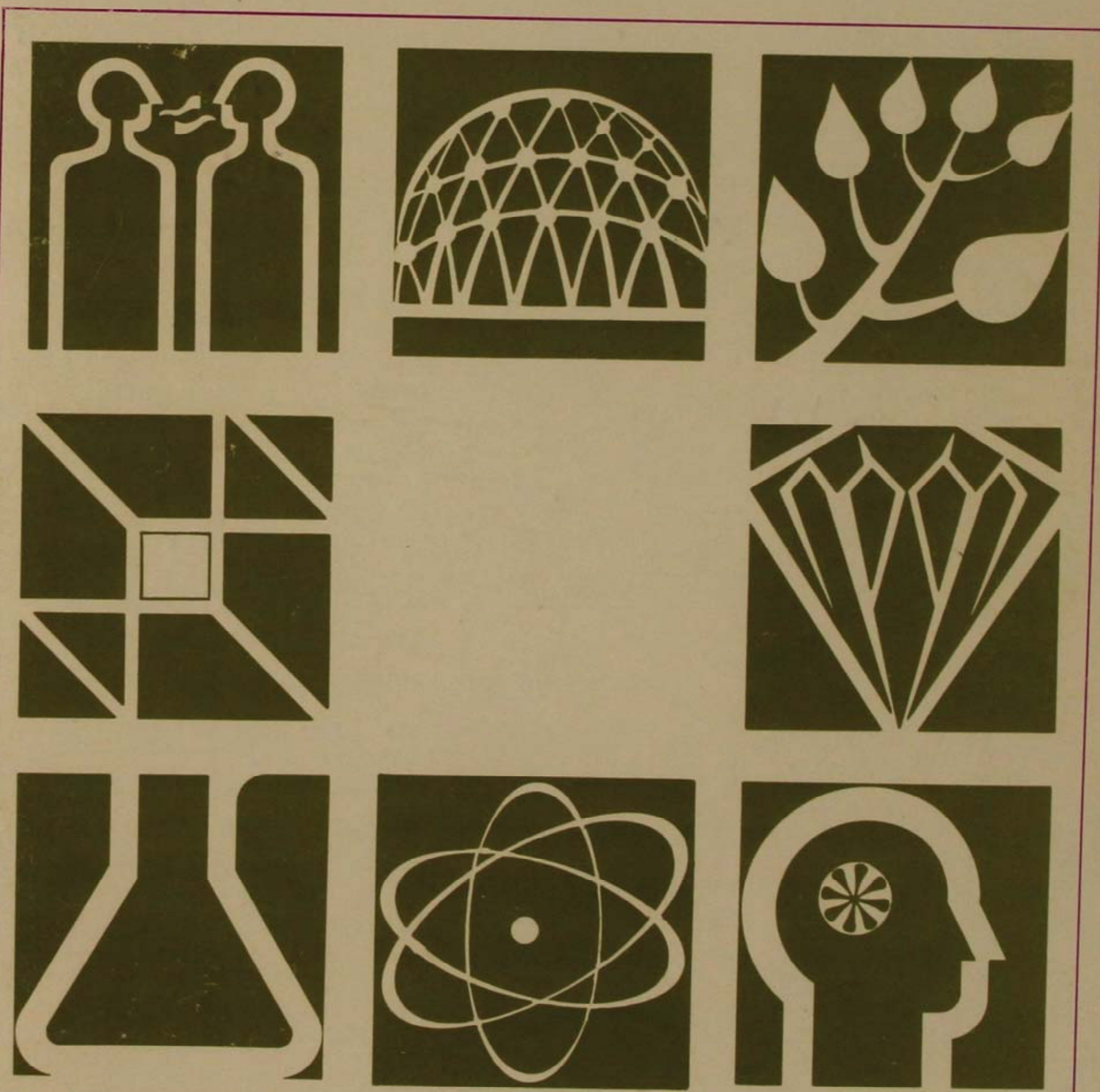
Volume 45, Number 2

Proceedings of the West Virginia Academy of Science

1973



Proceedings of the West Virginia Academy of Science 1973

Vol. 45—No. 2

THE FORTY-EIGHTH ANNUAL SESSION

Fairmont State College

Fairmont, West Virginia

APRIL 5, 6, 7, 1973

Printed by
McClain Printing Company
Parsons, West Virginia

Cover Design
Meredith Pearce
West Virginia University
Office of Publications
Morgantown

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."

Proceedings of the West Virginia Academy of Science

Published quarterly by the West Virginia Academy of Science, Inc. Manuscripts for publication should be sent to the Editor, Anthony Winston, Department of Chemistry, West Virginia University, Morgantown, West Virginia 26506. Proof, edited manuscripts, and all correspondence regarding papers for publication should be directed to the Editor.

Applications for membership in the Academy and dues should be sent to Joseph Glencoe, West Virginia Wesleyan College, Buckhannon, West Virginia 26201. Changes of address should be sent to Elizabeth Ann Bartholomew, Department of Biology, West Virginia University, Morgantown, West Virginia 26506. Correspondence concerning library exchanges should be directed to Director of Libraries, West Virginia University, Morgantown, West Virginia 26506.

Contents

Newsletter

The West Virginia Academy of Science Newsletter will now be included from time to time in the Proceedings. Only material of significant interest to the Academy, the State of West Virginia, or an appreciable segment of the membership will normally be included. All material for the Newsletter should be directed to Elizabeth Ann Bartholomew, Department of Biology, West Virginia University, Morgantown, West Virginia 26506.

OBITUARIES

Armand Rene Collett

Armand Rene Collett

Armand Rene Collett who devoted 41 years of his life to West Virginia University in capacities ranging from instructor in chemistry to dean of the college of Arts and Sciences, died on June 17, 1973 at the age of 78. Dr. Collett was dean during the decade from 1951 to 1961. When he left the deanship he returned to chemistry as professor and acting chairman for two years. Soon after, he retired, but was recalled to serve until 1965 in a part-time capacity as an assistant in Administration and Finance.

A native of Hartford City, Indiana, his studies at the University were interrupted by World War I military service but he returned to receive the A. B. degree with the Class of 1918, then went to Yale for graduate work.

Dr. Collett was a member of several scientific societies including the West Virginia Academy of Science, and was a member of the board of the Monongalia General Hospital for twenty years. Along with these many accomplishments he also published more than fifty scientific articles and was the author of a general chemistry laboratory manual.

Surviving are his widow, the former Dorothy Atwood, and two daughters, Dorothy Collett Vandelinde, Charleston, and Florence Collett Shreve, New Haven, Ohio.

Dr. Charles E. Lawall

Charles E. Lawall, charter member of West Virginia Academy of Science and twelth President of West Virginia University died at his home in Huntington on April 4, 1973 at the age of 81. Dr. Lawall was one of the most respected mining engineers in the history of the coal industry. Among his many distinctions he was awarded honorary doctorates by Lehigh, Waynesburg, and Morris Harvey College. In 1970, Dr. Lawall was given the highest award of the American Institute of Mining, Metallurgical and Petroleum Engineers, the Erskine Ramsey Gold Medal. He also received the Mineral Industry Education award in 1957 from the A.I.M.E., and the Bituminous Coal Research Award.

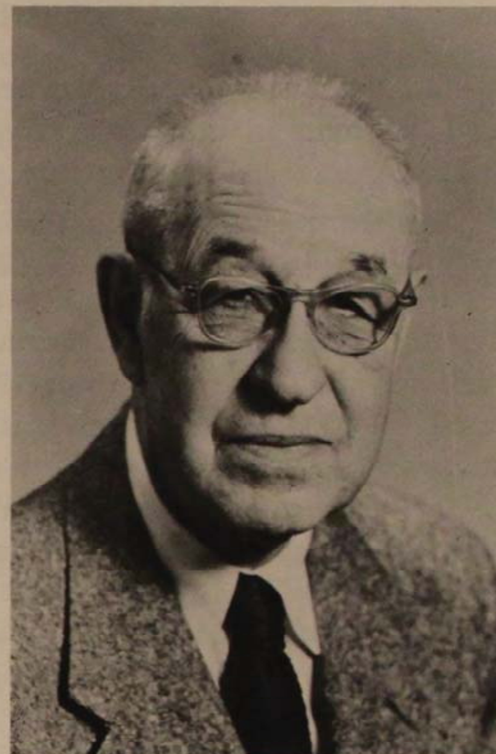
Dr. Lawall's educational background includes a bachelor of science degree in mining engineering with first honors at Lehigh in 1914. After serving in World War I he returned to Lehigh and received his master of science degree in mining engineering in 1921. After graduation, Dr. Lawall joined the faculty at West Virginia University and by the mid-twenties had advanced to the rank of professor and head of the Mining Engineering Department. In 1930, when the School of Mines was created, Dr. Lawall assumed the directorship. In 1938, Dr. Lawall was appointed acting president of the University and in 1939 was given full title.

Charles E. Lawall was very active in civic affairs, both state wide and locally. He was president of the West Virginia Coal Mining Institute and president of the Kiwanis Club. Also, he was active in the affiars of the Chamber of Commerce and a member of the First Presbyterian Church.

Dr. Charles E. Albert

One of our West Virginia Academy of Science members, Dr. Charles E. Albert, a prominent retired educator, died at the age of 90 on January 27, 1973, in Elkins. Dr. Albert had been in failing health since June, but his death was unexpected.

Dr. Albert was born January 10, 1883, in Pen Argyl, Pennsylvania, a son of the late Urabanus and Sarah Jones Albert. Following graduation from high school he received his degree in civil engineering in 1908 from Lafayette College. He took graduate work at North Carolina State and later at Penn State. On September 1, 1919, he married the former Jeanne Marstiller who survives him.



Dr. Charles E. Albert

After teaching in high school for six years, he went to Davis and Elkins College in 1911 as professor of mathematics and physics. In 1913, he went to Penn State University where he taught engineering and mechanics. He then became a civil engineer for Camden Irons in Camden, New Jersey, filling that post from 1916 to 1920.

From 1920 until 1922, Dr. Albert was instructor in strength of materials at Drexel Institute of Technology in Philadelphia. In 1922, Dr. Albert returned to Davis and Elkins College where he was dean of the college and professor of mathematics and physics, and later head of the Department of Physics and Engineering from 1946 until 1957. The Charles E. Albert Hall at Davis and Elkins College was named in his honor.

He had honorary degrees in Law from Southwestern University (1937) and Lafayette College (1939) and Doctor of Science from Davis and Elkins College.

Dr. Albert was a member of American Association of Engineering Education in which he held offices, National Society of Professional Engineers, West Virginia Society of Professional Engineers, and Registered Professional Engineers Association of West Virginia.

In addition to his widow, he is survived by one daughter, Jean (Mrs. William Lewis Abscott) of Daytona Beach, Florida. His son, Lt. Albert, Jr., was killed while serving in the U.S. Navy.

NATURAL LANDMARKS

West Virginia University has been asked by the National Park Service to evaluate nineteen sites in West Virginia that may be designated as U.S. natural landmarks. Only sites that possess national biological or geological significance are designated natural landmarks by the National Park Service. Such designations are made only if requested by the landowners, who agree to comply with basic conservation practices relating to management and protection. Cathedral State Park and the Cranesville Swamp Natural Preserve, which are administered by West Virginia University, are the only designated natural landmarks in West Virginia. Both are in Preston County.

The sites to be evaluated by West Virginia University are divided into two categories—inland wetland areas and eastern deciduous forests. Dr. Jesse F. Clovis of West Virginia University will administer the evaluations. The wetland areas are Big Run of Blackwater River (Tucker County), Blister Run Bog (Randolph), Blister Swamp (Pocahontas County), Canaan Valley (Tucker County), Dobbin Slashing (Tucker County), Fisher Spring Run (Tucker County), Moore Run (Randolph County), Red Run (Tucker County), Sinks of Gandy (Randolph County) and Yellow Creek (Randolph County). The hardwood forests are Bethany College Woods (Brook County), Cranberry Glades Botanical Area (Pocahontas County), Falls of Hills Creek Scenic Area (Pocahontas County), Fannie Bennett Hemlock Tract (Pendleton County), Gaudineer Scenic Area (Randolph County), North Fork Mountain Spruce Knob (Pendleton County), Shavers Mountain Spruce Patch (Randolph County), Tomlinson Run Park (Hancock County) and Virgin White Pine Stand (Greenbrier County).

ARBORETUM

West Virginia University's Arboretum adjoining the Coliseum is a nice place to take a stroll and relax. It's also an excellent outdoor classroom.

The Arboretum is becoming more important as a teaching area for West Virginia University biology, agriculture and forestry students. Last year more than 5,000 students used the Arboretum on class field trips—a nearly four-fold increase compared to 1965.

"These increases in the use of the Arboretum are due to the renewed interest in ecology, plus the fact that more and more people are learning that it's open to the public from dawn to dusk seven days a week," explained Dr. Roland L. Guthrie who is Arboretum director.

It is your editors opinion that every school should have an Arboretum. The way space is disappearing today it is best to get it as soon as possible—save the trees (and the wildlife that goes with them)!

Biology Section

The Ecological Distribution of Amphibians in Jackson County, West Virginia

James H. Meads II
Division of Foundation Studies
Glenville State College
Glenville, West Virginia 26351

Abstract

Distributional data, supplemented with ecological observations, for species of amphibians occurring within Jackson County, West Virginia, are presented. The study was based upon field research started in August, 1970, and concluded in June, 1972. Additional data were obtained from the records of the West Virginia Biological Survey Herpetological Collections.

Twenty amphibian species live in Jackson County. An additional twelve species which are of probable occurence are listed. This brings the total to thirty-two amphibian species in Jackson County out of the forty-one known to exist in West Virginia.

It was the purpose of this study to determine the distribution of the various species of amphibians in Jackson County, West Virginia, and where possible to study the ecology and life history of each species.

Due to poor transportation, mountainous terrain, and lack of interest in the herpetofauna, little scientific research has been accomplished in the western counties of Jackson, Roane, Calhoun, Gilmer, and Wirt. Until this study, little was known of the distribution of amphibians in Jackson County.

The herpetological collections of the Carnegie Museum at Pittsburgh, Pennsylvania, contain no specimens from Jackson County. In the thirty-eight years since the formation of the West Virginia Biological Survey, very few species have been collected from this county and no study has been made on the amphibian distribution of the Jackson County area. This study should serve as a guide to those interested in the amphibian distribution of Jackson County as to where each species is likely to be found and its habitat. It is the hope that this paper will serve as a stimulus to collectors in other counties where little or no information is available on the herpetofauna.

Description of the Area

Jackson County, comprising an area of 470.29 square miles, is a high plain or plateau averaging from 1100 to 1200 feet above sea level. Water erosion has reduced the original plain to slope land for the most part, and the streams flow in deeply cut V-shaped valleys.

Along the western edge, the Ohio River, which forms 27 to 28 miles of the county's boundary, has cut a gorge in the old plain one to two miles in width and 500 to 600 feet deep. Valley walls are generally steep but, on reaching the summit, the general surface is rolling (Soil Survey, 1961).

The county varies in elevation from 525 feet above sea level at the Ohio River to 1260 feet on the top of Garnes Knob, one mile southeast of Kenna. This is a range in elevation of 735 feet (Little Kanawha Regional Council, 1948).

Jackson County has a warm temperate climate. The annual mean temperature is 56°F. January and February have an average mean temperature of 32.5°F, while July and August have an average mean temperature of 76°F.

The precipitation, about 40 inches, is well distributed throughout the year, with the heaviest rainfall occurring during the growing season, June and July, and the lightest in September and October during the harvest season. Intense summer thundershowers of short duration occur within the county. Total snowfall is less than 20 inches per year. Usually this remains on the ground for only short periods and, therefore, furnishes little ground cover or protection (Krebs, 1911).

Materials and Methods

As each amphibian specimen was collected, it was placed in a saturated solution of chlorotone to relax the individual, and then preserved using a 10% solution of formalin. A label was attached showing the field identification number of the particular animal. The field identification number corresponds with the field notes kept for each specimen. Finally, the locality where each species was collected was charted on a Jackson County map so one could easily ascertain where future field work should be concentrated.

A research into the literature concerning Jackson County amphibian life was undertaken to compile all previous information collected on species within the county. The herpetological collection of the West Virginia Biological Survey was researched and each Jackson County specimen was recorded and included in this study.

Results

The field study established that twenty different amphibian species were found in the Jackson County area. An additional twelve species which are of probable occurence are listed. This brings the total to thirty-two amphibian species in Jackson County out of the forty-one known to exist in West Virginia.

Each species listed is represented by actual specimens in the author's collection or the West Virginia Biological Survey. Throughout this list when referring to localities, WVBS will represent West Virginia Biological Survey, and FN will represent the author's field number which was used to identify the specimen when it was collected.

Species Known to Occur in Jackson County

(1.) Mudpuppy. *Necturus maculosus maculosus* Rafinesque.

This thoroughly aquatic salamander is readily identified by its large size and bushy red external gills. It probably occurs in all larger streams in the county.

Little is known concerning parasitism in many amphibian groups. A series of tapeworms were collected from a captive *Necturus maculosus maculosus* in May, 1972. Localities: Ripley (FN 68); Staats Mill (FN 86); Evans (FN 101); Cottageville (FN 110); Sandyville (FN 130).

(2.) Red Spotted Newt. *Notophthalmus viridescens viridescens* Rafinesque.

This common amphibian is found in ponds, streams, and swamps throughout the area. Localities: Cottageville (FN 64); Frozen Camp (FN 65); Leroy (FN

72); Odaville (FN 75); Ravenswood (FN 107); Evans (FN 111); Junction of Rt. 33E and Joe's Run (FN 116); Ripley (FN 118).

(3.) Marbled Salamander. *Ambystoma opacum* Gravenhorst.

This is a woodland species that is found in drier situations than are suitable for most species of *Ambystoma.* They are often abundant in sandy and gravelly areas and in low grounds bordering ponds and slow streams (Bishop, 1943). The females deposit their eggs in the fall. Locality: Ravenswood (WVBS 2683).

(4.) Northern Dusky Salamander. *Desmognathus fuscus fuscus* Rafinesque.

This is an extremely abundant salamander throughout the county. Although Northern Dusky Salamanders may sometimes be found in moist woodlands, they are far more abundant under the stones and logs along small woodland streams. Localities: Ripley (FN 18); 4 miles SE of Ripley-Tug Fork (FN 19); Marshall (FN 21); 3 miles SE of Ripley-Tug Fork (FN 32); Staats Mill (FN 28); Kentuck (FN 37); Cedar Lakes FFA-FHA Camp (FN 36); 1½ miles SE of Ripley-Buffalolick Road (FN 54); 4.1 miles SE of Ripley (FN 56); Medina (FN 73); Goldtown (FN 109); Evans (FN 112); Romance (FN 120); 1 mile SW of Mt. Alto (WVBS 3132-3137).

(5.) Slimy Salamander. *Plethodon glutinosus glutinosus* Green.

All specimens were collected either in early spring or in autumn. In damp weather, the Slimy can be found under logs and stones in wooded regions. Localities: Ripley (FN 14); Evans (FN 67); Sandyville (FN 71); Cottageville (FN 74); Gay (FN 98); ½ mile N of Kenna (FN 99); Mt. Alto (FN 105); Medina (FN 125); 1 mile SW of Mt. Alto (WVBS 3129-3131).

(6). Wehrle's Salamander. *Plethodon wehrlei* Fowler and Dunn.

All specimens collected were under flat rocks on a moist hillside of mixed deciduous forest. Localities: Ripley (FN 58); Romance (FN 80); 1 mile SW of Mt. Alto (WVBS 3126-3128).

(7.) Ravine Salamander. *Plethodon richmondi richmondi* Netting and Mittleman.

This is a completely terrestrial species, most commonly encountered beneath thin flat rocks on or slightly embedded in the ground in open woodlands. They are most abundant on sloping hillsides and occur rarely on the flats. Localities: Cedar Lakes FFA-FHA Camp (FN 36); Ripley (FN 58); Evans (FN 76); Sandyville (FN 78); Mt. Alto (FN 105).

(8.) Northern Spring Salamander. *Gyrinophilus porphyriticus porphyriticus* Green.

This salamander frequents cool springs, and streams, or occupies little natural or excavated cavities beneath logs or stones at the margins. Localities: Ripley (FN 100); Goldtown (FN 109); Intersection of Rt. 33E and Joe's Run (FN 114); Given (FN 115); 1 mile SW of Mt. Alto (WVBS 3125).

(9.) Midland Mud Salamander. *Pseudotrition montanus diastictus* Bishop.

This species frequents muddy areas. It is most often encountered under stones or debris along a shallow, sluggish stream or spring run. Localities: Mt. Alto (FN 57); Odaville (FN 66); Ripley (FN 69); Goldtown (FN 106); Staats Mill (FN 108); Gay (FN 113); 1 mile SW of Mt. Alto (WVBS 3122-3123).

(10.) Northern Red Salamander. *Pseudotrition ruber ruber* Sonnini.

Specimen found under the shelter of a flat rock near the edge of a clear, cold stream. Locality: 6 miles N of Ripley (FN 121).

(11.) Northern Two-lines Salamander. *Eurycea bislineata bislineata* Green.

This species is widely distributed throughout the county. It occurs in damp woods, streams, and other aquatic situations. Localities: Ripley (FN 27); 4.1 miles SE of Ripley-Tug Fork (FN 56); Evans (FN 77); Odaville (FN 79); Sandyville (FN 104); Mt. Alto (FN 105); Junction of Rt. 33E and Joe's Run (FN 114); Romance (FN 120); Sandyville (FN 129); 1 mile SW of Mt. Alto (WVBS 3122-3123).

(12.) Long-tailed Salamander. *Eurycea longicauda longicauda* Green.

This species is mainly terrestrial and is found in and beneath old rotting logs and under stones. Often they abound in crevices of shale banks and beneath stones and rocks near the margins of streams. Locality: 6 miles N of Ripley on Rt. 21 (FN 132).

(13.) American Toad. *Bufo americanus americanus* Holbrook.

The American Toad is a widespread and abundant toad in Jackson County. This species is one of our most ubiquitous amphibians. Localities: Ripley (FN 3, 23, 40); Ravenswood (FN 8, 22, 51); Kenna (FN 11, 87); Mt. Alto (FN 17); Sandyville (FN 20, 50); 1½ miles SE of Ripley-Buffalolick Road (FN 54); Kentuck (FN 52); 4 miles E of Ripley-Gay Road (FN 41); 5½ miles SE of Ripley-Gay Road (FN 44); Gay (FN 47); 2 miles NE of Ripley-Sycamore Road (FN 49); Evans (FN 59); Medina (FN 60); Odaville (FN 75); 5 miles E of Ripley–Rt. 33 (FN 81); Rollins Lake (FN 82); Cottageville (FN 83); Staats Mill (FN 84); Rock Castle (FN 88); Kera Lakes (FN 92); Fairplain (FN 94); Romance (FN 97); Given (FN 128).

(14.) Fowler's Toad. *Bufo woodhousei fowleri* Hinckley.

This toad occurs throughout the county. It breeds in streams and more permanent pools at a later date than the American Toad. Localities: 2½ miles SE of Ripley-Staats Pond (FN 4); Ripley (FN 7); Ravenswood (FN 22); Goldtown (FN 62); Cottageville (FN 63); Sandyville (FN 94); Youngs Road off Rt. 21 (WVBS 3167).

(15.) Northern Spring Peeper. *Hyla crucifer crucifer* Wied.

Woods and thickets are the usual habitats, but individuals are rarely encountered there. Ordinarily they are noticed only in the spring when they gather in noisy breeding aggregations about shallow lakes, woodland pools, roadside ditches, and quiet streams. Localities: Ripley (WVBS 3189, FN 15, 16, 39); 2½ miles SE of Ripley-Staats Pond (FN 24); Evans (FN 25); Junction of Rt. 33E and Joe's Run (FN 26, 38); Ravenswood (FN 31); Kenna (FN 33, 35); Gay (FN 43, 47); Cottageville (FN 48); Silverton (FN 102); Fairplain (FN 103).

(16.) Eastern Gray Treefrog. *Hyla versicolor versicolor* LeConte.

This species is a woodland amphibian restricted to trees and brush except during the breeding season, when individuals congregate about woodland pools, farm ponds, impoundments, lakes, and streams. Localities: Ripley (FN 1, 61); 2½ miles SE of Ripley-Staats Pond (FN 2); 7.5 miles E of Ripley-Frozen Camp Road (FN 55); Cottageville (FN 122); 6 miles E of Ripley on Rt. 33 (FN 131).

(17.) Mountain Chorus Frog. *Pseudacris brachyphona* Cope.

This is a woodland species that emerges in early spring to congregate about springs, small woodland streams, pools, and roadside ditches in the woods or at woodland edges. Sometimes puddles in little-used roads serve as breeding pools. Localities: Sandyville (FN 9); Tug Fork (FN 29, 30); Gay Road (FN 41), 43, 46); Sycamore Road (FN 49); Buffalolick Road (FN 54); Frozen Camp Creek Road (FN 55); Fairplain (FN 123); Kenna (FN 124); Mt. Alto (FN 126); Ripley (FN 127).

(18.) Bullfrog. *Rana catesbeiana* Shaw.

The Bullfrog is highly aquatic and seldom ventures out of water for any distance. They prefer larger bodies of water than most frogs. Localities: Staats pond—2½ miles SE of Ripley (FN 4); Ravenswood (FN 5, 31); Cedar Lakes FFA-FHA Camp (FN 6); Junction of Rt. 33E and Joe's Run (FN 10); Sandyville (FN 12); Mt. Alto (FN 17; Buffalolick Run Road (FN 54); Frozen Camp Creek Road (FN 55); Evans (FN 59, 70); Rollins Lake (FN 90); Kenna (FN 93); Staats Mill (FN 117).

(19.) Green Frog. *Rana clamitans melanota* Rafinesque.

This frog is a chiefly aquatic species and a solitary one. It inhabits a variety of aquatic environments. Localities: Ravenswood (FN 2, 5, 13, 31); 2 miles NE of Ripley-Sycamore Road (FN 49); 6 miles SE of Ripley-Gay Road (FN 45); Evans (FN 59, 70); Medina (FN 60); Goldtown (FN 62); Cottageville (FN 63); 5 miles E of Ripley-Rt. 33 (FN 81); Rollins Lake (FN 82); Ripley (FN 85); Ripley Landing (FN 91); Given (FN 95); Staats Mill (FN 117); Junction of Rt. 21 and 56 (FN 119).

(20.) Pickerel Frog. *Rana palustris* LeConte.

This species is typically associated with cool, clear waterlakes, ponds, or streams; although it also occupies a variety of other habitats. In summer they often wander far from water and may be encountered in woods or grassy fields. Localities: 8 miles NE of Ravenswood (WVBS 3364); Cedar Lakes FFA-FHA Camp (FN 6, 34); Junction of Rt. 33E and Joe's Run (FN 10, 38); Ravenswood (FN 31, 51); Gay Road (FN 42, 46); 2 miles NE of Ripley-Sycamore Road (FN 49); Sandyville (FN 50, 53); 1½ miles SE of Ripley-Buffalolick Road (FN 54); Goldtown (FN 62); Evans (FN 70); Rollins Lake (FN 82, 90); Ripley (FN 85); Frozen Camp (FN 89); Given (FN 96); Junction of Rt. 21 and 56 (FN 119).

The following list of amphibians are those that have not been collected as of this writing but could possibly exist in the county due to favorable habitats and favorable avenues of migration such as river valleys and streams. The majority of the species recorded here have been collected in the counties surrounding Jackson.

Species That May Occur in Jackson County

(1.) Hellbender. *Crytobranchus alleganiensis alleganiensis* Daudin.

This huge, flattened, aquatic salamander is found in large streams and rivers where large stones provide daytime retreats and nesting sites. This species has been collected from the Ohio River in Cabell County and no doubt is found along the river in Jackson. Specimens have also been collected in Kanawha County.

(2.) Jefferson Salamander. *Ambystoma jeffersonianum* Green.

The habitat of this species is woodlands and is most common in low swamp areas. This salamander has been collected in Cabell and Wood Counties.

(3.) Spotted Salamander. *Ambystoma maculatum* Shaw.

These amphibians normally remain hidden beneath logs or piles of debris in woodland areas and are usually encountered only in early spring about their woodland breeding pools. A biology student at Ripley High School collected a specimen in the spring of 1970, but it was not preserved. Specimens have been collected in Kanawha, Mason, and Cabell Counties.

(4.) Small-mouthed Salamander. *Ambystoma texanum* Mattes.

This species is a spring breeder and is frequently found at that season under logs or other debris near ponds or swamps, in river bottoms, or other situations where moisture is abundant. It has been collected in neighboring Mason County.

(5.) Allegheny Mountain Salamander. *Desmognathus ochrophaeus ochrophaeus* Cope.

This is the most terrestrial of all the *Desmognathus*, and is commonly found beneath logs and rocks in moist woodland, sometimes near streams, but often some distance away. Specimens have been collected in neighboring Kanawha County.

(6.) Appalachian Seal Salamander. *Desmognathus monticola monticola* Dunn.

This salamander has been collected from neighboring counties of Roane, Wirt, Wood, and Kanawha. This amphibian prefers muddy areas and can be found in small streams, mountain brooks, and spring runs, usually in wooded areas.

(7.) Red-backed Salamander. *Plethodon cinereus cinereus* Green.

This animal is a completely terrestrial woodland species, often found far from any permanent water. It has been collected in Kanawha and Putnam Counties. The reason perhaps why a specimen has not yet been collected in Jackson County is the abundance of the Ravine Salamander. They seem to compete for similar habitats and are not usually found associated with each other.

(8.) Green Salamander. *Aneides aeneus* Cope and Packard.

The Green Salamander and its eggs have been collected by the author in neighboring Roane County. Specimens have also been reported from Kanawha. Narrow cracks and crevices along the face of sandstone cliffs are their habitat.

(9.) Eastern Spadefoot Toad. *Scaphiopus holbrookii holbrookii* Harlan.

The habitat of this amphibian is a terrestrial, subterranean burrow which it digs, backwards with the spurs on the hind feet. They are nocturnal in habit and breed only after heavy rains. The Spadefoot Toad has been collected in Cabell, Mason, Kanawha, and Wood Counties.

(10.) Blanchards Cricket Frog. *Acris crepitans blanchardi* Harper.

This frog has been collected in Mason County. Farm ponds, impoundments, lakes, and the large creeks and rivers are the usual habitat. Sunny mud flats and shallow water with some vegetative cover are necessary.

(11.) Eastern Wood Frog. *Rana sylvatica sylvatica* LeConte.

This is a solitary animal that lives in moist woodlands where there are permanent or at least semipermanent pools of water. It has been collected from Kanawha, Putnam, Wirt, and Wood Counties.

(12.) Northern Leopard Frog. *Rana pipiens pipiens* Schreber.

The habitat of the Leopard Frog is bodies of water that range from small creeks to rivers and farm ponds to lakes. The habitat of the Leopard Frog is similar to that of the Pickerel Frog. The Leopard Frog has been collected in Cabell, Kanawha, Putnam, and Mason Counties.

Acknowledgment

The author acknowledges Dr. N. Bayard Green for his encouragement and his helpful suggestions in the preparation of this manuscript.

Literature Cited

1. Bishop, Sherman C. 1943. Handbook of Salamanders, Comstock Publishing Company, Ithica, New York. p. 147.
2. Krebs, Charles. 1911. West Virginia Geological Survey, New Litho Company, Wheeling, West Virginia. pp. 46-48.
3. Soil Survey-Jackson and Mason Counties, Series 1957, No. 11. (Washington: U.S. Government Printing Office, 1961). p. 72.
4. The Human, Natural, Mineral, and Other Resources of the Area Comprising the Little Kanawha Regional Council, Little Kanawha Regional Council, Jan. 1, 1948. p. 2.

A New Species of *Octomacrum* (Trematoda: Monogenea) From the Stoneroller, *Campostoma Anomalum* (Rafinesque)

Karen N. Dansby* and Jon P. Shoemaker
Department of Biological Sciences, Marshall University
and
Research Service, VA Hospital,
Huntington, West Virginia 25701

Abstract

A new species of monogenetic trematode, *Octomacrum spinum,* was found on the gills of the Stoneroller, *Campostoma anomalum,* collected from Beech Fork, a tributary of Twelve Pole Creek, Wayne County, West Virginia. This is the fifth species in the genus, *Octomacrum* Mueller, 1934. It shows the closest relationship to *O. europaeum* Roman and Bychowsky, 1956, from which it differs in body shape and proportions, size, the presence of spines on the haptoral clamps, and the presence of setae and hooks on the body and haptor.

A previously undescribed species of *Octomacrum* Mueller, 1934, was found on the stoneroller, *Campostoma anomalum* (Rafinesque), during the summer and fall of 1971, bringing the total number of species reported in this genus to five. The following species and their hosts have been previously reported: *Octomacrum lanceatum* on *Catostomus commersonii* and *Erimyzon sucetta oblongus* (Mueller, 1934); *O. microconfibula* on *Notemigonus crysoleucas crysoleucas* (Hargis, 1952); *O. europaeum* on *Alburnoides bipunctatus* (Roman and Bychowsky, 1956); and *O. semotili* on *Semotilus atromaculatus* (Dechtiar, 1966).

*Present address: 108 Shawnee Place, Lexington, Kentucky 40506.

Stonerollers were obtained at two different sites on Beech Fork. Specimens of *O. spinum* were taken from 27.6% of the fish brought into the laboratory from one site. There were no parasites found on stonerollers near the second site, miles downstream. A similar situation was found to be true with *O. lanceatum* (Mueller, 1934). Two host fish brought into the laboratory harbored two specimens each of *O. spinum;* the other specimens occurred singly on the gills of their host. Several other species of fish from Beech Fork were examined, but none were parasitized by *O. spinum.*

Parasites were secured from the gills, fixed in A.F.A. under a coverslip, and mounted in glycerine jelly according to the method of Mizelle and Klucka (1953). Measurements were made with a calibrated filar micrometer. All measurements are in microns. The minimum and maximum measurements are given in parentheses after the mean measurements.

Description

Nine Mature Worms Measured

Small, body shape lanceolate, cuticula thick. Anterior end tapers to rounded tip. Body length 1117 (792 to 1515), greatest body width 190 (112 to 267), body width at oral suckers 112 (101 to 121). Prohaptor with pair of rounded oral suckers, 30 (24 to 35) wide and 30 (26 to 35) long. Intestinal crura do not extend beyond anterior margin of haptor. Distance from pharynx to genital sucker 121 (88 to 163). Genital sucker round, diameter 71 (62 to 88). Only one egg observed, accurate measurements could not be made because the egg was damaged in an attempt to remove it for examination. Hapter wider anteriorly, length 202 (154 to 267), width 190 (112 to 257). Two small hooks and few scattered setae on haptor. Setae on left side of third clamp and middle anterior margin of haptor. Setae present on ventral body surface, around genital sucker, and posterior to ovary. These setae appear in groups or clusters and vary in length (9 to 22). Two hooks situated between fourth pair of clamps on posterior margin of haptor, 27 (22 to 35) long. Clamp structure of *O. spinum* characterized by a pair of dorsal and ventral loops and a pair of dorsal and ventral clamps. Dorsal and ventral clamp pairs terminate in sharp processes. Second pair of clamps larger than other three pairs, both in width and length. Fourth pair of clamps smallest. On outside dorsal loop of clamps, there is a set of small spines. Clamps no. 1, 2, and 3 have five to six spines; clamp no. 4 may have three to four spines. Spines have slightly broadened base, vary slightly in length. There is greater interval between top two spines than between any other two spines.

Host: Stoneroller (Campostoma anomalum).

Site: Gills.

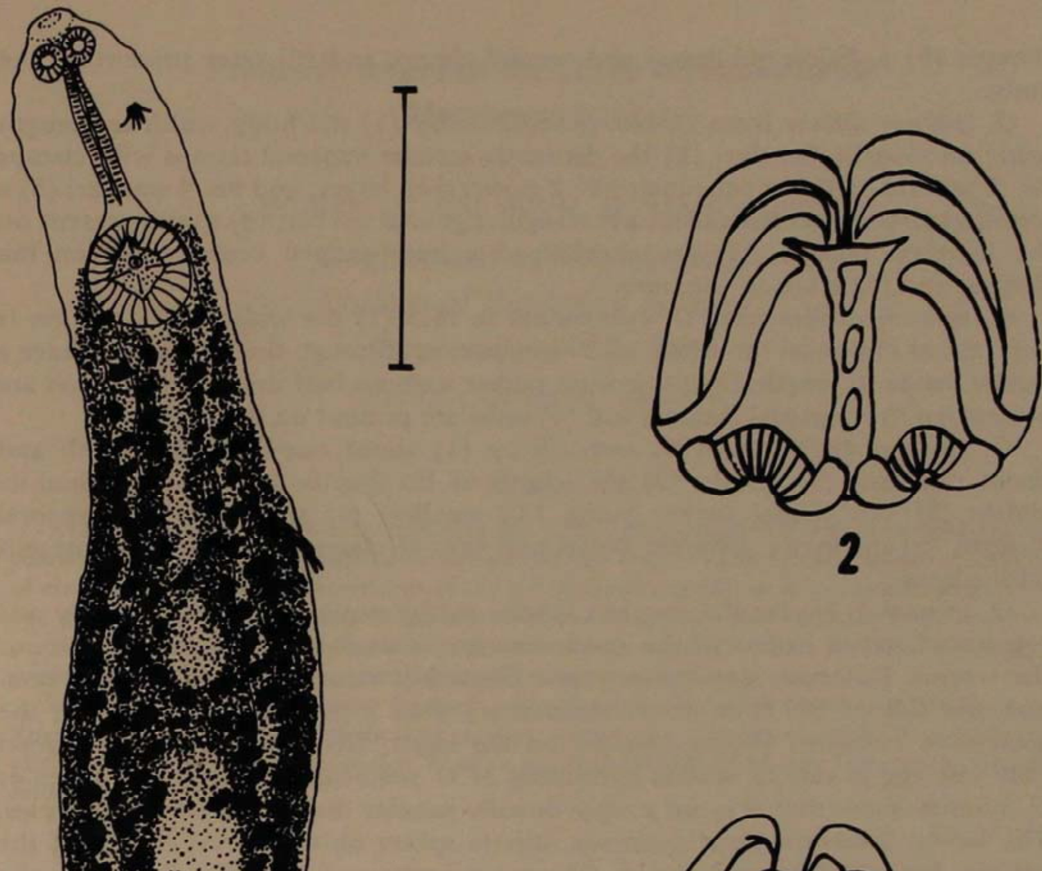
Locality: Beech Fork, Twelve Pole Creek, Wayne County, West Virginia.

Type: USNM Helm. Coll. No. 71060; paratype: USNM Helm. Coll. No. 71061.

Discussion

O. spinum is considered a new species because it differs in several significant morphological characters from the descriptions of the four known species.

O. spinum differs from *O. lanceatum* by having (1) a body size one-fifth the length and about one-tenth the width; (2) a haptor that is trapezoidal rather than rectangular; (3) a genital sucker 49 smaller; (4) spines on the haptoral



FIGURES 1-3. *Octomacrum Spinum* (scale value 200 microns for 1; 35 microns for 2 and 3).
1. Holotype, ventral view.
2. First haptoral clamp, ventral view.
3. Lateral view of fourth haptoral clamp showing set of 4 spines on outer dorsal loop.

clamps; (5) a disjointed dorsal and ventral clamp; and (6) setae present on the body.

O. spinum differs from *O. microconfibula* by (1) the body width and length being one-fourth the size; (2) the distinctly smaller haptoral clamps with clamps no. 1 and no. 3 being subequal; no. 2 noticeably larger, and no. 4 smaller; (3) a round genital sucker instead of a rectangular genital sucker; (4) spines present on the haptoral clamps; (5) the absence of a heart-shaped center piece on the clamps; and (6) setae on the body.

O. spinum differs from *O. europaeum* in that (1) the width of *O. spinum* is only about one-third the width of *O. europaeum* although the two species have a similar range in length; (2) the genital sucker is about half the size; (3) spines are present on the haptoral clamps; and (4) setae are present on the body.

O. spinum differs from *O. semotili* by (1) about one-third the length and about one-fifth the width; (2) the length of the haptor being greater than its width; (3) the genital sucker being 110 smaller; (4) spines on the haptoral clamps; (5) distinctly separate dorsal and ventral clamps; and (6) setae present on the body.

O. spinum is by far the smallest species in the genus. The fact that only one egg was observed in one of the specimens may cast doubt as to the maturity of the worms. However, specimens were collected at various times during the summer and fall of 1971. It would seem more than a coincidence that all of the specimens collected during this period are small. Dechtiar (1966) also found only one egg in one of several specimens of *O. semotili*. Measurements taken of *O. spinum* show that it is not proportionally smaller than the other four species. The newly described fluke possesses minute spines on the dorsal clamps of the haptor, for which it was named. There are numerous setae distributed on the haptor, near the genital sucker and the region over the ovary.

Acknowledgments

Thanks are due Judith G. Clark, Carl Olsen and John Dansby for their help in collecting the host fish. Also, much appreciation is expressed to Judith G. Clark for making the drawings, and to Clairous E. Smith for the photography. Special thanks are due Dr. Justus F. Mueller for his critical reading of the manuscript.

Literature Cited

1. Dechtiar, A. 1966. A new species of monogenetic trematode, *Octomacrum semotili*, from the creek chub, *Semotilus atromaculatus* (Mitchill), from Algonquin Park Lakes. *Canad. J. Zool.* 44:821-24.
2. Hargis, W. J., Jr. 1952. Monogenetic trematodes of Westhampton Lake fishes. I. Two new forms. *Am. Midl. Nat.* 47:471-77.
3. Mizelle, J. D., and A. R. Klucka. 1953. Studies on monogenetic trematodes. XIV. Dactylogyridae from Wisconsin fishes. *Am. Midl. Nat.* 49:720-33.
4. Mueller, J. F. 1934. Additional notes on parasites of Oneida Lake fishes including descriptions of new species. Part IV. *Roosevelt Wild Life Ann.* 3:336-73.
5. Roman, E., and B. E. Bychowsky. 1956. Sur un trematode monogenetique *Octomacrum europaeum* n. sp., parasite d' *Alburnoides bipunctatus* (Bloch). *Comun. Acad. Republ. Pop. Rom.* 6:901-4.

Freeze Fracture of the Outer Mitochondrial Membrane *in situ*

William J. Vail and C. R. MacKenzie
Department of Microbiology
College of Biological Science
University of Guelph
Guelph, Ontario, Canada

Abstract

After a 12 hr. incubation in 30% glycerol at 4C, large amplitude mitochondrial swelling was shown in unfixed rat heart tissue using both thin sections and freeze fracture techniques of electron microscopy. The cristae of the mitochondria appear only in the area adjacent to one side of the expanded outer membrane. Freeze fractured mitochondrial profiles show a smooth convex fracture surface with a particulate concave fracture surface. However in cross fractures both the outer membrane and cristae can be seen.

Evidence of complimentary fracture faces through the outer membrane is presented. Fracture face A shows a particulate region within the membrane of 2500 particles/μ^2 varying in size Ca. 44 Å or smaller. Possible structure of the membrane from freeze fracture evidence is postulated.

Introduction

The definitive works of Palade (1) and Sjöstrand (2) have clearly shown that the mitochondrion possesses 2 distinct and separate membranes by thin section techniques of electron microscopy. The outer membrane encloses the organelle, while the inner membrane invaginates to form cristae. Functionally, each membrane exhibits different enzymic activities (3) and possesses different degrees of permeability to molecules to the various compartments of the mitochondrion. According to Parsons *et al.*, (4) for isolated liver mitochondria, the outer membrane contains 0.83 mg phospholipid/mg protein while the inner membrane is 0.28 mg phospholipid/mg protein. Thus it appears that if the chemical composition varies between each membrane, the structural arrangement might likewise be different.

This work describes what appears to be a compliment fracture through the outer membrane of a rat heart mitochondrion in rat heart tissue.

Materials and Methods

Male rats from the same litter (250 g) were sacrificed by cerevical dislocation, the beating hearts removed, and washed twice in Ca^{++} free Krebs Ringer Phosphate medium (KRP) (5) on ice, the ventricles minced quickly on dental wax in KRP in 1 mm^3 or smaller and placed in 10 vols of 30% v/v glycerol at 4C for 12 hrs. with 3 changes of glycerol to ensure complete infiltration.

For freeze fractures, specimens were placed in gold cups, frozen in Freon 22, stored no longer than 5 min. in liquid N_2, and placed in a 4-place specimen table of a Balzer BA 360 freeze etch apparatus, precooled to -150C. Samples were fractured at -100C at 2 x 10^{-6} Torr and immediately shadowed with C-Pt and replicated with C. Replicas were prepared by the method of Vail and Riley (6), mounted on bare 75 x 300 mesh copper grids, and examined in a Philips EM 300 operated at 60 kV. The arrow in the lower left hand corner of the micrograph indicates the direction of the platinum deposit. All measurements were corrected for a platinum deposit thickness of 30 Å.

Thin sections were prepared by the method of Vail and Riley (6) from tissue incubated in 30% glycerol for 12 hrs. then fixed with 2% glutaraldehyde.

Results

Mitochondria respond as osmometers in hypotonic solutions. It was thought that if rat heart tissue was suspended in glycerol, the usual cryoprotectant for freeze fractures, the mitochondria would swell and maximumly stretch their outer membranes. Due to tissue restrictions, the outer membrane would not break. Thus the outer membrane would be more easily visualized with the freeze fracture technique. It can be seen in Figure 1A, using conventional thin section techniques of electron microscopy, mitochondria in tissue exposed to 30% glycerol shows large amplitude swelling. It can be seen that the cristae (at arrows) are still visible in most mitochondria. Figure 1B shows a freeze fracture replica of an unfixed preparation. In cross fracture, the mitochondrial cristae are still visible (at arrows) while the fracture surface of the plasma membrane (pm) surrounds the cell. Collagen (c) is present in the extracellular space while the myofibrils (mf) of the cardiac muscle elements are readily visible. Both convex ($\cap$) and concave ($\cup$) fracture surfaces of mitochondria can be seen. It seems that the convex fracture surfaces appear smooth while the concave fracture surfaces are particulate.

Figure 2A shows a convex surface of what appears to be a compliment fracture through the outer membrane of a rat heart mitochondrion. The fracture appears to be within the outer membrane and shows a particulate fracture face A with the compliment fracture surface B. The shadowed zone between each face indicates that the outer leaflet of the B face was folded over on the outer membrane during the fracture. Thus face B would be over and closely oppose face A in an unfractured membrane. Large arrows show linear marks on the surface of the membrane. Whether these marks were produced by the fracture or whether they are normally present has yet to be determined.

It would be expected that if face B were "peeled" from face A, the particles in face A might show depressions in face B where face B covered the particulate face A. Figure 2B shows a higher magnification of the compliment faces. It must be remembered that the platinum deposit (indicated by the arrow in the circle in the lower left hand corner of the micrograph) would coat a raised particle on the lower side while a white shadow would be behind the particle. This can be seen in fracture face A indicating raised particles. The compliment face B shows depressions since the white shadow is below the platinum deposit which indicates depressions. Thus, it is an opposite shadow pattern from fracture face A. The fold of the leaflet (at large arrows) still show depressions which extend on to face B. The small black arrows delineate the edge of the compliment leaflet which lies against the smooth surface of the outer membrane. A large part of the B leaflet has been removed with the fracture. However the upper edge of the fracture in face A seems to fit the edge of face B if it were folded back over again. This can be demonstrated if face B is cut from the photograph and placed on face A.

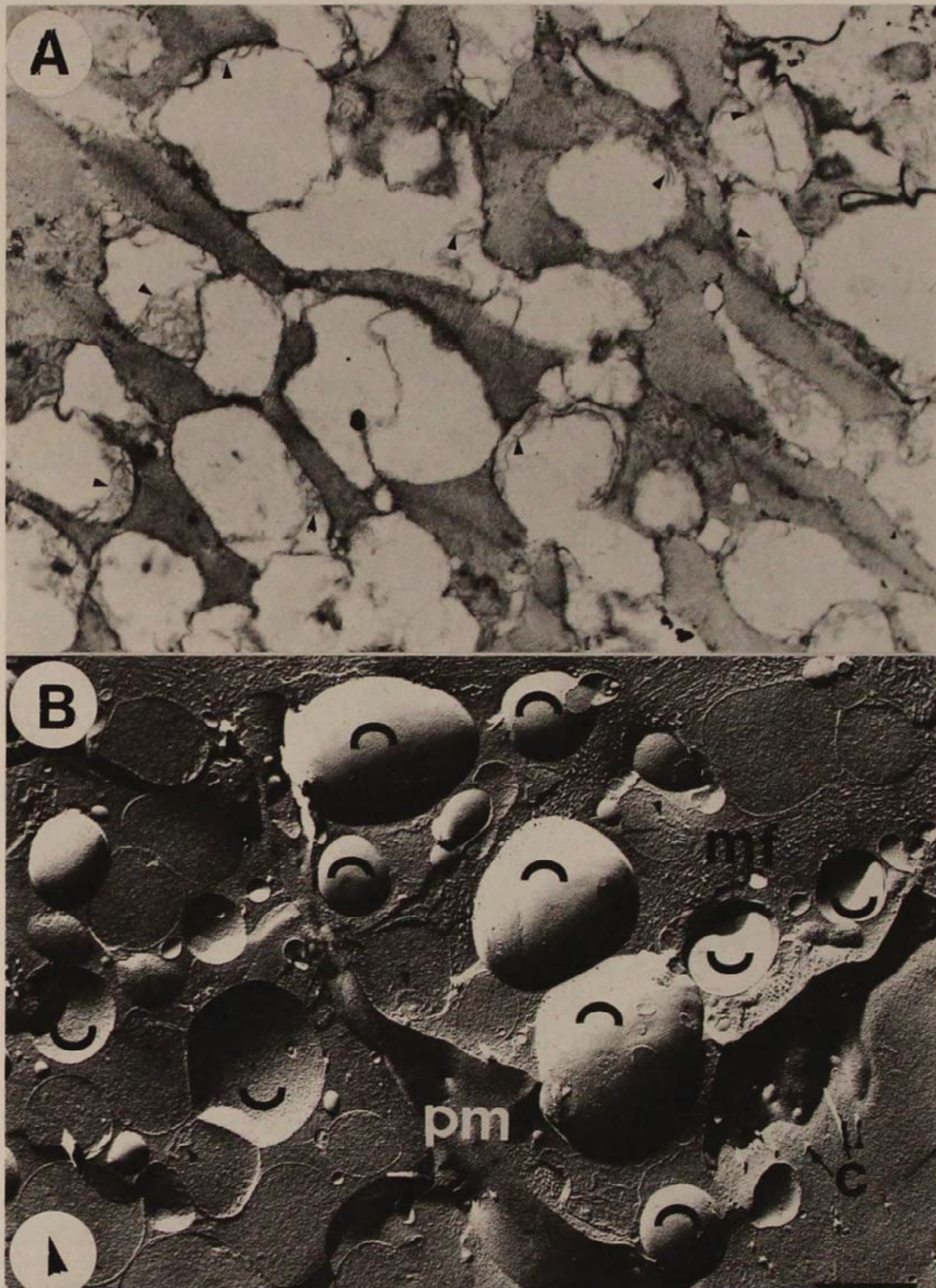


FIGURE 1. Rat heart tissue incubated for 12 hours in 30% glycerol at 4C. Magnification 15,600 x. Bar is 1μ meter. A. Thin section electron micrograph. Arrows show cristae in swollen mitochondria. (UGDM 3631). B. Freeze fracture replica. Arrow in lower left hand corner indicates the direction of the platinum deposit. Concave (∪) and convex (∩) fracture surfaces, (pm) plasma membrane, (mf) myofibrils, (c) collagen. Arrows show cristae in cross fractures. (UGDM 3146).

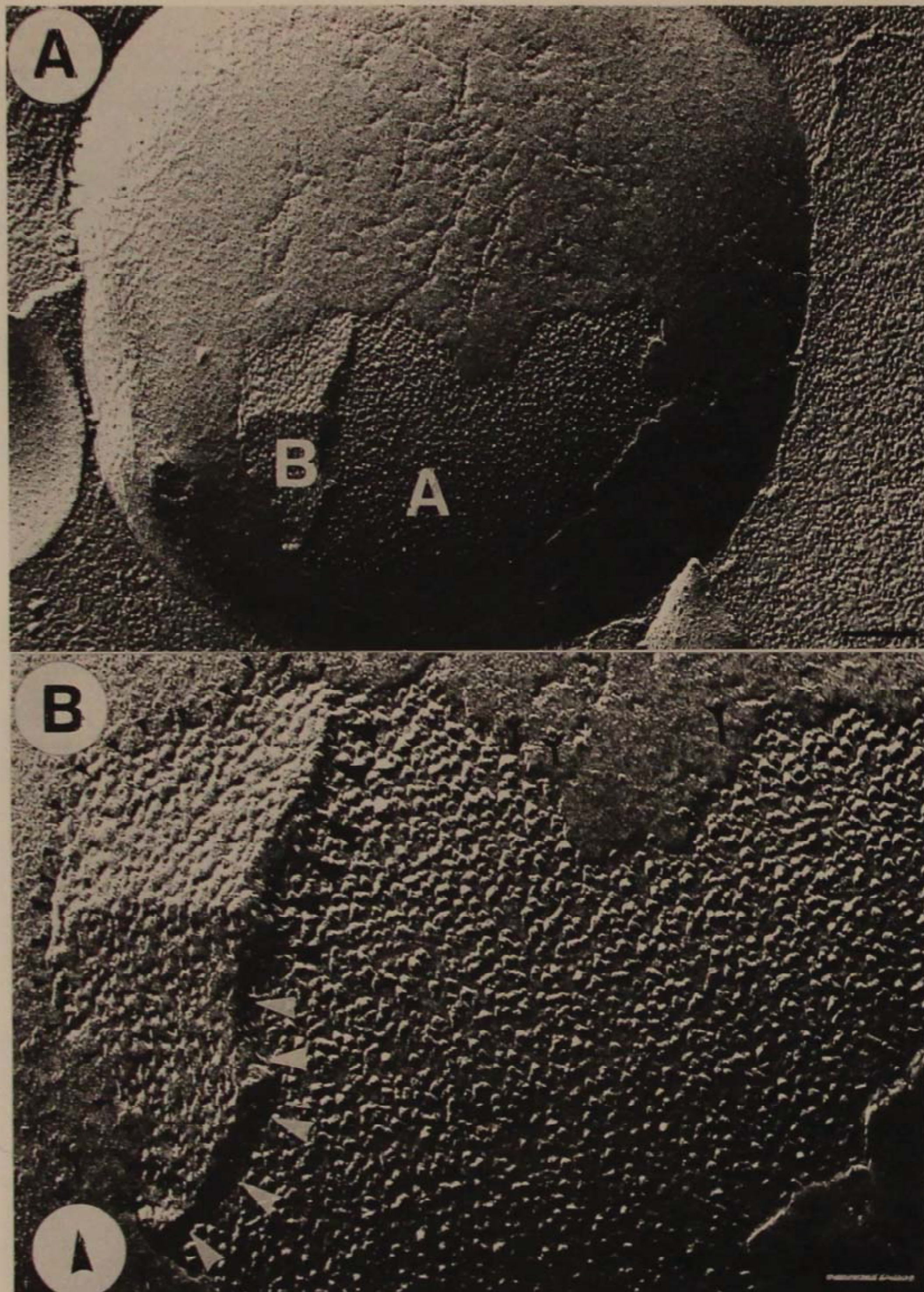


FIGURE 2A. Compliment fracture through the outer membrane of a rat heart mitochondrion from rat heart tissue using the freeze fracture technique. Fracture face A is particulate with the compliment fracture, face B showing holes or depressions which seem to fit the particles. Magnification is 47,800 x. Bar is 0.2 μM. (UGDM 3152). Figure 2B shows higher magnification showing fold of the leaflet (at larger arrows) with edge of the back-folded leaflet (at small arrows). Depressions are seen on the surface of the outer membrane (at arrows). Magnification 103,700 x. Bar is 0.1 μM. Arrow in white circle shows the direction of the platinum deposit. (UGDM 3571).

Discussion

In heart cells there exists extensive areas containing large numbers of mitochondria. These areas are closely associated with the myofibrils. Thus there are restrictions to high amplitude swelling for each mitochondrion due to the close association with other mitochondria and other cellular organelles which is not present when isolated mitochondria undergo large amplitude swelling.

It appears that convex fracture surfaces in glycerol infiltrated tissue show a smooth fracture surface while the concave fracture surface appears particulate. These surfaces could in fact represent surfaces of different membranes. The smooth outer convex surface could be the outer surface of the outer membrane while the particulate concave surface could be the matrix surface of the inner membrane.

In a recent communication, Vail *et al.,* (7) using unfixed isolated heavy beef heart mitochondria observed a smooth surface with what appeared to be particles embedded within it using the freeze fracture technique. They interpreted this surface to be the outer surface of the outer membrane. Using glutaraldehyde fixed rat heart tissue Vail and Riley (8) observed a similar surface. Some membranes were fractured so as to remove part of the outer membrane and revealed a particulate surface of the inner membrane. Both surfaces were convex fracture surfaces.

Compliment fracture surfaces are indeed rare using the single replica technique of freeze fracture. It was fortunate to observe a compliment fracture surface of the outer membrane. The smooth surface is interpreted as the true outer surface of the outer membrane with particles embedded within the membrane.

The particle density in fracture face A and the depression density in fracture face B is 2500 particles/μM^2 (an average of 10 fields of 0.01 μM^2). Since the particle density and the depression density are the same, it seems unlikely that they were derived from different membranes. Packer (9) examined isolated outer membrane fragments from rat liver mitochondria using the freeze fracture technique. He found a particle density of 2296 particles per μM^2 which nearly agrees with the particle density that has been determined here although the mitochondria were from a different tissue. From these data, it is assumed that face B was in intimate apposition with face A. The particle size distribution in face A varies from 44 Å or smaller, assuming a platinum deposit of 30 Å. No doubt smaller particles, presumably proteins, are present but the resolution of the technique is not sufficient to show them.

At least 2 other interpretations of Figures 2A and 2B are, however possible. The initial fracture could have removed the entire outer leaflet of the outer membrane as well as any particles on this face, while the particulate face A shows the outer surface of the inner membrane. Face B is then the inner leaflet of the outer membrane. If this would be the case, one might expect to see a ridge along the edge of the mitochondrion showing the remnants of the outer leaflet of the outer membrane. The replica was examined at high magnification and no evidence of a ridge could be discerned. The tight apposition between faces also speaks against the interpretation since conventional thin sections show an unstructured electron transparent region between the inner and outer membranes. Only rarely does one see tight apposition between both membranes and thus not over extensive areas even in swollen mitochondria.

A still further interpretation might be that the outer membrane has been removed by the fracture while the smooth surface is the true surface of the inner membrane, and fracture face A is the fracture face through the inner membrane with the compliment face B. If this were the case, one would expect to see some evidence of invaginations of the inner membrane to form cristae which are quite extensive in heart mitochondria as well as any remnants of a ridge for the outer membrane. No invaginations or ridge was observed. Hackenbrock (10) using the freeze fracture technique has shown extensive infolding or invaginations of mitoplasts, an inner membrane preparation derived from liver mitochondria.

The particles on fracture face A presumably are proteins within a hydrophobic interior of the membrane. The leaflet in face B could represent a phospholipid monolayer pulled out and folded back over on the surface of the outer membrane. The particles, presumably proteins, or lipoproteins, could be embedded in a hydrophobic centre above a second monolayer. Marker enzymes for the outer membrane have been enumerated by Ernster and Kuylenstierna (3) and perhaps some of these enzymes are visible as particles in face A.

The works by da Silva and Branton (11) and by Tillack and Marchesi (12) using protein markers for the membrane surface in erythrocyte ghosts has shown that the true surface of the ghost membrane is essentially smooth by the freeze etch technique. These works support this interpretation.

Acknowledgments

The authors thank the National Research Council of Canada which supported this work in part.

Literature Cited

1. Palade, G. E. 1953. An electron microscopic study of the mitochondrial structure. *J. Histochem. Cytochem.* 1:188-211.
2. Sjöstrand, F. S. 1953. Electron microscopy of mitochondria and cytoplasmic double membranes. *Nature* 171:30-32.
3. Ernster, L., and Krylenstierna, B. 1970. Membranes of mitochondria and chloroplasts. E. Racker, ed., p. 1972, Van Nostrand-Reinhold, New York.
4. Parsons, D. F., Williams, G. R., Thompson, W., Wilson, D., and Chance, B. 1967. Round table discussion on mitochondrial structure and compartmentation. E. Quaglieriello, S. Pappa, E. C. Slater, and J. M. Tager eds., p. 29, Adriatica Editrica, Bari.
5. Williams, C. H., Vail, W. J., Harris, R. A., Caldwell, M., Green, D. E., and Valdivia, E. 1970. Conformational basis of energy transduction in membrane systems VIII. Configurational changes of mitochondria *in situ* and *in vivo*. *Bioenergetics* 1:147-80.
6. Vail, W. J., and Riley, R. K. 1971. Ultrastructure of isolated heavy beef heart mitochondria revealed by the freeze-etching technique. *Nature* 231:525-27.
7. Vail, W. J., Riley, R. K., and Williams, C. H. 1972. The morphology and configurational states of isolated heavy beef heart mitochondria by the freeze fracture technique. *Bioenergetics* 3:467-79.
8. Vail, W. J., and Riley, R. K. 1972. Comparison of aggregated and orthodox rat heart mitochondria *in situ* using thin sections and freeze fracture techniques. Proc. W. Va. Acad. Sci. (in press).
9. Packer, L. 1972. Functional organization of intramembrane particles of mitochondrial inner membranes. *Bioenergetics* 3:115-27.
10. Hackenbrock, C. R. 1972. Energy-linked ultrastructural transformations in isolated liver mitochondria and mitoplasts. Preservation of configurations by freeze-cleaving compared to chemical fixation. *J. Cell Biol.* 53:450-65.
11. da Silva, P. P., and Branton, D. 1970. Membrane splitting in freeze-etching. Covalently bound ferritin as a membrane market. *J. Cell Biol.* 45:598-605.
12. Tillack, T. W., and Marchesi, V. T. 1970. Demonstration of the outer surface of freeze-etched red blood cell membranes. *J. Cell Biol.* 45:649-53.

Gene and Growth Dynamics of Chlorella Under Continuous Culture During Phenol Stress[1]

Alan Stein and E. C. Keller, Jr.
Department of Biology, West Virginia University
Morgantown, West Virginia 26506

Abstract

A system was devised to grow *Chlorella vulgaris* (Pratt) cultures under continuous culture conditions for a period of 35 days. Experimental cultures were grown in inorganic media (no carbon) containing 0.01 mg/l phenol and control cultures were grown in the same media without phenol. Marker genetic mutant cultures were used with reference to their resistance to various drugs such as: penicillin, tetracycline, actidione, neomycin, and chloromycetin. Several concentrations and combinations of these drugs were examined in an attempt to determine selection pressure of the pollutant (phenol). No significant differences in gene frequencies between control and phenol treated cultures were observed.

As a function of the treatment, significant changes in various correlations were detected among the several growth parameters: cell density, individual cell diameter, individual cell volume, and biomass. A significant positive correlation between biomass and cell density was observed in the control cultures, while the correlation in the phenol treated cultures was positive but not significant. Significant negative correlations were observed in the control cultures between biomass and cell diameter, between biomass and cell volume, and between cell density and cell size. However, in the phenol treated cultures, the correlation between cell density and cell size was negative while correlations between biomass and cell diameter and between biomass and cell volume were positive, although none were significant. Significant correlations were observed only in the control cultures among growth parameters. It was concluded that phenol significantly disrupted the associations among the growth parameters of the "normal" state (control cultures).

Introduction

The objectives of this study were to ascertain the effects of small amounts of phenol as a genetic selector, to ascertain the effects of phenol on growth parameters in the primary producer, *Chlorella vulgaris,* and to optimize an axenic continuous flow culture system, including sampling procedures, genetic assay, and apparatus for the growth of algae under genetic pressure.

Materials and Methods

The organism used in this study was *Chlorella vulgaris* Beijerinck (Pratt strain). The culture vessels were six modified Bellco No. 3002 continuous flow spinner flasks (Figure 1). The flask is described in detail by Stein (1973). The flask was inoculated with 10 ml of *Chlorella* suspension. The cultures were then allowed to grow for a period of three weeks before the continuous flow of nutrients was started. Then continuous flow conditions were started, with media flow rates of approximately 3 ml/hr. using a modified inorganic Beijerinck's solution. The working culture volume was 540 ml in each spinner flask. One set of three flasks contained 0.01 mg/l of phenol and the other set did not. The mutants, neomycin and actidione resistant, and tetracyline susceptible, were

[1]This work was supported by an Environmental Health Traineeship (HEW) 5 AID AH 0063804 and a Grant-in-Aid of Research, Society of Sigma Xi.

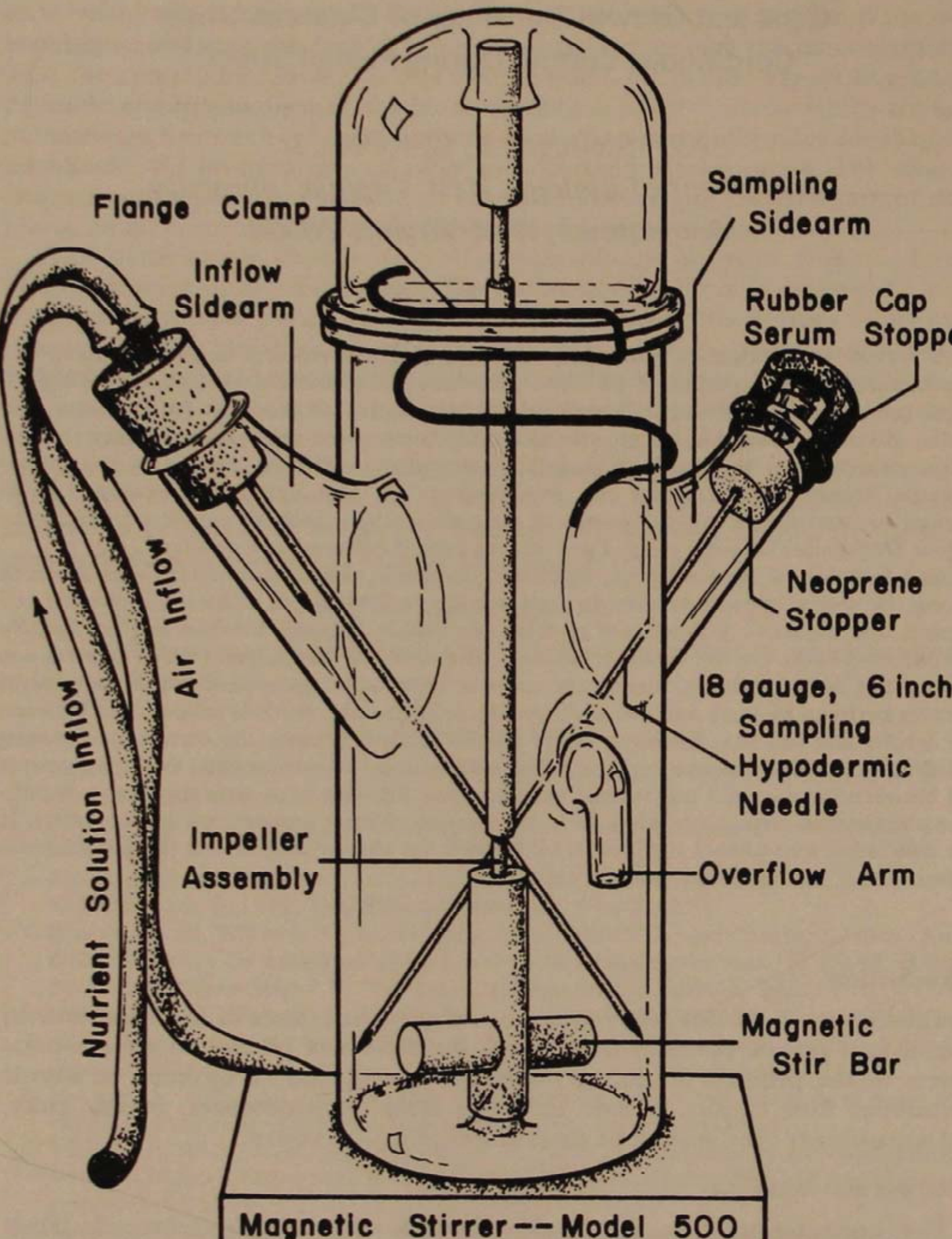


FIGURE 1. Modified Bellco Spinner Flask and Detail of Sampling Sidearm.

utilized for flasks. Sampling of the culture flasks was accomplished using a sterile 1 cc tuberculin syringe inserted through the serum cap stopper (Figure 1) in sampling sidearm of the spinner flask. A sample of 0.5 ml was withdrawn, diluted, and counted using a Model B Coulter Counter. The frequency of occurrence of genetic mutants in each of the cultures was determined by screening many strains from each flask using a replica plate technique. Average temperature range between flasks was between 31.1°C and 31.8°C. Growth parameters measured were: cell density, cell diameter, cell volume, and total biomass of the culture. The technique of continuous culture, as it exists today, was optimized by Monod (1950) and Novick and Szilard (1950). Myers and Clark (1944) grew a species of *Chlorella* under a continuous culture procedure different from the homogeneous open system used in this study. Several excellent reviews on the operation and theory of continuous culture are available (Monod, 1950; Novick, 1955; Moser, 1958; and Kubitschek, 1970). The methods of culture, sampling and assay are also described in detail by Stein (1973).

Results

The continuous culturing/genetic assay used here has been found to be adequate in conducting experiments on the "continuous culture" of the unicellular green alga *Chlorella vulgaris* and was adequate to meet the basic design criteria of the experiment. The algae were grown in spinner flasks (Figure 1) under continuous flow conditions for a period of 35 days during which time the cell numbers in most of the spinner flasks remained relatively constant, as required by the experimental design (Figure 2). It is evident from the graph that no upward or downward trend in cell number appears. Only one of the control flasks seemed to have cell counts that showed an upward trend in cell numbers.

At the end of the experimental period, when cultures were tested for bacterial contamination, contamination was found in all six cultures. Axenic culturing of the algae was, therefore, not accomplished successfully during the entire experimental period, but unialgal continuous culture of the cells without washout was successfully accomplished. Skinner (1972) found in batch cultures of *Chlorella* that if contamination occurred early, it did not significantly affect his results on cell numbers (as compared to replicate axenic cultures). Likewise, the multiple assay technique for screening large numbers of clones for the frequency of occurrence of certain genetic markers isolated from each of the cultures was successful.

No significant differences were detected among the growth parameters between the two treatments on either log or squared transformed or non-transformed data. However, trends were shown to exist (Figure 3) between the two treatments (control and phenol) with respect to cell counts obtained over the 35-day period of continuous flow. The average growth in the three replicate control flasks, as determined from cell counts, was greater than the growth in the three replicate phenol treated flasks (Figure 3). Within the trends that do exist, there was large variability among the replicates within treatments relative to cell number, especially among the control replicates (Figure 2). All analyses of the growth parameters were performed using cell density estimates but the presentations in Figures 2 and 3 are in terms of original Coulter Counter counts.

Correlation coefficients among the biomass estimators (determined only at the end of the experiment), two at a time, and daily cell counts (both log and squared transformed and non-transformed) were determined for both treatments together and for each treatment (control of phenol) separately. Significant linear correlations were present in three of five correlations where all of the cultures were considered together (Table 1). The significant correlations present in the combined data are probably a function of the four or five significant linear correlations present between the growth parameters in the phenol treated cultures. The absence of significant linear correlation coefficients in the phenol treated group clearly indicates major and significant differences due to the treatment effect (Table 1).

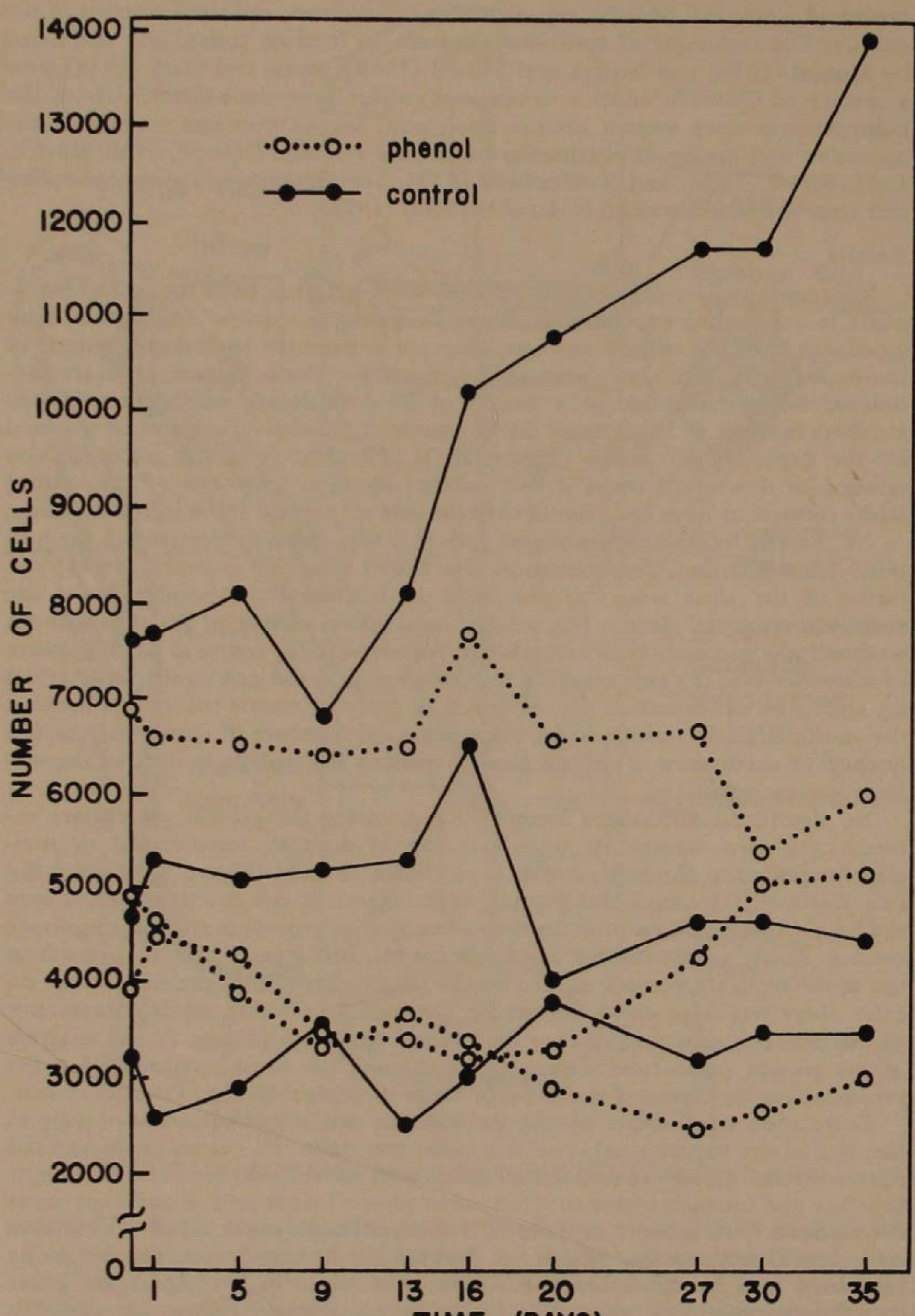


FIGURE 2. Cell Counts Plotted as a Function of Time for the Phenol and Control Cultures.

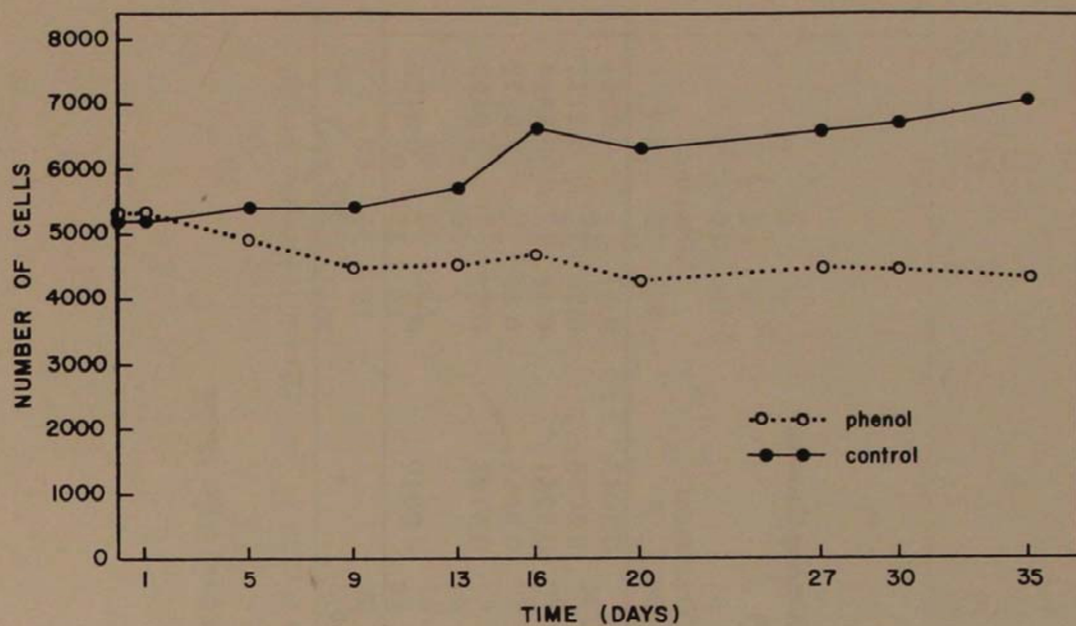


FIGURE 3. Cell Counts Plotted as a Function of Time for the Phenol and Control Cultures (average of three replicates for each point).

The populations in each flask were screened for mutants according to the method previously described by Stein (1973). Results of the screening are shown in Table 2. No statistically significant differences were detected in these latter analyses.

Visual observations were also made at the end of the screening period concerning the varying density of the culture suspension in the test tubes (in which the isolated clones were grown). Since it was not part of the experimental design, the optical density was not measured. Some tubes seemed to have the appearance of a deeper color than others. The Chi square test for contingency was again used to compare the distribution across the three growth categories (+,–,=) between the test tubes in the following two groups: group 1, cultures appeared darker and had a deeper green color; group 2, cultures were lighter in color and were more transparent. Forty-two cultures were each placed into one of the two groups, and were screened as to the amount of growth on the various drug assay plates. When the analyses were complete, a highly significant Chi square value for contingency (197.371) was obtained, thereby indicating that the amount of growth observed on the assay drug plates was dependent (at least partially) upon the amount of growth present in the test tubes.

Further analyses showed that more replicates were needed in order to detect a difference in the growth parameters at the five percent discriminatory level (Stein, 1973).

Discussion

A system for the continuous culture of algae and large scale genetic assay was constructed and tested. The system was found to be effective in maintaining a culture of algae in each of the culture vessels close to the steady state conditions. The system was unsuccessful, however, in maintaining the cultures axenically throughout the 35-day growth period.

Table 1. Correlations and Probability of $H_o: r=0$ Among Growth Parameters for the Two Treatments.

Correlation	Control Culture *r*	Control Culture *P*	Phenol Treatment *r*	Phenol Treatment *P*	Combined *r*	Combined *P*
Biomass x Cell Density	1.00	0.0261	0.89	0.3043	0.87	0.0244
Biomass x Cell Diameter	-1.00	0.0236	0.29	0.8028	-0.54	0.2712
Biomass x Cell Volume	-1.00	0.0386	0.33	0.7781	-0.48	0.3414
Cell Density x Cell Diameter	-1.00	0.0496	-0.17	0.8855	-0.86	0.0272
Cell Density x Cell Volume	-1.00	0.0665	-0.13	0.9128	-0.82	0.0459
(log) Cell Density x (log) Cell Volume	-1.00	0.0130	-0.15	0.9010	-0.83	0.0426
Proportion of Significant Linear Correlations	4/5		0/5		3/5	

Table 2. Growth of Cultures From Isolated Clones on Drug Agar Plates.

	Inoculum N=40			*Control Cultures* N=120			*Phenol Treatment* N=120		
Drug & Concentration	*%(+)*	*%(-)*	*%(=)*	*%(+)*	*%(-)*	*%(=)*	*%(+)*	*%(-)*	*%(=)*
Penicillin (100mg/l)	4	11	85	2	10	88	10	27	62
Chloromycetin (100mg/l)	3	12	85	2	9	88	12	22	65
Tetracycline (80mg/l)	6	13	81	2	7	91	15	37	47
Tetracycline (150mg/l)	3	8	83	2	4	94	7	20	72
Actidione (50mg/l)	5	10	85	4	10	84	17	12	70
Actidione (80mg/l)	2	6	92	1	7	92	5	15	80
Neomycin (100mg/l)	3	11	86	2	9	89	17	22	60
Neomycin (150mg/l)	1	9	90	0	8	92	12	20	67
Tetracycline (40mg/l) & Neomycin (50mg/l)	7	11	83	4	6	90	12	25	62
Actidione (40mg/l) & Neomycin (50mg/l)	6	7	85	2	7	91	10	30	60
Furadant in (100mg/l)	0	0	0	0	0	0	0	0	0
Sulfisoxazole (100mg/l)	0	0	0	0	0	0	0	0	0

(+)=Good solid perimeter to colony, good growth within perimeter.
(-)=Solid perimeter to colony, sparse growth within perimeter.
(=)=Broken perimeter to colony, poor growth within perimeter.

Cell counts, to determine cell density, were taken at various points during the 35-day period of continuous flow conditions. Since the cell counts obtained never showed a washout this would seem to indicate that the cells in most of the cultures were reproducing at close to a steady state.

The growth rate of *C. vulgaris* in this study was very slow and one complete changeover of culture volume (and thereby a doubling in cell number) took slightly over 7 days. This means that in the 35-day culturing period only about five complete changeovers in culture volume took place. When a population is placed under continuous culture conditions it generally undergoes several oscillations before it achieves equilibrium relative to cell number. Five changeovers of culture volume may not be sufficient time for the experiment to reach a stable equilibrium relative to cell number. The variability within treatment may have been due to the fact that each of the flasks began the continuous flow portion of the experiment with a different average cell number. When this is considered, the variability is decreased.

No significant differences in the growth parameters between the two treatments were detected but one reason may have been insufficient replication. There was large variability among the replicates within a treatment (Figure 2) relative to cell number and increased replications was demonstrated to be necessary in order to detect differences at the five percent discriminatory level. Since this was a pilot study in the lab, a need for increased replication is a most significant finding in this study.

The correlation coefficients between the growth parameters probably indicate that the introduction of phenol into the culture medium of the phenol treated flasks disturbed the normal associations among the growth parameters measured. For a better understanding of the effects of phenol or other pollutants all growth parameters should be determined throughout the experiment.

Recall the associations present among the growth parameters (Table 1). The results indicate the normal associations present among the growth parameters to be as biomass increases, cell density increases, and cell size decreases. In some cases phenol treated cultures showed a non-significant trend in the same direction as the control cultures while some showed a non-significant trend in the opposite direction. Clearly, though, the presence of phenol in the culture medium did upset the normal associations among the growth parameters.

One explanation may be that the cells became dormant, as compared to the cells in the control cultures, under the phenol stress conditions. If this is the case there would be a higher concentration of nutrients in the effluent from the phenol treated culture flasks because they were not used as rapidly. In a continuous culture study that attempted to determine the uptake of chlorinated hydrocarbons by *Chlorella*, biomass and cell density were also examined and found to be positively correlated (Sodergren, 1971). By changing the flow rate and thereby the growth rate of the algae, these correlations can be better examined and understood. According to theories that have been proposed concerning the control of continuous cultures, if the flow rate of fresh nutrients is increased, the microorganisms must react by maintaining a higher growth rate or they will be "washed out" of the culture. With a faster growth rate, the biomass parameter, as well as other growth parameters, will change. The cells may be smaller, and probably more numerous, and the total biomass of the culture may also change (this occurred in one control replicate).

The results clearly indicated that those cultures with a greater amount of growth in the test tubes demonstrated better growth on the assay drug plates.

This seemed to be true over all assay drug plates. The results of this analysis confirm the hypothesis suggested by the other data obtained in this study. In any future studies, the growth on the assay drug plates will be at least partially dependent on the growth found in the test tubes used in the screening procedure. This suggests a need for more replication in the number of clones isolated, to characterize a culture.

Conclusions

1. The growth/assay system described herein has confirmed the possibility of the evaluation of aquatic pollutant effects on genetic systems.
2. No significant differences were detected between the averages of the measured parameters for the phenol and control treatments.
3. Significant linear correlations were present between several of the biomass estimators in the control cultures but not in the phenol treated cultures, indicating that the presence of phenol significantly disturbed the relative relationship among the growth parameters.

Literature Cited

1. Kobitschek, H. E. 1970. *Introduction to Research with Continuous Cultures.* Prentice Hall Biological Techniques Series. Englewood Cliffs, New Jersey.
2. Monod, J. 1950. La Technique de Culture Continue. Theorie et Applications. *Ann. Inst. Pasteur.* 79:390-410.
3. Moser, H. 1958. The Dynamics of Bacterial Populations Maintained in the Chemostat. Carn. Inst. of Wash. Pub., 614.
4. Myers, J., and L. B. Clark. 1944. Culture Conditions and the Development of the Photosynthetic Mechanism. II. An Apparatus for the Continuous Culture of *Chlorella. J. Gen. Physiol.* 28:103-12.
5. Novick, A. 1955. Growth of Bacteria. *Ann. Rev. Microbiol.* 9:97-110.
6. Novick, A., and L. Szilard. 1950. Description of the Chemostat. *Science* 112:715-16.
7. Sodergren, A. 1972. Accumulation and Distribution of Chlorinated Hydrocarbons in Cultures of *Chlorella pyrenoidosa* (Chlorophyceae). *OIKOS* 22:215-20.
8. Stein, A. 1973. Gene and Growth Dynamics of *Chlorella* Under Continuous Culture During Phenol Stress. M.S. Thesis, West Virginia University.

The Effects of pH on the Growth of *Chlorella vulgaris*[1]

Diana Edens and E. C. Keller, Jr.
Department of Biology, West Virginia University
Morgantown, West Virginia 26506

Abstract

The pH of modified Beijerinck medium was adjusted to values of 2.5, 3.5, 4.5, 5.5, and 6.5 with sulfuric acid or hydrochloric acid. No combinations of the acids were used to adjust the pH. Modified Beijerinck medium (pH 6.8) without acid adjustment served as a control. The experimental cultures were maintained under continuous illumination at ambient temperature. Cell counts were made periodically using a Model B Coulter Counter.

Five growth parameters were evaluated: 1) population growth rate (popK); 2) specific growth rate (r_m); 3) maximum number of cells attained (Nmax); 4) generation time (genT); and 5) time required for a population to reach the midpoint of the maximum popK (G-50). Analyses of variance were performed on the pH groups. The effects of the two acids were tested separately. The effects of the two acids were then combined and the results of the five levels of pH evaluated. The level of pH significantly affected the popK, Nmax, genT, and G-50 final response levels.

Introduction

One of the major water quality factors being evaluated is pH. This factor is especially important in areas where streams receive acid mine water (AMW). Acid mine streams are usually characterized by low pH and high total acidity.

These extreme conditions of AMW affect the ecology of aquatic habitats. Of primary importance in streams is the growth of autotropic organisms which are the basic energy input for the other members of the aquatic food web.

It is the purpose of this study to evaluate the effects of pH on the growth of a unicellular alga, *Chlorella vulgaris*, under controlled laboratory conditions. The effects of pH are indicated by the following growth parameters: 1) population growth rate (popK); 2) specific growth rate (r_m); 3) maximum number of cells attained (Nmax); 4) generation time (genT); 5) time for the population to reach the midpoint of the maximum popK (G-50) (Skinner, 1972).

Literature Review

Hopkins and Wann (1926) found that the lower pH limit for the growth of *Chlorella* was 3.4 and that the growth rate was directly affected by hydrogen ion concentration below pH 5.7. Later, they found that the pH range for maximum growth of *Chlorella* was 4.6 to 7.0. However, Smith and Wiedeman (1964) believe that most algae prefer an alkaline environment.

Shoupp (1972) found that acid mine water significantly affected the growth parameters, r_m and Nmax, of *Chlorella vulgaris*. He also found that the principle component of variability in his experiments was an acid mine water concentration of 12.5% having a pH 3.62. Skinner (1972) found significant effects of acid

[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, WVU.

mine water on the growth parameters G-50 and genT of *Chlorella vulgaris* in low pH cultures.

The actual mechanism by which hydrogen ion concentration affects the cell is unknown. However, Kralovic and Wilson (1969) stated that pH may act on the protoplasmic membrane of the cell by preventing or causing the dissociation of molecules and thus controlling their absorption by the cell. Bennett (1969) found that acid mine water had a morphologically destructive effect particularly upon the chloroplasts and the chlorophyll of algae found in different levels of total acidity obtained from several streams of the Monongahela River system in West Virginia.

The initial pH of the culture medium either increases or decreases due to the growth of the algae (Hopkins and Wann, 1926, and Smith and Wiedeman, 1964). They believe the change in the pH of the growth medium is due to use of the nitrogen source available to the organisms. If nitrate is the nitrogen source, then the pH will increase due to its use. The opposite effect may be noted with ammonia as the nitrogen source.

Materials and Methods

The organism used in this experiment was *Chlorella vulgaris* Beijerinck Pratt strain. The stock cultures were axenically maintained in modified Beijerinck medium (Skinner, 1972). Four-day-old stock cultures were used in this study. They were checked for contamination before use by standard spread plating on plate count agar.

The experimental cultures were maintained in a block gradient at ambient temperature (31.5°C ± 1.463[2]). The culture modules and air delivery system were identical to that described by Shoupp (1972). Light intensities were adjusted to 14.0 ± 0.5 foot candles with a Weston Model No. 756 photometer.

The three media used in this study were: 1) modified Beijerinck medium; 2) Beijerinck medium with the pH adjusted with sulfuric acid; and 3) Beijerinck medium with the pH adjusted with hydrochloric acid. No combinations of the two acids were used to adjust the pH. By titrating the medium with .01 N H_2SO_4 and 0.1 N HCl the pH was adjusted to approximately 2.5, 3.5, 4.5, 5.5 and 6.5 using a Corning Model 7 pH Meter. Beijerinck medium without acid adjustment (pH 6.8) served as the control. The phosphate buffer system present in the Beijerinck medium was not modified. The medium was autoclaved and the pH once again determined. The experimental cultures consisted of 15.5 ml of medium inoculated with 0.5 ml of stock culture. There were two replicate cultures per pH level for both acid treatments and the control.

Counts of the number of cells of *Chlorella vulgaris* were made periodically on a Model B Coulter Counter as described by Skinner (1972). The experiment was terminated after 25 days. At this time, the cultures were examined for bacterial contamination and the *Chlorella* cells were examined for morphological changes. The final pH values of the cultures were also recorded.

The following growth parameters are used to characterize and compare the *Chlorella* populations: 1) popK, the population growth rate; 2) r_m, the specific growth rate; 3) Nmax, the maximal cell population attained; 4) genT, generation time; and 5) G-50, a measure of the length of the lag phase.

The population growth rate, popK, is the average rate of change in the number of organisms per unit time (Odum, 1971).

[2]This number represents the standard deviation.

The specific growth rate, r_m, is the average rate of change in the number of organisms per unit time per organism. This statistic, r_m, has been referred to as the intrinsic rate of natural increase (Lotka, 1925), the mean specific growth rate (Myers, 1953), the innate capacity for increase (Andrewartha and Birch, 1954), and the instantaneous coefficient of population growth (Odum, 1971). In this paper the statistic refers to the mean specific growth rate and may be used to determine quantitatively the effects on growth of a specific set of environmental conditions (Myers, 1953).

The growth parameter Nmax is the maximum number of individuals in a population, or the carrying capacity of the population (Andrewartha & Birch, 1954, Odum, 1971). It is simply the number beyond which no major increase in cell number will occur.

Generation time, genT, or doubling time is the time required for the population to double in number (Thomas, 1963; Fogg, 1965; Odum, 1971). Generation time is inversely related to the specific growth rate, r_m.

The time required for a population to reach the mid-point of the maximum population growth rate, popK, is referred to as G-50. It is a measure of the time of attainment of the average maximal growth rate.

Statistical Analyses

PopK and r_m values were obtained using the Conversational Program System at West Virginia University. G-50 was calculated from popK values and Nmax values were obtained from the actual counts. GenT was generated in the SAS computer program (Skinner, 1972).

Analyses of variance were performed on the growth response data by the Statistical Analysis System at the West Virginia University Computer Center (Barr & Goodnight, 1971).

Results

pH significantly affected the growth parameters Nmax and G-50 when the two acid treatments were analyzed separately (Tables 1 & 2). However, when the results of the levels of the acids were examined jointly there was a significant effect of pH level on popK, Nmax, genT, and G-50 (Table 3). There was no significant interaction effect of the kind of acid treatment across the various pH levels.

At pH 2.5, for both acids, the *Chlorella* population did not increase from the original number of organisms (Figures 1 & 2) and the typical sigmoid growth curve was never attained. Nmax for pH level 2.5 was about 10 fold less than the control and popK for this level was 60 fold less than the control. Also, generation time for pH level 2.5 was longer than the other pH levels, except the control (Table 4). At pH 3.5, for both acids, the growth curve exhibited a longer lag phase than those curves at the higher pH levels. This observation is verified by the high G-50 value for pH level 3.5 (Table 4). Even when the populations did increase, it was not on the order of the higher pH levels. The maximum number of cells attained at pH level 3.5 was about 5 fold less than the control and the popK value was 6 fold less than the control (Table 4). All other pH levels (4.5, 5.5, 6.5, and 6.8) exhibited typical sigmoid growth curves (Figures 1 & 2). In general, the higher pH levels produced higher population growth rates.

These data show that pH level did not produce a significant effect on r_m values. However, pH levels, on both ends of the range produced lower r_m values than the pH levels of the mid range (Figures 1 & 2, Table 4).

Table 1. ANOVA Results of the Effects of HC1 pH Levels on Growth Parameters (Model II)

		Nmax		*popK*		r_m		*G-50*		*genT*	
Source of Variation	*d.f.*	*M.S.*	*F value*	*M.S.*	*F value*	*M.S.*	*F value*	*M.S.*	*F value*	*M.S.*	*F value*
HC1	5	56500429	16.02*	149685516	2.13N.S.	6.99	1.11N.S.	57.59	6.38*	.000719	1.39N.S.
Residual	6	3527679		70124806		6.32		9.02		.000516	

Table 2. ANOVA Results of the Effects of H_2SO_4 pH Levels on Growth Parameters (Model II)

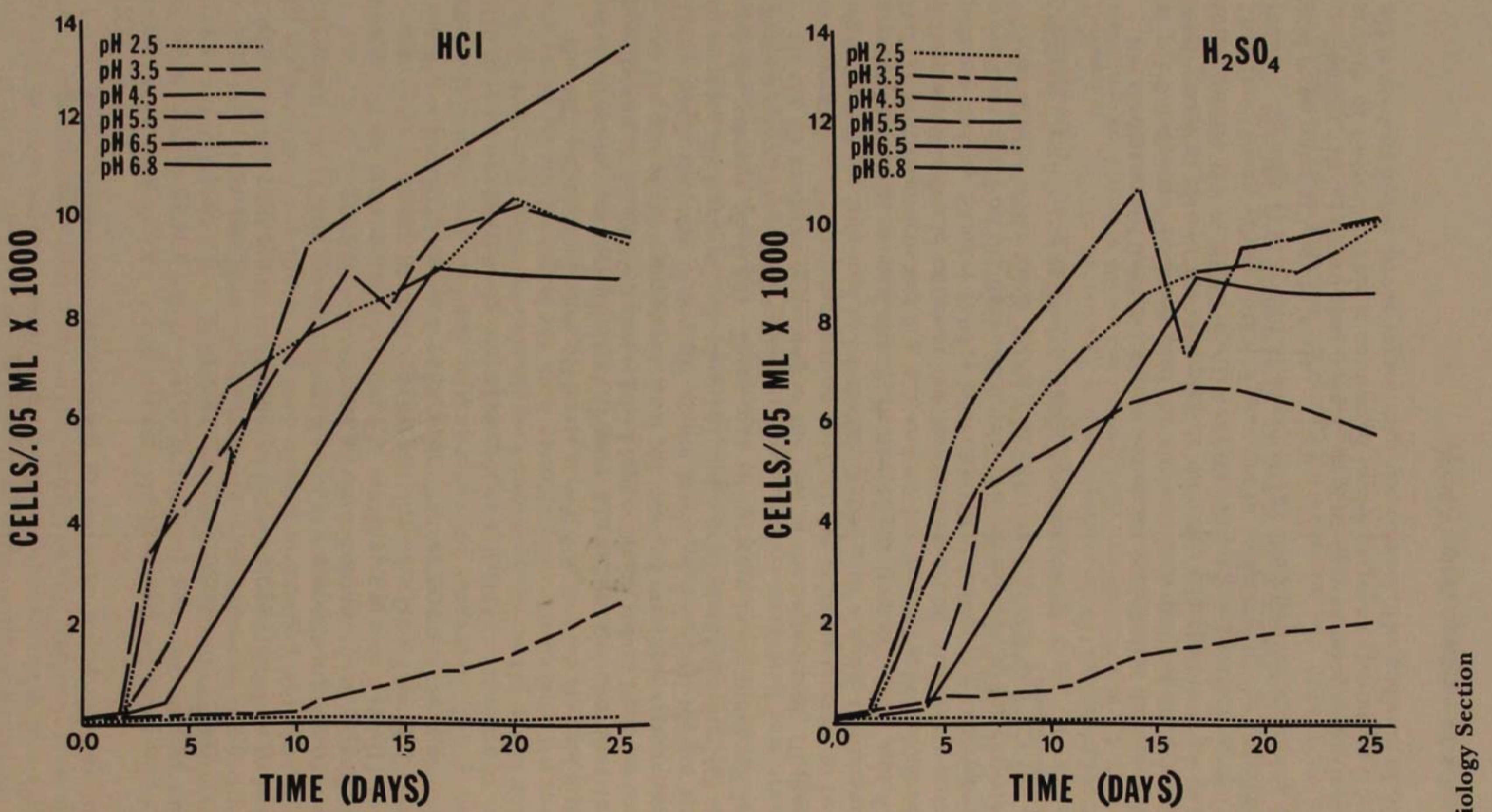
		Nmax		*popK*		r_m		*G-50*		*genT*	
Source of Variation	*d.f.*	*M.S.*	*F value*	*M.S.*	*F value*	*M.S.*	*F value*	*M.S.*	*F value*	*M.S.*	*F value*
H_2SO_4	5	38015380	6.24*	95372695	2.27N.S.	12.75	1.89N.S.	45.68	8.99*	.00203	2.93N.S.
Residual	6	6090623		41931893		6.73		5.08		.00069	

N.S.=No significant differences
* =P between .05 and .01
** =P less than .01

Table 3. ANOVA Results of the Effects on Growth Parameters When pH Levels of the Two Acids Were Grouped Across the Two Types of Acid (Model II)

		Nmax		*popK*		r_m		*G-50*		*genT*	
Source	*d.f.*	*M.S.*	*F value*	*M.S.*	*F value*	*M.S.*	*F value*	*M.S.*	*F value*	*M.S.*	*F value*
Acid Levels Grouped Together	5	87828872	15.92**	234370100	5.14**	15.044	2.66N.S.	95.85	11.30**	.00205	3.508*
Residual	16	5516129		45616527		5.658		8.48		.00059	

N.S.=No significant differences
* =P between .05 and .01
** =P less than .01



FIGS. 1 & 2. GROWTH CURVES FOR CHLORELLA IN ACID ADJUSTED MEDIUM

Table 4. Summary Table of Average Growth Parameter Values

	Nmax	*popK*	r_m	*G-50*	*genT*
HCl					
2.5	94.0	184.4	6.388	11.0	0.109
3.5	2551.6	2285.7	7.231	18.7	0.099
4.5	10671.3	15341.9	10.598	4.0	0.068
5.5	10300.7	13957.7	9.579	5.0	0.081
6.5	13844.1	23325.3	9.028	9.0	0.079
H_2SO_4					
2.5	106.6	249.5	4.900	8.0	0.146
3.5	2062.2	1904.5	9.230	16.5	0.077
4.5	10302.6	15337.9	10.681	5.0	0.072
5.5	6365.0	13155.5	8.334	5.0	0.089
6.5	9721.6	15779.6	11.201	3.0	0.062
Control					
(6.8)	9618.5	12483.2	5.975	7.0	0.116

The HCl populations with pH levels at 4.5, 5.5, and 6.5 reached higher Nmax values than the control pH level (Figure 1). Figure 2 shows that the H_2SO_4 populations at 4.5 and 6.5 also reached higher Nmax values than the control.

At the end of the experiment, final pH levels of the cultures were determined. There was a decrease in the pH at the levels of 6.5 (Δ-0.15) for the H_2SO_4 treatment, pH levels of 6.5 (Δ-0.14) and 5.5 (Δ-0.48) for the HCl treatment, and the control (Δ-0.24). There was an increase in all other pH levels. The increase ranged from 0.07 to 0.71.

Of the 23 modules remaining at the end of the experiment, 10 showed some contamination. The contamination in 7 of the cultures seemed to be bacteria, while 3 of them appeared to be contaminated with a mold. Contamination was present in at least one of the replicates of pH level 3.5, 5.5, and 6.5 for the H_2SO_4 acid treatment and 3.5, 4.5, and 6.5 for the HCl acid treatment but little difference was found between the contaminated and the axenic replicates. As pointed out by Skinner (1972) contamination during the experiment had no significant effect on the final outcome if it occurred later in the experiment.

Microscopic observation showed at pH levels of 2.5 and 3.5 the cells were clumped together and exhibited highly irregular shapes.

Discussion

Recall that at pH level 2.5 Nmax was about 10 fold less than the control and at pH 3.5 about 5 fold less than the control. Low cell counts in the lower pH levels could be due to the clumping of the cells. Clumped cells are counted as a single cell by the Coulter counter resulting in underestimation of cell numbers. Therefore, Nmax values obtained may not be indicative of the actual population present. Shoupp (1972) and Skinner (1972) also observed clumping of cells in acid mine water at low pH levels.

Skinner (1972) found that a small amount of acid mine water increased the growth of *Chlorella.* This is confirmed in the current experiment where several pH levels had higher Nmax values than the control. The addition of SO_4 and Cl might have had some favorable effect on the growth. However, it is difficult to evaluate the similarity of nutrient value of SO_4 and Cl. Also, statistically there was no effect of acid treatment on the growth. Hence, the prevalent differences in growth were primarily due to hydrogen-ion concentration and the major component of variability was the level of the hydrogen-ion concentration (pH 2.5 and 3.5).

The popK values for the lower pH levels may not be indicative of the actual population growth rate since the growth curves of the lower pH levels were not typical sigmoid growth curves. However, according to the results of the current experiment, popK is significantly affected by pH level and, again, the greatest component of variability is in the pH levels of the cultures between 2.5 and 4.5.

Even though the population growth rate was significantly affected by pH level, the intrinsic growth rate was not affected. Skinner (1972) also found that the main effect of acid mine water did not significantly affect r_m.

Skinner (1972) found a significant effect of acid mine water on the growth parameters G-50 and genT. Except for pH 6.5 and the control, the length of time *Chlorella* populations remained in lag phase is inversely related to pH level. Therefore, a high hydrogen ion concentration inhibits the capability of the organisms to reproduce. This is also shown by the growth parameter genT. The cultures of lower pH levels produced long generation times. Therefore, in this experiment pH level significantly affected both the length of the lag phase and the generation time.

The difference in the initial and final pH levels of the cultures may be explained on the basis of NH_4/NO_3 utilization by *Chlorella* (Smith and Wiedeman, 1964). Both NH_4 and NO_3 are present in the modified Beijerinck's medium. Possibly at higher pH levels there was a preference for NH_4 and, therefore, the pH of the medium decreased. The opposite may be true for the lower pH levels. In this experiment, the potential of the organisms to change the pH level of their environment may have been enhanced due to a poor buffering culture system at low pH levels.

Literature Cited

1. Andrewartha, H. G., and L. C. Birch. 1954. The distribution and abundance of animals. Univ. of Chicago Press, Chicago.
2. Barr, A. J., and J. H. Goodnight. 1971. Statistical Analysis System. Department of Statistics, North Carolina State University, Raleigh, N. C.
3. Bennett, H. D. 1969. Algae in relation to mine water. *Castanea* 34:306-28.
4. Fogg, G. E. 1965. Algal culture and phytoplankton ecology. Univ. of Wisconsin Press, Madison, Wisconsin.
5. Hopkins, E. F., and F. B. Wann. 1926. Relation of hydrogen-ion concentration to growth of *Chlorella* and to the availability of iron. *Bot. Gaz.* 81:353-76.
6. Kralovic, R. D., and H. A. Wilson. 1969. Survival and activity of sewage microorganisms in acid mine water. West Virginia University, Morgantown, West Virginia. 30 p.
7. Lotka, A. J. 1925. Elements of physical biology. Williams and Wilkins, Baltimore.
8. Myers, J. 1953. Growth characteristics of algae in relation to the problem of mass culture. In: J. S. Burlew (ed.), Algal culture from laboratory to pilot plant. Carnegie Inst. Wash. Publ., No. 600:37-54.
9. Odum, E. P. 1971. Fundamentals of ecology. Third edition. W. B. Saunders Co., Philadelphia.
10. Shoupp, W. J. 1972. The interaction of thermal and acid mine water loadings on the growth of *Chlorella*. M.S. Thesis. West Virginia University, Morgantown, West Virginia. 103 p.

11. Skinner, W. F. 1972. The interaction of sewage, thermal and acid mine water loadings on the growth of *Chlorella*. M.S. Thesis. West Virginia University, Morgantown, West Virginia. 182 p.
12. Smith and Wiedeman. 1964. A new alkaline growth medium for algae. *Can. J. Bot.* 42:1582-86.
13. Thomas, W. H. 1963. Physiological factors affecting the interpretation of phytoplankton production measurements. *In:* M. S. Doty, (ed.), Proceedings of the conference on primary production measurements, marine and fresh water. Univ. of Hawaii. 1961. Washington, D.C.
14. Wann, F. B., and E. F. Hopkins. 1926. Further studies on the growth of *Chlorella* as affected by the hydrogen-ion concentration. *Bot. Gaz.* 83:194-201.

Chlorella vulgaris Growth Response to Acid Mine Water Stress Under Conditions of Constant and Reduced Light[1]

R. Pisapia, F. Kopyta, and E. C. Keller, Jr.
Department of Biology, West Virginia University
Morgantown, West Virginia 26506

Abstract

Axenic cultures of *Chlorella vulgaris* (Pratt strain) were grown at ambient temperature using a modified Beijerinck's media and four concentrations of acid mine water (0/32, 1/32, 2/32, 3/32) in a light and a predominantly dark environment. Cell counts were made using an electronic particle counter. From these data, four growth parameters were estimated: 1) the maximum specific growth rate, r_m; 2) the maximum population growth rate, popK; 3) the average time for the population to reach the maximum growth rate, G-50, and 4) the maximum number of cells obtained, Nmax.

The maximum specific growth rate was shown to differ significantly between the effects of the light conditions and the level of acid mine water (AMW) concentration. Most of the significant variability among the four AMW concentrations for this parameter was attributable to those cultures grown in continuous light.

The difference between the light and reduced light conditions had a significant effect on the time measure of the lag phase of growth. For those cultures grown in the reduced light environment, the different concentrations of AMW showed no significant effect on the four growth parameters measured.

Introduction

The stimulation of growth by brief illumination of dark grown cells has been observed in algae. Killam and Meyers, 1955, using the Emerson strain of *Chlorella vulgaris* contributed the continued growth of this alga in darkness to a

[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, WVU.

"training" phenomenon which increased its sensitivity to small light dosages. The stimulation of the synthesis of some key materials required for cell division is believed to have occurred in this brief period. Finkle *et al.*, 1950, have shown that *Chlorella vulgaris*, Emerson strain, was unable to grow and divide actively on an inorganic carbon medium in the dark. However, Griffiths, 1961, reported that active cell division resumed when similarly treated cells were re-exposed to light after 12 days in the dark.

The growth of *Chlorella vulgaris*, Beijerinck (Pratt strain), used in this study, has been evaluated in several concentrations of acid mine water (Shoupp, 1972). Compared to cells grown on a completely inorganic Beijerinck's (control) medium, he reported higher maximum cell numbers for those cells grown in two of the acid mine water concentrations. This may be attributable to some growth factors which were not present in the inorganic culture medium.

This study was designed to determine the growth response of *Chlorella vulgaris* (Pratt strain) grown in a modified Beijerinck's medium and under acid mine water modification, to darkness after a brief exposure to a reduced light source.

Materials and Methods

An axenic culture of *Chlorella vulgaris*, Beijerinck (Pratt strain), originally obtained from Indiana Algae Culture Collection No. 398, was used in this study.

Acid mine water (AMW), having a pH of 2.85, was collected from Robinson Run, a small acid stream near Maidsville, West Virginia. Combined with the completely inorganic modified Beijerinck's medium by volume, the test medium was produced as follows: 1/32 AMW, contained 1 part AMW plus 30 parts modified Beijerincks media plus 1 part *Chlorella vulgaris* in a 4-day-old suspension = 32 parts. AMW concentrations were those used by Skinner, 1972, i.e., 0/32 (control), 1/32, 2/32, 3/32. Initial pH values for the cultures were 6.73, 6.60, 6.40, and 6.11, respectively. The average initial *C. vulgaris* cell diameter was 6.79 μ. Prior to inoculation with the algae, the test media were filtered and autoclaved. Final pH and cell diameter determinations were made at the termination of the experiment.

Eighteen replicates of each AMW concentration were grown in a continuous light environment. This consisted of an aluminum thermal gradient block (Shoupp, 1972) with a bank of 20-watt Gro-lux (Westinghouse Corp.) fluorescent tubes providing approximately 15-foot candles of illumination at the base of the culture tubes. Two replicates of each AMW concentration were grown in a light-free (dark) box constructed to create the reduced light environment. These dark grown cultures were exposed to the reduced light source on day 6 and day 12. The exposure time was less than one minute. Each dark culture sampled was sacrificed at each observation time due to its exposure to light during the sampling procedure.

Culture tube assemblies as described by Skinner, 1972, were employed to hold the growing algal cultures under both light conditions. Sterile humidified air was pumped through each culture for 20 seconds in every 20 minutes to facilitate gas exchange and to keep the cells suspended. Temperatures in both environments were recorded four times daily.

Growth response was obtained from cell counts of 0.1 ml samples, aseptically withdrawn from a culture and placed in a counting vial with 10.0 ml of Isoton (Coulter Electronics, Hialeah, Fla.) diluent. Cell counts were made with a Model B Coulter Counter over a twenty-one-day period with the light grown cultures, and a forty-one-day period for the dark grown cultures. To eliminate possible counting bias, five counts of 0.05 ml sub-samples were made from each sample with the highest and lowest counts being discarded and the average of the remaining three counts utilized for the growth analyses. The growth parameters employed are described in detail by Shoupp, 1972, and Skinner, 1972. However, a brief description of these parameters follows:

1) Maximum Specific Growth Rate, r_m—This is the average rate of change in the number of organisms per unit time per organism. This is the actual or realized growth rate as influenced by the experiment stress conditions as opposed to an intrinsic rate as found in optimal conditions. This point of r_m is found on a normal sigmoid growth curve at the inflection point where lag phase proceeds into log phase of growth.

2) Population Growth Rate, popK—This is the average rate of change in the number of organisms per unit time, i.e., the number of organisms added to the population per unit time. This is calculated by dividing the population increase by the time elapsed during the time interval measured.

$$popK = (N_f - N_i)/(t_f - t_i)$$

3) Average Time for the Population to Reach Maximum Growth Rate, G-50—This is the time required for a population to reach the midpoint of maximum population growth rate, popK. G-50 is a measure of the time of attainment of the average maximal population growth.

4) Maximum Number of Cells Obtained, Nmax—This is the number beyond which no major increase in cell numbers will occur or also called the carrying capacity of the population.

Results

Upon examination of the response values for Nmax, (Table 1) it was found that there was no great difference between the maximum number of cells attained in those cultures grown in continuous light and those illuminated for a brief period. It was also found that no significant differences existed for the population growth rate, popK.

Highly significant interaction effects of the AMW-Light Condition were noted on the maximum specific growth rate, r_m, as is shown in Table 2. In Figure 1, the r_m values were generally higher for the cells grown in the dark (except for 1/32 AMW) than for those cells grown in the light.

The variable light conditions also showed a significant main effect for the G-50 variable (Table 3). Figure 2 shows that a much shorter lag phase was observed for the light grown cultures than was noted for those organisms grown in the dark prior to their brief exposure to the reduced light source on day 6 and day 12.

Ambient temperature $29.7 \pm 0.6°C$[2] was not considered a major factor in this experiment. The generally slight reduction in pH for all cultures was similarly not considered to be significant from the work of Skinner, 1972.

Discussion

Since Nmax is simply the maximum number of cells attained, cell division occurred in the dark grown cultures as well as in those grown in the light. It may be that the continued growth of the algal cultures in the dark is due to a "training" phenomenon as has been suggested by Killam and Meyers, 1955. The

[2]Temperature with standard error of mean.

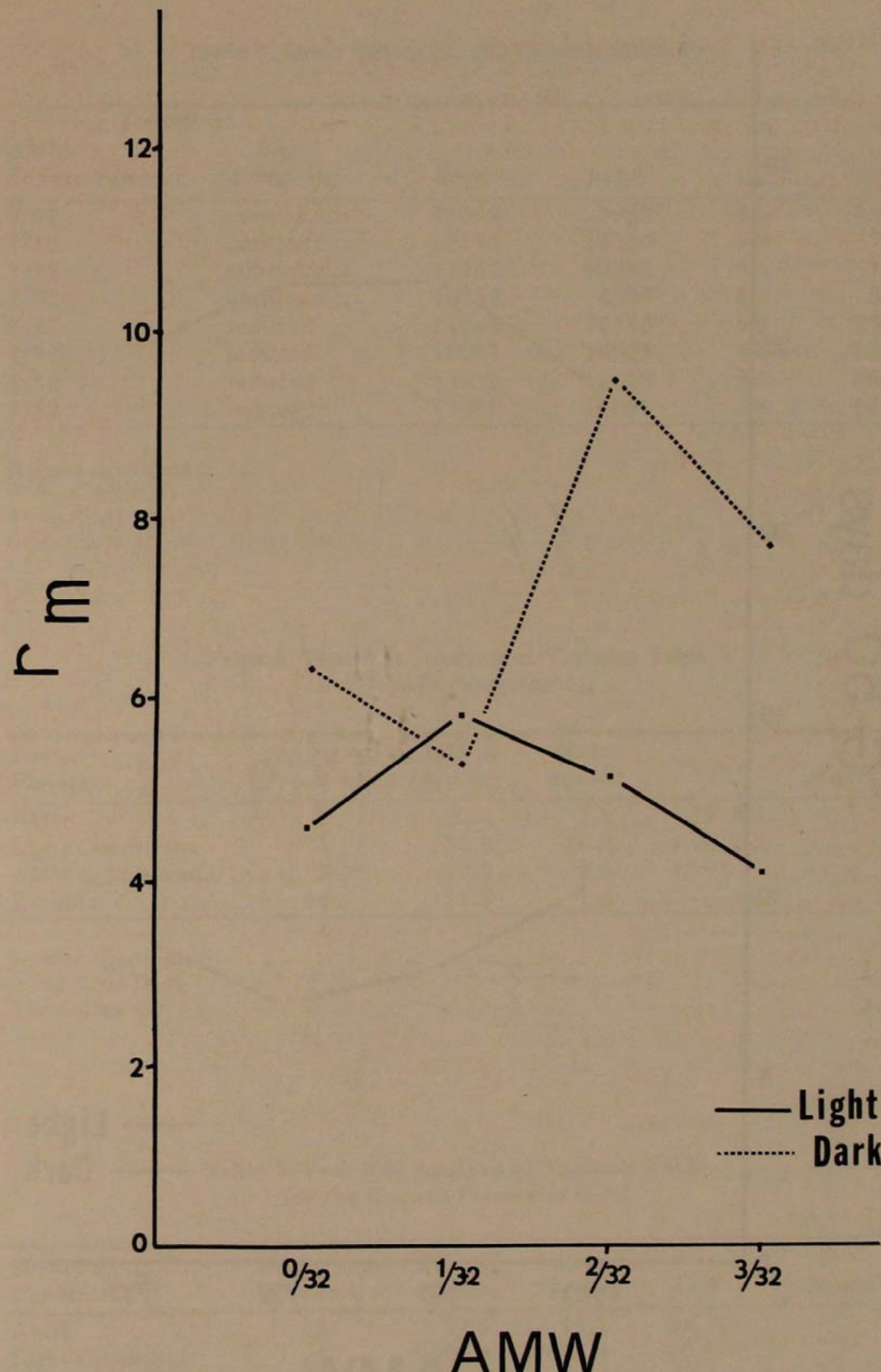


FIGURE 1. Average response of the maximum specific growth rate (r_m) of *Chlorella vulgaris* for each light condition across the variable AMW gradient.

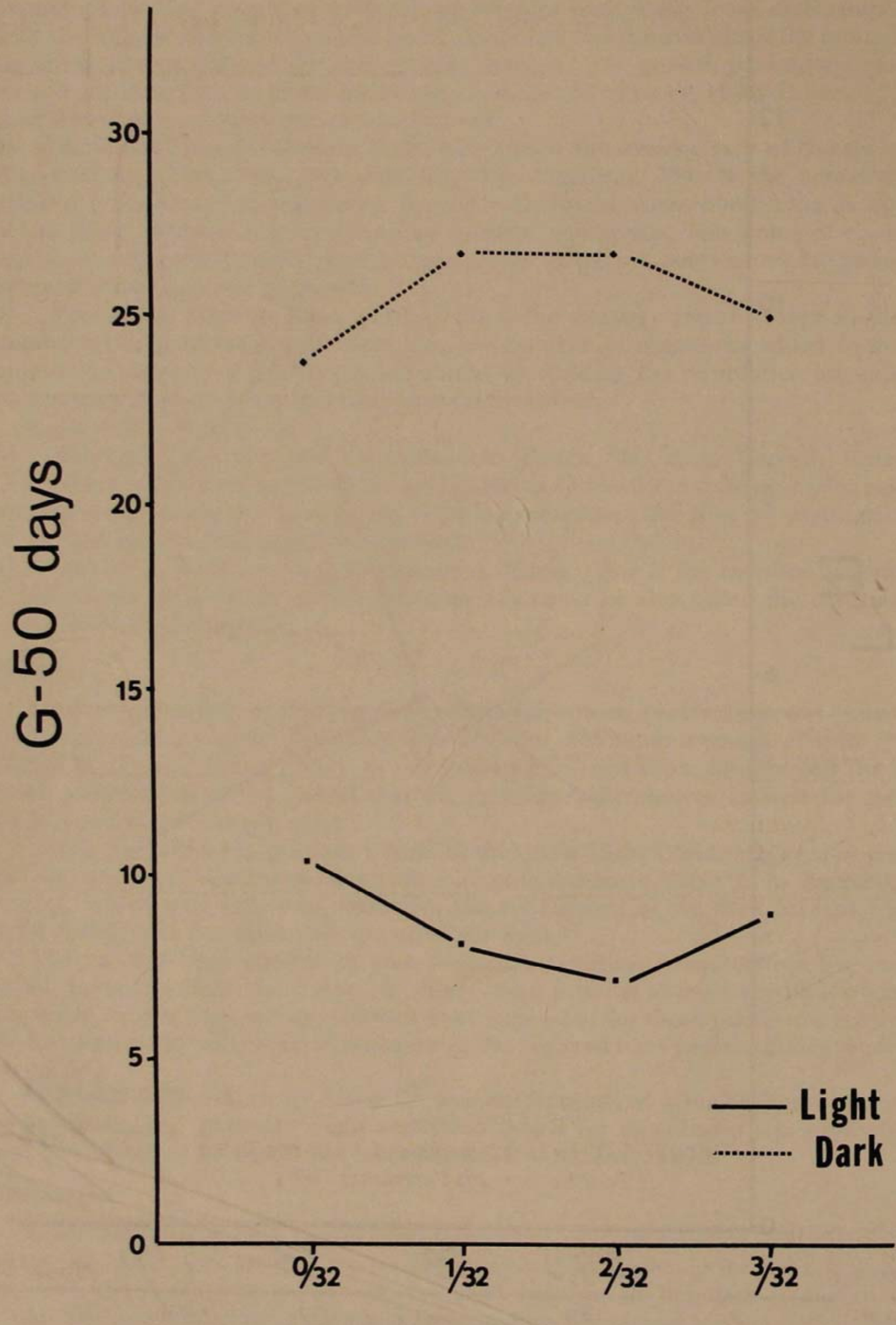


FIGURE 2. Time measure of the lag phase of growth (G-50) of *Chlorella vulgaris* for each light condition across the variable AMW gradient.

Table 1. Mean Values for the Growth Parameters

Treatment: AMW Concentration	Light Condition	*Nmax*	*popK*	r_m	*G-50*
0/32	continuous	11589	9802	4.6	10.3
1/32	continuous	12153	10556	5.9	7.9
2/32	continuous	11799	10283	5.1	7.2
3/32	continuous	10336	8533	4.1	8.9
0/32	reduced	11865	11440	6.3	23.8
1/32	reduced	16001	10925	5.3	26.5
2/32	reduced	11915	11679	9.5	26.5
3/32	reduced	13830	14590	7.8	24.7

NS=not significant
* =$p < .05 > .01$
** =$p < .01$

Table 2. Two-Way Analysis of Variance Table for Growth Parameter r_m

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F*	*Model II*
AMW	3	21.4	7.1	.79 NS	
Light Conditions	1	36.8	36.8	4.08 NS	
AMW & Li. Cond.	3	27.0	9.0	4.7**	
Residual	64	121.3	1.8		

NS=not significant
* =$p < .05 > .01$
** =$p < .01$

Table 3. Two-Way Analysis of Variance Table for the Growth Parameter G-50

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F*	*Model II*
AMW	3	57.5	19.1	0.6 NS	
Light Conditions	1	2004.4	2004.4	63.5 **	
AMW x Light Cond.	3	37.8	12.6	0.3 NS	
Residual	64	2019.2	31.5		

small dose of light may be enough to stimulate those key materials and systems necessary for cell division.

It may also be hypothesized that this continued growth in the dark after brief illumination may be due to a heterotrophic response to some utilizable growth factor contained in the AMW (Shoupp, 1972, reported increased growth in 1/32 and 2/32 AMW concentrations for light grown cultures). As compared to the cells grown on the inorganic media we also noted increased growth of *C. vulgaris* at the low levels of AMW (1/32, 2/32) in the light. Increased cell numbers for all levels of AMW (1/32, 2/32, 3/32) for the algal cells grown in the dark were obtained. However, since continued growth in the dark was also noted in the control cultures, i.e., without an AMW source, this theory of strictly heterotrophic growth in the dark does not warrant support.

It is more probable that a combination of the "training" phenomenon and an available heterotrophic energy source may be in operation in the dark grown cultures. This would account for the slightly increased cell numbers for those cells grown on the AMW-Beijerinck's media in the light as well as the continued growth of cultures grown in the dark.

The response variable, r_m, combines the effects of the environment (viz, pH, light, etc.) which influence the organism with the inherent growth characteristic of the organism and determines its actual or realized growth rate. As interpreted under the model II analysis of variance (Table 2), the two variables (AMW and Light Condition) are intricately involved in creating the variability observed in this parameter. However, upon further analysis, most of this variation appears to be attributable to the variable light conditions as shown in Figure 1. The light grown cells follow a growth response similar to that found by Shoupp, 1972. It is apparent that except for the 1/32 AMW, the cells grown in the dark have experienced a higher maximum specific growth rate. This increase in r_m is closely associated with the significant light condition effect shown for G-50 (Table 3).

The availability of a radiant energy source is obviously the determining factor for these two parameters. As may be expected, lack of an initial light source created an extended lag phase in the dark grown cultures. The sudden growth surge initiated through brief exposure to the light is believed to account for the major portion of the divergence among the variable light grown cultures. The increased growth of *C. vulgaris* at the low levels of AMW (1/32, 2/32 in the light compared to the cells grown on the inorganic medium alone) could indicate a possible deficiency in the inorganic medium (Becker & Keller, 1973) which is probably being compensated for by the iron ingredients.

We conclude that heterotrophic growth or stored growth potential is occurring since sufficient growth occurred prior to light exposure and further that minimal light exposure seems to enhance growth rate at higher AMW concentrations.

Literature Cited

1. Becker, A., and E. C. Keller, Jr. 1973. The Effects of Iron & Sulfate on the Growth of *Chlorella.* These Proceedings.
2. Finkle, B. J., O. Appleman, and F. K. Fleischer. 1950. Growth of *Chlorella vulgaris* in the dark. *Science* 3:309.
3. Griffith, D. J. 1961. Light-Induced Cell Division in *Chlorella vulgaris,* Beijerinck (Emerson strain). *Ann. Botany* 25(98): 85-93.

4. Killam, A., and J. Meyers. 1956. A special effect of light on the growth of *Chlorella vulgaris*. *Am. J. Botany* 43:569-72.
5. Shoupp, W. J. 1972. The interaction of thermal and acid mine water loading on the growth rate of *Chlorella*. M. S. Thesis, West Virginia University, Morgantown.
6. Skinner, W. F., and E. C. Keller, Jr. 1972. The growth of *Chlorella vulgaris* in sewage and acid mine water. These Proceedings.

The Effects of Iron & Sulfate Compounds on the Growth of *Chlorella*[1]

Anthony J. Becker, Jr. and E. C. Keller, Jr.
Department of Biology, West Virginia University
Morgantown, West Virginia 26505

Abstract

Physiological and morphological evidence is presented indicating the effects of two major chemical Acid Mine Water constituents, compounds of iron and sulfur, on the growth of *Chlorella vulgaris* (Pratt). Axenic algal cultures were grown in modified Beijerinck's medium to which one of the iron or sulfate-compound test substances had been added. Three different concentrations of each of the three iron compounds and three sulfate compounds were considered. Three replicates of each constituent concentration combination and the control culture were used. Culture tubes were maintained under constant light and ambient temperature conditions and were intermittently agitated by a humidified sterile air system. Five physiological growth parameters were considered: maximum population, maximum population growth rate, maximum specific growth rate, average time to attain maximum population growth rate, and generation time. Four morphological characteristics (cell size, shape, color and aggregate arrangement) were also observed. We conclude that: 1) the initial addition of iron results in an increase in population number, while the addition of sulfate results in a decrease, except in the case of the iron-sulfate interaction where the sulfate appears to enhance the effect of the iron, 2) continued increase in concentration of any of the test substances results in an accompanying decrease in population numbers, and 3) in high concentrations all of the test substances create conditions lethal to the algal population.

Introduction

The purpose of this study was to separate and consider the effects on *Chlorella* growth of the compounds of certain elemental constituents in Acid Mine Water (AMW), apart from field sampled AMW *in toto*. The importance of the determination of the effects of these compounds in AMW is in the relative ease in controlling these constituents for comparison, compared to the use of

[1]This project was supported, in part, by the Office of Water Resources Research, Department of the Interior, Grant #WVA-B001, and the Biology Department, W.V.U.

field sampled AMW which includes other possibly deleterious or growth enhancing components.

Materials and Methods

The two elemental constituents considered in this study were iron, Fe^{++} and Fe^{+++}, and sulfur (S) in the form of sulfate ($SO_4^{=}$). These are the major elemental constituents of AMW (as sampled in northern West Virginia), with concentrations of 700 ppm found for iron and 4024 ppm for sulfate. Much lower concentrations were detected for the other elemental constituents. In an effort to minimize the significance of the effects due to the various anions and cations used, three different anions for the iron were selected; nitrate (NO_3), chloride (CI), and sulfate (SO_4). Likewise, three different cations were chosen for the sulfate: potassium (K), calcium (Ca) and aluminum (Al). These anions and cations were chosen specifically because they all occur in AMW. Secondarily, they were chosen because the cations represent three different valence states and the anions form compounds with two different forms of iron, Fe^{++} and Fe^{+++}, thus attempting to obtain results attributable to a "generalized" iron compound and a "generalized" sulfate compound and minimizing the possibility of the results being related to a particular state of one of the ions.

The alga used in this study was *Chlorella vulgaris*, Biejerinck (Pratt strain; Shoupp, 1972). The species was first described by Biejerinck in 1890 (Prescott, 1962). The only known method of reproduction in *Chlorella* is autospore production, a mechanism in which 4 or 8 daughter cells are formed within the mother cell wall. *Chlorella vulgaris* of this laboratory has a range in size from 5 to 10 microns in diameter (Janeczek *et al.*, 1972).

A sub-culture was established from a stock culture of specimen #398 from the Culture Collection of Algae of the Department of Botany, Indiana University. Axenic isolation of the sub-culture was established by use of standard methods of agar plating.

Eighteen component/concentration combinations were used in the experiment. These consisted of 350, 700, 1400 ppm concentrations in the forms of Fe ($FeSO_4$, $FeCl_3$, and $Fe(NO_3)_3$) in liquid modified Biejerinck's medium as used by Skinner (1972) and 2012, 4024, and 8048 ppm concentrations of SO_4 in the forms of (K_2SO_4, $CaSO_4 \cdot 2H_2O$ and $Al_2(SO_4)_3 \cdot 18\ H_2O$) in liquid modified Biejerinck's medium. The second concentration in each case is that found occurring naturally in AMW sampled from a mine pump (Skinner, 1972). In addition to these eighteen component/concentration combinations, control cultures were used which consisted entirely of liquid modified Biejerinck's medium. Three replicates of each of the nineteen cultures were used, thus requiring fifty-seven axenic culture tubes.

Background counts of the nutrient media were made on a electronic particle counter (Coulter Counter, Model B, Coulter Electronics Inc., Hialeah, Florida) so that the initial counts could be considered and subtracted from subsequent cell counts (an external background count of from 20 to 60 counts/50 lambda of media counted was also obtained and was subtracted from the total count).

A dry bath thermal gradient was used in order to establish a stable uniform temperature regime throughout the gradient. This gradient is similar to that used by Keller *et al.* (1967). The culture system is identical to that used by Skinner (1972) and Shoupp (1972).

The fifty-seven culture tubes were filled with 14.5 ml of the respective medium and then autoclaved. The autoclaved culture tubes were then inoculated with 0.5 ml of a 4 day old sub-culture. Coulter counts were again made to determine the number of cells in the inoculum. A microsocpic examination of the cells in the inoculum was made to determine size, shape, color, and aggregate arrangement. Nutrient agar plating methods were again used to check for contaminants in the inoculum. The culture tubes were then randomly distributed throughout the eighty-eight locations in the gradient and were connected to the humidified sterile air system. Thereafter, counts were made at 48 hour intervals for a 30 day period. Methods of counting were identical to those described by Skinner (1972).

From the values obtained for cell counts, growth curves were plotted for each component/concentration combination and for the control. Five characteristics were then established for each of these populations: maximum growth obtained (N_{max}), maximum population growth rate (popK), maximum specific growth rate (r_m), generation time (genT), and average time to attain popK (G-50). All parameters were obtained in an identical manner to that described by Skinner (1972).

Results and Discussion

Each curve is compiled from points based on an average of the response of three replicate cultures. The growth curves for the nineteen populations considered in this study (Figures 1 & 2) are generally of a sigmoid nature. Figure 1, represents the growth curves for the Fe containing cultures and Figure 2, represents the growth curves for the SO_4 containing cultures. The control cultures, shown superimposed on each set of curves (grown only on modified Biejerinck's media, Skinner, 1972), demonstrated a lag phase of about 5 days and a log phase of about 18 days until on the 23rd day when they entered the stationary phase. Since temperature, light intensity, and other factors except for the composition and concentration of the elemental test substances were identical for all the cultures, the parameters of the control cultures are used as a standard against which the others can be compared.

The growth curves indicate that the three iron-containing test cultures (Fig. 1) (at least in the lower concentrations) showed curves comparable to the control cultures. However, the three test cultures without iron (Fig. 2) had very irregular curves and maximum populations much lower than those of the control cultures.

The growth curves, with the exception of those cultures with $FeCl_3$ added, also showed a general decrease in maximum populations which corresponded to an increase in concentration. In all cases, the cultures with the highest concentrations of Fe showed Nmax values greatly reduced from those of both the control cultures and the lower concentrations of the same additive constituent.

The values for the other growth related parameters are presented in Table 1. The first parameter considered, popK, was obviously affected both by the type of constituent added and by its concentration (Figure 3). Pop-K values were generally much higher for the iron-containing cultures (with an average of 7,500) than for the non-iron-containing cultures, with an average of 3,500. Also an inverse relationship between popK and concentration was apparent, similar to that existing between Nmax and concentration.

The second parameter, r_m, did not appear to be affected by the presence or absence of iron in the medium; however, it did appear to follow the same inverse relationship with concentration, as has been observed for Nmax and popK (Table 1).

G-50 had generally higher values for the iron-containing cultures (Table 1);

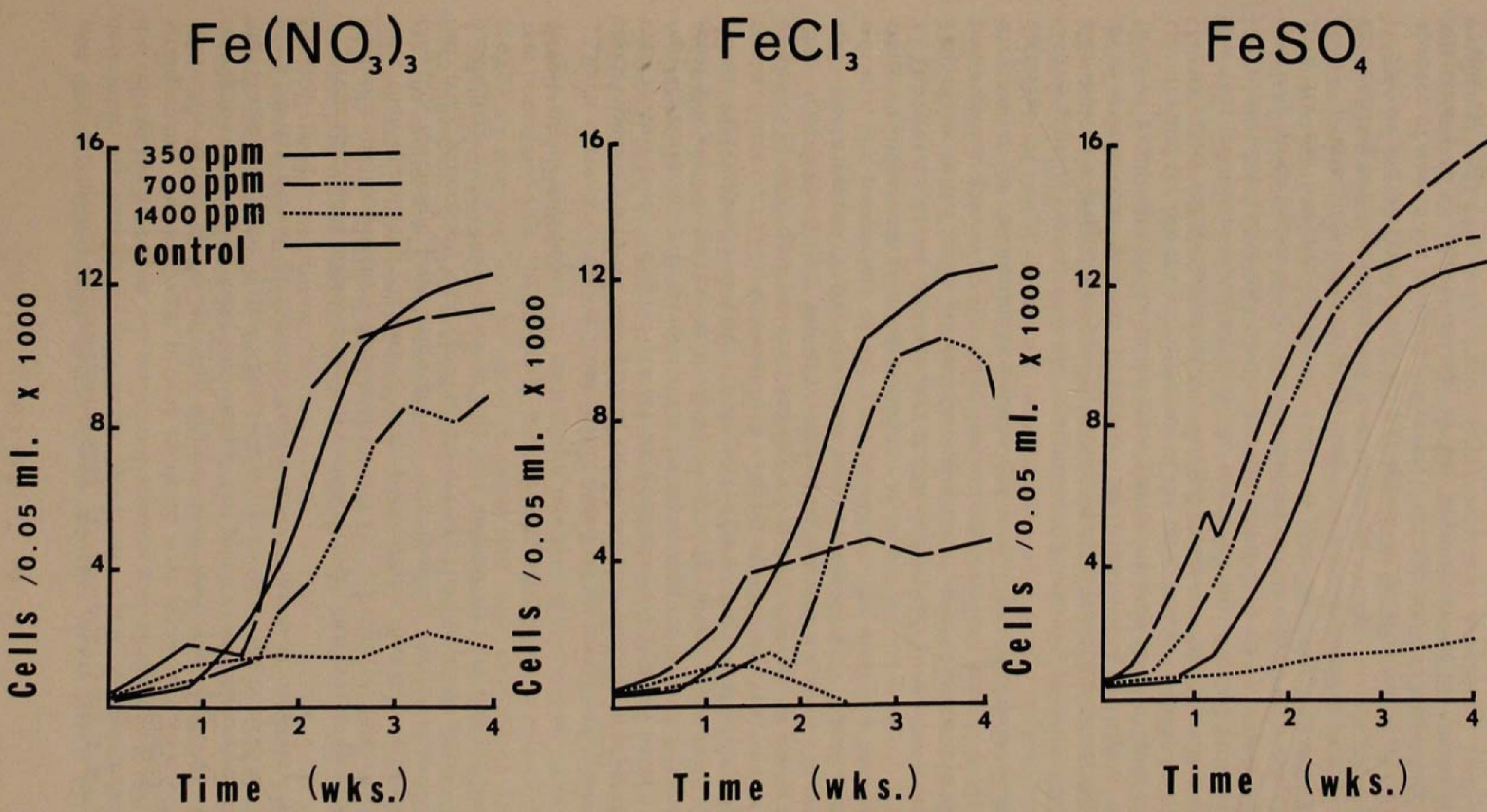


Fig.1 Fe Culture Growth Curves

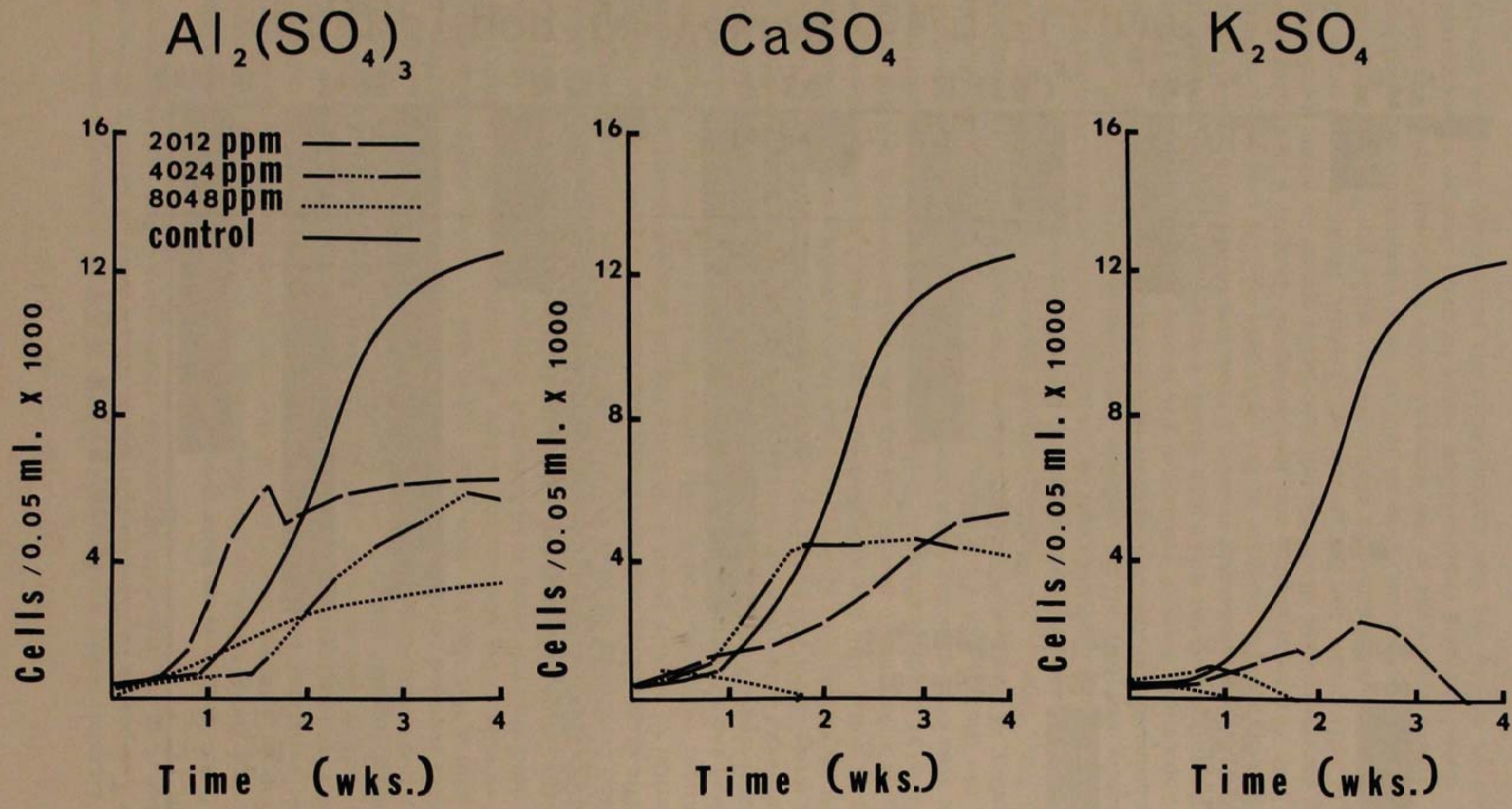


Fig.2 SO_4 Culture Growth Curves

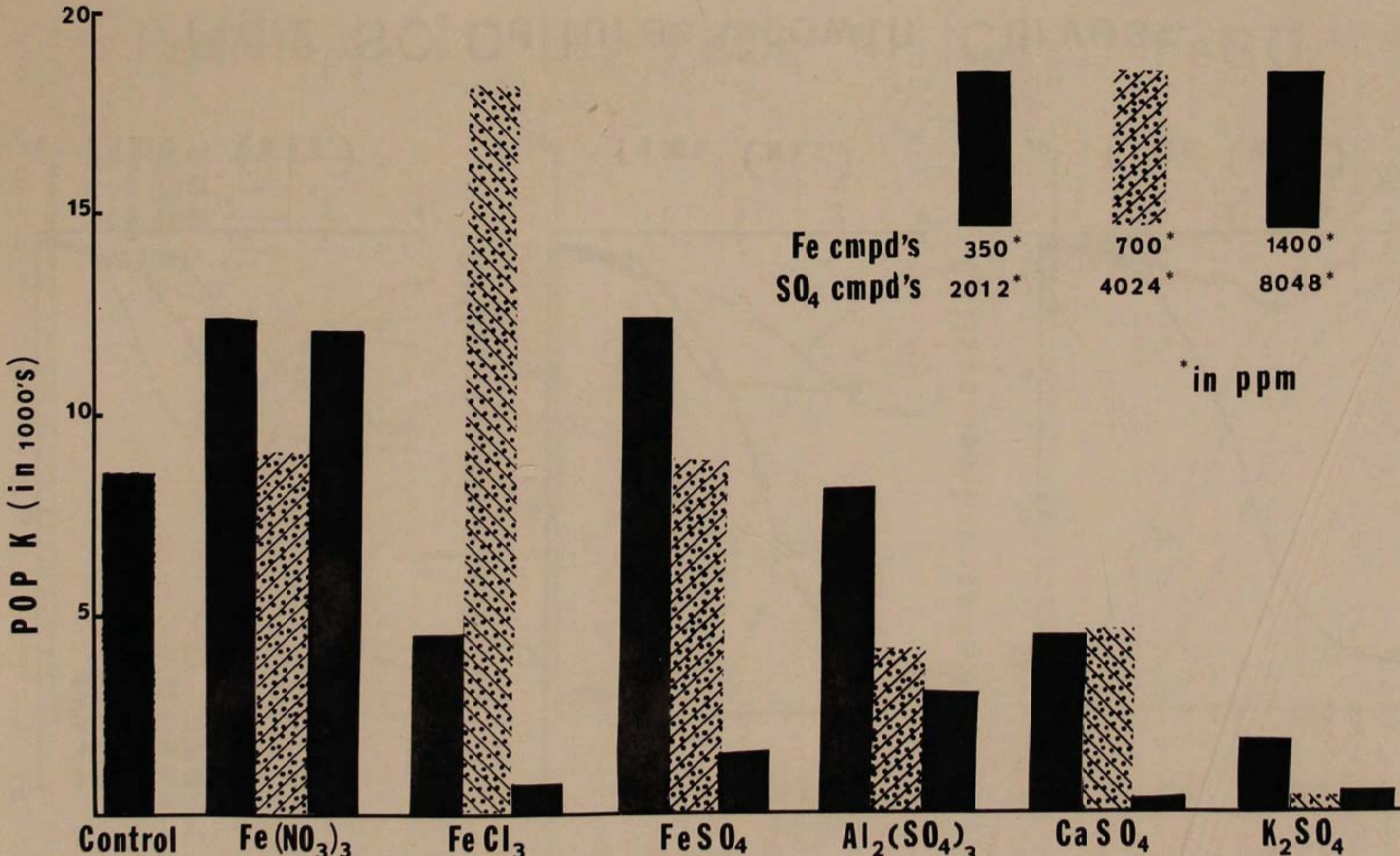


Fig. 3 pop-K Values

however, no relationship was apparent between G-50 and constituent concentration. Gen-T appeared to show no relation to the presence or absence of iron in the medium, but did demonstrate a positive relationship to the concentration of iron (Table 1).

Aside from the effects on these growth related parameters the test substance levels showed some effect on the morphology of the algal cells. Microscopic examination of samples from each of the cultures revealed that there were measurable variations in the four morphological characteristics examined: size, shape, color and aggregate arrangement. Microscopic examination of the initial inoculum culture had revealed cells 3-4.5 microns in diameter, spherical to slightly ovoid (regular margin), with a uniform flat green color, and occurring singly. At the termination of the experiment examination of the control replicate cultures revealed cells 5-7 microns in diameter, spherical to ovoid (regular margin), with a flat slightly deep green color, and occurring singly or in small (3-4 cell) clumps. Using the control culture as a standard, the test culture replicates revealed the following morphological changes: The iron containing cultures, except for the 700 ppm and 1400 ppm $FeNO_3$ which showed smaller cells (2-5 microns), showed cells of a slightly greater diameter (5.5-8 microns) while the non-iron containing cultures showed cells of essentially the same size as the control. The shape of the cells was fairly consistent for all the cultures ranging from spherical to slightly ovoid with fairly regular margins except in the higher concentrations of $CaSO_4$ and K_2SO_4 where the cells became very irregular in both outline and contour. Color changes were the most obvious and widespread of the morphological changes with only the lowest concentrations of $FeSO_4$ and Al_2SO_4 showing coloration identical to the control. The others ranged from a very dark green in the 700 ppm $FeSO_4$ culture to white in the 4024 ppm $CaSO_4$ and the 8048 $CaSO_4$ and K_2SO_4, with the iron containing cultures showing a lightening in color. The last morphological characteristic to be considered was the aggregate arrangement of cells; this was also very pronounced, but not nearly as widespread as the color changes. There was a definite tendency toward clumping of the cells corresponding to an increase in concentration of all constituents; however, the clumping in the $FeNO_3$, $FeCl_3$ and $FeSO_4$ cultures appeared to consist mainly of many small clumps, of from 3 to 8 cells, whereas the clumping in the Al_2SO_4, $CaSO_4$, and K_2SO_4 cultures appeared to consist mainly of a number of large clumps with as many as 50-100 cells in one aggregate.

Plating on standard methods agar at the end of the experiment revealed that approximately one half of the cultures were contaminated; however, microscopic examination at the termination of the experiment indicated that the bacterial populations were of substantial size in only 6 of the 57 cultures. These were in: 2 replicates of the 4024 ppm $CaSO_4$ containing culture, 1 replicate of the 700 ppm $FeNO_3$ containing culture, 2 replicates of the 8048 ppm Al_2SO_4 containing culture and 1 replicate of the 4024 ppm Al_2SO_4 containing culture.

From both the physiological and morphological changes, it is evident that increasing concentrations all of the test substances appear to create conditions increasingly unfavorable for the growth of *Chlorella*. Nmax, r_m, and popK all showed an inverse relationship to concentration, while gen-T shared a parallel relationship to concentration. Thus all four of these components indicated less favorable growth conditions as the iron and sulfur compound levels increased. Not only does increasing concentration result in these changes in population growth but its effects are also noticeable in the increasingly irregular growth

Table 1. r_m, popK, G-50, gen T and Nmax Values for Different Populations

TEST SUBS	*CONC. (in ppm)*	r_m	*popK*	*G-50*	*gen T*	*Nmax*
$FeNO_3$	350	4.79	12,536.74	12.0	0.1445	11,677
$FeNO_3$	700	4.60	8,910.82	14.0	0.1506	8,520
$FeNO_3$	1400	2.26	1,200.47	2.0	0.3067	1,560
$FeCl_2$	350	2.94	4,497.41	6.0	0.2357	4,750
$FeCl_2$	700	9.50	18,150.63	16.0	0.0729	7,992
$FeCl_2$	1400	1.81	759.48	6.0	0.3829	548
$FeSO_4$	350	5.28	12,344.25	2.0	0.1312	17,119
$FeSO_4$	700	3.34	8,735.82	2.0	0.2074	13,671
$FeSO_4$	1400	1.87	1,438.47	16.0	0.3706	1,744
$AlSO_4$	2012	5.59	8,196.83	6.0	0.1239	6,408
$AlSO_4$	4024	5.18	4,836.90	12.0	0.1334	5,483
$AlSO_4$	8048	2.94	3,664.42	2.0	0.2357	3,345
$CaSO_4$	2012	3.28	5,463.39	0.5	0.2113	5,668
$CaSO_4$	4024	4.32	5,477.39	8.0	0.1604	4,662
$CaSO_4$	8048	2.20	328.99	0.5	0.3150	293
K_2SO_4	2012	3.42	1,732.46	2.0	0.2026	1,121
K_2SO_4	4024	3.29	258.99	0.5	0.2106	197
K_2SO_4	8048	4.41	332.49	0.5	0.1571	247
Control		3.05	8,441.83	0.5	0.2269	12,288

curves and in their deviation from the normal sigmoidal curve. When the observations concerning the aggregate cell arrangement are considered with increases in the concentrations of the test substances, they show increasingly unfavorable growth conditions until at a given point they show conditions lethal to the test organism.

Not only do the growth curves for the iron-containing cultures more closely approximate those of the control cultures, but in many cases, several of the growth parameters (for the iron cultures) indicated considerably more favorable conditions than those of the control cultures. The values for popK in particular are particularly indicative of the importance of iron as a nutrient (Figure 3). In at least two instances (350 ppm and 700 ppm $FeSO_4$) the maximum populations attained were appreciably higher than the maximum populations for the control cultures (Table 1). These observations would indicate that the presence or absence of iron in the nutrient medium has a great influence on the growth of the population.

Iron and perhaps sulfur, it would appear, have a much more influential effect on growth potential than would ordinarily be expected for a micronutrient. It has been proposed, in fact, that iron can be considered as much a macro- as a micronutrient (Goldman, 1965). If this assumption is true (that iron is indeed a macronutrient) then the influence of AMW on the eutrophication of certain waters could be considerable. One study of extreme marl water indicated that "Iron alone frequently results in large stimulation of (algal) growth. . ." (Wetzel, 1965). Iron is, of course, an essential element of algal and metaphyte growth, since it serves as a co-factor for many essential enzymes, both pigment related (thus perhaps explaining the darker coloration noted for the cells in the iron-containing cultures) and in the cytochrome system, and although iron is not a part of the chlorophyll molecule it is necessary for the production of chlorophyll. In the last ten years, the response of algal photosynthesis to iron addition has been widely studied, in the Sargasso Sea (Menzel & Ryther, 1961), in a Michigan Lake (Schelski, 1962), in Lake Tahoe (Goldman, 1964) and in two Indiana lakes (Wetzel, 1965) and the hypothesis that iron is indeed a macronutrient is rapidly gaining acceptance, and our study supports this hypothesis.

Literature Cited

1. Andrewartha, H. G., and L. C. Birch. 1954. The distribution and abundance of animals. Univ. of Chicago Press, Chicago.
2. Ford, Richard F., and William E. Hazen. 1972. Readings in aquatic ecology. W. B. Saunders Co., Philadelphia.
3. Goldman, Charles R. 1969. Primary productivity in aquatic environments. Univ. of Calif. Press, Berkeley.
4. ———. 1965. Micronutrient limiting factors and their detection in natural phytoplankton populations. Pp 121-36 in C. R. Goldman (ed.) Primary productivity in aquatic environments. Mem. 1st. Ital. Idrobiol., 18 suppl. Univ. of California Press, Berkeley.
5. Hale, L. J. 1958. Biological laboratory data. Methuen and Co. Ltd., London.
6. Hopkins, E. F., and F. B. Wann. 1926. Relation of hydrogen-ion concentration to the growth of *Chlorella* and to the availability of iron. *Botanical Gazette* 81:353-76.
7. Karlander, Edward P., and Robert W. Krauss. 1966. Responses of heterotrophic cultures of *Chlorella vulgaris* Beijerinck to darkness and light. II. Action spectrum for and mechanism of the light requirement for heterotrophic growth. *Plant Physio.* 41(1):7-14.
8. Keller, E. C., Jr., C. S. Nagle, H. E. Keller, and D. C. Maxwell. 1967. The effects of saline-thermal-bacterial interactions on populations of primary producers. *Penna. Acad. Sci.* 41:63-72.
9. Krebs, Charles J. 1972. Ecology: the experimental analysis of distribution and abundance. Harper and Row Publ., New York.
10. Odum, E. P. 1959. Fundamentals of ecology. Second edition. W. B. Saunders Co., Philadelphia.
11. Prescott, G. W. 1954. The freshwater algae. Wm. C. Brown Corp., Dubuque, Iowa.
12. ———. 1968. The algae: a review. Houghton-Mifflin Co., Boston.
13. Rosowski, James R., and Bruce C. Parker. 1971. Selected papers in phycology. Dept. of Botany, Univ. of Nebr., Lincoln, Nebraska.
14. Shoupp, W. J. 1972. The interaction of thermal and acid mine water loadings on the growth of *Chlorella.* M.S. Thesis, West Virginia Univ. Library, Morgantown, W. Va.
15. Skinner, W. F. 1972. The interaction of sewage, thermal and acid mine water loading on the growth of *Chlorella.* M.S. Thesis, West Virginia Univ. Library, Morgantown, W. Va.
16. Wann, F. B., and E. F. Hopkins. 1927. Further studies on the growth of *Chlorella* and to the availability of iron. *Botanical Gazette* 83:194-201.
17. Wetzel, Robert G. Nutritional aspects of algal productivity in marl lakes with particular reference to enrichment bioassays and their interpretation. Pp. 137-57 in C. R. Goldman (ed.) Primary productivity in aquatic environments. Mem. 1st. Ital. Idrobiol., 18 suppl. Univ. of Calif. Press, Berkeley.

Some Observations on Channel Catfish Populations in the Ohio River Bordering West Virginia

Roger Schoumacher
West Virginia Department of Natural Resources
Elkins, West Virginia 26241

Abstract

Fish population sampling was carried on from 1966 to 1969 to determine the feasibility of establishing a commercial fishery in the Ohio River. A total of 24,028 fish, including 19,451 channel catfish, were taken with oak catfish traps and hoop nets. Numerous other fish were taken with other nets and toxicants. Channel catfish growth and condition factors were judged average when compared to other areas. Populations of this species suitable to support a commercial fishery were found in much of the Ohio.

Introduction

The Ohio River forms the western boundary of West Virginia for 277 miles and its 51,000 surface acres comprise 37 percent of the states total water area. The Eden-like atmosphere that Audubon (1836) described in the journal of his trip down the Ohio in 1808 was soon to be replaced by the dams, barges, smokestacks, buildings, and highways that characterize one of the greatest metropolitan and industrial complexes the world has ever seen—the Ohio River Valley. Changes in channel alignment and gross pollution in the late 19th and early 20th centuries drastically reduced the populations of many desirable species of fish. In more recent years, however, water quality has been improving and fish populations have responded.[1]

In 1966 a study was initiated to determine the feasibility of establishing a commercial fishery in the Ohio River bordering West Virginia. Previous investigations had indicated that channel catfish, *Ictalurus punctatus* (Rafinesque), were potentially the most valuable commercial fishery resource in the river; therefore, most of the sampling effort was directed towards that species, and this paper reports some aspects of that phase of the work.

Materials and Methods

Most netting activities utilized nylon hoop nets constructed of either ¾-inch or 1½-inch bar mesh. These nets were fished by staking them in the river bed utilizing a 4-foot piece of reinforcing rod and about 80 feet of No. 120 nylon line for a tailrope. They were baited with either cheese or soybean cake depending upon water conditions. The cheese generally works best in cooler water and the soybean at warmer temperatures. The nets were usually set near the shore in 4 to 15 feet of water and checked every two or three days.

Oak catfish traps were also used. These are one foot square and five feet long

[1] The most complete summary of fishery investigations in the Ohio River is contained in *Aquatic Life Resources of the Ohio River,* prepared in 1962 by the Ohio River Valley Water Sanitation Commission, 414 Walnut Street, Cincinnati, Ohio.

and constructed of white oak slats. They have two flexible throats, also constructed of oak, which allow the fish to enter but make escape difficult for all but very small fish. These traps may be baited and fished in the same manner as hoop nets, but are especially useful for taking spawning catfish. During the reproductive season, which in the West Virginia portion of the Ohio River runs from about mid-May to the first of July, channel catfish can be captured by attracting them into the traps with a live, sexually mature catfish of either sex as the attractant. In order to catch the initial fish to be used as the attractant a number of traps are set "blind"—empty—in likely looking spawning areas. Catfish, in their quest for a suitable spawning site, will enter one or more of the traps. Once this happens other catfish are attracted and they, in turn, can be used to bait the other traps. Catch rates are often low for these blind sets.

Traps nets (1½-inch bar mesh nylon with 50-foot leads) were also fished on a very limited basis.

Lock chambers on the Ohio River were sampled with rotenone in cooperation with the Federal Water Pollution Control Administration (now the EPA), the Bureau of Sport Fisheries and Wildlife, and the State of Ohio.

Fish were measured to the nearest 1/10 inch (total length) and weighed to the nearest 1/100 pound. Age and growth studies utilized cross sections of pectoral spines (Sneed, 1950). No back calculations were made. Condition factors were computed with the aid of conversion tables in Carlander (1953).

Results and Discussion

Netting Effort and Success

Netting activities were carried out at 13 areas on the Ohio River (Figure 1). Table 1 summarizes the netting effort and success during the 3 year period. A total of 24,028 fish, including 19,451 channel catfish, were taken with oak catfish traps and nylon hoop nets.

Oak catfish traps were the only gear available in 1966. They were fished 355 net days that year—210 net days for spawners and 145 net days in early September with bait. A total of 581 fish were caught at the rate of 1.64 fish per net day. This low catch rate was due to a considerable length of time fishing with blind traps, continuation of fishing for spawners past the spawning season, and, in September, to large numbers of small catfish entering the traps and eating all the bait. The length-frequency of fish taken at this time indicates that many fish under 6 inches were taken. Most fish this size escape from traps, which are designed to hold larger fish.

In 1967, 106 net days of fishing with catfish traps yielded 214 fish at the rate of 2.02 per net day. This catch rate also includes fishing blind traps to catch bait fish. Fishing with traps during 1966 and '67 was confined to the Pt. Pleasant and Gallipolis areas.

Extensive hoop netting was carried out in 1967 and 1968. In 1967, ¾-inch mesh hoop nets were fished for 355 net days, taking 12,166 channel catfish, 1,321 carp, 647 bullheads, and one flathead catfish. Channel catfish were caught at the rate of 36.31 fish per net day. In 1968, 111 net days yielded 5,618 channel catfish, 1,183 carp, and 86 bullheads. Channel catfish were caught at the rate of 50.61 fish per net day. Catch rates ranged from a low of 0.14 per day at Chester to 355 per day at Gallipolis.

In 1967, 1½-inch mesh hoop nets were fished for 240 net days, yielding 825 channel catfish, 496 carp, 371 bullheads, and four flathead catfish. Channel

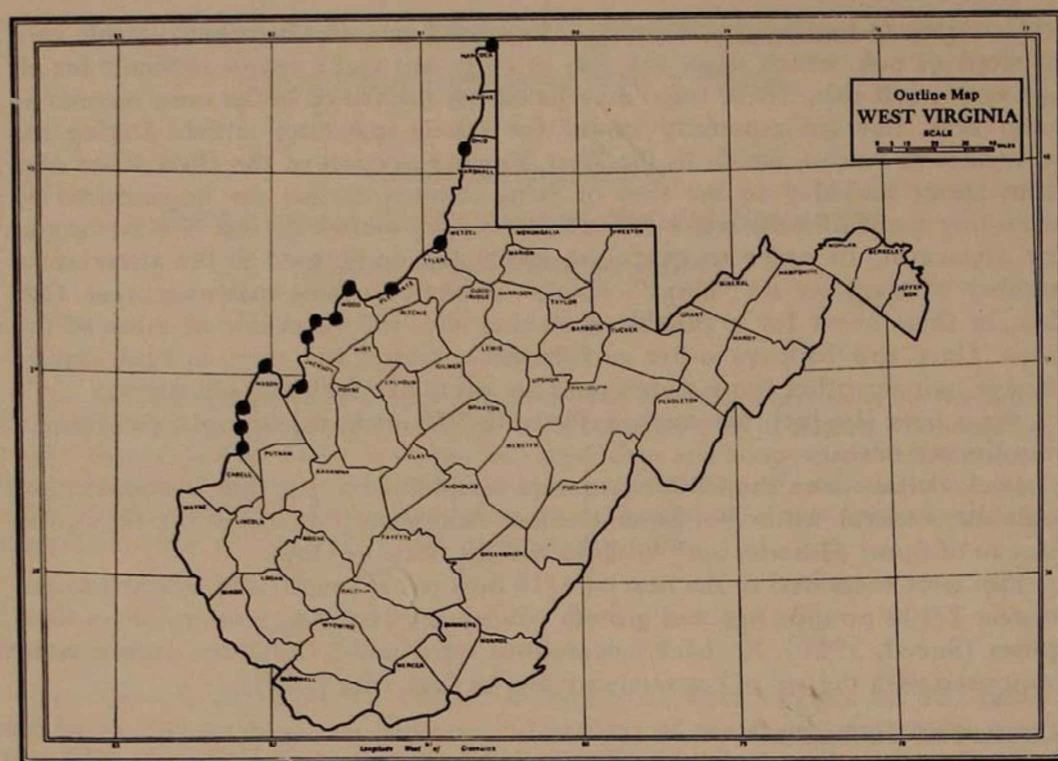


FIGURE 1. Location of test netting stations on the Ohio River.

catfish were caught at the rate of 3.43 per net day. In 1968, eight net days resulted in a catch of 47 channel catfish and 468 carp. Catfish were taken at the rate of 5.87 per net day.

Trap nets were fished for 13 net days in May, 1967, in flooded fields adjacent to Old Town Creek and the Ohio River near Pt. Pleasant. A total of 932 fish weighing 1,362.12 pounds was taken at the rate of 71.7 fish per net day (Table 2) and included 66 channel catfish weighing 82.55 pounds.

Some additional netting was conducted in the vicinity of Marietta, Ohio, which is not included here. This was done to collect fish for pesticide analysis in cooperation with the Bureau of Sport Fisheries and Wildlife and Ohio State University, and indicated an excellent population of channel catfish.

Length-frequency of Fish

Table 3 summarizes the length composition of channel catfish captured. Each of the three types of gear—oak catfish traps, ¾-inch mesh hoop nets, and 1½-inch mesh hoop nets—is selective for certain sizes of fish. The oak traps, when baited with a mature fish and set to catch spawners, are selective for sexually mature fish. When cheese or soybean bait is used, however, these traps will attract all sizes of catfish. Very small fish, however, can escape rather easily. Large numbers of small catfish were eating the bait from the traps during the September sampling and although many were captured the vast majority escaped. The ¾-inch mesh nets allow some escapement of fish in the 6-inch size group. These nets do catch large numbers of fish of all sizes from 6 inches up, and probably give the best picture of the size composition of the catfish population—at least that portion over six inches. The 1½-inch mesh nets allow escape-

Table 1. Summary of Fishing Effort and Success During 1966, 1967, and 1968 in the Ohio River Using Oak Catfish Traps and ¾-inch and 1½-inch Mesh Nylon Hoop Nets

Type of Gear	*Year*	*No. Net Days*	*Number of various species of fish caught*					*Ch. cat per net day*	*Total fish per net day*
			Channel catfish	*Carp*	*Bullheads*	*Flatheads*	*Total*		
Oak Traps	1966	355	581				581	1.64	1.64
	1967	106	214				214	2.02	2.02
Sub-total		461	795				795	1.72	1.72
¾-inch hoop nets	1967	335	12,166	1,321	647	1	14,135	36.31	42.19
	1968	111	5,618	1,183	86		6,887	50.61	62.04
Sub-total		446	17,784	2,504	733	1	21,022	39.87	47.13
1½-inch hoop nets	1967	240	825	496	371	4	1,696	3.43	7.06
	1968	8	47	468			515	5.87	64.38
Sub-total		248	872	964	371	4	2,211	3.51	8.91
Grand Total		1,155	19,451	3,468	1,104	5	24,028	16.84	20.80

Table 2. Results of 13 Net Days of Trap Netting in the Backwaters of Old Town Creek Near Pt. Pleasant, West Virginia, May 8-11, 1967

Species	*Number Taken*	*Weight taken (pounds)*
Carp	549	1118.00
Channel Catfish	66	82.55
Black Bullhead	10	5.99
Yellow Bullhead	2	1.50
Spotted Sucker	67	67.00
Carpsuckers	17	24.00
Buffalo	14	14.40
Bluegill	30	13.00
Black Crappie	50	39.99
White Crappie	107	75.02
Warmouth	6	3.00
Largemouth Bass	14	17.67
Total	932	1462.12

71.7 fish/day

Table 3. Length Frequency of Channel Catfish Taken From the Ohio River by Various Types of Gear, 1966-1968

Length Group in inches	*Oak Traps*		*¾" mesh*		*1½" mesh*		*Totals*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
4	56	7					56	Tr.
5	85	11	150	1			235	1
6	39	5	2180	14			2219	13
7	18	2	3880	26	1	Tr.	3899	23
8	11	1	2423	16	2	Tr.	2436	15
9	10	1	1616	11	1	Tr.	1627	10
10	21	3	1386	9	3	Tr.	1410	8
11	32	4	1003	7	7	1	1042	6
12	50	6	853	6	45	5	948	6
13	97	12	445	3	208	24	750	4
14	140	18	360	2	231	26	731	4
15	102	13	275	2	174	20	551	3
16	55	7	179	1	106	12	340	2
17	38	5	128	1	58	7	224	1
18	20	2	85	1	10	1	115	1
19	12	1	69	Tr.	18	2	99	1
20	4	Tr.	3	Tr.	13	1	20	Tr.
21	2	Tr.	8	Tr.	4	Tr.	14	Tr.
22	1	Tr.	11	Tr.	2	Tr.	14	Tr.
23	1	Tr.			1	Tr.	2	Tr.
24								
25	1	Tr.					1	Tr.
Total No. Fish	795		15,055		884		16,734	

Table 4. Condition Factors for Channel Catfish Collected From Various Locations on the Ohio River in 1967

	Location and dates							
Length group	*Pt. Pleasant 4/19-5/3*	*Pt. Pleasant 5/9-11*	*Pomeroy 5/2-4*	*Pomeroy 8/14*	*Ravenswood 7/14-18*	*Parkersburg 7/16-19*	*Marietta 6/1*	*Marietta 9/21*
6			2.46					
7			2.37	2.29	2.43			
8			2.53	2.30	2.44	2.93		
9			2.60	2.21	2.45			2.63
10			2.80	2.32	2.54	2.91		2.72
11		3.62	2.96	2.47	2.40			2.71
12	3.29		3.28		2.50	2.82		2.85
13	3.11	3.27	3.17	2.68	2.62	2.93	3.05	2.83
14	3.22	3.24	3.19	2.54	2.54	2.97	3.03	2.98
15	3.28	3.52	3.32	2.38	2.50	2.99	2.79	3.05
16	3.41	3.62		2.53	2.59		3.07	2.81
17	3.51		3.54	3.02	2.60	2.67	2.79	
18								
19	3.39							
20				3.09				
Total no. of fish*	147	53	201	85	130	64	60	65

*At least 5 individuals in each 1-inch size class.

Table 5. Observed Total Lengths of Channel Catfish of Various Ages From the Ohio River

No. annuli on spine	*Location and date*							
	Parkersburg 7/14-19/67	*Ravenswood 7/14-18/67*	*Pomeroy 8/14/67*	*Marietta 6/1/67*	*Marietta 9/21/67*	*Pt. Pleasant 4/14-19/67*	*Pt. Pleasant June 1966*	*Gallipolis 7/7/67*
III	8.4	7.8	7.6		11.1	6.7		
IV	11.0	10.7	10.1		13.6	9.6		10.8
V	12.9	13.1	13.6	14.9	14.6	11.5	13.6	13.0
VI	14.6	14.9	15.6	16.0		12.9	14.7	15.2
VII		15.3					16.1	
VIII							16.9	
Total No. of fish*	78	127	74	50	56	145	197	41

*At least 10 individuals in each age class.

Table 6. Per Cent of Channel Catfish in Various Age Classes. Fish Taken From Ohio River in ¾-inch Mesh Nets

	Location and date				
No. annuli on spine	*Gallipolis 7-7-67*	*Ravenswood 7-14, 18-67*	*Parkersburg 7-14, 19-67*	*Pomeroy 8-14-67*	*Pt. Plt. 4-14, 19-67*
II	13	5	14		
III	45	41	52	51	2
IV	21	19	14	31	59
V	8	22	12	13	24
VI	4	7	6	4	10
VII	5	5	1	1	4
VIII	2	Tr.	1		Tr.
IX	Tr.	1			Tr.
X		Tr.			Tr.
No. of fish	2579	3251	310	1537	3141

Table 7. Per Cent of Channel Catfish in Various Age Classes. Fish Taken From Ohio River in 1½-inch Mesh Nets

	Location and date			
No. annuli on spine	*Pt. Pleasant 4/14-19/67*	*Pomeroy 8/14/67*	*Marietta 6/1/67*	*Pt. Pleasant 4/27 to 5/11/67*
III				Tr.
IV	2	2	13	4
V	20	30	43	23
VI	38	44	34	40
VII	36	14	9	26
VIII	3	8		4
IX	1	1		2
X		1		Tr.
No. of fish	146	92	68	154

ment of some fish in the 13-inch size group. These nets are the type that would probably be used in a commercial fishery with a size limit of about 13 inches.

The majority of fish taken in the ¾-inch nets, then, were 6 to 12 inches long, whereas those in the 1½-inch nets were 13 to 17 inches. Spawners taken in traps were generally 13 to 18 inches in length.

Condition factors

Condition factors for channel catfish were judged "average" when compared to figures for other parts of the country (Carlander, 1953) (Table 4). There were the expected variations with size (maturity) of fish and season, as mature fish were heavier (higher "C" factor) just prior to spawning.

Table 8. Per Cent of Channel Catfish in Various Age Classes. Fish From the Wapsipinicon and Mississippi Rivers in Iowa (Schoumacher and Ackerman, 1967).

No. annuli on spine	*Percent of Fish*	
	Wapsipinicon R.	*Mississippi R.*
III		49
IV		45
V	23	
VI	17	
VII	25	
VIII	15	
IX	11	
X	6	
XI	2	
No. of fish	320	9415

Age and growth

Table 5 gives the average total lengths for fish of various ages from different parts of the river. The fish are growing at a rate that can be described as "average" when compared to growth elsewhere (Carlander, 1953; Schoumacher and Ackerman, 1967).

Age composition of channel catfish catch

Tables 6 and 7 give the age class composition of the catfish catch in ¾ and 1½-inch mesh hoop nets. The ¾-inch nets sample all ages from III up adequately; however, many age group II fish can escape. The 1½-inch nets probably don't sample any age class below VI without allowing significant escapement. Larger age V fish would be held, but smaller ones would not. Fish in age classes III to V dominate the catch in the ¾-inch mesh nets, whereas age classes V to VII dominate the catch in the 1½-inch mesh nets. Very few fish older than VII were taken.

Table 8 gives the age class composition for a catch from a commercially unexploited catfish population form the Wapsipinicon River in Iowa using 1½-inch mesh nets, and for the commercial catch from the Iowa portion of the Mississippi River where 1½-inch mesh nets are also used (Schoumacher and Ackerman, 1967). The Ohio and Wapsipinicon River data are somewhat comparable whereas the commercial fishing in the Mississippi River is obviously cropping off fish shortly after they reach the 13-inch size limit at age III and IV.

Lock chamber rotenone studies

Four lock chambers on the Ohio River bordering West Virginia were sampled with rotenone in 1967 in cooperation with the FWPCA. These studies yielded the following information of concern to this paper;

(1) The standing crop of fish in the West Virginia portion of the Ohio River generally increases as one moves downstream.
(2) The channel catfish population increases as one moves downstream.

Evaluation of the Feasibility of Establishing a Commercial Fishery

Netting data indicated good to excellent channel catfish populations in the vicinities of Pt. Pleasant, Ravenswood, Belleville, Washington, Parkersburg, and New Martinsville, West Virginia, and Pomeroy, Marietta, and Gallipolis, Ohio, good populations at Wheeling, West Virginia, and very low populations in the vicinity of Chester, West Virginia. Channel catfish populations in the river generally appear sufficient to sustain a commercial fishery.

Acknowledgments

This study was funded under the Commercial Fisheries Research and Development Act, Project 3-58-R, Investigations of the Commercial Potential of Fishery Resources in West Virginia (Schoumacher, 1969).

Literature Cited

1. Carlander, Kenneth D. 1953. Handbook of freshwater fishery biology with first supplement. Wm. C. Brown Company, Dubuque, Iowa, 429 pp.
2. Schoumacher, Roger. 1969. Investigations of the commercial potential of fishery resources in West Virginia. Completion report. W. Va. Dept. Natural Resources. Proj. 3-58-R. 51 pp., mimeo.
3. ———, and Gary Ackerman. 1967. Comparative age and growth of channel catfish from some eastern Iowa rivers. *Proceedings of the Iowa Academy of Science for 1965* 72:248-53.
4. Sneed, Kermit E. 1950. A method for calculating the growth of channel catfish. *Trans. Amer. Fish. Soc.* 80:174.

Water Quality, Nutrients, and Net Plankton Analysis of Summersville Lake

Bruce D. Lorenz
Department of Biology, West Virginia University
Morgantown, West Virginia 26506

Abstract

Analysis of chemical parameters and the net plankton community in Summersville Lake, West Virginia, were conducted during a year long limnological investigation. Primarily a flood control basin, the reservoir is subjected to extreme annual fluctuations in water level. Sampling was conducted on a semimonthly basis at a point near the dam. Samples for chemical analysis were taken at each five meter interval while those used for net plankton analysis were taken at each of the first fifteen meters and again at twenty meters. Two twenty meter vertical tows were taken on each date using a No. 25 Wisconsin net. These tows were used in the determination of net plankton volumes.

Chemical analysis involved the determination of nitrate and molybdate reactive orthophosphate. Net plankton samples were counted and identified to the species level.

The mean concentration of nitrate in the top twenty meters during the stratification period was 2.1 mg/1. The mean concentration of molybdate reactive orthophosphate in the top twenty meters during the same period was 8.5 ug/l. The net plankton community consisted primarily of copepods, cladocerans, rotifers, and non-filamentous green algae with copepods constituting the major portion. Seasonal alternation was apparent between the cyclopoid copepods *Cyclops bicuspidatus thomasi* and *Mesocyclops edax*, the latter becoming common during early summer. Seasonal succession of four species of *Daphnia* was also observed during the investigation. Flood conditions resulting from Hurricane Agnes exerted an obvious effect on all biological and chemical parameters investigated.

On the basis of nutrient concentrations and species composition, Summersville Lake appears to be mesotrophic.

This paper is the initial description of the net plankton population and levels of nitrate and molybdate reactive orthophosphate in Summersville Lake. Previously, only investigations concerning fish stock and physical parameters have been conducted (Pierce, 1970). With the ever increasing utilization of reservoirs, the effect of impoundment is an important consideration. Rodhe (1964) found increased concentrations of total nitrogen and phosphate following impoundment. Zooplankton volumes were also found to increase and reach levels never before recorded. The biological and chemical parameters are dependent upon the nature and course of the inflow and outflow. Wunderlich (1971) found the dynamics of stratified reservoirs to be such that inflowing waters could short circuit the system. Nutrient rich waters of a lower temperature may travel through the hypolimnion and be withdrawn from that level without ever entering the euphotic zone. Taylor *et al.* (1971) emphasized the importance of flushing in the examination of productive storage reservoirs and less productive main stream reservoirs of the Tennessee Valley. The fact that reservoirs have characteristics of both lakes and rivers becomes an important consideration in the evaluation of chemical and biological parameters.

The objectives of this study are to determine the concentrations and seasonal variation in the levels of nitrate and molybdate reactive orthophosphate, to determine the species composition and seasonal abundance of net plankton populations, and to determine the trophic status of Summersville Lake.

Description of Summersville Lake

Summersville Lake is located in central West Virginia. It was formed by damming the Gauley River, the only major inflow. Closure of the dam was completed and storage began in May of 1965. The primary function of the Lake is one of flood control. Reservoir maintenance involves dropping the water level one hundred thirty feet to obtain winter pool. This is begun in the fall and is completed by December. The water level is then raised in the spring and full pool is maintained throughout the summer. The lake is characterized by its steep sides and rapid fluctuation in water level. Depths in excess of fifty meters were often encountered during sampling.

Methods

Sampling of Summersville Lake was conducted on a semimonthly basis for a period of one year. Samples were collected at a station opposite the dam. Samples for chemical analysis were taken at each five meter vertical interval using an eight liter PVC VanDorn sampler. Samples for net plankton analysis were taken at each of the first fifteen meters and again at twenty meters using the same sampler. Each sample was then concentrated by passing it through a No. 25 Wisconsin net. All plankton samples were preserved with 10% formalin. On each date, two twenty meter vertical tows were taken using the No. 25 Wisconsin net. These vertical tows were used in the determination of net plankton volumes below a square meter for a twenty meter column.

Nitrate analysis were conducted using the phenoldisulfonic acid method outlined in Standard Methods (1971). Molybdate reactive orthophosphate was determined on filtered samples following the procedure of Golterman (1969). Samples for net plankton analysis were counted and identified in their entirety except in cases of extreme numbers of organisms. When this occurred, samples were diluted to a known volume and three replicate sub-samples were counted using the Sedgwick-Rafter counting cell. Plankton volumes of each vertical tow were determined using the mercury immersion method of Yentsch and Hebard (1957). A conversion factor was used to obtain square meter values for the twenty meter column.

Results

Figure 1 shows the mean concentration of nitrate in the top twenty meters during the period of stratification (May 13, 1972–September 30, 1972). The greatest concentration occurred on June 24, 1972. This was during the period in which Hurricane Agnes caused heavy rains resulting in flood conditions. The mean nitrate concentration in the top twenty meters during stratification was 2.1 mg/l.

Figure 2 shows the mean concentration of molybdate reactive orthophosphate in the top twenty meters during stratification, 1972. A slight rise in the concentration of molybdate reactive orthophosphate was noted during the flood. The greatest concentrations of phosphate occurred in late July and September. The July peak preceeded the largest net plankton volume of August 8, 1972. The mean concentration of molybdate reactive orthophosphate in the top

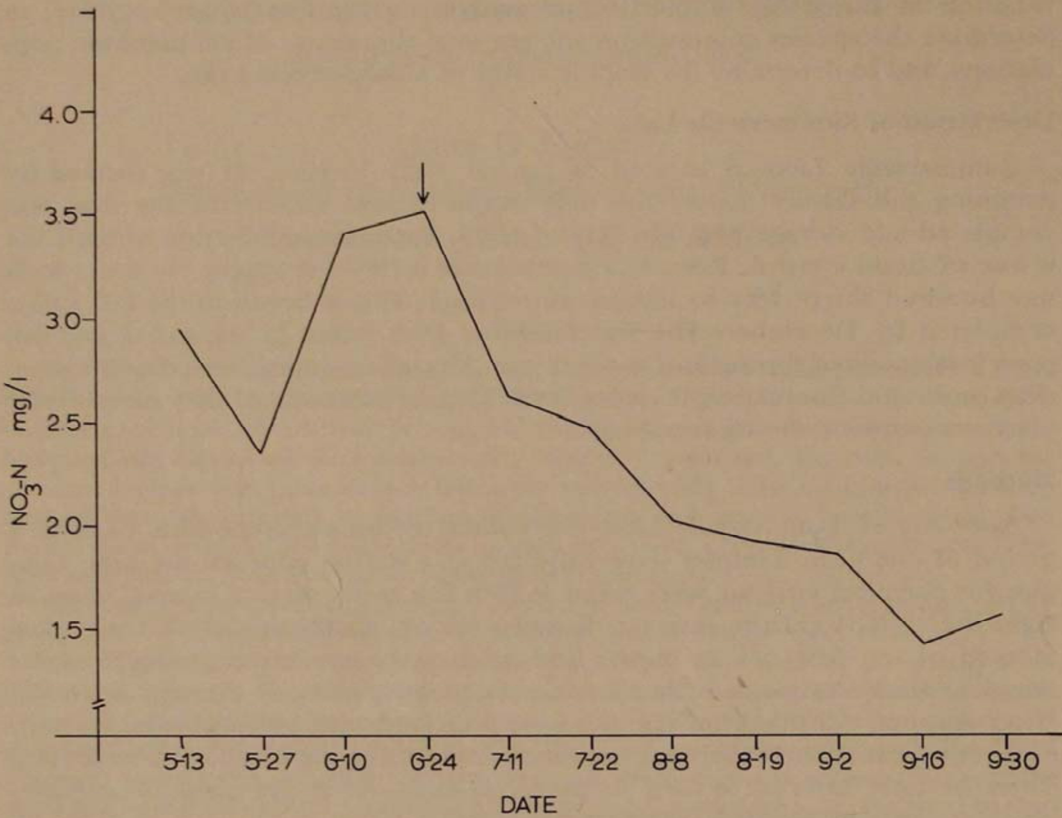


FIGURE 1. Mean values of NO_3-N for the top twenty meters during the stratification period, 1972, Summersville Lake, Station 1. Arrow indicates first sample after onset of Hurricane Agnes.

twenty meters was 8.5 ug/l during the period of stratification. Depletion of nitrate or phosphate never occurred.

Table 1 lists the net plankton species which were found during the investigation. *Mesocyclops edax* was the most abundant cyclopoid copepod and was present throughout the period of stratification. *Cyclops bicuspidatus thomasi* exhibited a seasonal alternation with *M. edax* as shown in Figure 3. Although *C. b. thomasi* replaces *M. edax* during the cold months, it was never as abundant as *M. edax.* Only one calanoid, *Diaptomus pallidus,* was present throughout the period of stratification and its population peaked in early July.

Cladocerans were represented by six species, including four species of *Daphnia.* Figure 4 shows the seasonal succession of the four species of *Daphnia.* There was a definite effect of the flood on the population of *D. ambigua* as shown by a decline in the population of this species on June 24, 1972. The distribution of *D. rosea, D. parvula, D. pulex* was very different from that of *D. ambigua. D. rosea* occurred only during October, 1971. *D. pulex* occurred only during November, 1971. *D. parvula* also occurred during November, 1971, but also in late September, 1972. *D. ambigua* was by far the most abundant cladoceran. Its population declined in November and was absent in December, 1971. *D. ambigua* reappeared early in May, 1972, and was represented by large populations throughout the period of stratification, reaching a maximum in late

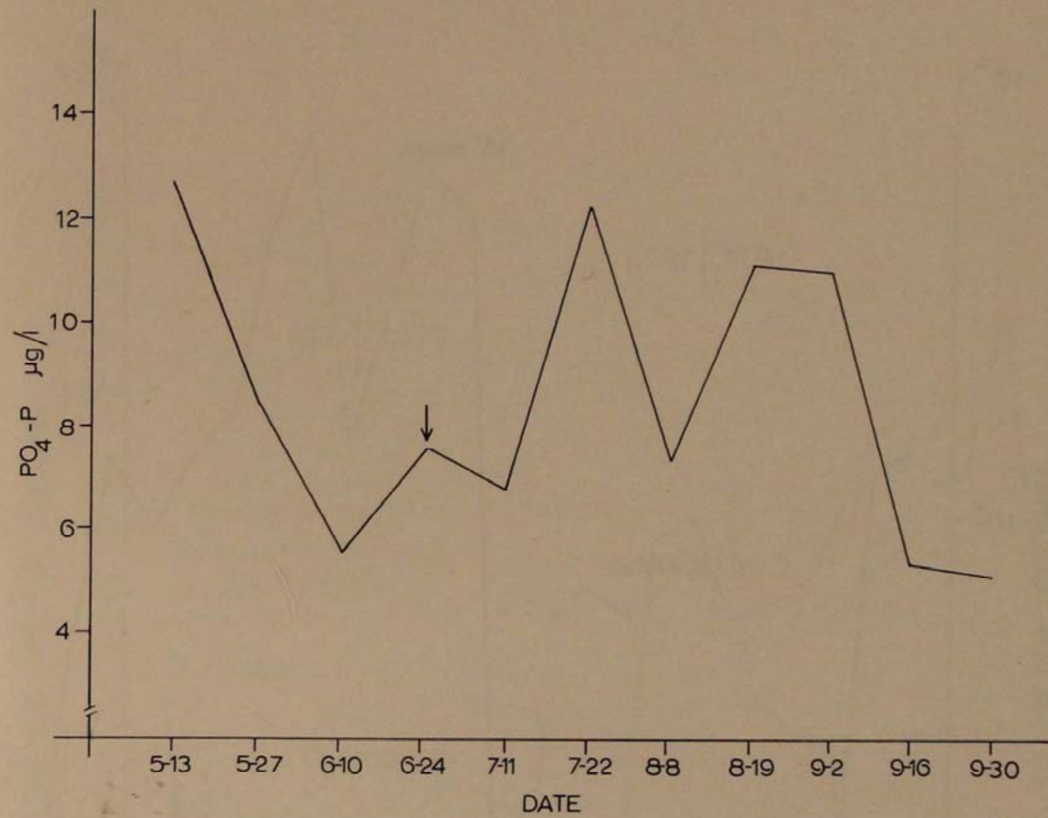


FIGURE 2. Mean values of PO_4-P for the top twenty meters during the stratification period, 1972, Summersville Lake, Station 1. Arrow indicates first sample after onset of Hurricane Agnes.

September. *Bosmina longirostris* was the only other cladoceran present throughout the stratification period. Although always present, this species was never very abundant.

Net phytoplankton consisted mainly of *Dinobryon sertularia, Ceratium hirudinella,* and *Staurastrum rotula. Dinobryon* occurred in great numbers during late July and September. *Ceratium* and *Staurastrum* were present from early July through September, 1972. Each of these species was present in great numbers in early August and September, 1972. A single blue-green, *Chroococcus limneticus,* occurred during late summer, reaching peak abundance in early August.

Figure 5 shows the pattern of net plankton volumes during the period of stratification. These are the volumes beneath a square meter for a twenty meter column. The greatest net plankton volume occurred in early August. This is the period during which *Ceratium* and *Staurastrum* were most abundant. Fraser (in prep.) reports August, 1972, as the time of maximum primary production for Summersville Lake. Of all parameters involved, the net plankton volumes showed the greatest response to the flood. During this period, net plankton volumes showed a decrease to levels which were recorded during the previous December. It was approximately a month before net plankton and *Daphnia*

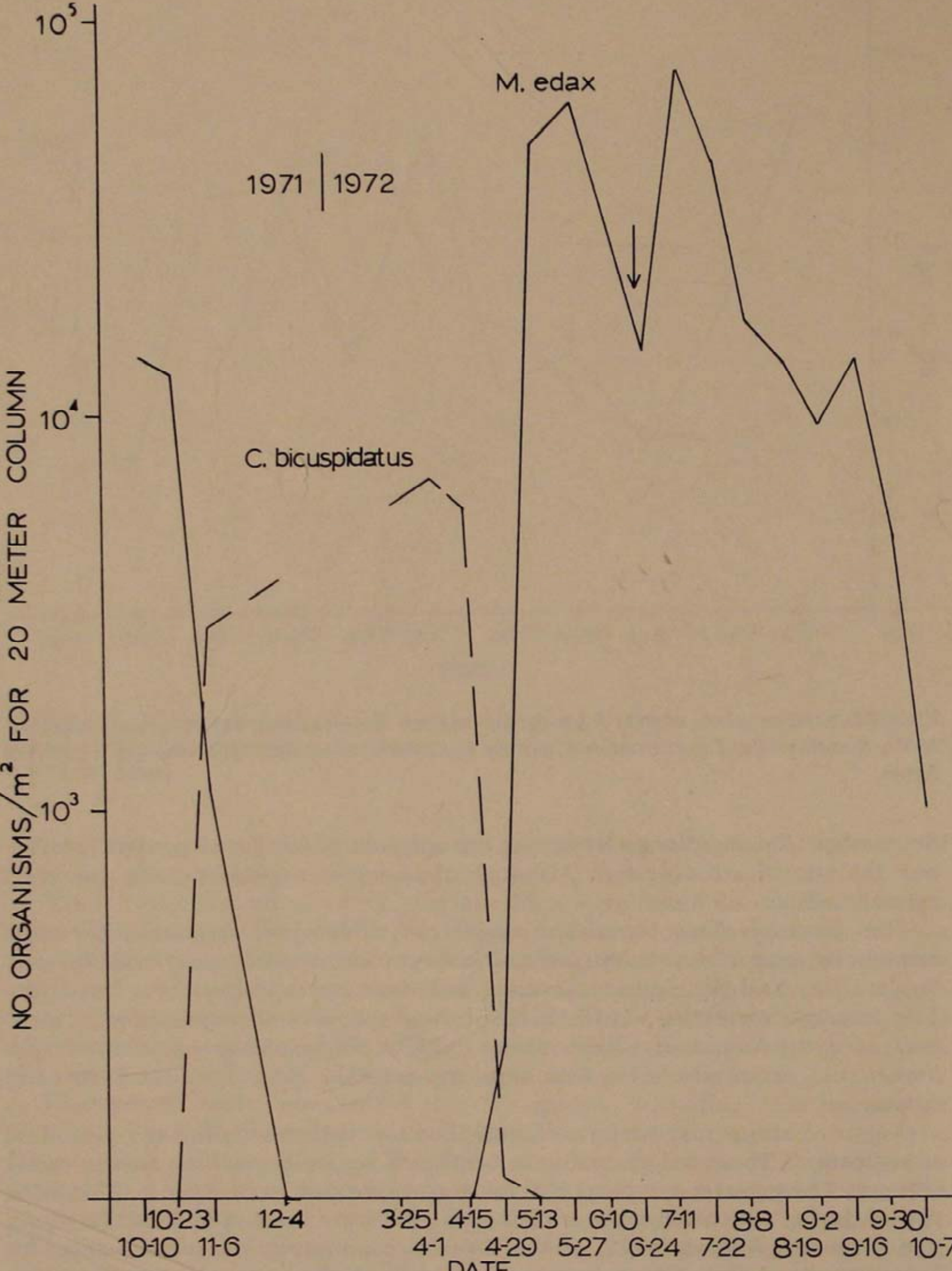


FIGURE 3. Population densities of the dominant cyclopoid copepods throughout the study, Summersville Lake, Station 1. Arrow indicates first sample after onset of Hurricane Agnes.

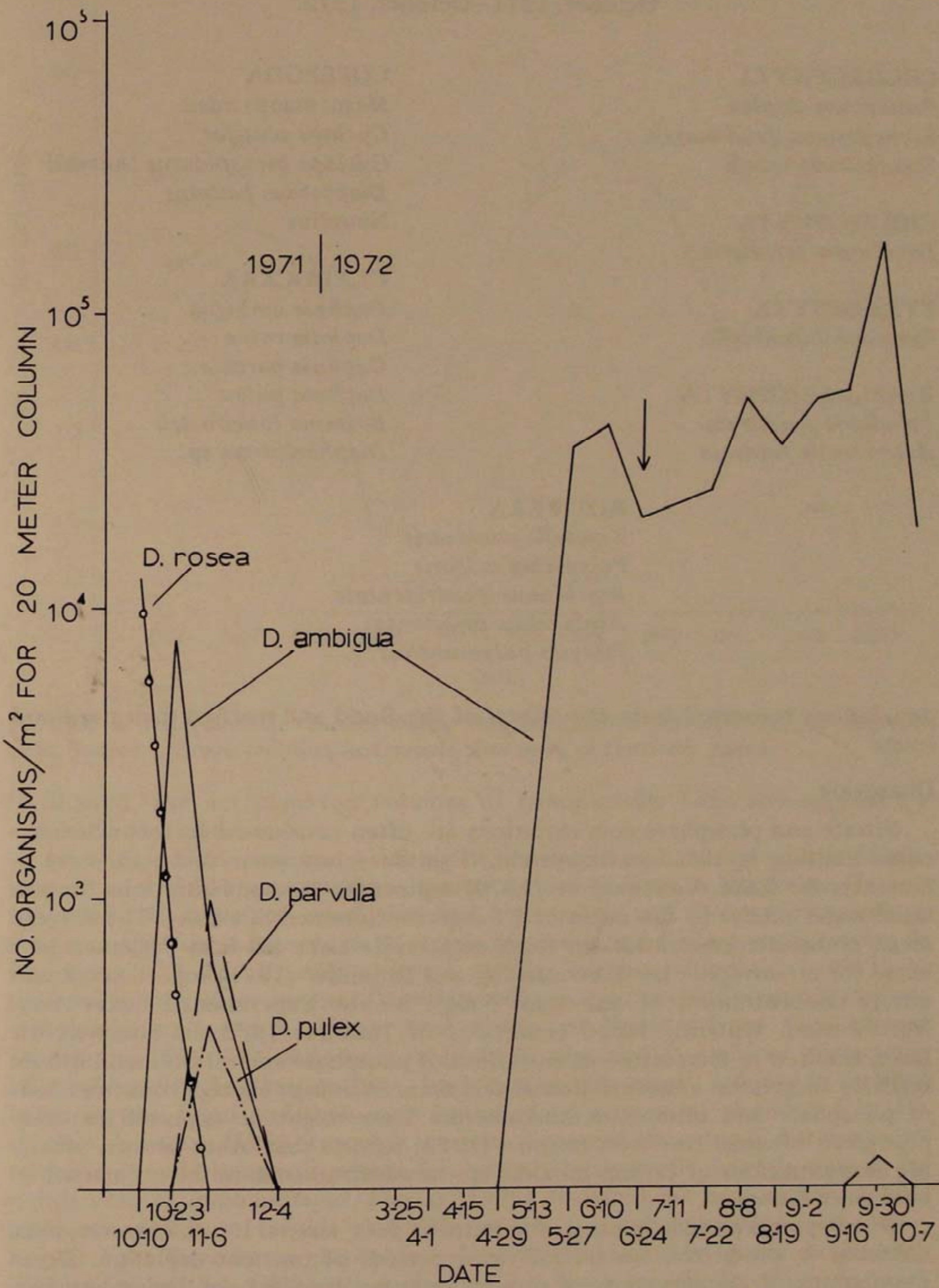


FIGURE 4. Population densities of four species of *Daphnia* throughout the study, Summersville Lake, Station 1. Arrow indicates first sample after onset of Hurricane Agnes.

Table 1. Net Plankton Occurring in Summersville Lake—October, 1971—October, 1972.

CHLOROPHYTA
Pediastrum duplex
Scenedesmus quadricauda
Staurastrum rotula

CHRYSOPHYTA
Dinobryon sertularia

PYRROPHYTA
Ceratium hirudinella

BACILLARIOPHYTA
Tabellaria fenestrata
Asterionella formosa

COPEPODA
Mesocyclops edax
Cyclops scutifer
Cyclops bicuspidatus thomasi
Diaptomus pallidus
Nauplius

CLADOCERA
Daphnia ambigua
Daphnia rosea
Daphnia parvula
Daphnia pulex
Bosmina longirostris
Diaphanasoma sp.

ROTIFERA
Keratella cochlearis
Polyarthra vulgaris
Brachionus quadridentata
Asplanchna priodonta
Platyias polyacanthus

populations recovered from the effects of the flood and reached their pre-flood levels.

Discussion

Nitrate and phosphate concentrations are often considered factors which become limiting in the aquatic system. This does not seem to be the case in Summersville Lake. Vollenweider (1970) reports nitrate concentrations for polluted water similar to the mean of 2.1 mg/l for Summersville Lake. The 8.5 μg/l mean phosphate concentrations for Summersville Lake fall into Vollenweider's range for mesotrophic lakes. Armstrong and Schindler (1971) report maximum nitrate concentrations of less than 2 mg/l for the Experimental Lakes Area, Northwestern Ontario. Flood conditions of June 24, 1972, in Summersville Lake, resulted in increased concentrations of phosphate and nitrate, with nitrate reaching its greatest concentration at this time. Such high average concentrations of phosphate and nitrate in Summersville Lake might be expected to cause blue-green blooms. However, Shapiro (1973) reports that other factors, including concentrations of carbon dioxide, are very instrumental in the occurrence of blue-green blooms.

Dinobryon is unusually present in nutrient poor waters. It has, however, been collected in productive waters following periods of nutrient depletion. Dense populations of *Dinobryon* were present in Summersville Lake during late July and September, 1972. Decreased levels of nitrate and phosphate preceeded each of these dates. Applegate and Mullan (1967) found net plankton populations of younger Beaver Reservoir to show a bimodal distribution while the older Bull Shoals Reservoir showed a unimodal distribution. If the flood and its effects are

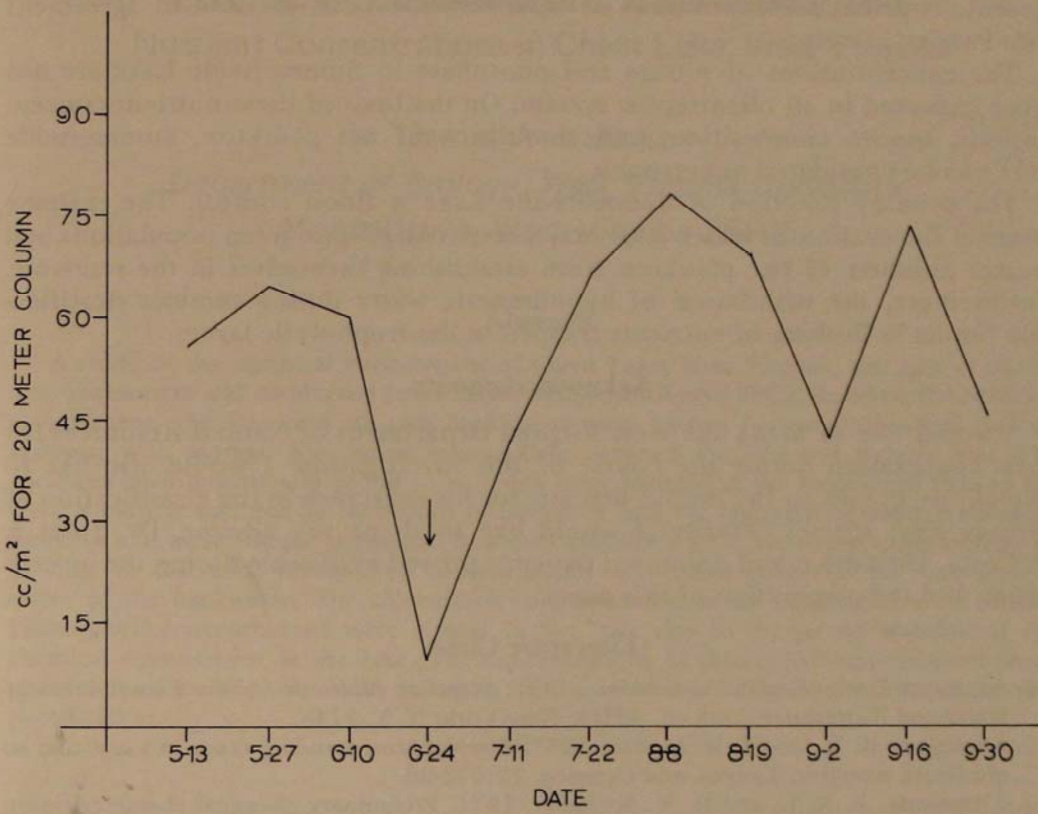


FIGURE 5. Net plankton volumes during the stratification period, 1972, Summersville Lake, Station 1. Arrow indicates first sample after onset of Hurricane Agnes.

considered, the net plankton volumes of Summersville Lake also approach a unimodal distribution.

All net plankton species occurring in Summersville Lake are widely distributed. Patalas (1971) reports the common species of lakes in Northwest Ontario to include *Mesocyclops edax*, *Bosmina longirostris*, and *Cyclops bicuspidatus thomasi*. A seasonal succession of certain copepods and cladocerans is evident in Summersville Lake. *M. edax* shows a definite seasonal alternation with *C. b. thomasi*, a cold water species. *D. ambigua* dominates the cladoceran population, completely replacing the other three species of *Daphnia* from April to mid September, 1972. The abundance of both *M. edax* and *D. ambigua* was greatly affected by flooding in late June. Each species was greatly reduced in number following the flood and then each increased to its greatest abundance in late summer. Yeatman (1956) reported seasonal succession involving *C. b. thomasi* to occur in Woods Reservoir within five years. Summersville Lake is now in its eighth year of impoundment. Patalas (1972) discussed the relationship of zooplankton species to trophic state. He reports a trend developing from oligotrophic Lake Superior to eutrophic Lake Erie. This is shown by the diminishing importance of the calanoid population and the increasing predominance of cyclopoids and cladocerans. Patalas cites *C. b. thomasi*, *M. edax*, and *B. longirostris* as species indicating transition from oligotrophy to eutrophy. Each of these species occurred in Summersville Lake and only one calanoid was present. Nutrient concentrations of Summersville Lake are also in agreement with Patalas' concept.

The concentrations of nitrate and phosphate in Summersville Lake are not those expected in an oligotrophic system. On the basis of these nutrient concentrations, species composition, and abundance of net plankton, Summersville Lake can be considered mesotrophic.

The primary function of Summersville Lake is flood control. The extreme seasonal fluctuation in water level may prevent large blue-green populations and greater numbers of net plankton from establishing themselves in the reservoir. Furthermore, the withdrawal of hypolimnetic water during summer stratification results in flushing of nutrients trapped in the tropholytic layer.

Acknowledgments

I would like to thank the West Virginia Department of Natural Resources for their cooperation during the course of this investigation. I would also like to extend my thanks to Dr. Harold Bennett for his assistance in the classification of various algal species. Finally, I would like to thank my advisor, Dr. John J. DeCosta. His advice and unlimited patience proved invaluable during the investigation and the preparation of this paper.

Literature Cited

1. American Public Health Association. 1971. *Standard Methods for the Examination of Water and Wastewater.* 13th ed. APHA, New York, N. Y. 874p.
2. Applegate, R. L., and J. W. Mullan. 1967. Zooplankton standing crops in a new and an old Ozark reservoir. *Limnol. and Oceanog.* 12:592-99.
3. Armstrong, F. A. J., and D. W. Schindler. 1971. Preliminary chemical characterization of waters in Experimental Lakes Area, Northwestern Ontario. *J. Fish. Res. Bd. Canada.* 28:171-87.
4. Fraser, J. E. in prep. Phytoplankton productivity in Summersville Reservoir. M. A. Thesis West Virginia University, Morgantown.
5. Golterman, H. L. 1969. *Methods for the Chemical Analysis of Fresh Waters, IBP Handbook No. 8.* Blackwell Scientific Publications, Oxford and Edinburgh. 166 p.
6. Patalas, K. 1971. Crustacean plankton communities in forty-five lakes in the Experimental Lakes Area, Northwestern Ontario. *J. Fish. Res. Bd. Canada.* 28:231-44.
7. Patalas, K. 1972. Crustacean plankton and the eutrophication of St. Lawrence Great Lakes. *J. Fish. Res. Bd. Canada.* 29:1451-62.
8. Pierce, B. E. 1970. Reservoir Investigations, Summersville Reservoir Investigations, West Virginia Department of Natural Resources. D-J Report. Project F-11R-7/8, 14 p. (mimeo).
9. Rodhe, W. 1964. Effects of impoundment on water chemistry and plankton in Lake Ransaren (Swedish Lapland). *Verh. Internal. Verein. Limnol.* 15:437-43.
10. Shapiro, J. 1973. Blue-green algae: Why they become dominant. *Science* 179:382-84.
11. Taylor, M. P. 1971. Phytoplankton productivity response to nutrients correlated with certain environmental factors in six TVA reservoirs. Reservoir Fisheries and Limnology, American Fisheries Society, Special Publication No. 8. Washington, D. C. 209-18.
12. Vollenweider, R. A. 1968. The scientific basis of lake and stream eutrophication, with particular reference to phosphorus and nitrogen as eutrophication factors. Tech. Rep. to O.E.C.D., Paris DAS/CSI/68. No. 27. 182 p.
13. Wunderlich, W. O. 1971. The dynamics of density-stratified reservoirs. Reservoir Fisheries and Limnology, American Fisheries Society, Special Publication No. 8, Washington, D. C. 219-31.
14. Yeatman, H. C. 1956. Plankton studies on Woods Reservoir, Tennessee. *J. Tenn. Acad. Sci.* 31:32-53.
15. Yentsch, C. S., and J. F. Hebard. 1957. A gauge for determining plankton volumes by the mercury immersion method. *J. Cons.* 22:184-90.

Chlorophyll Concentrations, Water Quality, and Nutrient Concentrations in Cheat Lake, West Virginia

James W. Yahnke
Department of Biology, West Virginia University
Morgantown, West Virginia 26506

Abstract

A study of the chemical environment of Cheat Lake, West Virginia, and two of its less acid backwaters was conducted from May through October of 1972. Based on the chemical environment, the lake and its two backwaters were shown to constitute three distinct systems, with Morgan Run being intermediate between the lake and Ruble's Run. The chemical environment was found to be much more variable in the backwaters than in the lake. Variation was due to incursions of lakewater into the backwaters, which were more frequent and more intense in Morgan Run than in Ruble's Run, due to the morphometry of the lake in that area. Chlorophyll concentrations were much greater in the lake than in either of the backwaters; the chlorophyll concentrations in the backwaters were similar. Chlorophyll concentrations were greater in the lake due to the greater stability of the chemical environment in the lake. The concentrations of chlorophyll and dissolved phosphate showed marked seasonality falling to their lowest levels following the onset of thermal stratification.

Phytoplankton populations are usually regulated by concentrations of inorganic nutrients. While the concentration of some nutrients are controlled by biological processes, the absolute amounts of others are dependent upon their physicochemical properties (Pomeroy, 1970). Gaseous elements are usually more available to phytoplankton than those which have their reservoir in the lithosphere, since exchange with the atmosphere occurs much more rapidly. Of all elements present in organisms, phosphorus is most likely to be limiting to phytoplankton populations in lakes because the ratio of phosphorus to other elements in organisms tends to be greater than the ratio in sources of biological nutrients (Hutchinson, 1957). The concentration of nitrogen can influence the amount of phosphorus required by phytoplankton. Algae can develop with a phosphorus concentration of only 20% of the normal requirement when nitrogen is present in excess (Strickland, 1960).

In the past both water quality and primary nutrients have been shown to be limiting in Cheat Lake with respect to algal productivity. When nitrate and phosphate were added to populations of *Chlorella vulgaris* cultured in Cheat Lake water, phosphate was found to be limiting, although the addition of either nitrate or phosphate enhanced algal growth (Burley, 1954). The high concentration of acid, which severely reduces the solubility of inorganic carbon species in water, was shown to inhibit primary production in Cheat Lake although there was no significant difference in productivity in the lake and two of its less acid backwaters (Volkmar, 1972). In Lake 227 in the Experimental Lakes Area of Northwest Ontario, phosphorus has been shown to be limiting to the standing crop of phytoplankton with no effect upon productivity (Schindler, 1971).

The objectives of this study are to provide data on the concentrations of primary nutrients and water quality variables in Cheat Lake. Relationships between these variables are to be examined along with their effects on algal biomass, indicated by the concentration of chlorophyll in the algal assemblage.

Description of the Lake

Cheat Lake, shown in Figure 1, is an impoundment on the Cheat River near the Pennsylvania-West Virginia state line. The reservoir is approximately 12.5 miles long and up to half a mile wide with a surface area of 1730 acres (Hall, 1968). The maximum depth is 26 meters. Three backwaters have been formed on the eastern side of the lake and are partially isolated from it by earthen dams.

Methods

Samples were collected periodically from May through October of 1972. All samples were collected through a water column with a van Dorn bottle at the five sampling stations shown in Figure 1. Samples varied from one every meter to irregularly spaced samples through the euphotic zone with one additional sample being collected from approximately one meter above the sediments. In the early stages of the study, the base of the euphotic zone was taken to be the Secchi disc depth. Later, the one percent light transmission level, as determined with a submarine photometer, was used. Temperature was recorded using a Whitney thermister.

Immediately upon returning to the laboratory, the samples were preserved as recommended by Golterman (1971).

Another one liter of sample was filtered through either .30 micron Gelman glass filters or .22 micron Millipore filters. Chlorophyll was extracted by the spectrophotometric method of Yentsch (Golterman, 1971).

Methods as outlined in Standard Methods were used to analyze for nitrate, by the phenoldisulphonic acid method, sulfate, by the turbidemetric method, and hot acidity and alkalinity by titrametric methods. The remaining analyses were performed by spectrophotometric methods as outlined in Golterman (1971), dissolved phosphate by the antimony-molybdate-ascorbic acid method on filtered samples, total phosphorus by the same method following hydrolysis and oxidation. Reactive silicate was determined by the molybdate-stannous chloride method. The bathophenanthroline method was used for total inorganic iron.

Results

Multivariate discriminant analysis was used to test whether there was a statistically significant difference between stations based upon all chemical variables and the chlorophyll concentrations. Discriminant analysis is a technique for studying the extent to which populations overlap or diverge from one another by computation of the generalized squared distance between means from the covariance matrix (Snedecor and Cochran, 1971). Results (Table 1) indicate that there is no significant difference between the three lake stations, but the lake as a whole does differ significantly from the two backwaters studied. A further discriminant analysis showed the two backwaters to be different from each other. On the basis of the chemical environment, the lake and the two backwaters constitute three distinct systems.

One of the more striking characteristics of Cheat Lake is the low pH during the summer. The mean pH for the duration of the study was 4.5. In the Morgan Run and the Ruble's Run backwaters, the mean pH values were 5.4 and 6.4 respectively. As can be seen in Table 2, at times of lakewater incursions, there is

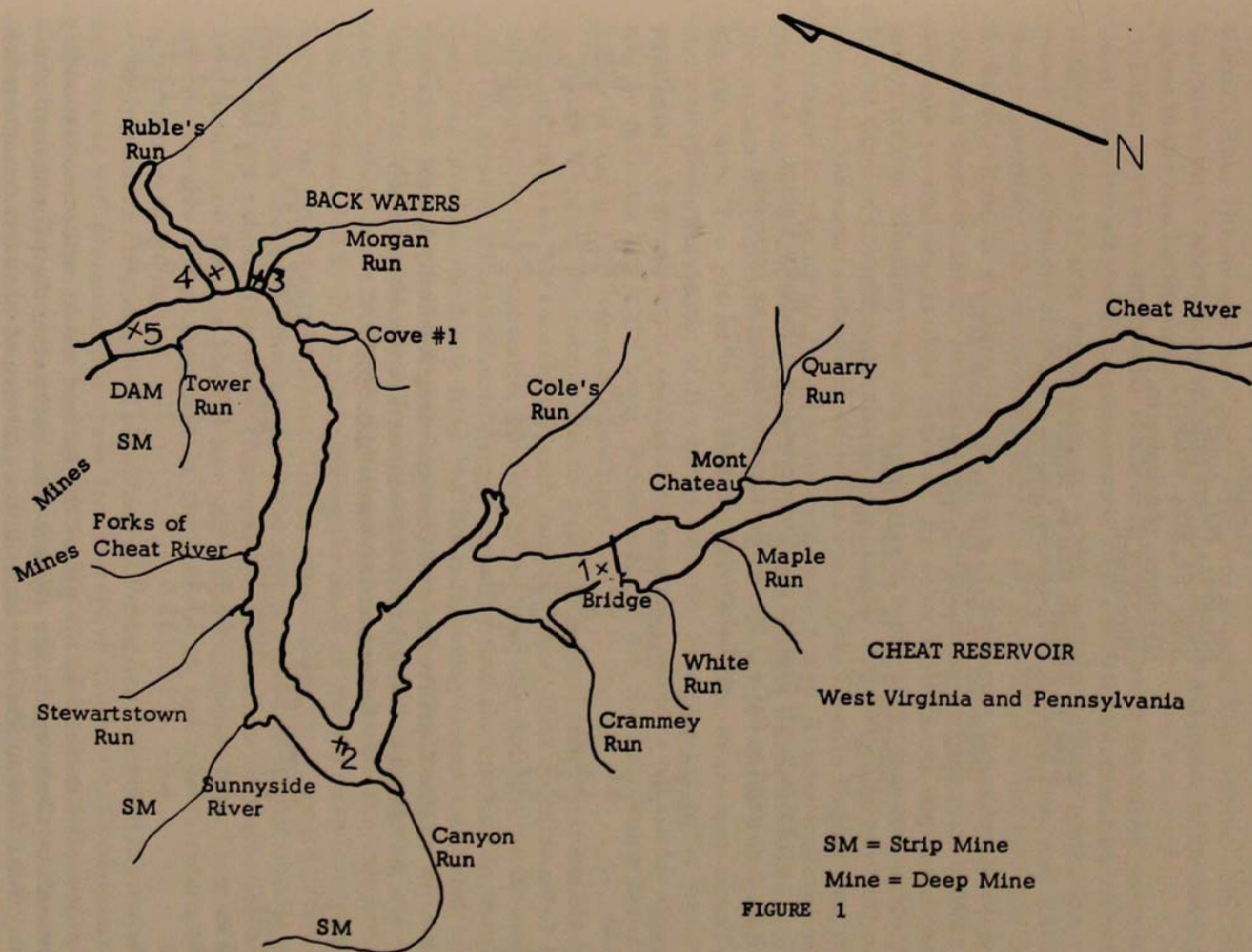


FIGURE 1. Map of Cheat Lake, West Virginia, showing sampling stations.

Table 1. Discriminant Analysis for Variables by Station, Cheat Lake, 1972

Variables
pH. No_3, PO_4, total P,
SiO_2, Fe, SO_4, acidity,
alkalinity, phaeophytin,
chlorophyll

Lake vs. Morgan Run vs. Ruble's Run
$D^2 = 373.8 \quad P > X^2 < 0.005$, d.f. 22.

Bridge Sta. vs. Slag Pile Sta. vs. Dam Sta.
$D^2 = 28.7 \quad P > X^2 = 0.15$, d.f. 22.

Morgan Run vs. Ruble's Run
$F = 4.51 \quad P > F < 0.001$, d.f. 11, 70.

Table 2. pH Profiles and Corresponding Alkalinity Values (mg/l as $CaCo_3$) for the Backwaters During an Incursion of Lakewater—August 15, 1972

	Morgan Run			*Ruble's Run*	
Depth	*pH*	*Alkalinity*	*Depth*	*pH*	*Alkalinity*
0 m	4.6	0.1	0 m	4.7	0.2
2	4.8	0.2	3	5.0	1.2
6	5.4	1.8	7	5.6	3.8
9	5.7	3.5	12	6.4	28.5
13	6.4	13.0	15	6.7	46.5

Table 3. pH Profiles and Corresponding Alkalinity Values (mg/l as $CaCO_3$) for the Backwaters in the Absence of Lakewater Incursions May 30, 1972.

	Morgan Run			*Ruble's Run*	
Depth	*pH*	*Alkalinity*	*Depth*	*pH*	*Alkalinity*
0 m	6.4	13.8	0 m	6.6	11.5
1	6.4	13.8	1	6.7	12.6
2	6.4	13.5	2	6.8	15.2
13	6.4	6.0	17	6.8	9.3

a pronounced decrease in the pH of the surface waters with a corresponding decrease in alkalinity. In the Ruble's Run backwater, the pH remained above that of the Morgan Run backwater in the presence of lakewater contamination, which creates a gradient of increasing pH and alkalinity from surface to bottom. Residual alkalinity in Ruble's Run was also greater than in Morgan Run, although Table 3 seems to indicate that alkalinity is greater in Morgan Run in the absence of lakewater contamination. Table 3 shows the only sampling date on which Morgan Run showed no incursion of lakewater. On two occasions lakewater was noted moving into Morgan Run while Ruble's Run was emptying into the lake. This difference in the direction of flow is due to a partially submerged spit which extends into the lake between the outlets of the two backwaters. The spit channels the lakewater into Morgan Run and, at the same time, away from Ruble's Run. The incursions of lakewater into Morgan Run without coincident incursions into Ruble's Run account for some of the difference in the mean pH values in the two backwaters.

Differences in water quality can also be observed using the sulfate concentrations, since the pH should be proportional to the sulfate concentration when the acidity is due to acid mine drainage, which includes sulfuric acid. Figure 2, shows the regressions of pH on sulfate for the lake, Morgan Run, and Ruble's Run respectively. The relationship between pH and sulfate in the lake and that in Ruble's Run, as exhibited by the slopes of the curves, is similar. In Morgan Run the slope is steeper, indicating that the pH changes much more rapidly in response to acid inputs than either of the other systems. As was noted previously, residual alkalinity was always greater in Ruble's Run than that in Morgan Run at times of coincident incursions of lakewater, indicating a greater buffering capacity. The mean sulfate concentration was slightly lower in Ruble's Run than in Morgan Run being 57.2 mg/l as opposed to 63.2 mg/l. Another indicator that the acid input was lower in Ruble's Run than in Morgan Run is the difference in the maximum concentrations, 99.2 mg/l in Ruble's Run versus 148.0 mg/l in Morgan Run. The pH in the lake responds less readily to changes in sulfate concentration than either of the backwaters do. Since the pH in the lake was usually low, a greater input of acid is necessary to exhibit a pH change equivalent to that at a higher pH range. Below a pH of 4.5, the relationship between inputs of acid and pH becomes curvilinear.

Figure 3 shows the comparison of mean chlorophyll concentrations for the lake and the two backwaters for each sampling date. Data on chlorophyll are unavailable for the backwaters on June 27 because the turbidity of the water was too great in Ruble's Run to permit extraction and the entrance to Morgan Run was blocked by debris from the flood following storms associated with Hurricane Agnes. On September 11, samples were not collected in the backwaters. In the lake chlorophyll concentrations exhibited strong spring and fall maxima, which were not so well defined in the backwaters. The chlorophyll concentrations fell to low levels following the onset of thermal stratification, but began to rise prior to its break-up. The maximum chlorophyll concentration in the lake was observed prior to the flood and was coincident with the lowest observed pH values. The mean chlorophyll concentrations in the backwaters were similar at 0.9 μg/l in Morgan Run and 0.8 μg/l in Ruble's Run. These values are considerably lower than that in the lake which had a mean chlorophyll concentration of 2.0 μg/l.

Molybdate reactive dissolved phosphate also exhibited spring and fall maxima but not as well pronounced as those by chlorophyll (Figure 4). The maximum concentrations occurred on June 27, following the flood. Dissolved phosphate fell to less than 1 μg/l on July 31, following the onset of thermal stratification, and was undetectable on August 15. Once thermal stratification broke down, the phosphate concentration returned to detectable levels at approximately 4.0 μg/l on September 11. No statistical relationship between the chlorophyll concentrations and dissolved phosphate was found although the lowest concentrations of both occurred at nearly the same time.

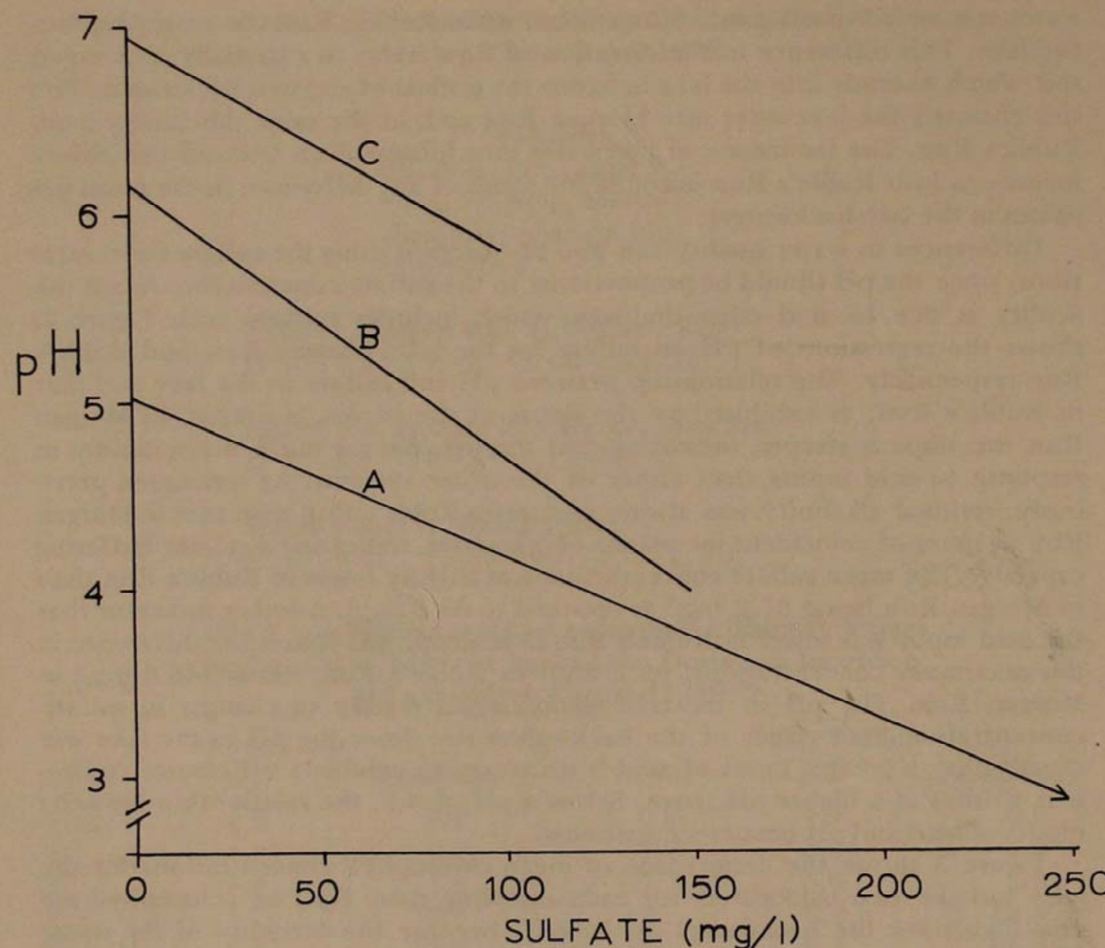


FIGURE 2. Linear regressions for pH on sulfate for A, Cheat Lake stations; B, Morgan Run; and C, Ruble's Run, 1972. Statistics for these regressions are: A, r=-.46, R^2=.21, P > F=0.0001, n=132; B, r=-.47, R^2=.22, P > F=0.0072, n=32; C, r=-.42, R^2=.17, P > F=0.0053, n=43.

Discussion

In 1971, the lake and its two backwaters were shown to constitute two distinct systems with Morgan Run somewhat intermediate between the lake and Ruble's Run but not significantly different from Ruble's Run on the basis of primary production and various water quality variables (Volkmar, 1972). In this study the lake and its two backwaters constitute three systems on the basis of the variables listed in Table 1, although there is no difference with respect to certain variables. The two backwaters are still more closely related on the basis of the chemical environment than either is to the lake. The major differences in the two backwaters are in the pH and alkalinity values, which differ most at times of non-coincident lakewater incursion. At times of coincident incursions, the effects are still more pronounced in Morgan Run, where the pH and alkalinity fall below that in Ruble's Run. Some of the difference is accounted for by a seemingly greater residual alkalinity in Ruble's Run at times of lakewater incursion. There is a limestone quarry in the drainage basin of Ruble's Run (Bible, 1972). However, it seems that the most important factor is morphometric. The spit at the outlets of the two backwaters causes differential incur-

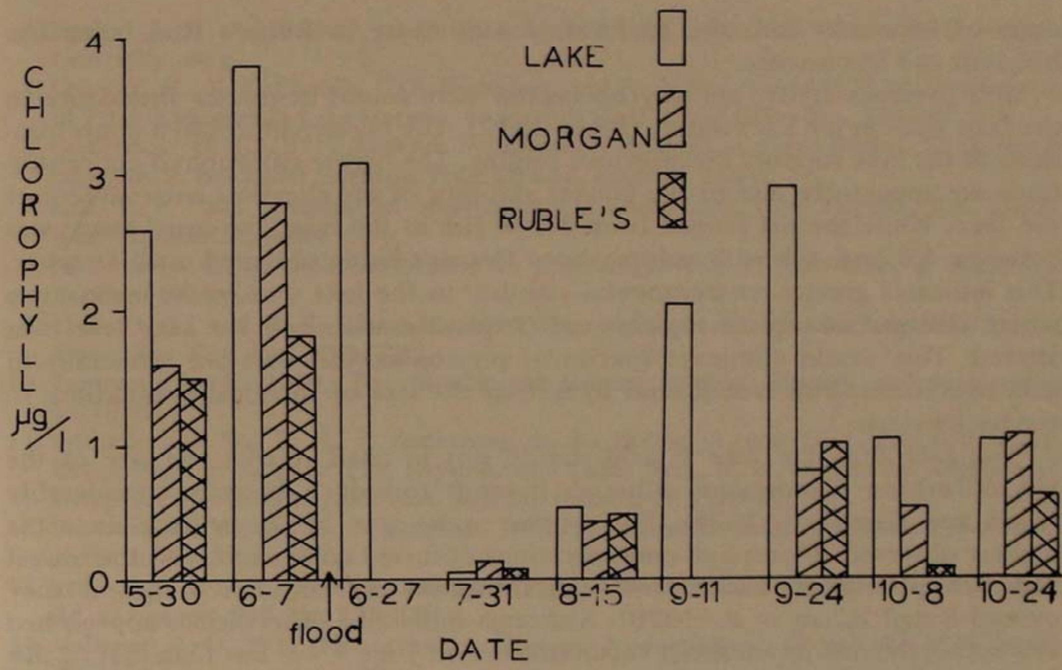


FIGURE 3. Mean chlorophyll values by sampling date for lake stations, Morgan Run and Ruble's Run, Cheat Lake, 1972. Arrow indicates the time of flood associated with Hurricane Agnes.

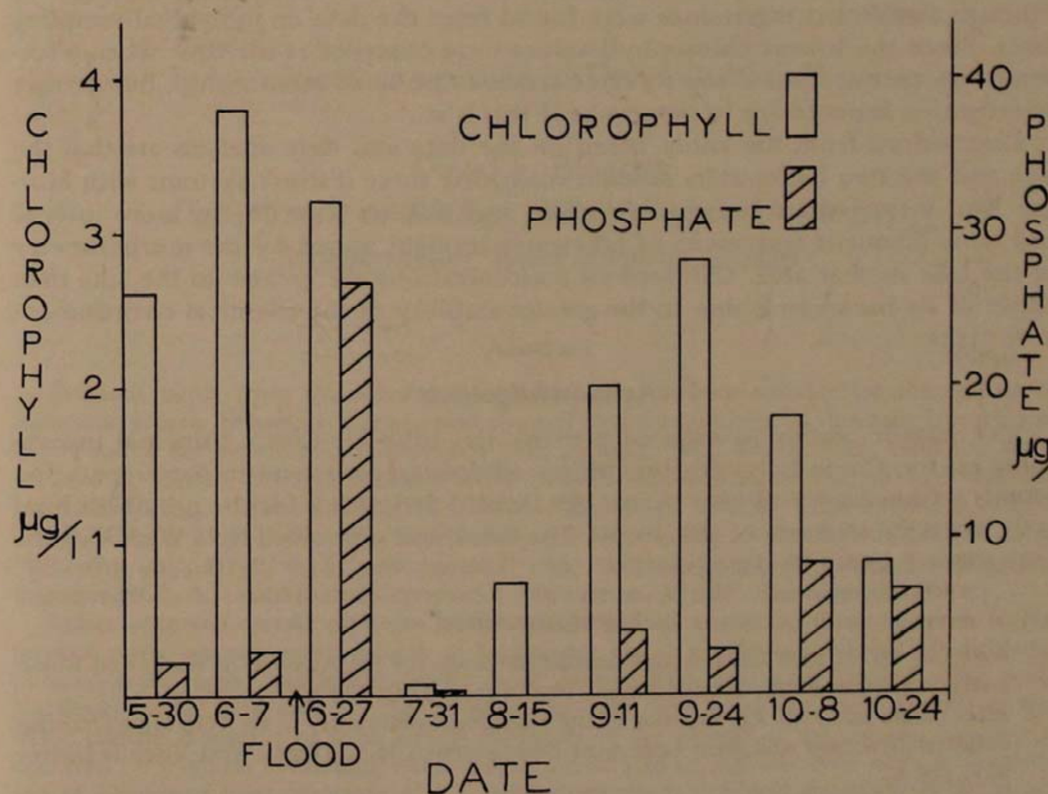


FIGURE 4. Mean chlorophyll and dissolved phosphate-phosphorus by date for lake stations, Cheat Lake, 1972. Arrow indicates the time of flood associated with Hurricane Agnes.

sions of lakewater into the backwaters with those in Ruble's Run being less frequent and less intense.

In a previous stydy, net phytoplankton were found in greater abundance in the lake than in the backwaters (Bible, 1972). The higher chlorophyll concentrations in the lake support this previous finding. The higher chlorophyll concentrations are apparently due to the greater stability of the chemical environment of the lake. While the pH ranged from 3.2 to 6.1 in the lake, the usual range was between 4.0 and 4.8 with values above five not being observed until October. This indicates greater environmental stability in the lake than in the backwaters where changes take place rapidly and frequently whenever the lake level was altered. This would eliminate species of phytoplankton that are intolerant of acid conditions. This would tend to restrict the size of the algal populations in the backwaters.

The fact that the lake is acid should not in itself restrict the size of the phytoplankton populations, although it tends to reduce diversity considerably (Lind and Campbell, 1970). This seems to be true in Cheat Lake since the highest observed chlorophyll concentrations occurred coincident with the lowest observed pH values. Chlorophyll concentrations are considered high if they exceed 8 μg/l (Chau *et al.*, 1970). Although individual observations approached this value, the maximum mean concentration on June 7 was less than half of this value.

If phosphate was the sole limiting factor, then a significant inverse relationship should be obtained by statistical analysis (Brydges, 1971). No statistical relationship was obtained from analyses between chlorophyll and phosphate, although significant regressions were found from the data on individual sampling dates. Since the lowest chlorophyll values were observed at the time when phosphate concentrations are lowest, there seems to be some relationship, but further investigation is necessary to determine if this is so.

Conclusions from the study based on the data and data analysis are that the lake and the two backwaters studied constitute three distinct systems with Morgan Run intermediate between the lake and Ruble's Run due to more intense and more frequent incursions of lakewater brought about by the morphometry of the lake in that area. Chlorophyll concentrations are greater in the lake than either of its backwaters due to the greater stability of the chemical environment in the lake.

Acknowledgments

The author wishes to especially thank Dr. John De Costa, principal investigator on the Cheat Lake project, for his advice and assistance in the preparation of this paper. I wish to also thank Mr. Donald Strimbeck for the use of his boat dock during the course of this study. The work was supported by a West Virginia University Faculty Senate Grant.

Literature Cited

1. A.P.H.A. 1971. 13th edition of Standard Methods for the Analysis of Water and Wastewater. American Public Health Assoc., Washington, D.C. 874 p.
2. Bible, Janie L. 1972. An Analysis of the Plankton Community in an Acid Mine Drainage Polluted Reservoir and Two Less Acid Embayments. M.S. Thesis. West Virginia University. 166 p.
3. Brydges, T. G. 1971. Chlorophyll-a-Total Phosphorus Relationships in Lake Erie. Proc. 14th Conf. Great Lakes Res. Internat. Assoc. Great Lakes Res. 185-90.

4. Burley, J. W. A. 1955. Algal Deficiencies in Cheat Lake. M.S. Thesis. West Virginia University. 50 p.
5. Chau, Y. K., V. K. Chawla, H. F. Nicholson, and R. A. Vollenweider. 1970. Distribution of Trace Elements in Lake Ontario. Proc. 13th Conf. Great Lakes Res. 659-72.
6. Golterman, H. L. 1971. *Methods for Chemical Analysis of Fresh Waters.* I.P.B. Handbook No. 8. Blackwell Scientific Publ. Oxford, England. 166 p.
7. Hall, R. P. 1968. Distribution of Sediments at Cheat Lake, West Virginia. M.S. Thesis. West Virginia University. 66 p.
8. Hutchinson, G. E. 1957. *A Treatise on Limnology.* Vol. 1. John Wiley & Sons, Inc. London. 1015 p.
9. Lind, O. T., and R. S. Campbell. 1970. Community Metabolism in Strip Mine Lakes. *Trans. Am. Fish. Soc.* 90:577-82.
10. Pomeroy, L. R. 1970. The Strategy of Mineral Cycling. *Ecology and Systematics* 1:170-90.
11. Schindler, D. W., F. A. J. Armstrong, S. K. Holmgren, and G. J. Brunskill. 1971. Eutrophication of Lake 227, Experimental Lakes Area, N. W. Ontario by Addition of Phosphate and Nitrate. *Jour. Fish. Res. Bd. Canada.* 28:1763-82.
12. Snedecor, G. W., and W. G. Cochran. 1971. *Statistical Methods.* The Iowa Univ. Press, Ames, Iowa, U.S.A. 593 p.
13. Strickland, J. D. H. 1960. Measuring the Production of Marine Phytoplankton *Fish. Res. Bd. of Canada. Bull.* No. 122. 172 p.
14. Volkmar, R. D. 1972. Phytoplankton Primary Productivity in Cheat Lake. M.S. Thesis. West Virginia University. 108 p.

Nicotiana glauca in Tissue Culture*

David F. Blaydes
Department of Biology, West Virginia University
Morgantown, West Virginia 26506

Abstract

Previous work from this laboratory resulted in a medium suitable for the culture of *Nicotiana glauca.* Subsequent study also showed that a cytokinin, in this case kinetin, was not required for continuous culture but that an auxin was. The medium used for stock cultures is Miller's 1965 modified White's medium minus kinetin. The medium contains (mg/l):KH_2PO_4, 300; KNO_3, 1000; NH_4NO_3, 1000; $Ca(NO_3).4H_20$, 500; $MgSO_4$:$7H_20$, 71.5: KCL, 65; $MnSO_4.4H_20$, 14.0; H_3BO_3, 1.6; $Cu(NO_3).8H_20$, 0.35; $(NH_4)6Mo_70_{24}.4H_20$, 0.10; meso-inositol, 100; nicotinic acid, 0.5; pyridoxine·HCL, 0.1; thiamine HCL, 0.1; naphthaleneacetic acid, 2.0; sucrose, 30,000; Bactoagar, 10,000.

Miller observed certain different effects of the various auxins on tissue cultures. In the present study we compared growth of *Nicotinana glauca* on the four auxins 2,4 dichlorophenoxyacetic acid, napthalene acetic acid, indoleacetic acid and indole-butyric acid. Napthaleneacetic acid and 2,4 dichlorophenoxyacetic acid produced more growth of the tissue culture according to fresh weight estimates of the growth of the culture. No growth occurred on medium containing either indole acetic acid or indolebutyric acid. A comparison of a series of concentrations of 2,4 dichlorophenoxyacetic acid showed that the maximum growth occurs at a lower concentration of 2,4 dichlorophenoxyacetic acid than of napthalene acetic acid.

*Supported in part by American Cancer Society Institutional Grant.

In conclusion, either napthalene acetic acid or 2,4 dichlorophenoxyacetic acid is suitable as an auxin for the culture of *Nicotiana glauca* tissue cultures.

The Effect of Plant Growth Substances and Natural Products on RNA and DNA Synthesis in Leukocytes*

Michael Farrow, David F. Blaydes,* and Knox VanDyke
Department of Pharmacology and Biology
West Virginia University
Morgantown, West Virginia 26506

Abstract

Plant growth substances inhibit and stimulate nucleic acid synthesis in higher plant systems. Phenolics are often inhibitory to plant growth; however, their role in plant metabolism is not clear. Little is known about the role of these compounds in human tissue culture systems. In this paper we present results of experiments of the effect of auxins, cytokinins, gibberellins and phenolics on 3H uridine and 3H thymidine incorporation into nucleic acids of human leukocytes cultures.

The biological system used is the leukocyte micro-screening system developed by Farrow and VanDyke.** It is based on the uptake, phosphorylation and incorporation of thymidine methyl 3H into DNA or Uridine 5 3H into RNA of the leukocytes in human whole blood. Phytohemaglutenin is included in the culture medium (Chromosome Medium 1 A Grand Island Biological).

The auxins, indole acetic, indole butyric and naphthalene acetic did not affect 3 H thymidine incorporation; however, all concentrations stimulated uridine incorpation. The gibberellins studied did not affect thymidine incorporation but gibberellic acid stimulated uridine incorporation. The cytokinins both inhibited and stimulated nucleic acid synthesis depending upon which type was used and on the concentration employed.

6 Methoxybenzoxazlinone, ferulic acid and naringenin were inhibitory to uridine incorporation.

In summary, the most consistent results were obtained with the auxins, all of which stimulated uridine incorporation. The pharmacologic significance of these results are being investigated.

*Supported in part by the American Cancer Society Institutional Grant, West Virginia University.

**Chemotherapy 16:76-84 (1971). A Micro-System for Screening Anti-Leukemic Drugs Utilizing Human Whole Blood.

First Record of the Alderfly *Sialis joppa* Ross (Megaloptera: Sialidae) in West Virginia

Donald C. Tarter
Department of Biological Sciences
Marshall University, Huntington, West Virginia 25701

Abstract

A new state distribution record for the alderfly *Sialis joppa* Ross is reported. Five adults (three males, two females) were captured near White Run, a tributary of Seneca Creek of the North Fork River of the South Branch of the Potomac River, on 27 May 1972 in Pendleton County, West Virginia. Additional notes are made regarding its taxonomy and distribution.

The alderfly, *Sialis joppa* Ross, is reported for the first time from West Virginia. On 27 May 1972, between 1200 and 1400 hours, five adult alderflies were captured near White Run, a tributary of Seneca Creek of the North Fork River of the South Branch of the Potomac River, in Pendleton County (Figure 1). Each adult was collected in flight within 5-7 feet of the stream. Subsequent identification yielded three males and two females, 8.0-12.0 mm and 13.5-16.0 mm body length, respectively. White Run has a meandering distance of 4.35 miles with a total fall of 1500 feet or a gradient of 344.82 feet per mile (WVGS, 1927). It has a drainage area of 6.07 square miles. White Run is less than 6 feet wide throughout its length, and the fine-graveled sandstone substrate ranges from 3 to 6 cm in size. The stream has an elevation of 2159 feet, and the air temperature on 27 May 1972 was 80 F. The pH is circumneutral. Previously, only two alderflies have been reported from West Virginia. *S. velata* Ross from Millville near the Shenandoah River in Jefferson County and *S. aequalis* Banks from Camp Creek in Wayne County were reported by Ross (1937) and Tarter and Woodrum (1973), respectively.

Taxonomy and Distribution

The genus *Sialis* Latreille contains 23 nearctic species (Ross, 1937; Townsend, 1939; and Flint, 1964). *S. joppa* was described for the first time in a monograph of nearctic alderflies by Ross in 1937. The male holotype was collected from Newfound Gap, Great Smoky Mountain National Park, North Carolina on 28 May 1934, by T. H. Frison. *S. joppa* has been previously reported from Illinois, Maine, New Hampshire, New York, North Carolina, Ohio and Pennsylvania by Ross (1937). Alderfly material in the United States National Museum added new state records from Maryland and Virginia (Flint, 1964). Minshall (1965) collected *S. joppa* near Morgan's Creek, an extensive karst topographic area, in Meade County, Kentucky.

Acknowledgements

A special thanks to Dr. Herbert H. Ross, Department of Entomology, University of Georgia, for identification of the alderfly specimens. A note of appreci-

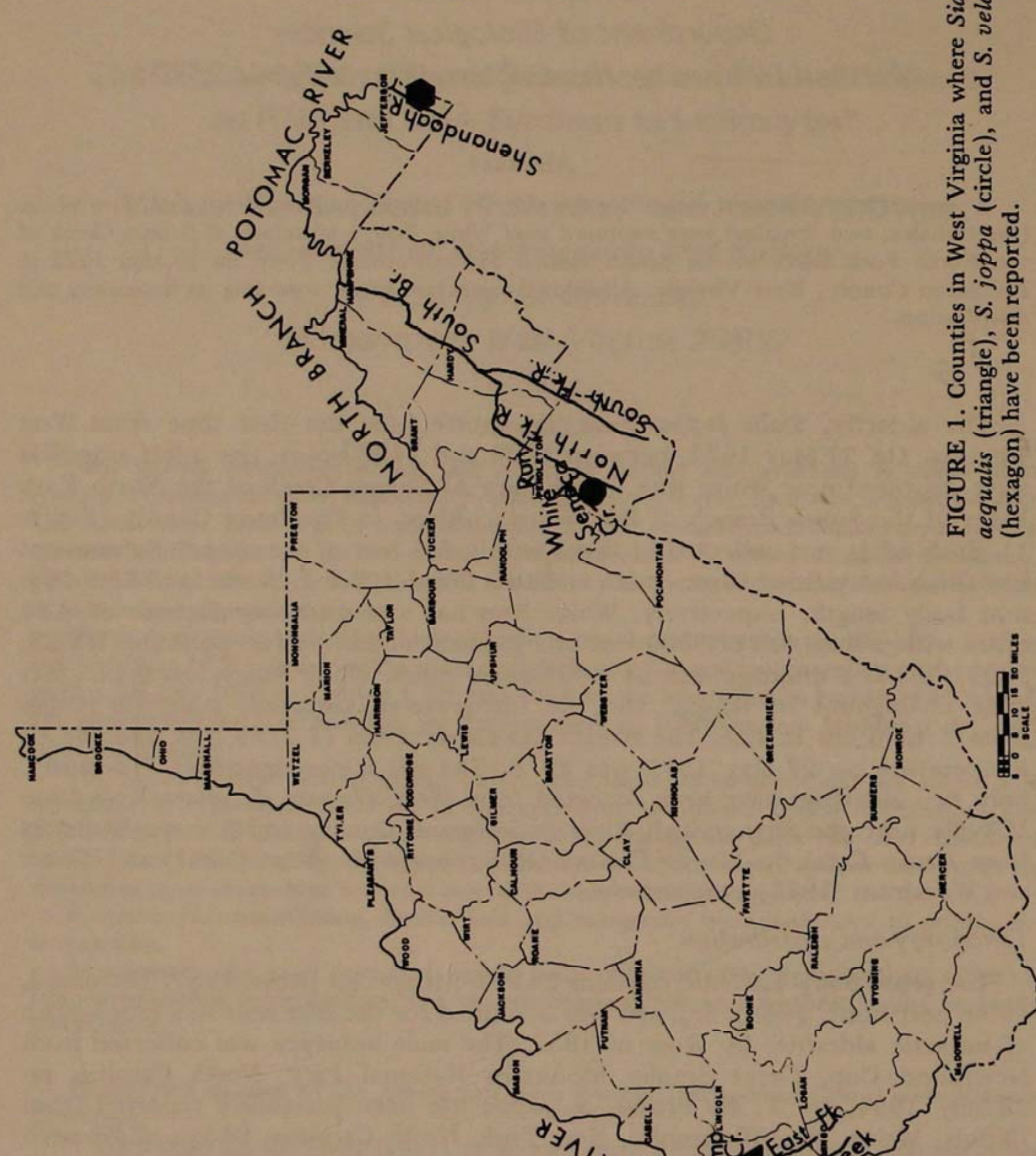


FIGURE 1. Counties in West Virginia where *Sialis aequalis* (triangle), *S. joppa* (circle), and *S. velata* (hexagon) have been reported.

ation to Dr. Oliver S. Flint, Jr., Smithsonian Institution, Washington, D. C., for critically reading the manuscript. Also, a word of thanks to Michael L. Little for help in the field work.

Literature Cited

1. Flint, O. S., Jr. 1964. New species and new state records of *Sialis* (Neuroptera: Sialidae). *Entomol. News* 75:9-13.
2. Minshall, G. W. 1965. Community dynamics and economics of a woodland springbrook: Morgan's Creek, Meade County, Kentucky. Unpublished Doctoral dissertation. University of Louisville, Louisville, Kentucky. 261 pp.
3. Ross, H. H. 1937. Studies of Nearctic Aquatic Insects, I. Nearctic Alder Flies of the genus *Sialis*. *Ill. Nat. Hist. Surv. Bull.* 21:57-58.
4. Tarter, D. C., and J. E. Woodrum. 1973. Distribution and new record of the alderfly *Sialis* (Megaloptera: Sialidae) in West Virginia. *Entomol. News* (IN PRESS).
5. Townsend, L. H. 1939. A new species of *Sialis* (Megaloptera: Sialidae) from Kentucky. *Proc. Ent. Soc. Wash.* 41:224-226.
6. West Virginia Geological Survey. 1927. Pendleton County. 384 pp.

Notes on Damselflies from West Virginia

Paul D. Harwood
Route 1, Box 346
Ashland, Ohio 44805

Abstract

Ten species of Odonata are reported. Eight species: *Archilestes grandis* (Rambur), *Lestes dryas* Kirby, *Lestes unguiculatus* Hagen, *Lestes vigilax* Hagen, *Enallagma boreale* Selys, *Enallagma cyathigerum* (Charpentier), *Enallagma geminatum* Kellicott, and *Nehallenia gracilis* Morse are new records for the state. *Lestes forcipatus* Rambur, and *Ischnura prognatha* (Hagen) are reported because of confusion surrounding knowledge of their distribution.

Introduction

During a survey of the Odonata of West Virginia, approximately 30 species have been found in the state that are not reported in the literature. A record of a species in the Calopterygidae new to the state was reported by Harwood (1973). The records of damselflies of the families Lestidae and Coenagrionidae are reported in this note. The collector and location of specimens are omitted if I collected the material.

Observations

Lestidae:—*Archilestes grandis* (Rambur), Rangoon, Barbour County, August 8, 1970, one famale, coll. Dr. Gerhardt Jurzitza; Valley Falls State Park, Marion County, July 25, 1971, several nymphs.

Lestes forcipatus Rambur, Cranesville Swamp, Preston County, July 31 and August 14, 1966, several adults, Carsten Ahrens (1968); Shady Spring, Raleigh County, August 13, 1968, adults.

Lestes dryas Kirby, Burlington, Mineral, June 15, 1931, 1 male in the Carnegie Museum, Pittsburgh, G. M. Kutchka.

Lestes unguiculatus Hagen, Wardensville, Hardy County, July 22, 1971, adults.

Lestes vigilax Hagen, Sleepy Creek Lake, Berkeley and Morgan Counties, August 30, 1969, 10 males, 1 pair, and 2 females.

Coenagrionidae:–*Enallagma boreale* Selys?, Red Creek, Dolly Sods Recreational Area, Tucker County, May 7, 1972, nymph which was reared.

Enallagma cyathigerum (Charpentier)?, Dolly Sods Recreational Area, Tucker County, June 27 and 28, 1960, adults; Spruce, Pocahontas County, August 7, 1971, adults.

Enallagma geminatum Kellicott, Sleepy Creek Lake, Berkeley and Morgan Counties, August 30, 1969; Cabell County, nymphs, Carl A. Olson (1972); Cacapon River, Hampshire County, August 5, 1966, 1 male; Burlington, Mineral County, June 9, 1971, male; Cedar Lakes, Jackson County, August 27, 1971, 1 mated pair.

Nehallenia gracilis Morse, Dolly Sods Recreational Area, Tucker County, June 28, 1960, adults.

Ischnura prognatha Hagen, Berkeley Springs, Morgan County, Osten-Sacken about 1835, (Hagen, 1861).

Discussion

A nymph of *Enallagma*, collected in the Dolly Sods Area May 7, 1972, emerged in the laboratory, was identified as *E. boreale*. It was sent to Mrs. Leonora Gloyd who reported that it is closer to *E. cyathigerum* (southeastern form) than to *E. boreale*. I have re-examined the specimen, finding her data exact, as always, but my interpretation of those same facts leads to a determination of *E. boreale*?.

I have collected several specimens of *E. cyathigerum* (southeastern form) from West Virginia. Since this form has been given this name in the literature, I am not creating a new synonym by using it here. However, I shipped many of my specimens to Mrs. Gloyd who is the authority on this group and she states that it is so constantly different from the typical circumboreal *E. cyathigerum* that it must be described as a new species. Until I have more material, particularly males reared from the nymphs, I reserve the right to use the former designation. I must thank Mrs. Gloyd for the attention she has kindly given this problem, and expect to agree with her.

Ischnura prognatha Hagen (1861) was reported from Berkeley Springs, Virginia. I have visited that locality, and believe it is now absent from its early habitat. While Berkeley Springs was in Virginia when Hagen wrote, the type locality is now in West Virginia, a fact overlooked by standard reference books.

Literature Cited

1. Ahrens, C. 1968. A list of Odonata taken in the Cranesville Swamp. Arboretum Newsletter, W. Va. Univ. 17(4):6.
2. Hagen, H. A. 1961. A synopsis of the Neuroptera of North America with a list of the South American species. Smiths. Misc. Coll., Odonata, pp. 55-187.

3. Harwood, P. D. 1973. *Calopteryx angustipenne* Selys in West Virginia. *Proc. W. Va. Acad. Sci.* 44:89.
4. Olson, C. A. 1972. A taxonomic survey of the larval odonate fauna of Cabell County, West Virginia. Thesis. Dept. Biol. Sci. Marshall Univ. Huntington.

Phytoplankton Generic Diversity and Biomass Estimates of a Monongahela River Acid Confluence[1]

D. Rankin and E. C. Keller, Jr.
West Virginia University, Department of Biology
Morgantown, West Virginia 26506

Abstract

Water samples were taken from the confluence of Robinson Run and the Monongahela River in northern West Virginia in order to obtain information on the effect of an acid stream on generic diversity. Samples at each station were examined to determine generic diversity, biomass, density and distribution of phytoplankton in relation to an acid stream. The parameters—pH, hot and cold acidity, dissolved oxygen, percent saturation of oxygen and water temperature—were also measured with analyses of variance and correlation performed on the data.

The generic diversity and density were found to be significantly decreased in the acid stream with *Euglena* being the only genus found. Both diversity and density slowly increased down river with increasing distance from the confluence. The highest generic diversities and densities were found upriver from the confluence. Significant differences in the chemical parameters of Robinson Run were also found. High acidity, as measured by hot and cold acidity values, appeared to be a significant factor in determining the diversity and density indices.

Introduction

The Monongahela River basin receives acid drainage from numerous coal mines situated within its drainage area. Most of the acid drainage enters the river from the many small tributaries along its course. It has been recorded that the Monongahela River carried an average net acid load of 235 tons per day at Star City, West Virginia (EPA, 1971). Much of the acid load was due to loadings from local creeks specifically, Deckers Creek, Dents Run, Scotts Run, and Robinson Run. The tributary water draining from the mines carries mineral acids, acid salts, and metallic ions such as iron and aluminum, thereby decreasing pH and increasing acidity (Warner, 1971).

Aquatic organisms, when placed in a stress condition, respond in various ways. Cairns and Lanza (1972) listed five responses by algal communities: "1)

[1]This project was supported, in part, by the Office of Water Resources Research, Department of the Interior, Grant #WVA-B001 and the Biology Department, WVU.

reduction in the number of species present, 2) increase in the range of numbers of individuals per species, 3) reduction in colonization rates, 4) changes in selective predator or parasite relationships upon particular segments of algal communities, and 5) a shift in the dominance within the community favoring some species over others." One method of assessing the effect of a stress on the aquatic communities is the use of diversity indices. Ransom and Dorris (1972) used diversity indices to analyze reservoir benthic communities. Herricks and Cairns (1972) studied the effect of acid drainage on stream macrobenthic communities using diversity indices and measuring chemical parameters. A change in diversity can be a good means of assessing pollution effects on aquatic communities because stress tends to reduce community complexity and many times reduces the total number of species, thereby effecting a decrease in organismic diversity.

These diversity indices measure the relative taxonomic complexity of the biological community being evaluated. In this study we have used these indices to demonstrate that there is a concordant shift in the biological community structure as the physical parameters shift. The generic diversity parameters are used here only as measures of the relative variability among the taxonomic groups present in this system.

This study was initiated in order to examine the effect of acid stress on phytoplankton communities associated with the confluence of Robinson Run and the Monongahela River and to evaluate possible correlative changes between certain water quality parameters and the diversity indices.

Site Description

Robinson Run is a small tributary flowing into the Monongahela River approximately one mile downriver from Star City, West Virginia. Its drainage basin receives acid discharges from various coal mines in the area. Between 1965 and 1968 Robinson Run received a net acid load of approximately 7,000 pounds per day from these mines (EPA, 1971). The stream has a characteristic "yellow boy" color. Seven stations located upriver, downriver, in the confluence, and in the stream were sampled. Station 1 was located 100 feet upriver from the confluence and station 2 was located thirty feet above the confluence in an upstream pool, while station 3 was located fifty feet upstream from the confluence and station 4 was located two feet upstream from the confluence. The remaining three stations were located downriver from the confluence. Station 5 was ten feet below the confluence, station 6 was fifty feet below the confluence, and station 7 was 100 feet below the confluence (stations 5 and 6 were located within a yellow plume produced by the stream).

Materials and Methods

Seven stations were sampled on October 15, 1972. Three replicate surface samples were taken approximately ten feet from shore. The chemical parameters of pH, hot and cold acidity, dissolved oxygen, and percent of dissolved oxygen were measured according to standard methods (A.P.H.A., 1971). Water temperature was measured with a thermistor.

Two liters of water sample were collected and preserved with merthiolate preservative solution (Weber, 1966). The phytoplankton were concentrated to a volume of ten milliliters using a Foerst electric centrifuge. A microscopic examination was made to identify the organisms to genus level and to determine the density of each genus. Only the first 100 organisms encountered were counted. The biomass was determined using the wet volume technique. The volume of each genus was computed by determining the volume for twenty-five organisms of a genus. Total biomass per liter was then calculated.

The generic diversity was calculated using a computerized Fortran IV diversity program developed by Keller and Silvester (1973). This program combines and calculates the following diversity indices: DBAR, H, EVEN, DSQ, VDQ, DELTA, EVDEL, and D (see Appendix I). Most of the above indices are also presented in the report by Mawson and Godfrey (1971).

Analyses of variance and correlation analyses were performed on all chemical and diversity parameters using the SAS system of the WVU Computer Center. Analysis of variance and correlation analysis were also performed on the river system data only (stations 1, 2, 5, 6, and 7) and on the stream-river systems only (stations 3, 4, 5, 6, and 7).

Results

The mean values and the coefficients of variation for chemical parameters are presented in Table 1. Stations 3 and 4 have a sharp deviation from all other stations and tend to give a higher or lower overall mean value for each parameter. The coefficient of variation between replicates of the chemical parameters are quite reasonable when taking into account the stress of the system sampled.

Cold acidity was extremely high in the acid stream stations and decreased downstream. Hot acidity which is related to cold acidity followed the same pattern. The pH values were low in Robinson Run but they approached neutrality in the downstream stations. Dissolved oxygen decreased to less than one milligram per liter in stations 3 and 4 and returned to values equal to those found in stations 1 and 2. Percent saturation of dissolved oxygen followed the same pattern as dissolved oxygen. Water temperature was found to be 10°C on all river stations but showed a drop of two degrees at the confluence and four degrees in the upstream pool of station 3.

The mean values for biomass are also presented in Table 1. The coefficient of variation was found to be 12.24 which was somewhat higher than those shown for chemical parameters. Stations 3 and 4 contained the highest biomass while the two upriver stations had a slightly higher biomass than the three downriver stations. There was an increase in biomass moving from station 5 in the acid stream to the downriver station 7.

Station 3 and station 4 diversity means were found to be zero (single genera present). All coefficients of variation for the diversity indices were found to be under fifty percent variation which is the accepted value for diversity parameters. H, HMAX, and EVENESS remained relatively the same for all river stations but dropped to zero in the stream stations, 3 and 4.

Discussion

At the confluence of the acid stream and the Monongahela River all chemical and physical parameters were drastically changed. Hot and cold acidity increased approximately 100 times that of the river stations due to heavy acid loadings from various mines located in the Robinson Run drainage basin. The pH of the stream was lowered to a value of 3.3 which is considered highly acidic. The natural buffering system of the stream can stabilize pH levels even though total acidity increases. Dissolved oxygen and percent saturation of dissolved oxygen were reduced to almost zero at stations 3 and 4. Robinson Run showed the

Table 1. Average Values and Overall Coefficients of Variation for all Sites of Parameters Evaluated at the Confluence of Robinson Run and the Monongahela River

	MEANS								
Site	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*Overall*	*C.V.*
BIOMASS	0.388	0.957	2.652	2.249	0.198	0.283	0.353	0.925	12.24%
pH	6.47	6.63	3.30	3.30	6.57	6.83	7.23	5.76	1.07%
HOTACID	8.00	5.33	830.13	823.60	4.67	3.83	4.00	239.94	2.12%
COLACID	11.33	12.00	1454.93	1453.00	13.33	11.67	11.33	423.94	1.00%
WATTEMP	10.0	10.0	6.0	8.0	10.0	10.0	10.0	9.1	—
DO	9.73	9.87	0.20	0.20	9.37	9.40	9.77	6.93	1.91%
PCTSAT	85.7	86.8	1.0	3.0	80.7	82.7	85.8	60.8	2.5%
DBAR	1.99	1.99	0.00	0.00	1.81	1.93	1.89	1.38	7.92%
H	1.38	1.38	0.00	0.00	1.25	1.33	1.31	0.95	7.95%
HMAX	2.479	2.480	0.000	0.000	2.476	2.476	2.478	1.770	0.02%
EVEN	0.56	0.56	0.00	0.00	0.50	0.54	0.53	0.38	7.94%
DSQ	0.68	0.69	0.00	0.00	0.64	0.65	0.66	0.47	7.04%
VDQ	0.01	0.01	0.00	0.00	0.02	0.02	0.02	0.01	37.10%
DELTA	0.44	0.44	0.00	0.00	0.41	0.42	0.42	0.30	9.66%
EVDEL	0.61	0.62	0.00	0.00	0.56	0.58	0.59	0.42	9.70%
D	1.225	1.210	0.000	0.000	1.300	1.294	1.261	0.898	1.15%

characteristic "yellow boy" color which is caused by the precipitation of ferric hydroxide. The ferrous compounds can act as strong reducing agents and tend to denude the stream of its dissolved oxygen (Blum, 1956). This chemical process can also increase total hardness and sulfate, while tending to shift the pH values even lower. Water temperature was found to be four degrees colder in the stream as a result of the cold subsurface mine water being pumped into the stream. The water was only two degrees cooler at the confluence due to the mixing with the warmer river water.

The genera found in the stream-river community consisted of the Chlorophytas: *Chlorella, Chlamydomonas, Ankistrodesmus, Scenedesmus, Pediastrum, Gloeocystis,* and *Coelastrum;* the diatoms: *Synedra, Navicula,* and *Gyrosigma;* and the Euglenophytas: *Euglena* and *Phacus.* In earlier studies of acid mine streams in West Virginia, Bennett, 1969; Lackey, 1938; Warner, 1971; and Mackenthum, 1969, found *Chlamydomas, Euglena* and *Navicula* to be dominant organisms in an acid situation. The most abundant organisms found in the river stations were *Chlorella, Chlamydomas, Ankistrodesmus,* and *Scenedesmus.* The stream rocks and substrate were covered with a green slime. This slime was found to be the organism *Euglena* which was the only organism found in Robinson Run during the sampling. *Euglena* was not found at an upriver station, but it was found at all downriver stations. *Pediastrum* was not found at any downriver station.

The greatest density of organisms per liter was found at stations 1 and 2, while the lowest density of organisms per liter was found at stations 3 and 4 (Table 2). The density began to increase with increased distance from the confluence to the downriver stations indicating recovery (or dilution) was taking place. A previous study of macroinvertebrates by Herricks and Cairns in 1972 showed that density increased as one moved downriver from an acid stream. Mackenthum (1969) also found that in streams degraded by acid mine water there was a drastic reduction of biomass in the acid stream stations, while the lowest biomass was found at station 5. High biomass estimates for the acid stream were due to *Euglena* being the dominant organism. Using the wet volume biomass

Table 2. Average Density of Genera at Each Station. (Number of Organisms per ml)

	Station						
Genus	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Chlorella	360	385	0	0	241	248	300
Chlamydomonas	235	221	0	0	122	126	168
Ankistrodesmus	119	130	0	0	74	67	72
Scenedesmus	16	27	0	0	16	7	18
Pediastrum	0	3	0	0	0	0	0
Gloeocystis	16	5	0	0	3	12	3
Coelastrum	16	11	0	0	5	7	3
Synedra	21	5	0	0	8	13	16
Navicula	3	0	0	0	2	0	4
Gyrosigma	0	5	0	0	2	0	0
Phacus	8	5	0	0	0	0	0
Euglena	0	0	307	280	2	10	12
Total	794	797	307	280	475	490	596

method *Euglena* had a biomass ten to forty times greater than that of other phytoplankton found due to the great amount of water contained in the organism's biomass. Biomass, like density, increased going downriver from the confluence. Patrick (1968) also found decreased density and changes in biomass production due to influence of an acid mine stream. A study of streams in West Virginia and Pennsylvania found reduced population size in acid mine streams (Mackenthum and Ingram, 1967).

Two different diversity programs were used to examine the diversity of the aquatic community. Total volume diversity was calculated using number of organisms per genus per liter. Total count diversity was calculated using number of organisms per genus per 100. Both programs showed similar results; thus, only total volume diversity was used in all later statistical tests. Zero values for all diversity indices were obtained for stations 3 and 4 (Table 1). The zero values were obtained because only one genus was found to exist in the acid stream; therefore, there was no diversity in the acid stream. At the river stations the diversity values were essentially equal. A study made on Scott's Run (located upriver from Robinson Run) showed a slight increase in diversity as one moved downriver from the confluence of the acid stream (Pisapia, personal communication). Herricks and Cairns (1972) showed that diversity was lowest in acid stream and increased moving downriver. The diversity parameters for the Monongahela River stations showed a low diversity for all indices indicating that the ecosystem was under stress produced from other sources. A correlation analysis between the diversity and chemical parameters showed that these two groupings of variables were significantly correlated with each other P= .01 (Table 3).

In the Model II analysis of variance high F values were encountered. F ratios were all found to have P values less than 0.0001. An analysis of variance and correlation were performed on the river system using stations 1, 2, 5, 6, and 7; and the stream system using stations 3, 4, 5, 6, and 7. The stream system parameters were all correlated at the 0.1% level, while only a few parameters were significantly correlated in the river system. The same results were found in the analysis of variance for the separate systems as those found in the correlations. It can, therefore, be stated that there was an effect on diversity and chemical parameters in water influenced by the acid drainage.

Conclusions

The effects of acid drainage on water quality were: a decrease in pH, a decrease in dissolved oxygen, and percent saturation, and an increase in hot and cold acidity levels.

The effects of acid drainage on the aquatic communities were: a reduction in diversity of organisms, reduction of density of organisms, a reduction in the number of genera, and an increase in the density and the dominance of a tolerant species.

Recovery (or dilution) was evidenced by an increase in generic diversity, an increase in density and generally "better" water quality as one sampled downriver from the confluence.

The low generic diversity of the Monongahela River indicated that all of the communities studied are under considerable stress.

Appendix I. Species Diversity Parameters

H, HMAX, DBAR and EVEN are analogous to the uncertainty involved in predicting which species an animal would be confronted with by the next random

Table 3. Linear Correlation Coefficients Among Parameters Evaluated in the Confluence of Robinson Run nd the Monongahela River

	Biomas	*PH*	*Hotacid*	*Colacid*	*Wattemp*	*DO*	*Pctsat*	*DBAR*	*H*	*HMAX*	*Even*	*DSQ*	*VDQ*	*Delta*	*Evdel*	*D*
Site	.20	.27	.16	.16	.20	.14	.14	.11	.11	.16	.11	.13	.51*	.11	.11	.20
Biomas	–	.98**	.99**	.99**	.96**	.98**	.98**	.98**	.98**	.99**	.98**	.98**	.78**	.98**	.98**	.99**
PH		–	.99**	.99**	.92**	.98**	.99**	.98**	.98**	.99**	.98**	.98**	.77**	.98**	.98**	.99**
Hotacid			–	1.0**	.98**	1.0**	1.0**	.99**	.99**	1.0**	.99**	.99**	.76**	.99**	.99**	1.0**
Colacid				–	.93**	1.0**	1.0**	.99**	.99**	1.0**	.99**	.99**	.76**	.99**	.99**	1.0**
Wattemp					–	.93**	.94**	.92**	.92**	.93**	.92**	.93**	.71**	.92**	.92**	.93**
DO						–	1.0**	.99**	.99**	1.0**	.99**	1.0**	.74**	.99**	.99**	1.0**
Pctsat							–	.99**	.99**	1.0**	.99**	.99**	.74**	.99**	.99**	.99**
DBAR								–	1.0**	.99**	1.0**	1.0**	.70**	1.0**	1.0**	.99**
H									–	.99**	1.0**	1.0**	.70**	1.0**	1.0**	.99**
HMAX										–	.99**	.99**	.76**	.99**	.99**	1.0**
Even											–	1.0**	.70**	1.0**	1.0**	.99**
DSQ												–	.71**	1.0**	1.0**	.99**
VDQ													–	.69**	.69**	.79**
Delta														–	1.0**	.98**
Evdel															–	.98**
D																–

*=statistical significance with P between .05 and .01 **=statistical significance with P less than .01

encounter assuming it wanders freely over the whole community (Godfrey and Mawson, 1971).

H = Pielou's Index or Shannon Weaver Index

HMAX = Information Index with maximum diversity

DBAR = Wilhms Index

EVEN = Ratio of H/HMAX

DSQ and VDQ are the probability that organisms drawn from the same sample will not belong to the same species if they are drawn at random (Ibid.).

DSQ = Simpson's Index

VDQ = Variance of Simpson's Index

DELTA and EVDEL are the distance of the sample from the origin where the number of individuals in each species represents coordinates in an S-dimensional hyperspace (Ibid.).

DELTA = McIntosh's Index

EVDEL = Eveness of McIntosh's Index

D is the ratio of the number of species minus one to the log of the total number of species.

D = Species Richness Index

Literature Cited

1. A.P.H.A. 1971. Standard Methods for the Examination of Water and Wastewater. 13th Edition. A.P.H.A., Washington. 874 pp.
2. Bennett, H. D. 1969. Algae in Relation to Mine Water. *Castanea* 34:306-28.
3. Blum, J. L. 1956. The Ecology of River Algae. *Bot. Rev.* 22:291-341.
4. Cairns, J., Jr., and G. R. Lanza. 1972. Pollution Controlled Changes in Algal and Protozoan Communities. In Water Pollution Microbiology.
5. Environmental Protection Agency. 1971. Second Session of the Conference in the Matter of Pollution of the Interstate Waters of the Monongahela River and Its Tributaries. Pittsburgh, Pa. 175 pp.
6. Herricks, E. E., and J. Cairns, Jr. 1972. The Recovery of Stream Macrobenthic Communities from the Effects of Acid Mine Drainage. Fourth Symposium on Coal Mine Drainage. Mellon Institute, Pittsburgh. 435 pp.
7. Keller, E. C., Jr., T. Becker, H. Bennett, D. Edens, D. Garrison, W. A. Janeczek, Edward Keller III, H. Keller, F. Kopyta, N. Kowal, R. Pisapia, D. Rankin, W. J. Shoupp, J. A. Silvester, W. F. Skinner, Alan Stein. 1973. Micro-systems Simulation of Primary Production in Thermal and Acid Mine Water Loadings Related to Water Use in the Monongahela River. Final Report, OWRRI.
8. Lackey, J. B. 1938. The Flora and Fauna of Surface Waters Polluted by Acid Mine Drainage. *U.S. Public Health Reports* 34:1499-1507.
9. ———. 1939. The Aquatic Life in Waters Polluted by Acid Mine Waste. *U.S. Public Health Reports* 54:740-46.
10. Mackenthum, K. M. 1969. The Practice of Water Pollution Biology. Federal Water Pollution Control Administration, Washington. 126 pp.
11. Mackenthum, K. M., and W. Ingram. 1967. Biological Associated Problems in Freshwater Environments. Federal Water Pollution Control Administration, Washington, 126 pp.
12. Mawson, J. C., and P. J. Godfrey. 1971. Diverse A Fortran IV Program to Calculate Diversity Indices of Stream Bottom Organisms. Water Resources Research Center, Amherst, Mass. 16 pp.
13. Patrick, R., N. A. Roberts, and B. Davis. 1968. The Effect of Changes in pH on the Structure of Diatom Communities. *Not. Nat. Acad. Nat. Sci. Philadelphia* 416:1-13.
14. Ransom, J. D., and T. C. Dorris. 1972. Analysis of Benthic Community Structure in a Reservoir by Use of Diversity Indices. *The American Midland Naturalist* 87:434-47.

15. Warner, R. W. 1971. Distribution of Biota in a Stream Polluted by Acid Mine Drainage. *Ohio Journal of Science* 71:202-15.
16. Weber, C. I. 1966. Methods of Collection and Analysis of Plankton and Periphyton Samples in the Water Pollution Surveillance System. Federal Water Pollution Control Administration, Washington, 27 pp.