular factors on individual amphibian and reptile species.

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New Records of Odonata in West Virginia

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Abstract

Gomphus (Stylurus) scudderi Selys and *Dromogomphus spoliatus* Hagen have been identified from larval specimens collected for the first time. A short description of the specimens and their habitats are included following a review of the literature on Odonata of West Virginia.

A review of the literature on Odonata of West Virginia reveals a dearth of information. Needham and Westfall (1955) noted 20 species occurring in the state; Kormondy (1960) added 8 species to the list; Cruden (1962) has provided the most extensive faunal list, noting 74 species, including the species mentioned above; and Harwood (personal communication, 1971) added 11 species to the known fauna in the manuscript done by Needham in 1930 which will soon be in print.

Distribution

A thorough examination of the larval odonate collection of Marshall University and this author's extensive collection of odonates of Cabell County revealed two new species heretofore unrecorded in West Virginia. Both species, *Gomphus scudderi* Selys and *Dromogomphus spoliatus* Hagen, members of the family Gomphidae, were collected in Wayne County, while the latter species was also collected in Cabell County.

Morphology and Habitat

G. scudderi Selys is a member of the subgenus *Stylurus,* a group of rather large gomphines. The larval specimens collected were in an early instar, but still exhibited the key morphological structures needed for classification. These included the sharply incurved end hooks on the lateral lobes of the labium, the burrowing hooks on the fore and middle tibiae, lateral abdominal spines on segments 6 to 9, and one feature usually not found on this species, a dorsal hook in the shape of a triangle on abdominal segment 9. The body is elongate and has a yellowish hue to it. The specimens were collected near Rich Creek, East Fork of Twelvepole Creek, Wayne County, West Virginia. The substrate from which they were taken was entirely sand, with very little vegetative cover present.

The larvae of *D. spoliatus* Hagen are elongate, the abdomen being somewhat depressed and tapering caudad, the tip usually sharply upturned. Small, flattened, dorsal abdominal hooks are present on segments 3 to 9. Burrowing hooks are well developed on the fore and middle tibiae. The labium is short and flat, having a truncated median lobe, and the lateral lobes are roundly incurved and armed with minute teeth on the inner edges. These larvae frequented the following wide range of habitats in Cabell and Wayne counties: Camp Creek, Wayne County, a creek with a pH range of 4-5 due to acid mine drainage, various lotic sites in Cabell County with pH ranges of 7-8, and even some lentic (ponds) sites with an alkaline pH range. Generally they were taken from piles of leaf and twig litter or trash which covered a sandy bottom. They were always taken in standing water, either pools before a riffle or near the shore of ponds.

Acknowledgments

The author is indebted to Drs. Paul D. Harwood, Ashland, Ohio, and Minter J. Westfall, Jr., University of Florida, for their help in the identification of the specimens, and to Dr. Donald C. Tarter, Marshall University, for his assistance in field work to obtain this data and in the preparation of this paper.

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A Pre-impoundment Investigation of the Limnology of East Fork of Twelvepole Creek, Lincoln, Mingo, and Wayne Counties, West Virginia

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Abstract

The fish populations at 10 collecting stations in Twelvepole Creek were sampled by rotenone, in order to determine the species composition prior to impoundment. Seven families represented by 38 species of fish were collected during the study. A total of 1,474 fish which weighed about 64 pounds were collected above the dam site. Game fishes comprised 25.4 per cent of the total number of fishes and 33.6 per cent of the total weight; rough fishes, 14.1 per cent by number and 36.1 per cent by weight; and forage fishes 60.5 per cent by number and 30.3 per cent by weight. Maximum and minimum standing crops were 221.3 and 16.6 pounds per acre, respectively; the average was 87.97 pounds per acre.

The benthic invertebrates at the 10 collecting stations were sampled with a bottom dredge. A total of 707 organisms were collected which represented 9 orders, 24 families, and about 36 species. The following benthic taxa were ranked according to percentage frequency by number; Ephemeroptera (42.7), Diptera (13.8), Decapoda (13.8), Trichoptera (10.6), Odonata (7.4), Coleoptera (6.7), Plecoptera (2.3), Mollusca (1.6), and Megaloptera (1.1).

The following physical and chemical parameters were determined: dissolved oxygen, $\bar{x}$ = 8.8 (7.1-11.0) mg/l; pH, $\bar{x}$ = 6.6 (6.3-7.0); total hardness, $\bar{x}$ = 32.0 (20.0-75.0) mg/l $CaCO_3$; total alkalinity, $\bar{x}$ = 32.0 (15.0-60.0) mg/l $CaCO_3$: iron, $\bar{x}$ = 0.9 (0.2-2.0) mg/l; sulfate, $\bar{x}$ = 12.0 (7.0-16.0) mg/l; and temperature, $\bar{x}$ = 21.6 (17.0-28.0) C.

East Fork of Twelvepole Creek has its source at the base of Guyan Mountain in the northern part of Mingo County, West Virginia, and flows in a northwest direction through Mingo and Lincoln counties to its junction with the West Fork of Twelvepole Creek about 1 mile south of Wayne, West Virginia, in Wayne County. The length of the East Fork is approximately 50.0 miles and the average gradient is 13.5 feet per mile.

The U. S. Army Corps of Engineers, Huntington District, is constructing a dam on the East Fork of Twelvepole Creek in Wayne County, 1.6 miles south of East Lynn, West Virginia. Initial impoundment is tentatively scheduled for the spring 1972. The dam will control a drainage area of 133 square miles. The proposed reservoir will have a surface area of 823 acres during the winter months, and a surface area of 1,005 acres will be maintained from May through September for recreation and fish and wildlife purposes (USACE, 1969). Flood control storage capacity will vary from 65,300 acre-feet during the summer to 70,800 acre-feet during the winter-spring flood season.

The primary objectives of this investigation were to survey the populations of fishes and benthos and to delineate the primary chemical and physical parameters of the East Fork of Twelvepole Creek and its tributaries, providing a baseline for impoundment and post-impoundment studies in the future.

Materials and Methods

Fishes were sampled at 10 collecting stations (Figure 1) with rotenone. The collecting station was blocked off with seines, and the toxicant was applied to the upper end of the area at a concentration of 0.5 to 1.0 ppm. Potassium permanganate was added in the lower end of the collecting station to detoxify the rotenone. Specimens were fixed immediately in 10 per cent formalin, and later washed and preserved in 50 per cent isopropyl alcohol. Collections are presently stored in the Department of Biological Sciences, Marshall University.

Benthic invertebrates were sampled at the 10 collecting stations (Figure 1) with a bottom dredge. The bottom net, with an opening of 18 x 8 inches, was held immediately downstream while the substrate was agitated causing the organisms to be dislodged. The specimens were fixed in 10 per cent formalin, and later preserved in 70 per cent ethanol.

The following chemical parameters were measured in the field at each collecting site using a Hach "Direct Reading" Portable Water Engineer's Laboratory, Model DR-EL: dissolved oxygen, pH, total hardness, total alkalinity, iron, and sulfate. Water temperature was determined by placing a Celsius thermometer in the water for several minutes.

Results

A total of 1,474 fish which weighed about 64 pounds were collected from the study area; 38 species, comprising 7 families, were represented in the collections (Tables 1, 2, and 3). Nomenclature follows that of the American Fisheries Society (1970). All fishes were generally grouped into the following 3 categories (Tables 1 and 2): game, forage, and rough fishes. Game fishes, based on the above categories, represented the family Centrarchidae; the forage fishes included the families Atherinidae, Cyprinidae, Percidae, and Petromyzontidae; and the rough fishes contained the Catostomidae and Ictaluridae. Game, forage, and rough fishes comprised 25.4, 60.5, and 14.1 per cent, respectively, of the total number of fishes, and 33.6, 30.3, and 36.1 per cent, respectively, of the total weight (Table 3). The most abundant 7 species in the study are in order of

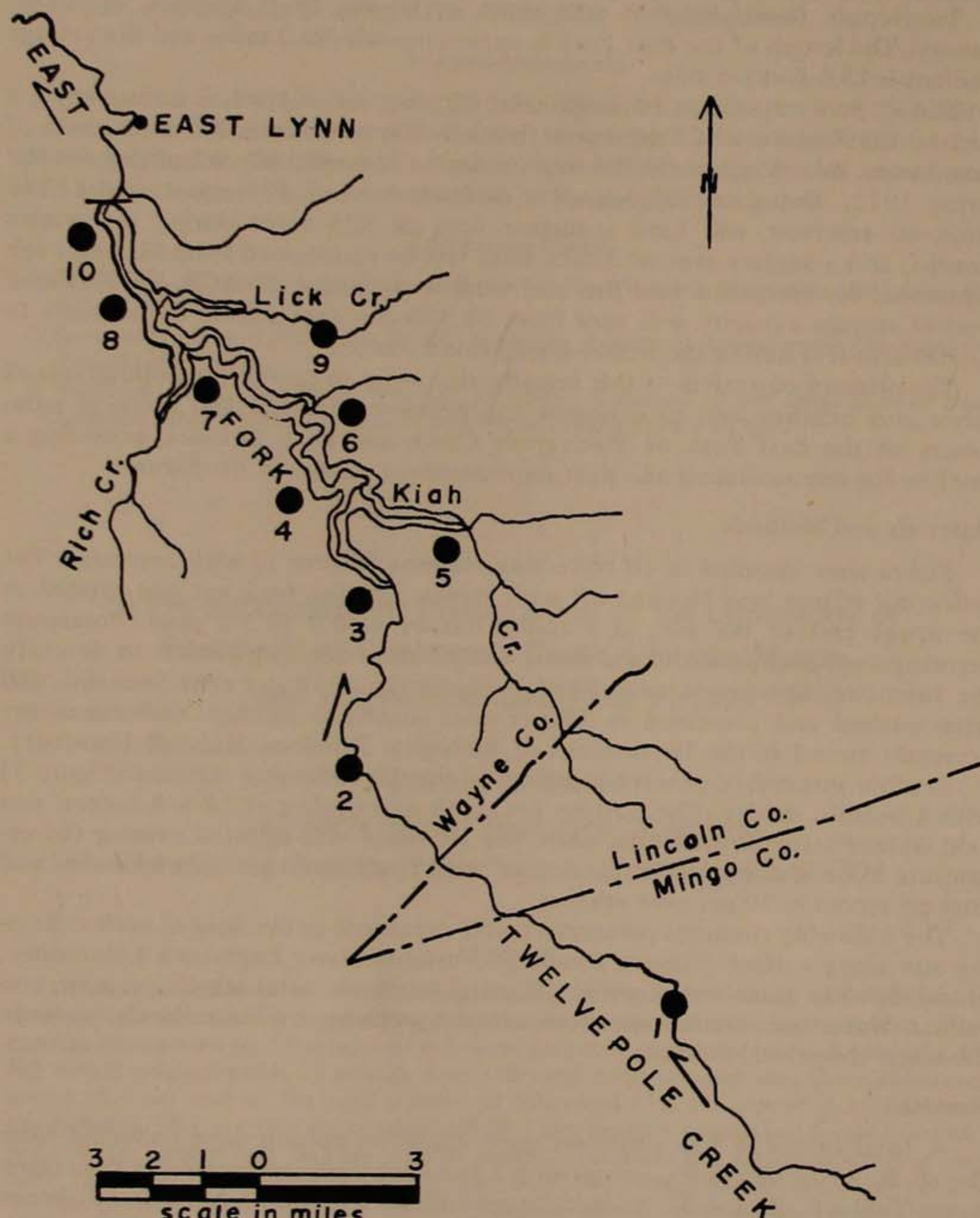

FIGURE 1. Map of East Fork of Twelvepole Creek, Lincoln, Mingo, and Wayne counties, West Virginia, showing locations of collecting stations, and extent of East Lynn Lake. The line through East Lynn Lake shows the course of the East Fork prior to impoundment (U. S. Army Corps of Engineers, Huntington, West Virginia).

Table 1. List of fishes collected from East Fork of Twelvepole Creek, 1970.

GAME FISHES	
Family Centrarchidae—Basses and Sunfishes	
Ambloplites rupestris (Rafinesque)	Rock bass
Lepomis cyanellus Rafinesque	Green sunfish
L. macrochirus Rafinesque	Bluegill
L. megalotis (Rafinesque)	Longear sunfish
Micropterus dolomieui Lacepede	Smallmouth bass
M. punctulatus (Rafinesque)	Spotted bass
M. salmoides (Lacepede)	Largemouth bass
FORAGE FISHES	
Family Atherinidae—Silversides	
Labidesthes sicculus (Cope)	Brook silverside
Family Cyprinidae—Minnows	
Campostoma anomalum (Rafinesque)	Stoneroller
Ericymba buccata Cope	Silverjaw minnow
Hybopsis amblops (Rafinesque)	Bigeye chub
H. micropogon (Cope)	River chub
Notropis cornutus (Mitchill)	Common shiner
N. photogenis (Cope)	Silver shiner
N. rubellus (Agassiz)	Rosyface shiner
N. spilopterus (Cope)	Spotfin shiner
N. stramineus (Cope)	Sand shiner
Pimephales notatus (Rafinesque)	Bluntnose minnow
Rhinichthys atratulus (Hermann)	Blacknose dace
Semotilus atromaculatus (Mitchill)	Creek chub
Family Percidae—Perches	
Etheostoma blennioides Rafinesque	Greenside darter
E. caeruleum Storer	Rainbow darter
E. flabellare Rafinesque	Fantail darter
E. nigrum Rafinesque	Johnny darter
E. variatum Kirtland	Variegate darter
E. zonale (Cope)	Banded darter
Percina caprodes (Rafinesque)	logperch
P. maculata (Girard)	Blackside darter
P. sciera (Swain)	Dusky darter
Family Petromyzontidae—Lampreys	
Lampetra aepyptera (Abbott)	Least brook lamprey
ROUGH FISHES	
Family Catostomidae—Suckers	
Catostomus commersoni (Lacepede)	White sucker
Hypentelium nigricans (LeSueur)	Northern Hog sucker
Minytrema melanops (Rafinesque)	Spotted sucker
Moxostoma anisurum (Rafinesque)	Silver redhorse
M. erythrurum (Rafinesque)	Golden redhorse
Family Ictaluridae—Freshwater catfishes	
Ictalurus melas (Rafinesque)	Black bullhead
I. natalis (LeSueur)	Yellow bullhead
Noturus miurus Jordan	Brindled madtom

Table 2. Composition, number (parentheses), and collecting stations of fishes in East Fork of Twelvepole Creek, 1970.

GAME FISHES–298

- Basses
  - Rock bass (89) 1,2,3,4,5,6,7,8,10.
  - Smallmouth bass (9) 2,4,5,6,8.
  - Spotted bass (29) 1,2,3,4,5,6,7,8,10.
  - Largemouth bass (1) 5.
- Sunfishes
  - Green sunfish (20) 3,6,10.
  - Bluegill (13) 5,9,10.
  - Longear sunfish (137) 1,2,3,4,5,6,9,10.

FORAGE FISHES–884

- Silversides
  - Brook silverside (14) 1,2,3,7,8,10.
- Minnows
  - Stoneroller (27) 3,4,5,6,9.
  - Bigeye chub (49) 2,3,4,6.
  - River chub (14) 3,4,6.
  - Silverjaw minnow (29) 1,2,3,4,5,6,9.
  - Common shiner (205) 1,2,3,4,5,6,7,8,9,10.
  - Silver shiner (32) 1,2,3,4,5,8.
  - Rosyface shiner (25) 3,4,6.
  - Spotfin shiner (11) 6.
  - Sand shiner (26) 1,3,4,5,6,7.
  - Bluntnose minnow (121) 1,2,3,4,5,6,7,8,9.
  - Blacknose dace (2) 4.
  - Creek chub (134) 1,3,4,5,6,7,8,9.
- Perches
  - Greenside darter (13) 2,3,4,5.
  - Rainbow darter (17) 1,3,4,5.
  - Fantail darter (26) 1,2,3,4,5,8.
  - Johnny darter (13) 3,4,5,6.
  - Variegate darter (2) 5.
  - Banded darter (5) 4.
  - Logperch (36) 1,2,3,4,5,6,7,8,9.
  - Blackside darter (53) 2,3,4,5,6,7,8,10.
  - Dusky darter (1) 7.
- Lampreys
  - Least brook lamprey (29) 2,3,4,5,7,10.

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- Suckers
  - White sucker (71) 1,3,4,5,6,7,8,9,10.
  - Northern hog sucker (37) 1,2,3,4,5,6,7,8,9.
  - Spotted sucker (15) 2,3,4,7,8.
  - Silver redhorse (4) 1,7,8.
  - Golden redhorse (42) 3,5,6,7,8.
- Freshwater catfishes
  - Black bullhead (3) 6,8.
  - Yellow bullhead (16) 2,3,5,7,8,9.
  - Brindled madtom (104) 1,2,3,4,5,6,7,8.

Table 3. Percentage Frequency of Total Numbers and Weight of Game, Forage, and Rough Fishes in East Fork of Twelvepole Creek, 1970. N = Total Numbers and Weight.

Station	*Percentage Frequency (Numbers)*			*Percentage Frequency (Weight-lbs.)*		
	Game	*Forage*	*Rough*	*Game*	*Forage*	*Rough*
1	28.1	37.4	24.5	35.4	13.7	50.9
2	31.5	64.8	3.7	59.5	21.4	19.1
3	21.1	69.5	9.4	28.5	25.6	45.9
4	7.0	86.5	6.5	12.6	58.6	28.8
5	16.0	76.7	8.3	33.0	38.1	28.9
6	18.3	69.9	11.8	21.4	34.9	43.7
7	11.6	62.8	25.6	28.4	18.3	53.3
8	10.8	52.3	36.9	31.8	21.8	46.4
9	8.5	78.2	13.3	11.5	65.6	22.9
10	91.2	8.3	1.5	74.3	5.0	20.7
Total (1-10)	25.4	60.5	14.1	33.6	30.3	36.1
N		1474			64	

Table 4. Estimated Total Pounds of Fishes Per Acre Collected From East Fork of Twelvepole Creek, 1970.

Station	*Weight (Pounds)*	*Area (Acres)*	*Pounds/Acre*
1	9.31	0.042	221.3
2	3.06	0.058	52.8
3	9.37	0.104	90.0
4	6.57	0.046	142.9
5	11.15	0.065	171.6
6	4.49	0.077	58.3
7	6.96	0.366	19.3
8	9.40	0.140	67.1
9	0.91	0.055	16.6
10	2.79	0.070	39.9
		Average	87.97

decreasing abundance were *Notropis cornutus, Lepomis megalotis, Semotilus atromaculatus, Pimephales notatus, Noturus miurus, Ambloplites rupestris,* and *Catostomus commersoni* (Table 2). The maximum and minimum standing crops for these 10 stations were 221.3 and 16.6 pounds per acre, respectively; the average was 87.97 pounds per acre (Table 4).

A total of 707 benthic invertebrates were collected which represented 9 orders, 24 families, and about 36 species (Tables 5 and 6). The following benthic taxa were ranked according to their numerical percentage frequency (Table 5): Ephemeroptera (42.7), Diptera (13.8), Decapoda (13.8), Trichoptera (10.6),

Table 5. Numerical Percentage Frequency of the Total Number of Benthic Taxa Collected From All Stations in East Fork of Twelvepole Creek, 1970.

Taxon	*Number*	*Percentage Frequency*
Ephemeroptera	302	42.7
Diptera	98	13.8
Decapoda	98	13.8
Trichoptera	75	10.6
Odonata	52	7.4
Coleoptera	47	6.7
Plecoptera	16	2.3
Mollusca	11	1.6
Megaloptera	8	1.1
Total	707	

Odonata (7.4), Coleoptera (6.7), Plecoptera (2.3), Mollusca (1.6), and Megaloptera (1.1). The most abundant 5 species in order of abundance were *Stenonema vicarium, Cambarus* sp., *Chironomus* spp., *Orconectes* sp., and *Cheumatopsyche* sp. (Table 6).

The following physical and chemical parameters were characteristic of good water quality (USDI, 1968): dissolved oxygen, $\bar{x}$ = 8.8 (7.1-11.0) mg/l, 86-118 per cent saturation; pH, $\bar{x}$ = 6.6 (6.3-7.0); total hardness, $\bar{x}$ = 32.0 (20.0-75.0) mg/l $CaCO_3$; total alkalinity, $\bar{x}$ = 32.0 (15.0-60.0) mg/l $CaCO_3$; iron, $\bar{x}$ = 0.9 (0.2-2.0) mg/l; sulfate, $\bar{x}$ = 12.0 (7.0-16.0) mg/l; and temperature, $\bar{x}$ = 21.6 (17.0-28.0) C (Table 7).

Discussion

Game fishes comprised about 25 per cent of the total number of fishes collected from the pre-impoundment area. Hoyt et al. (1970) reported that game fishes represented about 22 per cent of the collections sampled in the pre-impoundment area of the upper Salt River in Kentucky. Game fishes were rather rare in the nearby pre-impoundment area of Beech Fork of Twelvepole Creek, representing about 5 per cent of the total collected (Olson, 1970). Forage fishes represented the highest percentage (60.5) of fishes collected during the study period. Olson (1970) and Hoyt et al. (1970) reported that forage fishes composed 85 and 50 percent, respectively, of the fishes in their pre-impoundment studies. Forage species also made up the greatest percentage of total numbers in pre-impoundment surveys of 6 Kentucky streams (Turner, 1959). Rough fishes constituted the highest percentage (36.1) of total weight of the East Fork collections. The average standing crop (87.97 pounds per acre) in the study area was similar to the average standing crop (90 pounds per acre) in the upper Salt River in Kentucky (Hoyt et al., 1970). Electroshocking and seining were used to sample the fishes in the upper Salt River. In Kentucky, the average standing crops were 108,47, 30, and 40 pounds per acre in the Barren, Middle Fork of the Kentucky, Nolin, and Rough rivers, respectively (Carter, 1968). Olson (1970) reported the average standing crop to be 17 pounds per acre in the nearby pre-impoundment area of Beech Fork. Probably the deficiencies of electrofishing were partly responsible for the low standing crop in Beech Fork.

Table 6. Composition, number (parentheses), and collecting station of the benthic fauna in East Fork of Twelvepole Creek, 1970.

EPHEMEROPTERA (302)
- *Ameletus lineatus* Traver (1) 9.
- *Caenis* sp. (14) 1,4,5,6,9.
- *Ephemera guttulata* Pictet (1) 4.
- *Hexagenia limbata* (Serville) (7) 2,6,8.
- *Isonychia* sp. (38) 1,3,4,5,7,8,9.
- *Pseudocloeon punctiventris* (McDunnough) (2) 7.
- *Stenonema heterotarsale* (McDunnough) (2) 1,8.
- *S. tripunctatum* (Banks) (13) 1,6,7.
- *S. vicarium* (Walker) (224) 1,2,3,4,5,6,7,9,10.

DIPTERA (98)
- *Chironomus* spp. (96) 1,2,4,5,6,8,9,10.
- *Tipula* sp. (2) 4,9.

DECAPODA (98)
- *Cambarus* sp. (98)
- *Orconectes* sp. (93) 1,2,4,5,6,7,8,9,10.

TRICHOPTERA (75)
- *Cheumatopsyche* sp. (67) 1,3,4,5,9.
- *Chimarra* sp. (8) 3,4.

ODONATA (52)
- *Zygoptera* (damselflies)
  - *Agrion maculatum* Beauvois (1) 9.
  - *Argia violacea* (Hagen) (1) 9.
  - *Enallagma civile* (Hagen) (3) 6,8.
- Anisoptera (dragonflies)
  - *Aeschna umbrosa* Walker (1) 7.
  - *Basiaeschna janata* Say (1) 8.
  - *Boyeria vinosa* Say (6) 6,9.
  - *Cordulegaster maculatus* Selys (2) 9,10.
  - *Dromogomphus spoliatus* Hagen (3) 2,6,7,8.
  - *Gomphus scudderi* Selys (5) 7.
  - *Hagenius brevistylus* Selys (4) 7,8.
  - *Macromia illinoiensis* Walsh (24) 2,6,7,8.
  - *Progomphus obscurus* Rambur (1) 7.

COLEOPTERA (47)
- *Helichus* sp. (35) 1,3,4,7,9.
- *Psephenus herricki* DeKay (1) 5.
- *Stenelmis* sp. (11) 1.

PLECOPTERA (16)
- *Perlesta placida* (Hagen) (6) 7.
- Unidentified (10) 7,9.

MOLLUSCA (11)
- *Helisoma* sp. (6) 2,6,8.
- *Sphaerium* sp. (5) 7.

MEGALOPTERA (8)
- *Chauliodes* sp. (1) 3.
- *Corydalus cornutus* (Linnaeus) (2) 3,4.
- *Sialis* sp. (5) 7,8.

Table 7. Water Analyses, East Fork of Twelvepole Creek, 1970.

Sta.	Temp. °C	pH	Dissolved Oxygen mg/l	Dissolved Oxygen % sat.	Alkalinity mg/l ($CaCO_3$)	Hardness mg/l ($CaCO_3$)	Fe mg/l	SO_4 mg/l
1	17.0	7.0	11.0	118.0	40.0	35.0	1.1	14.0
2	22.0	6.7	9.3	110.0	30.0	30.0	0.3	7.0
3	18.0	6.5	8.3	90.0	25.0	20.0	0.6	12.0
4	20.0	6.7	9.5	108.5	25.0	25.0	0.5	12.0
5	27.0	6.9	8.2	105.0	15.0	20.0	0.2	7.0
6	28.0	6.4	8.0	104.0	35.0	35.0	1.5	8.0
7	17.0	6.5	8.3	88.0	30.0	25.0	0.8	15.0
8	20.0	6.5	9.6	108.5	60.0	75.0	2.0	14.0
9	24.0	6.6	7.1	85.6	30.0	20.0	0.6	15.0
10	23.0	6.3	9.1	109.0	30.0	35.0	1.8	16.0
Av. (1-10)	21.6	6.6	8.8	102.8	32.0	32.0	0.9	12.0

Immatures of dipterans, ephemeropterans, and trichopterans constituted about 17, 34, and 22 per cent, respectively, of the total number in a pre-impoundment shallow-water benthic investigation of Watts Bar Reservoir area in Tennessee (Lyman, 1943). In East Fork of Twelvepole Creek, immatures of dipterans, ephemeropterans, and trichopterans composed about 14, 43, and 11 per cent, respectively, of the total number of benthic organisms. Pierce (1969) reported that ephemeropterans composed 30 per cent of a pre-impoundment survey on the Elk River in West Virginia. Olson (1970), in a pre-impoundment study of Beech Fork of Twelvepole Creek in West Virginia, collected 2,106 benthic invertebrates which represented 14 orders, 40 families, and about 60 species. He noted that immatures of ephemeropterans, trichopterans, and plecopterans comprised the highest percentage frequencies, 29, 29, and 18, respectively, of the total number of benthic organisms.

The fish and benthic fauna and certain environmental parameters have been delineated prior to impoundment in the East Fork of Twelvepole Creek in West Virginia. Eutrophication, siltation, and water quality in the proposed reservoir will greatly change the extant lotic fish and benthic fauna, and gradually many of these species will diminish or disappear and be replaced by different lentic populations.

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Comparison of Aggregated and Orthodox Rat Heart Mitochondria *In Situ* Using Thin Sections and Freeze Fracture Techniques

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Abstract

Visualization of 2 distinct mitochondrial configurational states *in situ* is reported using 2 different techniques of electron microscopy. There appears to be a correlation between the aggregated state and couplied mitochondria while the orthodox state results from the addition of an uncouplier of oxidative phosphorylation. The mitochondrion in the aggregated state has tubular cristae while the cristae in the orthodox state are linear with the matrix space expanded. Fracture surfaces of glutaraldehyde fixed mitochondria show a smooth outer membrane surface, a particulate outer surface of the inner membrane, while the matrix surface shows numerous 90-100 Å particles, thought to be ATPase. No fracture through the center of the inner membrane was ever observed in glutaraldehyde fixed tissues.

Configurational changes have been observed with isolated liver mitochondria (1), with isolated beef heart mitochondria (2), with rat heart mitochondria *in situ* (3), and with mitochondria *in situ* from a variety of tissues (4). These

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changes seem to reflect the energy state of mitochondria. Vail and Riley (5,6) have shown using isolated heavy beef heart mitochondria that the configurational changes can be observed using both conventional thin sections and freeze etch or freeze techniques of electron microscopy.

We wish to present evidence here that configurational changes of rat heart mitochondria *in situ* can be observed using freeze fracture techniques as well as thin sections from the same sample with glutaraldehyde fixed specimens.

Materials and Methods

Male adult albino rats from the same litter were sacrificed by cerevical dislocation, and the beating hearts removed, washed twice in ice cold Ca^{++} free Krebs Ringer Phosphate solution (KRP) containing 0.123M in NaCl, 5mM in KCl, 1 mM in $MgSO_4$ and 16 mM in sodium phosphate, pH 7.4. The ventricles were minced with a razor blade on dental wax in KRP and placed in the aggregated or orthodox configurational state, and fixed by the method of Williams *et al.* (4). The fixed tissue was washed in 10 vols KRP with 60 min incubation in each wash and suspended in 30 per cent v/v glycerol overnight at 4C.

For freeze fractures, the tissue was placed in gold cups in 30 per cent glycerol, frozen quickly in Freon 22, placed on the specimen table of a Balzer BA360 freeze etch apparatus precooled to -150C. Freeze fractures were made at -100C at a vacuum of 2×10^{-6} Torr and replicas prepared by the method of Vail and Riley (5). For thin sections, the glycerol treated glutaraldehyde fixed samples were stained and embedded by the method of Williams *et al.* (4). All specimens were examined in a Philips EM 300 operated at 60 kV. The direction of the shadow for freeze fracture micrographs are shown by an arrow in the lower left hand corner.

Results

Figure 1 shows thin sections of rat heart tissue. Numerous mitochondria are present. Figure 1A shows the dense aggregated mitochondria while the less dense orthodox mitochondria are present in Fig. 1B. Note that nearly all of the population of mitochondria are in the same configurational state.

Figure 2 shows the mitochondria at higher magnification. Figure 2A shows mitochondria in the aggregated state having tubular cristae with a dense matrix (space within the tubes). The orthodox state (Fig. 2B) shows a linear arrangement of the cristae while the matrix is much less dense. We have interpreted the difference between the states as a swelling of the matrix in the transition between the aggregated to the orthodox states.

Figure 3 is freeze fracture replicas showing numerous mitochondria in cross section at arrows (Fig. 3A) in the aggregated state. The muscle elements surrounding the mitochondria show linear myofibrils with particles either on or within the myofibrils. The replica is comparable with Figure 1A. Figure 3B is a higher magnification of several mitochondria. The outer membrane (OM) appears as a smooth surface which we interpret as the outer surface. There are convex surfaces (at arrows) which we interpret as the inner membrane matrix surface with numerous 90-100 Å particles on or projecting from the membrane. These particles could represent the ATPase enzyme.

Figure 4 shows freeze fracture of rat heart tissue placed in the orthodox state. Mitochondria (at arrows) show linear arrays of cristae (Fig. 4A). The surface of cross sections show particles. Figure 4B is a high magnification of a mitochondrion showing at least 3 different fracture planes through the membrane. The

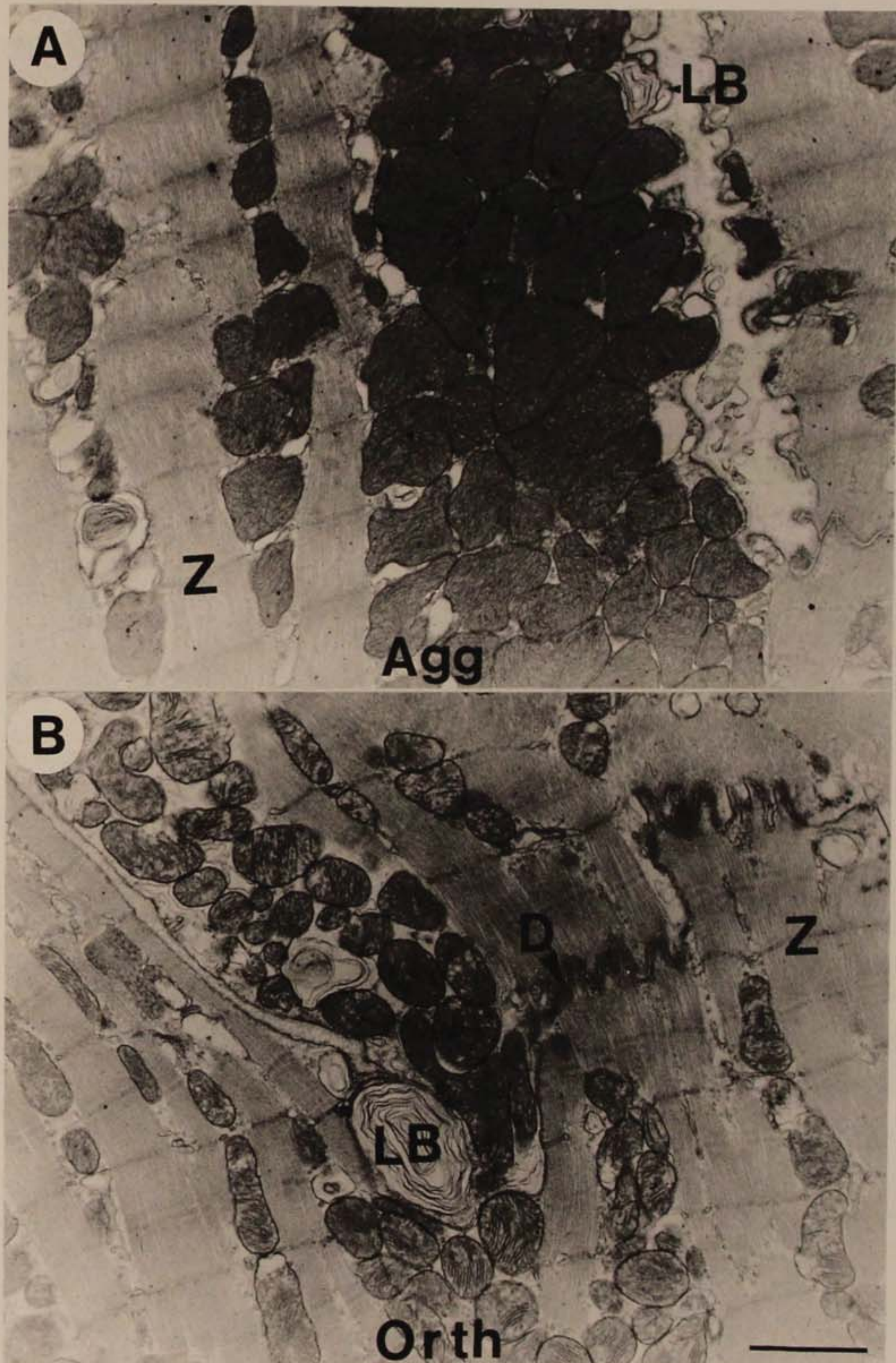

FIGURE 1. Rat heart tissue showing numerous mitochondria. Z lines in muscle, LB-lamellar bodies or myelin figures, D-desmosome. Total magnification: 20,200 x. Bar is 1.0 μ meter.

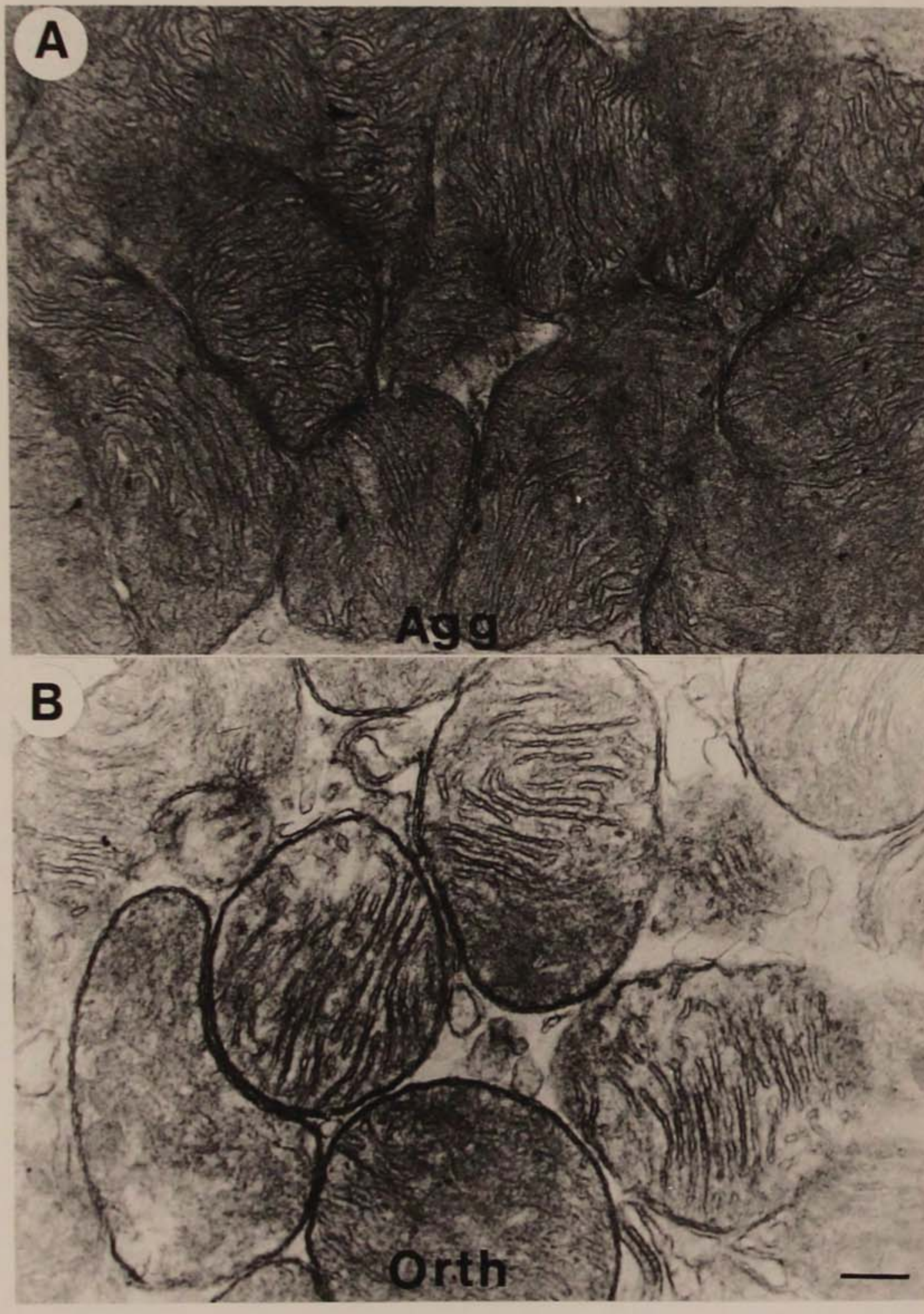

FIGURE 2. High magnifications of mitochondria. Total magnification: 62,500 x. Bar is 0.2 μ meter. A. Aggregated mitochondria showing the tubular cristae. B. Orthodox mitochondria showing the expanded linear cristae.

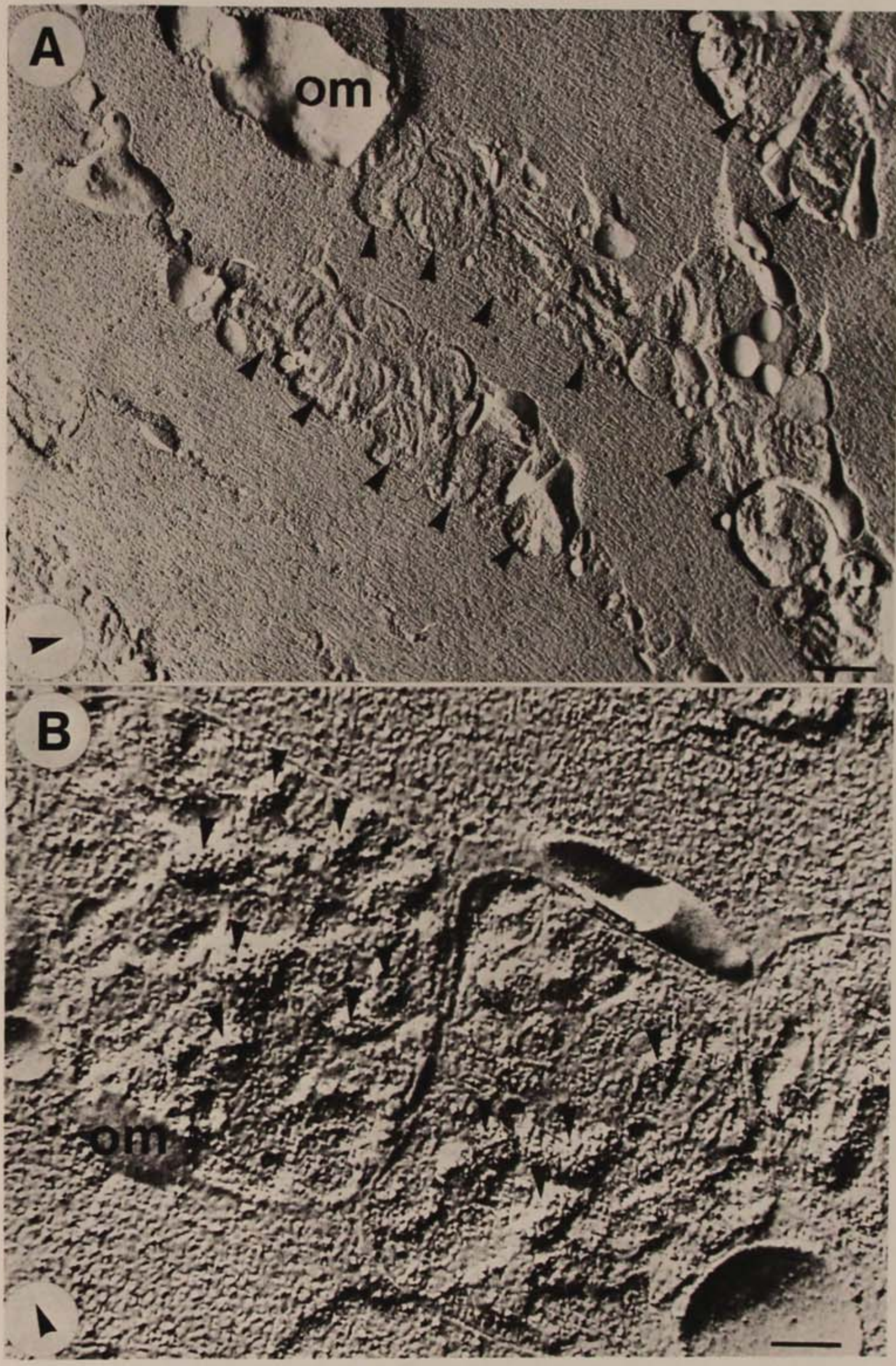

FIGURE 3. Freeze fracture of rat heart tissue. Mitochondria are aggregated and cristae appear tubular. A. Arrows show individual mitochondria fractured in cross section. OM-outer surface of the outer membrane. Total magnification is 25,500 x. Bar is 0.5 μ meters. B. High magnification of several mitochondria. Arrows show the convex tubular fracture of the cristae which have a particulate surface. OM-outer membrane. Total magnification: 62,500 x. Bar is 0.2 μ meters.

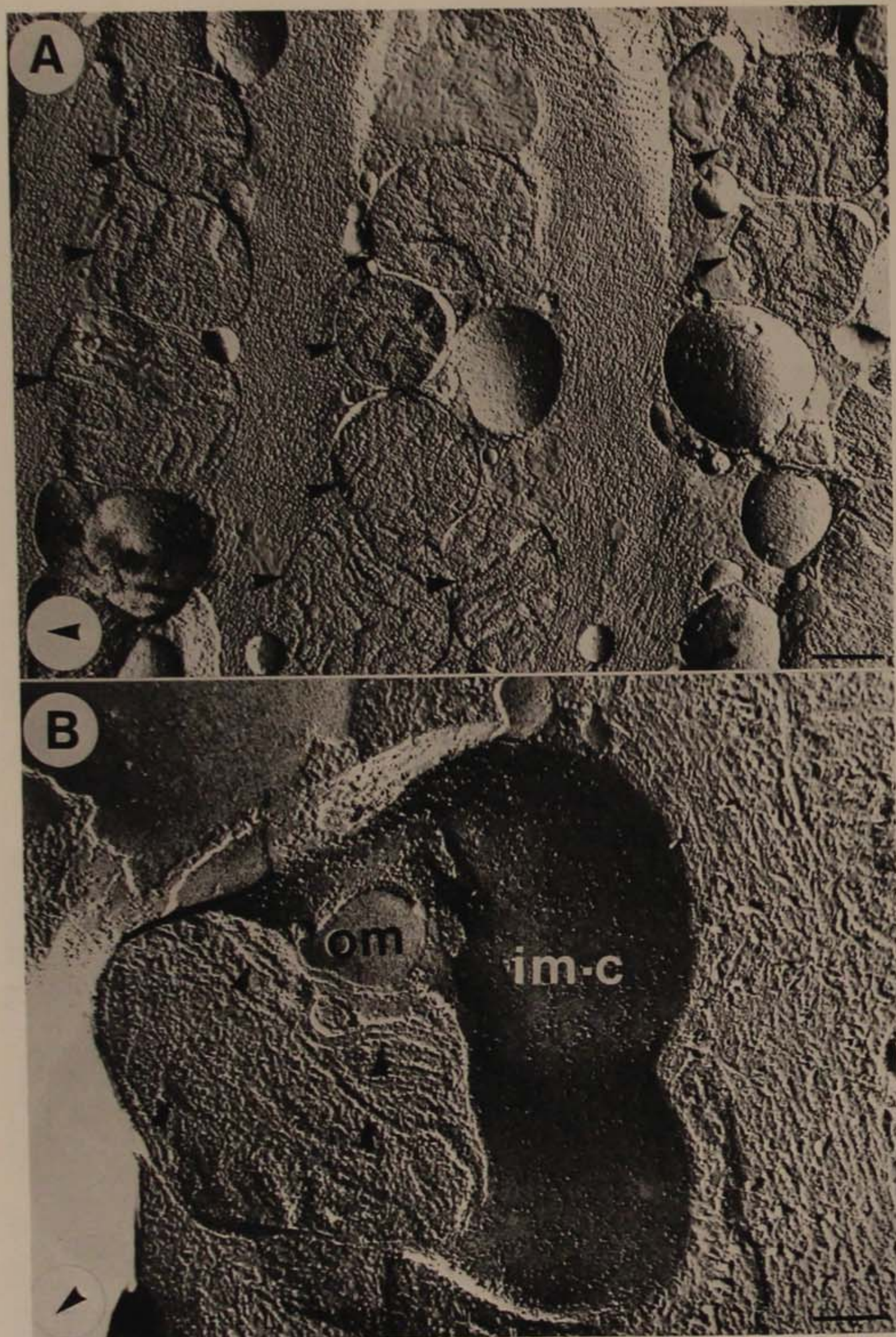

FIGURE 4. Freeze fracture of rat heart tissue. Mitochondria are in the orthodox configurational state while cristae appear linear. A. Arrows show individual mitochondria fractured in cross section. Total magnification: 25,200 x. Bar is 0.5 μ meters. B. High magnification of a single mitochondrion showing 3 fracture planes. OM is the smooth outer surface of the outer membrane, IM–C is the outer surface of the inner membrane (cytochrome *c* side) showing numerous particles in a circular arrangement. Arrows show the cristae. Total magnification: 62,500 x. Bar is 0.2 μ meters.

smooth surface is interpreted as the outer surface of the outer membrane while the surface having a circular arrangement of particles is the outer surface of the inner membrane or the cytochrome *c* side (IM-C). This surface is continuous with the cristae (at arrows), which are invaginations of the inner membrane.

Discussion

Biological membranes seem to have distinct fracture planes. Using red blood cell ghost membranes with membrane surface markers, da Silva and Branton (7) and Tillack and Marchesi (8) found that the fracture surface was particulate. They interpreted that the fracture plane occurs through the interior of the membranes in either unfixed or partially fixed membranes. We have observed fractures which revealed a smooth surface of the outer membrane which we have interpreted as the true membrane surface in freeze fractured specimens. Thus, unlike red blood cell ghosts, mitochondrial membrane surfaces are exposed by the fracture.

Wrigglesworth *et al.* (9) has shown fractures of isolated rat liver mitochondria which were unfixed. They interpreted the particulate surfaces as fractures through the center of the membranes revealing particles, most probably proteins, which were embedded within the interior of the membrane. In cross fractures of the inner mitochondrial membrane Vail (10) has observed large 150 Å particles which appear to extend through the membrane and project into the intra-cristal space. He has interpreted that the 80 Å particle on the intracristal surface of the inner membrane is in reality 80 Å of a 150 Å particle of which 70 Å is embedded in the membrane. Since the particles extend through the membrane, fractures would normally occur around the particles and expose the surface of the inner membrane. Vail and Riley (6) found that unfixed mitochondria, whether *in situ* or isolated, undergo large amplitude swelling which may disrupt the integrity of the membrane. Likewise, glutaraldehyde fixation seems to stabilize the membrane by cross linking adjacent proteins.

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New Records of Rare Opiliones (Arachnida) from West Virginia

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Abstract

Three species of rare Opiliones (harvestmen, daddy-longlegs) have been newly discovered in West Virginia in Mercer, Summers, Pocahontas, Pendleton and Randolph Counties. *Siro exilis* Hoffman (Sironidae) is recorded from Mercer, Summers, Pendleton and Randolph Counties; it was previously known only from two localities in Virginia. *Crosbycus dasycnemus* (Crosby) (Nemastomatidae) is recorded from Pocahontas Co.; it is also found at scattered localities in upper New York, Michigan, Indiana, North Carolina, Wisconsin and Missouri. *Sabacon cavicolens* (Packard) (Sabaconidae) is recorded from Mercer Co.; it usually occurs at high elevations or in cool microclimates, south of central Pennsylvania. The confused taxonomic situation of the latter two species is briefly discussed.

Not much collecting has been done in the central Appalachians by persons interested in arachnids. Thus the collection of three species of rare opilionids in the area is of considerable interest, and suggests that the fauna is much richer than has been supposed heretofore. The detailed records and some explanatory notes follow:

Siro exilis *Hoffman*

New Records: WEST VIRGINIA: *Mercer Co.:* 0.75 mi. N. of Athens, Berlese sample of oak mull from bank of Laurel Creek, 8 December 1967, W. A. Shear, ♀; Athens, ruins of old waterworks, Berlese of oak-hickory-*Rhododendron* mull and humus, 5 July 1967, W. A. Shear, ♀♀. *Summers Co.:* Bull Falls Recreation Area, Bluestone Reservoir, Berlese of wet humus and shale from spring seep, 17 June 1971, W. A. Shear and D. Bard, ♂♂♀♀. *Pendleton Co.:* Spruce Knob, elev. 3500′, Berlese of litter, 8 June 1967, S. Peck and A. Fiske, ♂. *Randolph Co.:* Bickle Knob E. of Elkins, elev. 3800′, Berlese of wet rotted wood, 19 June 1968, S. Peck, ca. 20 juveniles.

Hoffman (1963) described *S. exilis* from wooded ravines in Alleghany and Montgomery Counties, Virginia. As in the above records, all collections were made with Berlese extraction techniques. Hoffman (1963) supposed that *S.*

exilis might be a widespread species in the central Appalachians, but local in occurrence; the above data support his view. The species probably requires constant high humidity and does not occur where any significant drying of the mull and humus layers takes place. I have never found *S. exilis* by sifting litter, and assume that the animals spend all or most of their lives deeper in the mull and humus layers. The type of substrate, lime or shale, does not appear to be important. Other North American sironid species are found in Florida, and in the Pacific Northwest; Juberthie (1969) provides a key to genera.

Crosbycus dasycnemus *(Crosby)*

New Records: WEST VIRGINIA: *Pocahontas County.:* Hills Creek Falls Scenic Area, Berlese of birch litter from narrow floodplain just above middle falls, 19 June 1971, W. A. Shear, ♀♀.

Crosbycus dasycnemus was described as a species of *Nemastoma* by Crosby in 1911; Roewer (1923) later established the genus *Crosbycus* for it. The species has been recorded in the literature only from Columbia, Missouri (the type locality), New York and Michigan, but I have collected it in North Carolina and have seen specimens from Indiana and Wisconsin. In Michigan and New York, the species is often taken from sphagnum moss; elsewhere it has been found in leaf litter. The animals are exceedingly small (less than a millimeter long) and mitelike, and are not likely to be collected except as part of a Berlese sample or by a collector who knows exactly what to look for. All known North American specimens of *Crosbycus* are females. Rambla (1968) has speculated that the species might be parthenogenic.

Rambla (1968) has also shown that the European "species" of *Crosbycus* described by Roewer (1951) are all based upon immature examples of various species of *Nemastoma. Crosbycus goodnightorum* Roewer (1951) from Fountain Cave, Virginia, is likewise not a *Crosbycus.* The description suggests the western species of *Hesperonemastoma;* in particular the size (2.5 mm long), the presence of dorsal spines and the lack of curly recumbent setae on the legs place it in that genus rather than in *Crosbycus.* However, the divided scutum is confusing. The type may have been immature; Roewer never illustrated and rarely examined the genitalia of species he described.

The family placement of the genus *Crosbycus* is also in question. Traditionally placed in the Nemastomatidae, there is some evidence (Gruber, 1970) that like the related *Hesperonemastoma* it may belong in the Ischyropsalidae. The resolution of this question awaits a detailed revision of the entire group in America.

Sabacon cavicolens *(Packard)*

New Records: WEST VIRGINIA: *Mercer Co.:* Athens, under rocks in moist oak woods, 2 July 1966, 22 July 1967, W. A. Shear, juveniles; Camp Creek State Forest, in litter from *Rhododendron* thicket near Mash Fork Falls, 4 December 1971, W. A. Shear, ♂.

For the past 50 years, all North American *Sabacon* species have been synonymized under *Sabacon crassipalpe* (Koch), a Siberian species. I have closely examined material from several parts of the United States, and can readily distinguish at least three species, none of which would seem to be a synonym of *S. crassipalpe.* All material from the eastern United States from New England south to Georgia and west to central Ohio can be assigned to one species, for which the oldest available name is *Sabacon cavicolens* (Packard). *Sabacon occidentalis* (Banks) occurs on the Pacific Coast, and at least one additional species is found in the lower midwestern United States, from Arkansas and Alabama to Illinois. Perhaps this latter species can be called *S. jonesi* Goodnight and Goodnight. Data supporting these conclusions will be supplied in my forthcoming revision of North American *Sabacon*. Dresco (1969) has removed the species of *Sabacon* from the family Ischyropsalidae and placed them in the new family Sabaconidae, a procedure that certainly seems justified.

My experience in collecting *Sabacon* in West Virginia, North Carolina, Pennsylvania and Vermont has led me to the conclusion that mature specimens can be obtained only very late in the year from late October to December, depending on the latitude and altitude. Very early spring collecting will sometimes yield a few adults. Large series of adults can be taken from spruce-fir forests on the tops of mountains, from beneath rocks and logs; individuals appear to occur only sporadically at lower elevations. This affinity for cool microhabitats and the attainment of maturity in winter suggest that *Sabacon cavicolens* in the central Appalachians is a boreal relict.

Acknowledgments

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Allometric Changes with Instar in *Leptoneta* sp. (Araneae, Leptonetidae)

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The genus *Leptoneta* of the family Leptonetidae has a wide distribution. The genus is well represented in caves in Europe (Fage, 1913) and is found in the United States in caves and in epigean situations. Little has been published about the biology of species of *Leptoneta.*

Specimens of an undescribed species of *Leptoneta* used in this study were collected in Camp Creek State Forest, Mercer County, West Virginia, the farthest north specimens of the genus have been collected in eastern North America. When time permits, this species will be compared to *L. silvacultrix* Crosby and Bishop, since that species has been collected nearer West Virginia than any other.

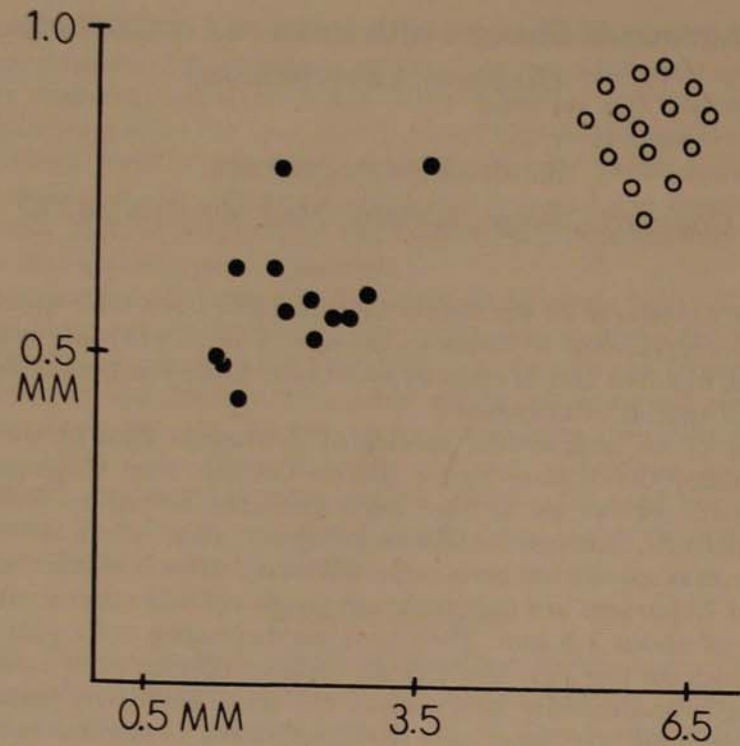
Species of *Leptoneta* are pale pink and cream-colored spiders with an average body length of about 1.5 mm. They have no distinctive color pattern, but have iridescent scales on the legs. The legs are long relative to body size and give the spiders a fragile and delicate appearance. The most distinctive feature is the eye pattern. Aside from the blind, cave dwelling forms, *Leptoneta* species have six eyes, with four in the front row and two in the back row. Species are usually separated on the basis of differences in the sexual organs and their accessories (see Komatsu, 1970).

In this study, I attempted to determine allometric growth patterns in *Leptoneta* sp., and the reliability of some characters often used in general spider taxonomy.

All measurements were taken by myself with a compound microscope and eyepiece micrometer. The measurements taken were: Length and width of carapace, length and width of ocular area, length and width of femur, length and width of tibia, length and width of metatarsus and tarsus, length of leg I (all leg measurements taken on leg I), and total lengths of the legs of one side. Time did not permit the measurements to be taken more than once.

The animals were grouped into five instars based on discontinuities in carapace length. It is not certain that the smallest instar is present in the collection, however. Statistical parameters were calculated (mean, standard deviation, standard error) for instars II (14 specimens) and V (18 adult specimens). Scatter diagrams and Hubbs-o-grams were made from these computations. Fig. 1 is a scatter diagram of the length of leg I plotted versus the length of the carapace. The points do not appear to follow a single regression line. Fig. 2 is a scatter diagram of tibia I length plotted versus tibia I width. These two groups of points also do not follow the same regression line. Fig. 3 is a Hubbs-o-gram of the ratio of tibia I length over tibia I width; Fig. 4 a Hubbs-o-gram of the ratio of leg I length over carapace length. There is no standard error overlap in either.

Allometric growth, a change with age in the ratio between two dimensions, can be shown by a scatter diagram of the dimensions if the two groups of points plotted do not appear to follow a single line. This was the case in the pairs of dimensions plotted in Figs. 1 and 2, demonstrating the more rapid growth of the first leg when compared to the carapace, and of the length versus the width of tibia I. Thus the legs of older specimens are longer with respect to the carapace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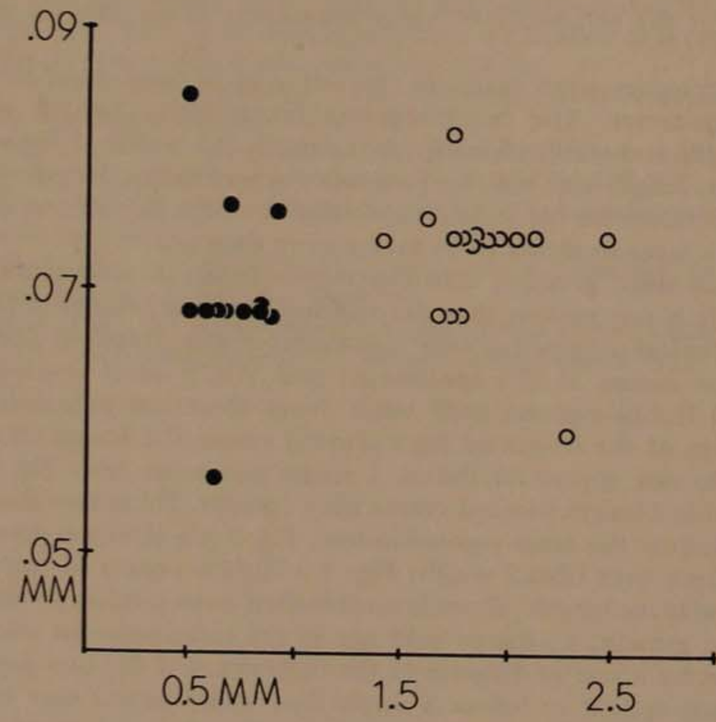
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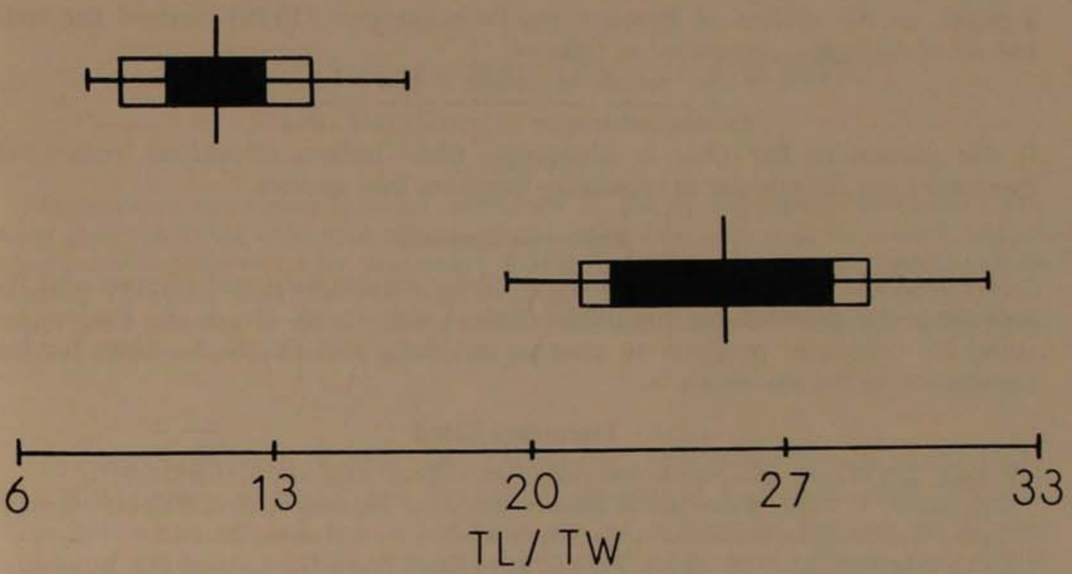
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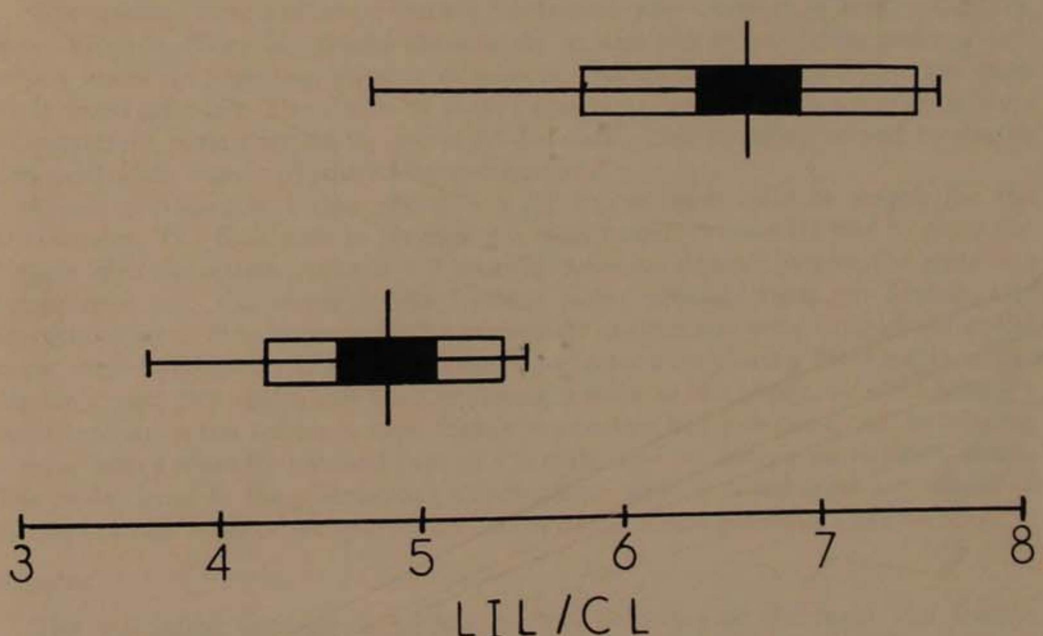
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length than the legs of young spiders, and the tibia becomes longer and thinner with age. Furthermore, comparison with other measurements indicated that the main increase in leg length takes place in the tibia.

The fact that the growth of the tibia is allometric is of taxonomic interest. In a paper on the spiders of Porto Rico, Petrunkevitch (1929) devised the well-known tibial index, computed as follows:

$$\frac{\text{width of patella X 100}}{\text{Combined length of patella and tibia}}$$

If the growth of the tibia is allometric, tibial indices calculated from adult specimens are of little use in separating juveniles into species.

Acknowledgments

I would like to thank Dr. William A. Shear for his help and interest and for preparing the illustrations for publication. I also thank Dr. L. E. Bayless for using his computer program to process my data, and Dr. E. K. Blatt for her comments on the manuscripts.

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Observations of Courtship Behavior in *Lycosa Carolinensis*

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Abstract

Seventy-one encounters between individuals of *Lycosa carolinensis* Walckenaer have been observed. Using adult spiders, male-female encounters, male-male encounters, and sex pheromone experiments were performed. Foreleg extensions, palpal movements, and abdominal vibrations were observed to be the major aspects of the male's courtship display. Males were observed to court other males and to court each other simultaneously. Some evidence is presented in support of a sex pheromone.

Lycosa carolinensis Walckenaer (Lycosidae), commonly known as the Carolina Wolf Spider, is distributed throughout the eastern part of the United States. This species is one of the larger members of the genus *Lycosa;* the female attaining a length of 22-35 mm and the male of length of 18-20 mm. Very little has been published concerning the mating behavior and the actual courtship of this species. This study establishes a specific account of the courtship procedure of *L. carolinensis* males and examines various aspects of the courtship behavior.

Materials and Methods

The spiders were collected during September and October in Mercer County, West Virginia. They are found abundantly at night in grassy fields and pastures which maintain high populations of insects. The specimens were housed in cages built from pint jars. The floors of these cages were covered with a half-inch layer of plaster of paris containing activated charcoal. This covering served to absorb and neutralize wastes produced by the spiders.

Clear, rectangular boxes about 5 x 12 inches were used as arenas for the encounters. The first step in arranging a male-female encounter was to place the female into the arena. After a 1-2 minute "calming-down" period, the male was introduced into the arena at the farthest point possible from the female. Observations were then recorded. The male-male encounters were carried out in the same way. Pheromone experiments were performed by placing filter paper in the female's cage overnight and then exposing a male to the paper, by introducing a male into an arena where a male-female encounter had just occurred, by placing a male into a recently vacated cage of a female, and by using a partitioned arena. The males used in the pheromone experiments were isolated from any visual or tactile contact with other spiders for twenty-four hours previous to their use.

Results

The following account is of the normal behavior of the male and female during an encounter. It is based on average times and specifics which occurred most often in twenty-eight separate male-female encounters.

At the beginning of an encounter the female would generally assume a certain position in the arena before the male was introduced and remain there motionless. Upon the introduction of the male into the arena, she would usually maintain her position while he moved about.

A major movement of the female when the male was within a few inches or an actual contact between the two spiders usually initiated immediate reactions from the male. In the many cases where the female maintained her position after the male was introduced it was found that the male began courtship without any movement or contact from the female in a mean time of 3.1 minutes; the actual times varying from 1-15 minutes.

The display of the male began when he assumed a characteristic courtship posture. The most noticeable feature of this posture is the anterior vertical extension of the forelegs. This position is held throughout courtship. The forelegs, when held in this position, may be observed to be flexed in a very specific way. Both legs are held above the body and are nearly parallel to each other. The tarsus points upward at an angle of about seventy-five degrees in relation to the substrate. The metatarsus, tibia, and patella are held along a straight line and at an angle of about forty-five degrees to the substrate. The femur is positioned also at about seventy-five degrees, although this position is not held as rigidly throughout the courtship procedure as are the others. The legs are held relatively fixed in their vertical position, but there is a horizontal, simultaneous waving of them in opposite directions outward from and back to their normal parallel position. From the time that the leg-raised position is assumed and throughout the courtship, the body is held slightly above the substrate on the three posterior pairs of legs. The male maintains this position throughout the courtship sequence.

After the posture is assumed, the male begins other movements. Almost immediately there is a forward movement of the palps, away from the body. They are not raised much during this time and may even be touching the substrate. The palps move simultaneously and proceed from a position where they are tucked near the body forward to the position where they are normally held at times other than courtship and finally to a point where they are extended away from the body. This movement produces no sound audible to the human ear. This palpal extension takes less than one second. As soon as the palps reach their most extended position, there is a vibration of the abdomen and the palps return to the tucked position. This abdominal vibration consists of two distinct movements. The first movement is a downward twitch at which time the tip of the abdomen is quickly lowered near the substrate but does not strike it. The second movement is a vibrating return of the abdomen to its original position. This entire abdominal movement takes less than one second. These vibrations occur throughout the courtship and with each vibration there is a corresponding palpal movement.

After the male assumes the courtship posture, he usually remains in a fixed position until two or three vibrations and palpal movements occur. This series of movements is then followed by a short approach toward the female, or the movement of the body to orient the head region in a different direction, or sometimes nothing at all other than a pause of 8-10 seconds. After this, there generally followed another sequence of two or three vibrations with the same event(s) occurring afterwards. This alternation of sequences occurs throughout the male's approach. The time between each vibration at the beginning of the courtship is somewhere between 6-10 seconds, and the time between each set of vibrations is anywhere from 5-20 seconds, depending on the male's actions.

The characteristics of the male's approach depends greatly on the female's actions. If the female remains motionless, the approach of the male is steady and deliberate; if the female moves suddenly away from the male, then the male moves toward her quickly; or if the female moves toward the male, his approach becomes slow and cautious. When the male gets within reaching distance of the female, about 30 mm, he proceeds carefully to make contact. This contact is made by probing the female (generally at the region which is closest to him) with either one or both of the raised forelegs. This probing is done quickly, with the leg extended and withdrawn in a rapid fashion. The only part of the leg which actually touches the female is the tip.

The female responds to this action in one of two ways. She may turn on the male and fight. The fighting usually consists of an entwining of the legs and wrestling. Only once has the wrestling been observed to result in the death of either spider; this spider was a male. The female usually flees after fighting, and the male will follow and continue the courtship movements. Sometimes, however, the female becomes the aggressor and pursues the male. In this case the male flees and ceases courtship. Another way in which the female may respond to the probing is to flee immediately from the male. In this case the male continues to react and to follow the female. It appears that the main objective of the female in these encounters has been to get away from the male.

Other observable actions, possibly of importance, were seen in some courtships. The courtship movements of the male seem to increase in intensity as the encounter proceeds. The vibrations and palpal movements occur every 6-10 seconds at the onset. As they continue they speed up and eventually occur every 2-3 seconds. Also, contact between the male and female seems to speed the sequences in a similar manner, no matter at what point in the encounter the contact occurs. The sequences will also speed up if a male's forelegs are touched with an inanimate object during courtship. This indicates that these movements are responsive to touch. The males also exhibited random movements of the palps on the substrate and other erratic movements when they apparently lost sight of the female. This usually resulted in a re-orientation in the female's direction. During this movement the vibrations and palpal movements ceased, but the courtship posture was maintained. When the male was re-oriented the sequences began again.

Twelve male-male encounters were performed and courtship occurred in nine of them. The males were observed to begin courtship upon making contact with another male and in some cases upon seeing another male. Simultaneous courtship occurred in two of the tests.

Thirty pheromone tests were performed and ten of them proved to be positive. In eight of these positive tests the males performed normal courtship; that is they showed all of the normal movements. In the other two tests the males performed only a partial courtship. One assumed the posture but performed no sequences. The other performed the sequences but did not assume the posture.

Discussion

The courtship procedure of *Lycosa carolinensis* described in this paper contains three definite behavioral aspects, and possibly a fourth. The use of the forelegs in lycosid courtship seems to be a common action. In the case of *L. carolinensis* both legs are elevated simultaneously and are waved. Rovner (1968) describes an extension of one or the other forelegs of *L. rabida* Walckenaer during courtship, but never a simultaneous extension.

Another of the most noticeable characteristics of courtship is the vibration or twitching of the abdomen. Rovner (1968) describes a similar movement in *L. rabida*, and Kaston (1936) speaks of similar actions in two other species of *Lycosa*. Harrison (1969) mentions the production of sound audible to the human ear caused by the vibration of the abdomen of *L. gulosa* Walckenaer. The vibration of *L. carolinensis* produces no detectable sounds.

The third major aspect of the courtship is the scraping movement of the palps. Rovner (1968) mentions the drumming of the palps of *L. rabida* during courtship and the production of an audible sound. Palpal drummings are also described for the species *L. gulosa* and *L. helluo* Walckenaer (Kaston, 1936). The palpal movements of *L. carolinensis* occur at the same time as the abdominal vibration. The palps may or may not be touching the substrate, and there is no sound produced that is audible to the human ear.

The fourth aspect which is possibly part of the courtship is the presence of a sex pheromone. Although some evidence was compiled in favor of a pheromone, the results are not conclusive. Hegdekar and Dondale (1969) completed more positive studies concerning pheromones in several species of lycosid spiders.

The male-male encounters indicated that sight plays an important role in courtship but that the specific sight of the female is not needed to incite courtship. The males were observed to court simultaneously. Rovner (1968) observed courtship in male-male encounters of *L. rabida* but never a simultaneous courtship. The fact that males will court other males seems to be an indication of poor vision in *L. carolinensis*.

Acknowledgments

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Somatic and Genetic Effects of Vibration on *Drosophila Melanogaster*

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Abstract

Pupae from an inbred wild strain of *Drosophila melanogaster* Meigen were exposed to a low frequency (mechanical) vibration treatment consisting of a ± 10 G acceleration and a sweep frequency of 50 to 100 Hz for four minutes. Somatic and genetic effects of the vibration treatment were evaluated between vibrated and control groups by progeny comparisons within and between generations.

The absence of gross developmental abnormalities in the vibrated P_1 generation indicated that there were no significant somatic effects from the vibration treatment. However, the presence of a significant number of morphological abnormalities in the F_1 generation vibrated group may indicate that dominant mutations were produced by the vibration treatment; but further testing is needed to confirm that the F_1 abnormalities are mutants.

The genetic effects of the vibration treatment were indicated by modifications of the egg-laying capacities, sex ratios, and survivals of progeny in the vibrated group. The higher egg-laying capacity of the vibrated F_1 females indicated a heterotic effect resulting from the combination of vibration altered chromosomes and wild type chromosomes. The F_2 generation vibrated group showed a significantly decreased egg-laying capacity, slightly decreased survival rate, and a slightly altered sex ratio (favoring females). The significantly decreased egg-laying capacity indicated that deleterious effects of vibration were masked in the F_1 females. The slightly decreased survival and slightly altered sex ratio was attributed to the presence of recessive sex linked lethals and small polygenic changes. The F_3 generation exhibited a significantly reduced survival rate which indicated deleterious genetic modifications were present. These changes probably resulted from the vibration treatment but were masked in earlier generations and transmitted to their progeny.

Accumulation of Air Borne Particulates in the Lungs Of House Sparrows (*Passer domesticus*)

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Abstract

This investigation was undertaken as a prelimary study to determine the amount of accumulation and proportion of area containing air borne particulates in the lungs of two different house sparrows populations. Twelve birds, six each, from Montgomery and Mount Storm, West Virginia were collected from February, 1971 to March, 1971. Lungs were excised and slides were made and examined for particulate matter. A grid method was used to determine the concentration and proportional amounts of tissue containing particulates. And a weighing method was also used to determine the proportional percent of tissue containing particulates.

These two populations were significantly different from one another. The amount of air borne particulates, measured as micrograms per cubic meter, was 1.50 times greater in Montgomery than that near Mount Storm. The amount of tissue containing particulate matter was 1.87 times greater in birds from Montgomery than those from near Mount Storm. Also as determined by weight method the total area containing particulate matter in the tissue was 1.74 times greater for Montgomery birds than those near Mount Storm. The concentration of particulates in the lungs of the two populations were also different. Forty-six per cent of the tissue of the birds from Montgomery containing particulates was rated high, 20 per cent medium, and 34 per cent low; whereas in, the birds from Mount Storm only 21 per cent of the tissue containing particulates was rated high, 33 per cent medium, and 46 per cent low.

In birds, particulate matter was found predominately in the tissue surrounding the air passage ways of the lung. The appearance of the tissue containing particulate matter was different from that not containing particulate matter.

The Incidence of Intestinal Parasites Found in Students in a Small West Virginia Community

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Abstract

Seventeen per cent of three hundred eighty-two students from Head Start and the tenth grade in a southern West Virginia community were found to harbor parasites. Among the

older students *Ascaris lumbricoides* was prevalent while in the younger students, *Entamoeba histolytica* cysts were most numerous.

The purpose of this study was to determine the prevalence of intestinal parasites in students in a small community of southern West Virginia and correlate the findings with observations made in the examination of children entering the Head Start program and with the national average of parasitema.

Ascaris lumbricoides is cosmopolitan. Stoll (1947) estimated that there are 644 million human infections in the world, of which 3 million are found in North America and 421 million in tropical America, 59 million in Africa, 488 million in Asia, 32 million in Europe, 19.9 million in Russia and 500,000 in the Pacific Islands.

Adult ascarids live in the small intestine of man where they may be capable of puncturing intestinal walls in addition to feeding on chyme. Egg production is prolific, one female laying 27 million eggs. A fertilized egg is 60-70 μ by 40-50 μ and has a thin transparent shell covered by an irregular coat of albumin that is usually brownish in color. Both fertilized and unfertilized eggs are found in feces. The unfertilized egg is less ovoid and the albuminous coat is less prominent or absent and the zygote is not well formed.

Enterobius vermicularis was found to infect 32.93 per cent of the pre-school children from a wide geographic range in the United States. (Cheng, 1960. *E. vermicularis* requires no intermediate host. Both males and females are attached to the epithelial wall within the host's intestines. After copulation, the gravid females migrate posteriorly at night and lay their eggs in the perianal folds. After ovipositing eggs containing motile larvae, the females migrate back into the large intestine. Ingested eggs hatch once they reach the duodenum, and the larvae migrate posteriorly, molt three times and become mature upon reaching the large intestine. It generally takes two months to reach maturity. Eggs remain viable one week or more under cool humid conditions.

Entamoeba histolytica is the causative agent of amoebic dysentery, or amoebiasis, and is universally distributed. The incidence of infection has varied from 1.4 per cent in Tacoma, Washington (Cresswell and Wallace, 1934) to 36.4 per cent in rural Tennessee. On the average, 8.1 per cent of Americans serve as hosts.

Cysts of *E. histolytica* are spherical and surrounded by a cyst wall. The size varies between 3.5 and 20 μ in diameter. The cysts contain four prominent vesicular nuclei and elongate chromatoidal bars that possess rounded ends.

Entamoeba coli has a similar cycle as that of *E. histolytica*, but it does not ingest or invade host tissues. The mature cyst includes eight vesicular nuclei with eccentrically placed endosomes, and the chromatoidal bodies are irregular with jagged ends. Cysts of this amoeba are found in diarrheic feces, however, it is believed to be nonpathogenic.

The community surveyed is a small town located in mountainous terrain with the majority of the homes built on steep river valley hills. The economy is based chiefly on railroads, lumber and tourism. Local industry is non-existent; most businesses are small, and locally owned. The economic level of the majority of persons living in this community is below the poverty level as defined by the Nixon Administration. There are 1681 jobs in the county that net gross their workers an average of $1,810.00 per annum. The county supports on its welfare roll, 1000 families with an average of $1,750.00 per annum.

Methods

Permission for this survey was obtained from the county Board of Education, principal of the school, faculty, and from the participants' parents.

After acquainting the students with the importance of medical research and the value of such project, each was given a small brown paper bag, 3 Tomac tongue blades and 1 half-pint Sealkraft Nestyle Sealright fecal sample cup. They were told to use the toilet as they normally do and to use a tongue blade to cut and lift a small piece of sample (about the size of a thumb) and place it in the collection box, put the lid on and place the boxed sample in the bag and store in a cool place until morning when they would bring them to school to be collected and examined. The sample was marked with "T" if the student lived within the city limits and with "R" if rural.

The collected samples were numbered for identification, placed in an ice chest and transported to the laboratory where they were refrigerated until they could be prepared. A small piece of feces was taken from each cup and stirred in a drop of 0.4 percent saline on a slide. This direct smear was then covered and examined microscopically under high power (300X) by starting at one corner of the cover slip and examining all of the area. All findings were carefully recorded and the samples put in refrigeration until all smears were examined. The next step was to perform a concentration on each sample by use of D'Rivas Concentration Procedure.

A third piece of sample (about 1 oz.) was placed in a dropper-type bottle and stored in Merthiolate Iodine Formalin preservative for future reference.

Results

A thorough examination of the fecal samples of 382 children revealed very little difference in the per cent of infection between the five year old and the fifteen year old groups; 18 per cent as opposed to 16 per cent respectively. The 34 per cent incidence of infection in rural areas is almost five times that of the 7 per cent obtained in town areas. The most common parasite found in the fifteen year old group was *Entamoeba* (59 percent incidence) while the younger group had a larger incidence of *Ascaris* (38 percent). (Tables I, II, III, and IV).

Discussion

This survey shows that for the small segment of the population represented by this sampling, the rural residents harbor a greater incidence of parasitemia. This difference may be accounted for by the disposal of sewage. The town ordinances do not allow raw sewage disposal in city limits and all city housing must be linked to the sewage disposal treatment system maintained by the city. Eighty per cent of the rural homes in the area have inadequate sewage disposal, most families simply dump raw sewage, which may reach local water tables without much modification.

The two most prevalent parasites, *Entamoeba* and *Ascaris* are associated with poor sanitation disposal systems. When we consider that these eggs must be ingested either directly from feces or via infested water or foodstuffs fertilized with infected feces, the high incidence of these two parasites indicates that the sanitation systems of the rural subjects are inefficient.

The 17 per cent total infection found in this survey is greater than the national incidence of approximately 10 per cent, therefore, this study should alert local health authorities that a problem does exist.

Table 1. Number of Students Infected With Parasites In a Small Southern West Virginia Community.

Parasite	*Town*	*Rural*
Entamoeba histolytica	0	1
Entamoeba coli	4	5
Ascaris lumbricoides	1	5
Enterobius vermicularis	0	1

Table 2. Number of Children Infected With Parasites Entering the Head Start Program In Southern West Virginia Community.

Entamoeba histolytica	9
Ascaris lumbricoides	20
Enterobius vermicularis	3

Table 3. Comparison of Intestinal Parasites In Public School Students and Head Start Participants.

	School	*Head Start*
Subjects Studied	103	279
Subjects Age	15	5
Subjects with Parasites	17 (16%)	52 (18%)
Total No. Rural Subjects	35 (34% of total studied)	* (approx. 40% of total studied)
Total No. Town Subjects	68 (66% of total studied)	* (approx. 60% of total studied)
Rural Students Infected	12 (34% infection)	* ------
Town Students Infected	5 (7% infection)	* ------

*Data Not Available.

Table 4. Relative Frequency. Total No. of Each Parasite Found Divided by the Total No. of Parasites Found.

	Students	*Head Start*
Entamoeba histolytica & *E. coli*	59%	17%
Enterobius vermicularis	6%	6%
Ascaris lumbricoides	35%	38%
Other	0%	39%

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