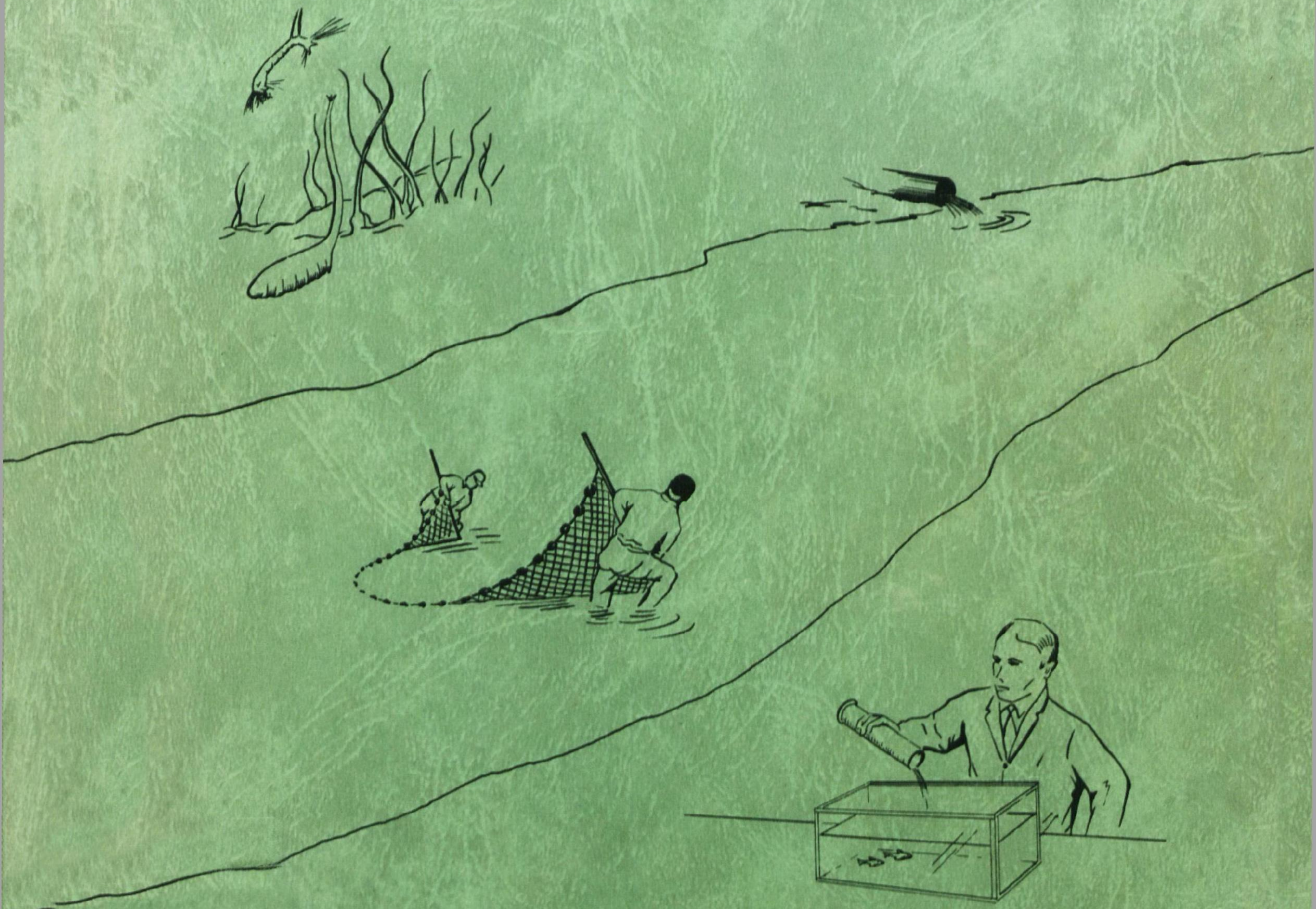
BIOLOGICAL PROBLEMS IN WATER POLLUTION



U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
Public Health Service

1957

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BIOLOGICAL PROBLEMS
IN
WATER POLLUTION

Transactions of a Seminar on Biological Problems
in Water Pollution held at the
Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio
April 23 - 27, 1956

Compiled and Edited

by

CLARENCE M. TARZWELL

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
Public Health Service
Bureau of State Services
Division of Sanitary Engineering Services
Robert A. Taft Sanitary Engineering Center

1957

PREFACE

During the past few years a number of State Health Departments and Water Pollution Control Boards have initiated or expanded investigations in the field of sanitary biology. Universities and other research organizations are showing increased interest in biological problems connected with the detection and abatement of stream pollution. A few universities are now giving courses directed toward the training of sanitary biologists, and several are considering the establishment of curricula for the training of aquatic biologists for work in the water works, sewage treatment, and stream pollution fields. In recent years industries have added sanitary biologists to their staffs, and several aquatic biologists have undertaken consultant activities.

Biologists engaged in pollution investigations and research often work alone and are somewhat isolated. For some time, therefore, there had been recognized a need for a conference of those engaged in the study of biological problems in water pollution control, to acquaint them with current developments and new methods of approach, and to enable them to become acquainted with other workers in the field. The first such gathering was held as a seminar at the Robert A. Taft Sanitary Engineering Center, April 23 - 27, 1956. The meeting was well attended, as ninety persons were registered at the Seminar. Representatives were present from twenty-eight states, the District of Columbia, and the Provinces of Ontario, Quebec, and New Brunswick, Canada. The following states were represented: Alabama, California, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Louisiana, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Montana, New York, North Carolina, Ohio, Oregon, Pennsylvania, Rhode Island, South Carolina, Tennessee, Texas, Utah, Washington, Wisconsin, and Wyoming. Participants included workers from industry, State Conservation Departments, Universities, State Boards of Health, and Stream Control Commissions. Representatives were also present from the Ohio River Valley Water Sanitation Commission, the Illinois State Natural History Survey, the Atomic Energy Commission, the U. S. Naval Radiological Defense Laboratory, and the Fish and Wildlife Service. A total of twenty-three were in attendance from the Public Health Service, six from outside the Cincinnati Area.

The Dow Chemical Company, Atlantic Refining Company, Sulphite Pulp Mfg. Research League, Institute of Paper Chemistry, General Electric Company, and Pantech, Inc. had representatives at the Seminar. Representatives were present from Conservation Departments of the following states: Alabama, Idaho, Indiana, Iowa, Michigan, Mississippi, Missouri, Montana, New York, Ohio, Pennsylvania, Tennessee, and Wyoming.

Representatives were present from sixteen universities as follows: Pennsylvania State University, University of Florida, Massachussets Institute of Technology, University of Missouri, University of Wisconsin, Michigan State University, University of Utah, Ohio State University, University of

Michigan, University of Miami, University of Southern California, University of Montreal, University of Minnesota, University of Cincinnati, University of Toronto, and University of North Carolina. The State Boards of Health of Florida, Missouri, and Illinois sent representatives as did the Stream Control Commissions of Louisiana and Wisconsin. Michigan led in the number of representatives present, having seven; Ohio was second with six; the Province of Ontario, Pensylvania, and California were third with five each; Wisconsin, Florida, and Alabama each had four; while Iowa and Missouri sent three.

The seminar consisted of panel discussions and was planned so that most of the time was devoted to commentary from the floor with only short presentations by panel members. Subjects discussed were: (1) Use and Value of Bioassays; (2) Use and Value of Biological Indicators of Pollution; (3) Current Investigations in Water Pollution Biology; (4) Water Quality Criteria for Aquatic Life; and (5) Training of Sanitary Aquatic Biologists. Discussions of each subject were lead by a panel chairman and 4 to 9 panel members, each of whom presented a phase of the problem in 10 to 20 minutes. The remainder of the day was then devoted to discussions from the floor. All presentations and discussions were off the record in order that the participants could feel free to express their opinions. All members of the panel on Biological Indicators of Pollution were asked to prepare papers to be included in the transactions of the meeting. These papers could be discussed in their panel presentations if they wished or their presentations could be entirely different. All panel members and all those in attendance were invited to submit papers for inclusion in the transactions. The transactions, therefore, are not a record of the discussions at the meeting, and the papers contained therein may or may not have been presented during the panel discussions, but are the results of the prospect or the events of the sessions.

During the meeting, discussions were free and critical. It is believed they were valuable in stimulating those in attendance and will serve to advance research and investigations in the field of water pollution. Numerous letters have been received expressing approval of the meeting and inquiring as to when another similar meeting would be held. It is planned to hold a seminar on biological problems in water pollution every three years; hence, the next meeting will occur in 1959. It is requested that all those interested in such a meeting submit their suggestions by June 1958. Suggestions are solicited as to time and length of meeting, program, and manner of carrying on the discussions.

C. M. Tarzwell

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PROGRAM

SEMINAR ON BIOLOGICAL PROBLEMS IN WATER POLLUTION

April 23 - 27, 1956

DATE & TIME	SUBJECT
Monday April 23	
8:00 - 9:00	REGISTRATION
9:00 - 9:15	WELCOME - Harry G. Hanson, Director Robert A. Taft Sanitary Engineering Center
9:15 - 9:30	PLAN OF THE SEMINAR AND ANNOUNCEMENTS - Clarence M. Tarzwell Chief of Aquatic Biology
9:30 - 4:30	Panel Discussion - Use and Value of Bio-Assays Chairman, George Burdick, Dept. of Conservation Albany, New York Extending Acute Toxicity Data to Indicate Toxicity Under Continuous Exposure George Burdick Department of Conservation Albany, New York Bio-Assays for Determining the Tainting of Fish Flesh A. W. Winston Dow Chemical Company Midland, Michigan Use of Bio-Assays in Industry W. B. Hart Director, Industrial Waste Eng. Div. Pantech, Inc. Folcroft, Pennsylvania

DATE & TIME	SUBJECT
Monday April 23 (cont'd)	Methods of Assav for Paralytic Shellfish Poison Earl F. McFarren Public Health Service Cincinnati, Ohio Application Factors to be Applied to the Bio-Assays for the Safe Disposal of Toxic Wastes Croswell Henderson Public Health Service Cincinnati Ohio
8:00 P.M.	Social Evening at Residence of C. M. Tarzwell 780 Ivy Ave. Glendale, Ohio
Tuesday April 24	
8:30 - 4:30	Panel Discussion - Use and Value of Biological Indicators of Pollution Chairman, Dr. A. R. Gaufin, University of Utah, Salt Lake City, Utah Problems in Identification of Microorganisms Dr. Wm. Bridge Cooke Public Health Service Cincinnati, Ohio Bacteria Dr. Paul Kabler Public Health Service Cincinnati, Ohio Protozoa Dr. James Lackey University of Florida Gainesville, Florida

DATE & TIME	SUBJECT
Tuesday April 24 (cont'd)	Algae Dr. C. Mervin Palmer Public Health Service Cincinnati, Ohio Fungi Dr. Wm. Bridge Cooke Public Health Service Cincinnati, Ohio Mollusca Dr. William Ingram Public Health Service Cincinnati, Ohio Macro-Invertebrates Dr. A. R. Gaufin University of Utah Salt Lake City, Utah Fishes Dr. Peter Doudoroff Public Health Service Oregon State College Corvallis, Oregon Indicators of Lake Pollution Eugene Surber Fish and Wildlife Service Atlanta, Georgia
Wednesday April 25	
8:30 - 4:30	Panel Discussion - Water Quality Criteria for Aquatic Life Chairman, Dr. O. Lloyd Meehean Assistant to the Director, U.S. Fish and Wildlife Service

DATE & TIME	SUBJECT
Wednesday April 25 (cont'd)	Oxygen Requirements Dr. William Spoor University of Cincinnati Cincinnati, Ohio Effects of Turbidity and Silt John Wilson Public Health Service Portland, Oregon Requirements for Aquatic Life Dr. O. Lloyd Meehean Fish and Wildlife Service Washington, D. C. The Status of Water Quality Criteria Dr. C. M. Tarzwell Public Health Service Cincinnati, Ohio
6:00	Group Supper - Millcroft Inn, Milford, Ohio
Thursday April 26	
8:30 - 4:30	Panel Discussion - Current Investigations in Water Pollution Biology Chairman, Kenneth M. Mackenthun, Committee on Water Pollution, Madison, Wisconsin Investigations and Problems in the Missouri Basin Dr. J. K. Neel Public Health Service Kansas City, Missouri Investigations and Problems in Alabama Dr. I. B. Byrd Alabama Dept. of Conservation Montgomery, Alabama

DATE & TIME	SUBJECT
Thursday April 26 (cont'd)	Biology and Water Pollution in Great Britain Thomas W. Beak Consulting Biologist Hawkesbury, Ontario Investigations at Oregon State College Charles Warren Department of Fish and Game Management Oregon State College Corvallis, Oregon Investigations and Problems in Ontario John Neil Ontario Dept. of Health Toronto, Ontario, Canada
4:30 - 6:00	Program Review and Tour of the Robert A. Taft Sanitary Engineering Center
6:30	Dinner - Center Cafeteria Speaker - Harry A. Faber, PHS, Washington, D.C. The PHS Research Grant Program
8:00 P.M.	Panel Discussion - The Training of Aquatic Sanitary Biologists Chairman, Dr. Herbert Jackson, Public Health Service, Cincinnati, Ohio Panel Members: Dr. Curtis L. Newcombe U.S. Naval Radiological Defense Laboratory San Francisco, California Dr. Lloyd Smith University of Minnesota St. Paul, Minnesota Dr. Charles Renn Department of Sanitary Engineering and Water Resources Johns Hopkins University Baltimore, Maryland Dr. T.H. Langlois Ohio State University Columbus, Ohio

DATE & TIME	SUBJECT
Thursday April 26 (cont'd)	
	Dr William Spoor University of Cincinnati Cincinnati Ohio
Friday April 27	
9:00 - 4:30	Panel Discussion - Current Investigations in Water Pollution Biology Chairman, Dr. A. F. Bartsch, Public Health Service, Cincinnati, Ohio Use and Value of Sewage Lagoons Dr. A. F. Bartsch Public Health Service Cincinnati, Ohio Investigations and Problems in Florida Mr. William M. Beck State Board of Health Jacksonville, Florida Investigations and Problems in Illinois Dr. William C. Starrett Illinois Natural History Survey Urbana, Illinois Investigations and Problems in the Disposal of Radioactive Wastes Dr. J. J. Davis General Electric Company Richland, Washington Investigations and Problems in Ohio John Ries Division of Wildlife Dept. of Natural Resources Columbus, Ohio

DATE & TIME	SUBJECT
Friday April 27 (cont'd)	
	The Relationship of the Polychaetous Annelid *Capitella capitata* (Fabricius) to Waste Discharges of Biological Origin Donald J. Reish Dept. of Biology & the Allan Hancock Foundation University of Southern California Los Angeles, California

List of Registrants

Seminar on Biological Problems in Water Pollution

April 23-27, 1956

Public Health Service, R. A. Taft Sanitary Eng. Center

Cincinnati, Ohio

Dr. Bertil G. Anderson	Pennsylvania State University	University Park, Pennsylvania
J. B. Anderson	PHS, Region 7	Dallas, Texas
Robert F. Balch	Inst. of Paper Chemistry	Appleton, Wis.
Dr. Robert C. Ball	Div. of Conservation Michigan State College	E. Lansing, Mich.
P. G. Barnickol	Missouri Conservation Comm.	Columbia, Mo.
Dr. A. F. Bartsch	PHS	Cincinnati, Ohio
T. W. Beak	4 Hamilton Street	Hawkesbury, Ont.
William M. Beck, Jr.	Bureau of San. Eng. Florida State Bd. of Health	Jacksonville, Fla.
B. B. Berger	PHS	Cincinnati, Ohio
L. Berner	University of Florida	Gainesville, Fla.
K. E. Biglane	Stream Control Comm.	Baton Rouge, La.
Mario Boschetti	Lawrence Experiment Station	Lawrence, Mass.
Clifford E. Bosley	Game and Fish Commission	Cheyenne, Wyo.
Arthur Bradford	Pennsylvania Fish Comm.	Harrisburg, Pa.
G. E. Burdick	Conservation Department	Albany 1, N. Y.
I. B. Byrd	Dept. of Conservation	Montgomery, Ala.
Dr. Robert S. Campbell	University of Missouri	Columbia, Mo.
Robert Cleary	Biology Bldg.	Okoboji, Iowa
Dr. Wm. Bridge Cooke	PHS	Cincinnati, Ohio
J. Davis	General Electric Co.	Richland, Wash.
Dr. Peter Doudoroff	PHS	Corvallis, Ore.

Robert J. Ellis	Inst. for Fisheries Research Michigan Dept. of Conservation	Ann Arbor, Mich.
Harry Faber	Eng. Resources Program, PHS	Washington, D. C.
Dr. Frederick Fish	PHS	Atlanta, Ga.
Dr. George P. Fitzgerald	University of Wisconsin	Madison, Wis.
Dr. Paul O. Fromm	Michigan State University	E. Lansing, Mich.
Dr. Arden R. Gaufin	University of Utah	Salt Lake City, Utah
Dick Graham	Dept. of Fish and Game	Helena, Montana
G. Hamlin	ORSANCO	Cincinnati, Ohio
Harry Hanson	PHS	Cincinnati, Ohio
Harry Harrison	Biology Building	Okoboji, Iowa
W. B. Hart	Industrial Waste Eng. Div. Pantech, Inc.	Folcroft, Pa.
Forrest Hauck	Dept. of Fish and Game	Boise, Idaho
Croswell Henderson	PHS	Cincinnati, Ohio
Karl E. Herde	AEC	Aiken, S. Carolina
John Hester	Dept. of Conservation	Montgomery, Ala.
Dr. Frank F. Hooper	Michigan Dept. of Conservation	Ann Arbor, Mich.
W. Charles Howard	PHS	Cincinnati, Ohio
Dr. Wm. Ingram	PHS	Cincinnati, Ohio
Dr. Herbert Jackson	PHS	Cincinnati, Ohio
Dr. Paul Kabler	PHS	Cincinnati, Ohio
H. D. Kelly	Dept. of Conservation	Montgomery, Ala.
Dr. M.H.A. Keenleyside	Fish. Research Bd. of Canada	St. Andrews, N.B.
Harry Kramer	PHS	Cincinnati, Ohio
Dr. James B. Lackey	University of Florida	Gainesville, Fla.
Dr. Thomas H. Langlois	Ohio State University	Columbus, Ohio

Dr. George H. Lauff	University of Michigan	Ann Arbor, Mich.
Bernard Lueck	Sulphite Pulp Mfg. Res. League	Appleton, Wis.
Kenneth M. Mackenthun	Comm. on Water Pollution	Madison, Wis.
Earl McFarren	PHS	Cincinnati, Ohio
Kneeland McNulty	Marine Laboratory	Coral Gables, Fla.
H. E. McReynolds	Ind. Dept. of Conservation	Versailles, Ind.
Dr. O. Lloyd Meehean	Fish & Wildlife Service	Washington, D. C.
R. H. Millest	Dept. of Lands and Forests	Toronto, Ontario
John L. Mohr	Univ. of Southern California	Los Angeles, California
D. D. Moss	Dept. of Conservation	Montgomery, Ala.
J. K. Neel	PHS, Region 6	Kansas City, Mo.
John H. Neil	Ontario Dept. of Health	Toronto, Ontario
Dr. Curtis L. Newcombe	U. S. Naval Radiological Def. Lab.	San Francisco, Calif.
Dr. H. P. Nicholson	PHS, Region 6	Kansas City, Mo.
George Paine	PHS	Cincinnati, Ohio
Ralph Palange	PHS	Cincinnati, Ohio
Dr. C. Mervin Palmer	PHS	Cincinnati, Ohio
Quentin Pickering	PHS	Cincinnati, Ohio
Gustave Prevost	University of Montreal	Montreal, Canada
R. Raneri	PHS	Cincinnati, Ohio
John N. Reis	Aquatic Biology Laboratory	Delaware, Ohio
Dr. S. C. Rittenberg	University of Southern Calif.	Los Angeles, Calif.
Earl T. Rose	Biology Building	Okoboji, Iowa
Donald J. Reish	University of Southern Calif.	Los Angeles, Calif.
J. D. Roseborough	Lands and Forests	Toronto, Ontario

E. R. Roth	Atlantic Refining Co.	Philadelphia, Pa.
C. E. Ruhr	State Game & Fish Comm.	Nashville, Tenn.
Robert Schiffman	Michigan State University	E. Lansing, Mich.
Lloyd L. Smith, Jr.	University of Minnesota	St. Paul, Minn.
Wesley E. Smith	Bur. of Public Health Eng. Division of Health	Jefferson City, Mo.
William G. Spence	Game and Fish Commission	Jackson, Miss.
Dr. Wm. Spoor	University of Cincinnati	Cincinnati, Ohio
John Sprague	University of Toronto	Toronto, Ontario
Dr. William C. Starrett	State Nat. Hist. Survey Div.	Urbana, Illinois
Dr. Robert E. Stevenson	University of Sourthern Calif.	Los Angeles, Calif.
Harold Streeter	ORSANCO	Cincinnati, Ohio
Eugene W. Surber	Fish and Wildlife Service	Atlanta, Georgia
Dr. C.M. Tarzwell	PHS	Cincinnati, Ohio
W.W. Towne	PHS	Cincinnati, Ohio
William J. Tucker	Dept. of Public Health	Springfield, Ill.
John E. Watson	Fish and Wildlife Service	E. Greenwich, R.I.
Charles M. Weiss	University of North Carolina	Chapel Hill, N.C.
John N. Wilson	PHS	Portland, Oregon
A.W. Winston, Jr.	Dow Chemical Company	Midland, Michigan
Charles B. Wurtz	Commercial Trust Building	Philadelphia, Pa.

1. John Wilson
2. John N. Reis
3. S. C. Rittenberger
4. Robert E. Stevenson
5. Donald J. Reish
6. Quentin Pickering
7. John Hester
8. H. D. Kelly
9. I. B. Byrd
10. D. D. Moss
11. George H. Lauff
12. T. W. Beak
13. Croswell Henderson
14. Mario Boschetti
15. J. B. Anderson
16. Clifford E. Bosley
17. William M. Beak, Jr.
18. Bernard Lueck
19. A. F. Bartsch
20. J. D. Roseborough
21. Charles M. Weiss
22. John H. Neil
23. William C. Starrett
24. John Sprague
25. James B. Lackey
26. Karl E. Herde
27. H. E. McReynolds
28. Arthur Bradford
29. J. J. Davis
30. A. W. Winston
31. Kneeland McNulty
32. George E. Burdick
33. M. H. A. Keenleyside
34. Kenneth M. Mackenthun
35. Arden R. Gaufin
36. Harry Faber
37. George P. Fitzgerald
38. Lloyd L. Smith, Jr.
39. R. H. Millest
40. Bertil G. Anderson
41. C. Mervin Palmer
42. Clarence M. Tarzwell
43. Curtis L. Newcombe
44. William J. Tucker
45. John L. Mohr
46. William M. Ingram
47. Peter Doudoroff
48. William G. Spence
49. George Paine
50. Gustave Prevost

USE AND VALUES OF BIOASSAYS

DERIVATION OF THE THRESHOLD VALUE FOR TOXICITY AND THE EQUATION OF THE CURVE BY A GRAPHICAL METHOD

G. E. Burdick
Senior Aquatic Biologist
New York State Conservation Department
Albany, N. Y.

The curvature found in graphs of toxicity when time is plotted against concentration on logarithmic paper is introduced by the failure of the effective range to conform to the axes of the paper.

For many years workers in the field of fish toxicology have been plagued by the curvilinear nature of the graphs of data that cover a wide range of concentrations. The equation must then be formulated by means of multiple degree equations, or, alternatively, the data must be transformed so it may be fitted somewhat approximately by a straight line.

Many methods have been suggested to avoid the necessity of considering the data as curves, such as the use of the reciprocal of time (Powers, 1917, and a number of English authors); the use of only that part of the data conforming most closely to a straight line (Herbert and Merkens 1952, and others) and the conventional plotting as powers, roots, or natural logarithms.

In their TL_m method Hart, Doudoroff and Breenbank (1945) avoid the production of a curve by the use of single values for specified times. However, in their estimation of biologically safe concentrations, which involves the relationship between the 24 and 48-hour TL_m an approximate straight line relationship appears to be assumed.

Shepard (1955) straightened his data on the tolerance of brook trout to low oxygen by applying as a corrective the time for death at zero oxygen.

In most cases concentrations have been plotted as logarithms and consideration of the spread of the data when times for death of individual fish are noted, also indicates time to be logarithmically distributed. When so plotted a curve results which usually can be fitted only approximately by multiple degree equations.

This observation led to an analysis of various methods of plotting and the procedures commonly used to straighten curvature. As a result of this study the hypothesis which was stated at the start of this paper was formulated.

Time would not permit the complete discussion of the reasoning and calculations which led to this supposition and the suggested method for graphical translation. This has previously been prepared and submitted to the N. Y. Fish and Game Journal for publication.

Briefly, concentration can affect time only in that range which lies between the threshold value of concentration and a minimum time for death which is independent of actual concentration. Only if both of these approached zero would the data conform to the axes of the paper. If the values were known, translation of the data could readily be accomplished by subtraction. This is not generally feasible.

Usually the minimum time for death at any concentration is small and if there is sufficient spread, this correction becomes negligible at low concentration. Progressive subtraction of different assumed values from the plotted curve in this range will produce a straight line, which is extended to cover the entire spread of the data. Only one such line can be produced on logarithmic paper and the concentration subtracted when it is formed represents the threshold value. Undercorrection produces a curve in the same direction as the original, overcorrection a curve in the reverse direction. If the subtraction is then continued over the remainder of the curve, the points gradually deviate from the straight line and approach the original curve. The space between the straight line and this produced curve represents the minimum time for death. The straight line represents a translation of the data to the axes of the paper. In order to use this method the data must include the part of the curve having maximum inflection.

The procedure also provides a method of deriving an equation representing accurately the original plotted curve. The fit on those curves tested appears better than that given by the use of multiple degree equations, while the labor is obviously much less. The equation of the straight line is first found by any of the usual methods. In this equation, the log (x - the threshold value) is substituted for log X and the log (y - the minimum time) is substituted for log Y. This then represents the equation of the original data and closeness of fit can be determined by substituting selected values of x and solving for y.

While the data to which it has been applied appear to confirm the hypothesis, it cannot yet be considered completely proved, since it should be applied to many toxicity curves. The indications are that it may prove a useful tool for the determir tion of threshold concentrations, since these are derived not as extrapolated values, but as a function of the plotted curve. Use of the straightline relationship and replotting or re-use of the corrected data would appear also to open the way for the use of a number of statistical analyses which could not be applied to curvilinear data.

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Hart, W. B., Peter Doudoroff and John Greenbank (1945)
The evaluation of the toxicity of industrial wastes, chemicals and other substances to fresh-water fishes. The Atlantic Refining Co., Philadelphia, Pa. 317 pp.

Herbert, D. W. H. and J. C. Merkens (1952)
The toxicity of KCN to rainbow trout. Jour. Exp. Biol., 29:632 - 649.

Powers, Edwin B. (1917)
The goldfish as a test animal in the study of toxicity. Ill. Biol. Monographs, 4: No 2, U. of Ill., Urbana, Ill. 73 .

Shepard, M. F. (1955)
Resistance and tolerance of young speckled trout (Salvelinus fontinalis) to oxygen lack, with special reference to low oxygen acclimatization. J. Fish. Res. Bd. of Canada, 12 (3): pp. 387-446.

INTERIM PLAN* FOR STANDARDIZING THE BIOASSAY OF PARALYTIC SHELLFISH POISON BY USE OF A REFERENCE STANDARD

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
Public Health Service
Washington 25, D. C.

INTRODUCTION

Prevention of poisoning due to eating toxic shellfish has been a problem of concern to health and fishery authorities for many years.

On May 26, 1955, the Public Health Service sponsored a conference to discuss recent developments in the assay for shellfish toxicity. The principal objective of the conference was establishment of a uniform procedure for bioassay of shellfish poison.

State and Federal agencies having representatives at the Conference included: Department of the Army, Fish and Wildlife Service, Food and Drug Administration, Massachusetts Department of Public Health, Canadian Department of National Health and Welfare, State of New York Conservation Department, and U. S. Public Health Service.

The most significant development in shellfish poison assay was reported by Dr. E. J. Schantz, Chief, Chemistry Branch, Army Chemical Corps biological research laboratory. Dr. Schantz and his associates were engaged in this field of research for several years and succeeded in isolating the poison in pure form. In working with the purified poison they also found certain color reactions, such as the Jaffe and Benedict-Bahre tests, which could serve as the basis of chemical assay for the poison. The conferees agreed that: (1) purified poison should be used as a tentative reference standard, and (2) results of future bioassays should be reported in terms of weight of poison.

The Army Chemical Corps has provided the Public Health Service with a limited quantity of purified shellfish poison for initiating standardization of the assay procedure. This plan outlines the manner in which the Public Health Service will distribute the reference standard to laboratories interested in standardizing their bioassay procedures.

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* This plan has been developed jointly by representatives of the Public Health Service and the Chemical Corps. Among those primarily responsible for preparing the technical details are Dr. E. J. Schantz, Chief, Chemistry Branch, Fort Detrick, Md.; Mr. E. T. Jensen, Acting Chief, Shellfish Sanitation Section, Milk and Food Program, Washington, D. C., Dr. K. H. Lewis, Chief, and Mr. E. F. McFarren, Chemist, Milk and Food Research, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. The background data discussed by Mr. McFarren at the seminar will be presented in a series of papers to be published elsewhere.

SECTION A

OBJECTIVES

Primary objectives as stated at the 1955 Conference on Shellfish Toxicology are: (1) National and international standardization of laboratory techniques used in assay of paralytic shellfish poison, and (2) determination of the weight of purified shellfish poison equivalent to one mouse-unit. Schantz and associates have found that approximately 0.2 µg of purified poison is equivalent to one mouse-unit. This relationship needs to be established in each laboratorv that employs the reference standard.

Secondary objectives are: (1) Accumulation of data which might be used to evaluate the statistical reliability of the bioassay, and (2) development of a chemical test to supplement or replace the bioassay.

SECTION B

DISTRIBUTION OF REFERENCE STANDARD

Laboratories interested in utilizing the purified shellfish poison for standardizing the bioassay procedure should direct their requests to Public Health Service, Washington 25, D. C., Attention: Shellfish Sanitation Section. Requests from Canadian laboratories can be considered only when endorsed by the Food and Drug Directorate, Department of National Health and Welfare, Ottawa.

In making application for the reference standard the laboratory director agrees:

(1) To use this material only for standardization of assay procedures used in connection with control or research activities on paralytic shellfish poison.

(2) To make no secondary distribution of the reference standard to other laboratories except those which are under his administrative control.

(3) To include in the standardization of the assay procedures the methods recommended herein.

(4) To furnish the Public Health Service with the accumulated standardization data and typical results of assays employing the standardized procedures.

The Public Health Service will send participating laboratories 5 to 10 ml of a reference standard containing 100 µg of poison per ml in an acidified aqueous solution. Each lot of solution will be assayed prior to mailing. Additional supplies of the standard are contingent upon receipt of data noted in item (4) above. Copies of a standardized report form (See page 30)are available from the Public Health Service.

Investigations of paralytic shellfish poison will be continued by the Public Health Service. Emphasis will be given to improvement of chemical assay methods which may supplement or replace the presently used bioassay techniques.

Requests for information or consultation on technical problems related to utilization of the reference standard or development of assay procedures should be directed to the Robert A. Taft Sanitary Engineering Center, Cincinnati 26, Ohio, Attention: Chief, Milk and Food.

SECTION C

OBLIGATIONS OF PARTICIPATING LABORATORIES

Participating laboratories have three obligations under this program:

(1) Determination of the weight of poison per mouse unit for their own laboratory's conditions. When this relation has been established, assay results can be reported in terms of micrograms of poison per 100 grams of shellfish meats.

(2) Use the purified poison as a periodic check on their operating procedures. (Experience to date indicates that CF valued 1 should not deviate from the mean by more than ± 20%. A recheck is recommended if this value is consistently exceeded).

(3) Fulfill the agreement noted in Section B.

SECTION D

PROCEDURE FOR BIOASSAY OF REFERENCE STANDARD SOLUTION

(1) Select healthy mice weighing 19 to 21 grams from the stock to be used for routine assays. If mice weighing less than 19 grams or more than 21 grams are used a correction factor must be applied to obtain the true death-time. 2/

(2) Place one ml of the Standard Reference Solution in a 100 ml volumetric flask or graduated cylinder and dilute to 100 ml with water. If kept at 3 to 4° C this solution is good for several weeks.

(3) Dilute aliquots of the above solution with distilled water until intraperitoneal injection of 1 ml doses into a few test mice causes the median death time to fall between 5 and 7 minutes. The following dilutions are suggested as a guide:

Parts of poison solution	Parts of water	ug poison per ml
10	10	0.500
10	15	0.400
10	20	0.330
10	25	0.286
10	30	0.250

1/ $CF = \frac{\text{ug poison per ml}}{\text{mouse unit poison per ml}}$

2/ See page 29 for weight correction factors.

Preparation of smaller increments of dilution, as indicated by these preliminary tests, will be necessary to obtain satisfactory results. For example, when 10 parts of poison solution in 25 parts of water kill the initial test mice in 5 to 7 minutes additional dilutions should be tested which contain 10 parts of poison solution in 24 parts of water and 10 parts of poison solution in 26 parts of water. The pH of the dilutions should be between 2 and 4 for assay and must not be higher than 4.5.

(4) Use 10 mice on each of the 2 dilutions--preferably 3 dilutions-- that fall within the 5 to 7 minute median death period. Give a 1 ml dose to each mouse by intraperitoneal injection and determine the death time as the time elapsed from completion of the injection until the last gasping breath of the mouse.

(5) Repeat the assay one or two days later on the dilutions as prepared under (3) above.

(6) Repeat the entire procedure starting with step 1.

(7) Take the median death time of the ten mice for each dilution within each of the 4 trials and determine the number of mouse units contained in one ml of each dilution from the Sommer Tables (page 11). Divide the micrograms of poison in one ml by the mouse units in one ml. The result is a conversion factor (hereafter termed the CF value) expressing the micrograms of poison equivalent to one mouse unit. Compute the average of the individual CF values and use this average value as a reference point to check routine assays. This CF value will vary from one laboratory to another depending on differences in animals and techniques. The individual CF values may be expected to vary significantly within a laboratory if techniques and mice cannot be rigidly controlled. The latter situation would require the continued use of the reference standard or a secondary standard depending upon the volume of assay work undertaken by the laboratory.

SECTION E

PROCEDURE FOR USING THE REFERENCE STANDARD WITH ROUTINE ASSAYS OF SHELLFISH PRODUCTS

The conversion factor (micrograms of poison per mouse unit), determined as indicated in Section D-7 and termed the CF value, is used to calculate the micrograms of poison in a sample of shellfish by multiplying the number of mouse units found in 100 grams of sample by the CF value.

A periodic check on the CF value should be made as follows: If shellfish products are assayed less than once each week, a check run should be made each day assays are performed by injecting 5 mice with the reference standard. If assays are made on several days during a week only one check need be made each week. The check run should be carried out on a dilution of poison such that the median death time falls between 5 and 7 minutes. The CF value thus determined should check the average CF value within ± 20%. If it does not check within this range complete a group of ten mice by adding 5 more mice to the 5 mice you have already injected, and inject a second group of 10 mice with the same dilution of poison. The CF value determined for the second group should be averaged with that of the first group and the resulting value taken as the new CF value for the conversion of mouse units to micrograms of poison. A variation of more than 20% represents a significant change in the response of the mice to the poison, or in technique of assay. Any changes of this type should be compensated by a change in the CF value.

Repeated checks of the CF value ordinarily produce consistent results within the limits prescribed above; if wider variations are encountered frequently, the possibility of uncontrolled or unrecognized variables in the test procedure should be investigated before proceeding with routine assays.

SECTION F

PROCEDURE FOR BIOASSAY OF CLAMS OR MUSSELS FOR SHELLFISH POISON 3/

(1) Preparation of Sample

a. Shucking

Thoroughly clean the outside of the shellfish with fresh water. Open by cutting the adductor muscles. Rinse the inside with fresh water to remove sand or other foreign material. Remove the meat from the shell by separating the adductor muscles and the tissue connecting at the hinge. Do not use heat or anaesthetics preparatory to opening the shell, and do not cut or damage the body of the mollusk at this state of the procedure. Collect the meats in a glazed dish until about 100-150 grams of material are obtained.

b. Draining

As soon as possible after shucking, transfer the shellfish meats to a ten-mesh sieve without layering and allow to drain for 5 minutes. Discard the drainings.

c. Grinding

Grind the meats in a meat grinder of the household type which has 1/8 to 1/4 inch holes in the grinding face, or macerate in a blender until a homogeneous mixture is obtained.

3/ Modification of a procedure adopted November 19, 1943, by the U.S. Public Health Service, U.S. Fish and Wildlife Service, and the Federal Food and Drug Administration, with excerpts from a procedure used by the Laboratory of Hygiene, Department of National Health and Welfare, Canada.

(2) Extraction

a. Weighing
Weigh out 100 grams of the well mixed material into a tared beaker.

b. Acidification
For all species of clams add 100 ml of 0. 1 N hydrochloric acid, for sea mussels add 80 ml of 0. 1 N hydrochloric acid and 20 ml of distilled water. Stir thoroughly.

c. Digestion
Heat the mixture and boil gently for 5 minutes, remove from the heat and allow to cool to room temperature.

d. pH adjustment
Adjust the cooled mixture to pH 4. 0 to 4. 5 as determined by B. D. H. Universal Indicator, brom phenol blue, congo red paper or a pH meter. To lower the pH add 5 N hydrochloric acid drop by drop with stirring. To raise the pH add 0. 1 N sodium hydroxide dropwise with constant stirring to prevent local alkalinization and consequent destruction of the poison. After adjustment of pH make the volume up to 200 ml.

e. Clarification
Stir the mixture to homogenity and allow to settle until a portion of the supernatant liquid is translucent and can be decanted off free of solid particles large enough to block a 26 gauge hypodermic needle. If necessary it may be centrifuged (5 minutes at 3000 r.p.m.) or filtered through filter paper. It is necessary to obtain only enough liquid to carry out the bioassay.

(3) Mouse Test

a. Inoculation
Select mice weighing between 18 and 22 grams, if possible, and never over 25 grams. The mice should be of the same strain as used in the standardization procedure (Sections D and E). Inoculate each test mouse intraperitoneally with 1 cc of the acid extract obtained in F-2 above. Note time of inoculation and observe mice carefully for time of death as indicated by the last gasping breath. Record time by means of a stop watch or clock with a sweep second hand. One mouse may be used for the initial determination but 2 or 3 are preferred. If the death time or median death time of several mice is less than 5:00 minutes, make a dilution so as to obtain death

times between 5 and 7 minutes. If the death time of one or two mice injected with an undiluted sample is greater than 7 minutes but one or more mice do die, a total of at least three mice need to be inoculated in order to determine the toxicity of the sample with confidence. If large dilutions are necessary, the pH of the dilution should be adjusted by addition of dilute hydrochloric acid (0.1 or 0.01 N) dropwise so that the pH is between 3.5 and 4.5 (and never higher than 4.5). Inoculate three mice with the dilution that gives death times between 5 and 7 minutes (death determined as the time of the last gasp).

b. Calculation of Toxicity

Determine the median death times4/ of the mice, and from Sommer's table determine the number of mouse units corresponding to the median death time. If test animals weigh less than 18 grams or more than 22 grams, a weight correction must be made for each mouse by determining the mouse units corresponding to the death time for that mouse from Sommer's table, multiplying this value by the correction factor for that mouse from Sommer's table, and then determining the median mouse unit for the mice. To determine the amount of poison per 100 grams of meat multiply the median mouse-unit by the dilution factor used in obtaining this unit and then by 200, since the original clam extract was made up to 200 ml. Convert the mouse units to micrograms of poison per 100 grams of meat by multiplying by the CF value.

4/ Include survivors in determination of median death time.

TIME - DOSAGE RELATIONS FOR PARALYTIC SHELLFISH POISON (ACID) 5/

TIME	DOSE	TIME	DOSE	WEIGHT OF MICE	DOSE
		5:00	1.92	10 gm.	0.50
		05	1.89	10-1/2	.53
1:08	100	10	1.86	11	.56
10	66.2	15	1.83	11-1/2	.59
15	38.3	20	1.80	12	.62
20	26.4	30	1.74	12-1/2	.65
25	20.7	40	1.69	13	.675
30	16.5	45	1.67	13-1/2	.70
35	13.9	50	1.64	14	.73
40	11.9	6:00	1.60	14-1/2	.76
45	10.4	15	1.54	15	.785
50	9.33	30	1.48	15-1/2	.81
55	8.42	45	1.43	16	.84
2:00	7.67	7:00	1.39	16-1/2	.86
05	7.04	15	1.35	17	.88
10	6.52	30	1.31	17-1/2	.905
15	6.06	45	1.28	18	.93
20	5.66	8:00	1.25	18-1/2	.95
25	5.32	15	1.22	19	.97
30	5.00	30	1.20	19-1/2	.985
35	4.73	45	1.18		
40	4.48	9:00	1.16	20	1.000
45	4.26	30	1.13		
50	4.06	10:00	1.11	20-1/2	1.015
55	3.88	30	1.09	21	1.03
3:00	3.70	11:00	1.075	21-1/2	1.04
05	3.57	30	1.06	22	1.05
10	3.43	12:00	1.05	22-1/2	1.06
15	3.31	13	1.03	23	1.07
20	3.19	14	1.015	23-1/2	1.075
25	3.08			24	1.08
30	2.98	15	1.000	24-1/2	1.085
35	2.88			25	1.09
40	2.79	16	0.99	26/27	1.10
45	2.71	17	0.98	28/29	1.11
50	2.63	18	0.972	30	1.12
55	2.56	19	0.965		
4:00	2.50	20	0.96		
05	2.44	21	0.954		
10	2.38	22	0.948		
15	2.32	23	0.942		
20	2.26	24	0.937		
25	2.21	25	0.934		
30	2.16	30	0.917		
35	2.12	40	0.898		
40	2.08	60	0.875		
45	2.04				
50	2.00				
55	1.96				

5/ These data are for IP injections as determined by Dr. Sommer, and are based on the data furnished by him to workers in the field.

FORM FOR REPORTING ASSAY DATA ON REFERENCE STANDARD SHELLFISH POISON SOLUTION

Log Number Reference Standard or RSS* ______________________

Laboratory ______________________

Date of Assay ______________________

Date Reference Standard Solution was prepared or received ______________________

Date of preparation of 1 to 100 dilution of RSS* ______________________

Name of Assayer ______________________

Strain of mice ______________________

Data on individual mice ______________________

		Dilution		
		1	2	3
g poison per ml				
pH of dilution				
Mouse No.	Mouse Weight (gm)	death time (seconds)**	death time (seconds)**	death time (seconds)**
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
Median death time***				
Mouse units per ml				
CF value = $\frac{\text{g poison per ml}}{\text{Mu poison per ml}}$				

Send 3 copies of data sheets to: Sanitary Engineering Center, Cincinnati 26, Ohio Attn: Chief, Milk and Food.

*RSS- Reference Standard Solution.

** Death time from completion of injection to last gasp.

***Include survivors in determination of median death time.

APPLICATION FACTORS TO BE APPLIED TO BIOASSAYS FOR THE SAFE DISPOSAL OF TOXIC WASTES

Croswell Henderson

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The tremendous expansion of the chemical industry, especially that dealing with petrochemicals, the newer metals, synthetic fibers, insecticides, and detergents, presents new and difficult problems in the protection of aquatic life from effects of industrial pollution. Necessary chemical methods for the detection and measurement of many substances in these complex wastes have not been developed. Even when chemical methods are available, toxicity information generally is not, nor can toxicity always be attributed to one or more simple materials. In complex wastes there may be a number of different toxicants or there may be synergy or antagonism between substances so that the toxicity of final effluents may be entirely different from that of its components.

By using bioassays correctly, the effect on aquatic life of complex industrial wastes may be determined directly. Relatively simple bioassay methods have been devised and are being currently used. The procedures recommended by the subcommittee on toxicity of the Sewage and Industrial Wastes Federation (1) which are based in part on research previously reported by Hart, Doudoroff, and Greenbank (2) are quite satisfactory.

The basic bioassay procedure consists essentially of preparing different concentrations of an effluent or other test material with a selected dilution water, adding the test fish and observing their reactions over a definite time period. A logarithmic series of concentrations is generally most convenient.

For effluents of unknown toxicity, it is desirable to make exploratory or small scale tests to determine the approximate toxic range. Two fish are added to 2 liters of each test solution over a wide range of concentrations; e.g., 100, 10, 1, and 0.1 percent effluent. Observations over a short time period will indicate the necessary concentrations for the full scale experiments.

For the full scale tests, it is desirable to use a minimum of ten fish for each test concentration. This may be conveniently done in five gallon wide mouth glass bottles, using five fish in ten liters with duplicate samples. A series of intermediate concentrations are set up in the range indicated by the exploratory tests. For example, if the exploratory tests indicate an effect on fish between 10 and 1 percent, concentrations of 10, 5.6, 3.2, 1.8, and 1.0 percent, or in some cases intermediates between these are set up and fish added to each.

The dilution water used should be water from the receiving stream above the effluent outlet, if suitable for fish, or water of similar characteristics, particularly with respect to pH, alkalinity, and hardness.

The test fish should be a species adaptable to laboratory conditions such as temperature, feeding and handling, should be of relatively small size and readily available. Fathead minnows (Pimephales promelas) and bluegill sunfish (Lepomis macrochirus) from 1-1/2 to 2-1/2 inches long and weighing from 1 to 2-1/2 grams have been satisfactory for work in several laboratories. Many other species may be suitable. In many cases it is preferable to use a species native to the receiving water or at least one directly comparable.

The above tests are designed so that no oxygenation or aeration is generally necessary. Atmospheric absorption of oxygen by the exposed water surface adequately takes care of the requirements of the fish during the test period. However, if strong concentrations of high oxygen demand effluents are being tested, oxygen or air may be needed. Such methods as solution renewal at definite time intervals, introducing air or oxygen at a specific rate and maintaining an oxygen interface over the solution in the test jar, have been used successfully. Physical and chemical determinations such as temperature, dissolved oxygen, pH, alkalinity, acidity, hardness, etc., are made periodically during the course of the bioassays.

Observations as to the fish reactions are generally made for a 96 hour period. Twenty-four, 48, and 96 hour TL_m (median tolerance limit-concentration which causes 50% mortality) values are estimated through straight line graphical interpolation.

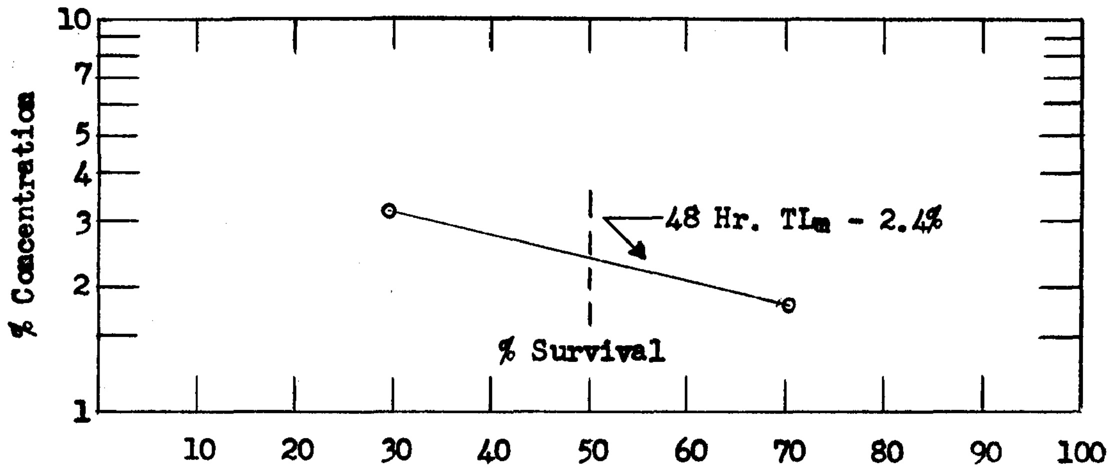
Example - 5.6% - All fish dead in 48 hrs.

3.2% - 3 of 10 fish survive 48 hrs.

1.8% - 7 of 10 fish survive 48 hrs.

1.0% - All fish survive 48 hrs.

Controls - All fish survive 48 hrs.



Once the TL_m value has been obtained, the next step is to determine how to use it. Obviously a concentration that will kill 50% of a test species is not safe for aquatic life. Liberal application factors (sometimes erroneously called safety factors) must be applied. Methods of applying laboratory bioassays have received very little attention. Industry and others are now using bioassays to some extent and more are ready to use them if provided a relatively simple bioassay procedure and reasonable methods of applying the results.

Stepwise, a proposed method of applying bioassays to meet specific industrial waste problems is as follows:

(1) Perform laboratory bioassays to obtain TL_m values.

(2) Calculate the dilution water required.

Example: 48 hour TL_m - 2.4%

Effluent flow - 10 cfs

Dilution Ratio - 2.4:97.6 or 1:40

10 cfs effluent would require 400 cfs dilution water from the receiving stream to produce a condition which would kill half of the test fish in 48 hours.

(3) Develop and apply a numerical factor that will provide safety to aquatic life in the receiving water. This can be in the form of a whole number when applied to dilution water needed or a fractional value of the TL_m when applied to the effluent.

There are many individual factors to be considered in developing a single application factor for specific wastes. The major ones may be grouped as follows:

A. Relating laboratory bioassays to actual conditions.

(1) Conversion from 50% to 100% survival.

(2) Conversion of acute or short term toxicity to possible long term or chronic effects of a toxicant.

(3) Conversion of toxicity in non-renewed or static test solutions to continuous flow conditions.

B. Relating test fish to other aquatic life.

(1) Effect on test fish versus other more sensitive fish species that may be present in the receiving stream.

(2) Other stages in the life cycle such as fry or eggs may be more sensitive.

(3) Some of the major fish food organisms may be more sensitive.

C. Variability in the toxicity of effluents which may fluctuate considerably over a period of time. Bioassays with 24 hour composite samples of effluents from various industries over a three month period, have produced the following results:

Type of Industry		No. of Samples	48 hr. TL_m % Concentration Maximum Toxicity	Minimum Toxicity	Average Toxicity
Petrochemical		3	2.6	22	9.6
Oil Refinery		2	37	40	38.5
By products coke		4	3.7	13.5	9.0
Sewage plant (containing industrial wastes)		4	3.3	22	10.2
Chemical & dye	1	6	4.4	16	11.9
"	2	6	13.5	28	20.0
"	3	7	4.2	24	8.5

It is evident that any one sample taken for bioassay at a particular time may give erroneous results. Either a large number of samples would have to be tested or some factor developed to provide for conditions of maximum toxicity. The above factors are generally of concern in all cases and are not provided for in the experimental procedures.

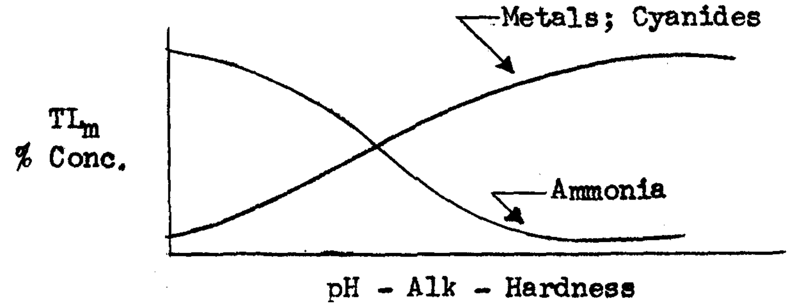
D. Other individual factors may have considerable effect on toxicity but are generally provided for in the tests. Any major differences between test and actual conditions in the following characteristics must be considered.

(1) Change in water quality characteristics

Temperature and Dissolved oxygen - Toxicity is generally greater at higher temperatures and lower dissolved oxygen.

pH, Alkalinity, Hardness - These characteristics are somewhat interdependent. The buffering capacity would determine the pH which may have an effect on the toxicity.

Example - The toxicity of metals, cyanides, ammonia, and many other toxicants is greatly influenced by pH and other characteristics.



(2) Synergy and antagonism - A combination of materials may produce a more toxic or less toxic effect. It has been reported that copper and zinc in the same solution will produce an effluent eight times more toxic than copper alone. Likewise a material such as calcium may have an antagonistic effect (3).

The addition of several different effluents to a receiving water may give an entirely different toxicity picture from that of the separate effluents. For example, one industry may release an effluent containing metals into a hard water stream. The metals may precipitate or complex into insoluble compounds with materials in the stream waters and become relatively non toxic to aquatic life. Further downstream, another industry may release an acid effluent, lowering the pH and causing the metal to go into solution and become highly toxic. Bioassays with receiving waters would indicate the toxicity but the toxic materials may be attributed to the wrong industry. For this and other reasons, a knowledge of the chemical composition of the effluents and what reactions may be expected are of extreme importance.

If the above factors are carefully examined and related to established experimental evidence, sizeable application factors are apparent. An application factor of 10 times the 48 hour TL_m has been tentatively suggested. Circumstances in a particular study may raise or lower this value. Some individual factors, as already mentioned, may add to the above figure. Others which may detract some from the magnitude of the figure and must be considered are natural purification, loss of volatiles, oxidation or hydrolysis to non toxic products, precipitation, complexing, antagonism, etc.

The following example may illustrate a means of arriving at an application factor, providing the necessary information is available or can be obtained:

Factor	How Derived	Numerical Value
A. Test vs Actual conditions	Experiments Research	2
B. Test fish vs other aquatic life	Experiments Research	3
C. Variability of effluents	Experiments Research	2
D. Other variables (see page 34)	Provided for in tests	-

Application Factor = 2 x 3 x 2 = 12

Using the previous example of dilution water required for 10 cfs of an effluent having a 48 hr. TL_m value of 2.4%.

400 x 12 = 4800 cfs dilution water required for safety to aquatic life.

It is evident that considerable research is necessary before definite factors can be assigned which will be accepted. Good experimental evidence and a definite basis for the derivation of these factors will have to be available. Factors should be suggested which will adequately do the job and yet not cause undue expense for treatment.

Several methods of approach to this problem are suggested. The laboratory fundamental research program may give some definite answers or at least methods of mathematical expression. These could consist of long time continuous flow experiments with different wastes or waste components using various species of fish, different life history stages, and other aquatic organisms. The information could be mathematically related to our short term static bioassay. Other fundamental knowledge needed which could be obtained is the effect on toxicity of changes in water quality characteristics such as turbidity, temperature, dissolved oxygen, pH, alkalinity, hardness, etc. Programs to obtain this information could be and to some extent are being carried out here at the Sanitary Engineering Center, as well as in states, universities, industrial laboratories, and other research institutions.

Another approach which may give partial but more immediate answers is the field approach of working with actual effluents under field conditions. Bioassays could be made of the effluents from a particular industry or group of industries. Operating conditions, effluent flows, stream flows, and other conditions could be obtained. A study of the biota of the receiving waters could detect what is happening under present operating conditions. Study of several plants connected with a particular type of industry may give evidence as to the magnitude of the application factor needed. The information may also be valid for related industries with similar types of effluents.

An ideal situation for a study of this type would be one continuously operating plant on a stream which may lagoon or regulate waste flows. The effect on the biota may be determined at different flow rates and the application factor estimated which affords protection to aquatic life.

These problems can be worked on for an extended period and still no mathematically precise answer obtained as with certain physical and chemical phenomena. However, a reasonably accurate estimate may be obtained. With proper application factors conditions may be estimated which are reasonably safe for aquatic life. How satisfactory they are can only be determined by studying the stream biota after a period of operation when they are in effect.

There are many ways in which industry can use bioassays. Toxicity of final effluents and the probable effects on receiving waters can be determined. Toxic components can be traced in process effluents, which may permit the treatment of much smaller quantities of waste. The effectiveness of treatment processes may be established. In the location of new plants, the amount and quantity of dilution water necessary or the degree of treatment of wastes may be ascertained in advance of construction.

It is believed that the greatest use will be made of bioassays if the methods are relatively simple and easy to use. Reliable and suitable application factors should be developed.

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USE AND VALUE OF BIOLOGICAL INDICATORS OF POLLUTION

SOME PROBLEMS IN THE IDENTIFICATION OF MICROORGAMISMS

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In studying the populations of algae and fungi on high-rate and standard-rate trickling filters recently, it was found that within groups of well known organisms, such as algae, growing in this habitat there were problems of identification which were difficult if not impossible to solve using preserved materials. For instance, in the case of a coccoid green alga, would you feel confident in determining from a pickled sample whether the cells belonged to Chlamydomonas, Chlorococcum, Protococcus, Palmellococcus, some other coccoid genus, or were only early palmelloid stage cells of Stigeoclonium? Under certain conditions of observation after preservation of a sample in the field any one or several of these names could be applied to a collection. In the case of blue green filamentous algae, could you tell from a preserved specimen which had been broken up in a Waring Blendor so that filament length or branching were not factors in the collection, whether you had Oscillatoria, Phormidium, Lyngbya, or some other form? Yet, we had such problems to answer. Several specialists in the field of green algae were consulted on the identification of the coccoid organisms, and only after prolonged study and as a result of familiarity with the groups did G. W. Prescott come up with an answer which admittedly was only tentative. Dr. Drouet was sent material of the filamentous blue-green, which turned out to be an Amphithrix.

This experience led me to the idea that it might be well to develop a discussion of the problem of the identification of organisms that it is hoped may be used as indicative of pollution by their presence or absence in a stream. Some of the pitfalls in the identification of such organisms may be mentioned.

In the taxonomy and systematics of any group of organisms, plant or animal, there are usually two schools of thought. In the colloquial language of the trade of taxonomy these are referred to as the splitters and the lumpers. Modern taxonomy has shown that there is room for both, but in the past the matter has had the finality of either/or. On the basis of minute variations, some species of organisms have been split into many species, while without visible differences but with microscopic variation not visible to a casual observer, species have been lumped together.

In the early years of systematic biology the cataloging of the animals and vegetation of the earth's surface was of prime importance. As time went on, it became important to integrate the studies so begun, and for various regions floras and faunas were produced; lists, annotated or not, of various organisms were developed; and monographic treatments of genera, families, orders, and even classes were attempted. As mountains of materials were built up in the herbaria, museums, and culture collection of the world, more and more such synthesizing of our knowledge could be endeavored. Much of this work was done with the assumption, stated or implied, that certain barriers existed between species, barriers which prevented exchange of genetic materials between populations. When such barriers were based on political lines, as in the older works and the earlier catalogues, they were unnatural and the assumption of separate populations invalid within certain groups. Depending on the members of the populations, many geographic barriers may be considered valid, others not.

Where specimens were not available to a student of a group, where reliance had to be placed on records regardless of reliability, the development of generic monographs without regard to unnatural barriers resulted in burdensome lists of species in excess of numbers which could be supposed with little effort to by synonymous. Where specimens were available and critical interpretation of parallel geographic species was carried out, the lists became shorter. Where undue emphasis was placed on minute detail as valid bases for species separation, lists of species became quite lengthy. Where minute detail was considered a non sequitur for species criteria but the result of individual variation, species lists shrank. Based on specimens of leaves from one tree, a species has been based on the shade leaves of the common beech, another on the sun leaves.

In the genetics laboratory, an elementary problem is occassionally presented in which the common fruit-fly, *Drosophila melanogaster*, is used. A group of females of one type is mated with a group of males of another. The types are chosen with the idea of developing the concepts involved in the statistical study of Mendelian ratios. The progeny of such crosses are obviously different from or similar to their parents. Yet if a taxonomist of the pre-Mendelian school were to observe these he could easily find the bases for several species of *Drosophila*, if not for new genera.

These introductory remarks are used to indicate that as our knowledge of life and our ability to classify its various aspects progress with the increasing knowledge from various fields such as morphology, cytology, anatomy, physiology, genetics, etc., the concepts of species and other categories change. As we find more and more specimens of organisms, some of which appear to vary markedly from known species, our concepts of various groups change. Various myths of taxonomy become exposed as additional materials are obtained. For instance, Dr. W. M. Ingram mentioned recently that many species of Hawaiian snails were shown to have been invalidly proposed when it was discovered that the basic character, that of color of shell, was found to have been altered for gain by those paid to collect additional color variants.

The use of dried specimens of plants, skins of animals, prepared mounts of micro-fauna, dried and otherwise prepared materials of algae, fungi and bacteria, has its place in modern taxonomy. At one time these were the only type of material on which concepts of species were based. More and more, modern taxonomy is using such materials as "type" materials or as records of past accomplishment and historical fact. The type specimen is the basis on which the species is founded, but the living specimen is the basis on which species in many groups of organisms are best known and studied today. The geneticist needs living material for various types of genetic manipulation, as do the physiologist, the experimental morphologist, and the other scientists who base their work on the types of activity used by the first three mentioned. The systematic botanist today more and more uses genetic techniques for proving or denying the existance of one species or another in developing generic monographs. Experimental taxonomy is being actively used in many fields of botanical and zoological classification as well as in bacteriological and mycological systematics. In many cases, not only taxonomy and genetics are being learned in this way, but, at the same time and with the same organisms, many bits of information useful in "pure" and "applied" science are being discovered.

The present state of systematic identification of micro- and macro-organisms in the average field laboratory is based largely on well established texts in which a limited number of organisms are described and illustrated. Such texts may be considered comparable to a mushroom handbook in which only the most obvious form is illustrated with a photo taken more or less recently or a painting or a pencil sketch made more or less accurately within the last half century. Such description and nomenclature as may be used do not take into consideration the wide variation possible within the species, nor do they consider the fact that two species of mushroom obviously identical to the naked eye may be quite different both in respect to edibility and to micromorphology. The process of revising a genus of bacteria, fungi, algae, protozoans, mollusks, diatoms, or any other group, is long and laborious. Unless it is a genus which includes species of great economic importance such as Penicillium or Aspergillus, the work must be carried out in the "ivory tower" of the University or the Academy, otherwise it will interfere with or conflict with the interests of the employer whose business is the practical application of one process or another, of one species or another, or the development of one product or another.

In the development of a taxonomy which is adequate to the needs of the student of the biological indicators of pollution, not only at the level of specialization represented by the members of this panel but also at the level of usability of the man in the field, many factors will have to be taken into consideration. Not the least of these is the development of monographic studies at the genus or family level, in which by use of the techniques of experimental taxonomy a species may be given a more sound definition comprehensible to the man in the field whether he be biologist, engineer, or chemist, for it is rare that all three professions will be represented adequately on any field staff. Such dynamic rather than static taxonomic studies should be carried out in all fields in which organisms occur that may be found under varying conditions and types of pollution. The studies should overlap into other fields, since organisms occurring in or prevented from occurring in polluted waters may also be of importance in agriculture, forestry, food processing, industrial production, and other fields. Information from the laboratory should be available concerning the pollution tolerances of such species. Following the production of the monographic studies, the information contained therein of interest to the pollution biologist should be gathered together and made available to him in language understandable by a relatively unspecialized college graduate.

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Such studies, properly presented, should enable a pollution biologist to interpret readily the role of the more important organisms in the stream, sewage treatment plant, or possibly even water treatment plant under his jurisdiction, in terms of work load or indicator value of which those organisms are capable, much as the forester can determine the possibilities of his forest in terms of board feet of lumber available and potential crop replacement.

USE AND VALUE OF BACTERIAL INDICATORS OF POLLUTION

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Normal Intestinal Bacteria

Following Pasteur's epic work and Kock's discovery of solid media, rapid progress was made in the isolation and description of the bacterial species commonly found in the intestinal tract.

S. typhosa (B. typhosus) described by Eberth in 1880.

Vibrio cholera described by Kock in 1884.

E. coli (Bacillus coli) described by Escherich in 1886.

It was clearly shown that E. typhosa, V. cholera, and the Salmonellae were associated with enteric disorders and it was hoped that suitable procedures for the identification of these and other etiological agents of human disease might be developed for use as indices of drinking water quality. Several ingenious techniques were developed and remain with certain modifications as valuable research tools. There are many instances on record in which specific pathogenic bacteria have been isolated from suspected water sources, however, such attempts almost always ended in failure when used as a routine test for water quality. It is, there fore, apparent that attempts to isolate pathogenic microorganisms from a water supply are not recommended in the routine examination as an index of pollution for the following reasons:

1. Present available methods are tedious, laborious, and require high technical skill.

2. By the time a pathogen is recovered from a water the harm has long been done.

Subsequent to the demonstration by Escherich that E. coli was a normal intestinal organism, numerous investigators showed that E. coli and related organisms were consistently present in sewage, polluted streams, lakes and wells and in contaminated soil. These data showed that:

1. Coliform organisms are normally present in the feces of all warm blooded animals.

2. Some coliforms are to be found on plants and grains.

3. Feces contain from 5 to 500 million coliforms per gram, about 60 to 95% of which are E. coli.

4. A. aerogenes usually numbers 10,000 to 500,000 per gram of feces.

5. Coliform organisms are rarely found in virgin soil, but may be numerous in cultivated soils. From 65 to 80% may be A. aerogenes.

6. Coliforms may survive weeks or months in fresh water, the predominating type varying with environmental conditions.

7. Coliforms may live in dry soil 45 to 100 days, but in moist soil may survive a year or more.

Because the coliform group is constantly present in alimentary discharges, and because of the comparative ease of enumerating them, the coliform organisms have become the accepted indicator of fecal pollution Also because of the fact that water containing fecal pollution may contain intestinal pathogens, the "coliform test" has assumed importance as a criterion in judging the sanitary quality of water.

The Coliform Group

"The coliform group shall include all of the aerobic and facultative anaerobic Gram-negative nonspore-forming bacilli which ferment lactose with gas formation in 48 hrs. at 35° C." (Standard Methods for the Examination of Water and Sewage, 10th edition, p. 375).

The first coliform test was devised at the New York State Department of Health Laboratories by Theobald Smith in 1893 using dextrose broth and first edition of "Standard Methods" was published in 1905. The current edition (10th) of Standard Methods provides for no differentiation of "fecal" and "nonfecal" types. It is stated, "Such differentiation is of little moment in determining the suitability of water for human consumption, as contamination with either type of waste renders the water potentially unsatisfactory and of unsafe sanitary quality". Current methods have certain recognized imperfections as to time required, specificity and reproducibility. Continuous efforts have been directed toward development of better methodology. Some improvements have been:

1. Substitution of lactose for glucose in the presumptive medium, which materially reduced the number of presumptive false positive tests.

2. Churchman's observation that some dyes will selectively inhibit certain species or group of Gram-positive and of spore-forming organisms.

3. Modifications of incubation times, amount of gas, media ingredients and incubation temperatures.

    a. Bile salts by MacConkey (1908).

    b. Perry and Hajna modified Eijkmann test (1933).

    c. McCrady, brilliant green bile (1937).

    d. Mallmann, lauryl sulphate tryptose broth (1941).

The adoption of brilliant green lactose bile broth as a confirmatory medium is perhaps the most important advance in methods in the last fifty years. The use of lauryl sulfate tryptose broth in the presumptive test is also an important improvement.

Recently suggested (tentative method only) membrane filter techniques may prove to have far reaching applications.

Other Organisms as Pollution Indicators

Streptococci

A leading "pretender" as an indicator of pollution is the *Streptococcus* (Enterococcus).

The *Streptococci* as a group have wide distribution. Many of them are pathogenic for man and animals. Some strains are strict parasites while others are saprophytic in existence. Many are fastidious in their growth requirements while others will grow with ease.

The sewage *Streptococcus* or *Enterococcus* have been proposed as an indicator organism on the basis that:

1. They are present in feces and sewage and are found in known polluted waters.

2. They are not found in pure waters, virgin soil and sites out of contact with human and animal life.

3. They do not multiply outside the animal body (except in such media as milk). (Suckling, Exam. Waters & Water Supplies [1943], Blakiston & Co.)

There is incomplete agreement on these three points. Evidence indicates the *Enterococci* do not multiply in water. Whether they persist longer in water or disappear at about the same rate as coliforms is a disputed point.

The sewage *Streptococci* are never present in as large numbers as coliforms. The *Streptococcus* coliform ratio varies from 1/100 down to 1/10. As the *Streptococcus* detection methods are apparently no more sensitive than coliform methods, and as the pollution density is less, this would have the practical effect of reducing the sensitivity of the method.

Streptococcus classification methods have attempted to distinguish between human and animal strains. Mannitol-positive strains "do appear more common in human feces and raffinose-positive strains more common in bovine feces," but separation on this basis has no place in evaluating the sanitary quality of a water supply.

Streptococcus methods offer a promising line of investigation. At this time the *Streptococcus* index in not a satisfactory substitute for the coliform group. Its greatest value is as a corroborative test where the coliform datum is suspected.

The Anaerobes

Spore-forming, lactose-fermenting anaerobes have been used as indicator organisms in water. The anaerobic organism is usually referred to as Clostridium welchii in England and Clostridium perfringens in America.

The spore-forming Clostridium is unsuitable as an indicator organism for the following reasons:

1. They are extremely resistant to destruction and survive for long periods.

2. They are abundant in animal manure, cultivated soil, and decomposing organic material.

3. They fail to correlate with results of sanitary surveys.

4. There are small differences in their numbers in heavily polluted and pure waters and they give no evidence of the degree of pollution.

The major interest in anaerobes at present centers about the occasional false positive presumptive test and its carry-over to brilliant green bile broth tube with gas and decolorization. The anaerobe can be eliminated by its failure to grow in formate ricinoleate broth.

The problem of finding a better indicator organism is one of paramount interest. Some show promise, but none at present compete with the fifty odd years of experience with the coliform estimation and its correlated data.

Escherichia coli

Originally Bacterium coli was considered to be a single species. As new methods and tests were devised, this so-called single species was divided into more and more species. Now this group of organisms is considered under the collective term of "coliform organisms"

Hundreds of research workers have added their contributions to the studies to determine the characteristics of the bacteria included in the coliform group, so that the ideal bacterial criterion of pollution might be defined. From this mass of data has evolved a classification of the coliforms into the coli group, the intermediate group, and the Aerobacter group. These differences are not sharp, well-defined distinctions, but are rather differences that shade from one variety to another on the basis of the interpretation of results from multiple testing procedures. It is further complicated by the question of what intermediate varieties to include with the E. coli as pollution indicator organisms.

Lewis and Pittman found no significant difference in the ratio of E. coli to coliforms in polluted water and in water of high sanitary quality. Ruchhoft, et al., found the ratio of E. coli to coliforms in a sewage effluent and at the pumping station to be about the same although the coliform M. P. N. counts were 13,815 and 0.219 per milliliter, respectively. Taylor found the E. coli ratio of no help in estimating the sanitary quality of water in English lakes.

A summation of the evidence of many workers warrants the following conclusions.

1. The ratio of E. coli to that of other coliforms is not an index of water quality.

2. Presence of E. coli usually is no more significant than other members of the coliform group.

3. The additional labor involved in the routine identification of E. coli in the presence of other coliforms is not justified on the basis of the information attained.

4. The sanitary quality of water is dependent on the total number of coliform organisms present. Density is an indication of pollution.

Summary

From a bacteriological point of view the coliform group is currently the best available indicator for use in the estimation of pollution of waters and in the sanitary evaluation of pure waters of potable quality. The coliform test is a quantitative test. Significance of its interpretation is dependent on determining the density (or most probable number). Only when the count exceeds a normally expected minimum number of coliform organisms is pollution indicated. Few samples of water are completely free from coliforms when a sufficiently large sample is examined. The established standard of the Public Health Service for drinking water recognizes the possible occurrence of an occasional coliform.

In the foregoing discussion it has been evident that pathogenic organisms cannot be used as indicator organisms. Their unsuitability is based on infection of the public before their discovery, their abnormal occurrence in the feces of a small percent of any group, and their small number in comparison to other intestinal type organisms.

It is freely admitted that there are normally found in the intestinal tract of man many types of organisms not belonging to the "coliform group". Some of these organisms at various times have been considered as "indicator organisms". The available data eliminated each for one or more reasons.

The substitution of E. coli for the present coliform group is unsuitable because it gives no additional information for the increased labor and because there are not sufficient epidemic studies to interpret the E. coli index.

Butterfield stated that ". . . . while it is desirable to seek for better bacterial criteria of pollution in water, and these researches should be continued, the results of such studies to date have not produced a criterion which may be considered to have satisfactorily replaced the coliform group." This summation is equally true today.

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PROTOZOA AS INDICATORS OF THE ECOLOGICAL CONDITION OF A BODY OF WATER

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Introduction

The topic as assigned by the program chairman was limited to protozoa. "Protista" would be a better term for several reasons. One is that much of this work has been concerned with various species of colored flagellates, whereas only a few colorless flagellates today are assigned to the protozoa and even these are regarded as colorless algae by some workers. Another is that very few ciliates are regarded as indicator species - some ciliates seem to prefer anaerobic habitats and others aerobic, but there is little to indicate a marked preference for pollution by any of them. The third reason is that the Sarcodina have been far too little investigated in respect to pollution. There is some indication that a small group of the minute amoebas are characteristic of certain stages of domestic sewage treatment but the larger naked amoebas are rarely abundant anywhere. Decloitre (1) is currently tending to show that the thecate amoebas are cosmopolitan, whereas there has been a tendency to regard most of them as sphagnum bog types. Foraminifera and Radiolaria we know are marine - the former in the ooze, the latter pelagic. For these reasons, any discussion should be more widespread than the colorless flagellates, the ciliates and the amoebas.

The Varying Nature of Environments

An organism generally occupies a given niche permanently because that niche offers the most favorable environment for it. Such occupation implies choice on the part of the organism. It does not take into account crowding or other competition, changing the environment as by shutting off light, being forcibly removed or retained as by a current, etc. In other words, we too frequently assume an environment to be relatively constant, whereas it is anything but constant and the organisms in it either must be adaptable to change, or migrate, or die. New kinds, suited to the new environment, may replace them and our concept must be flexible to take care of such changes. There was a lake in Florida which was often referred to as "a good bass lake." Within one growing season, it became covered with water hyacinths and the bass disappeared, migrating downstream. This did not alter the concept implied by the phrase "good bass lake," but it certainly showed that such concepts are applicable to transient situations.

Actually, we talk as if we are stating a constant. Over a broad environmental range, such relative constants actually exist. Thus a coal mine stream, among other things may constantly contain enough sulfuric acid to be highly acid a great part of the time. At such times, it is favorable to a small group of organisms, the most striking being Euglena mutabilis. This Euglena has been noted as abundant also in iron seeps where ferric hydroxide was concentrated and is occasionally found in a variety of other situations, some of them alkaline. What favors Euglena mutabilis in the acid coal mine stream? Does it have a greater physiological tolerance to sulfuric acid than does, say Euglena polymorpha? Or is there less competition for food and sunlight? Or is there a particular food substance in such a stream? Until we have answers to some such questions as these, we are on dangerous ground in talking of indicator organisms. Euglena polymorpha is one of several species of this genus which increase markedly in streams receiving effluents from sewage treatment plants. It has been referred to as an indicator species of recent fecal pollution. But it also occurs as dense blooms in certain types of swamp waters, such as the cedar swamp at Woods Hole, Massachusetts. These two situations must have in common some other factor than fecal pollution.

Ubiquitous Organisms

Some organisms tolerate such a wide range of environmental variation as to almost defy identification of limiting factors. Man is the most conspicuous of these. In our case, we can adapt to extremes of heat, cold, moisture, dryness, pollution of the atmosphere, and so on through a wide range. The blue crab, Callinectes sapidus, occurs along the Atlantic coast from Main to the Florida Keys, as well as the Gulf Coast - a geographic range within which there is a wide fluctuation of temperature, food, enemies, salinities and other factors. In Florida it migrates up the rivers and into some of the fresh springs, or those almost fresh. But what of the small ciliate, Cyclidium glaucoma, which apparently is truly cosmopolitan and which Finley has shown (2) can transfer from fresh to sea water or vice versa? It rarely attains huge numbers anywhere but in a hay infusion it may. The only indicator value it has in such a situation is to indicate that the bacteria on which it feeds are very abundant and that competition for this bacterial food is at a low ebb. This is easily amenable to experimental proof - one has only to grow Cyclidium with and without competing predatory ciliates.

The number of such ubiquitous species may be large. Undoubtedly, we often fail to recognize them. For example, Didinium nasutum, rarely common anywhere, but used by Beers (3) as a fresh water experimental ciliate, is frequent in samples from the high seas. Some Vorticella species belong in this category also and apparently Chlorella among the green algae. Peranema trichophorum and Anisonema ovale of the colorless Euglenophyceae are others.

Organisms of Somewhat Limited Distribution

It seems an easy matter to make a long list of organisms which occur in either salt or fresh water but not both. Or, for marine

TABLE I

	Probable Oxygen Relationship Of Species			Species Of Frequent Occurrence or May Occur in Large Numbers in						
	Aerobe	Anaerobe	Faculative	Sewage treatment plants	Streams of waters enriched by treated or untreated sewage	Waters with organic enrichment other than sewage	Waters fertilized by agricultural drainage - largely inorganic enrichment	Deliberately fertilized ponds, as for fish production	Other pollutants such as acid mine drainage, etc. Inorganic	Unpolluted waters
Rhizopoda										
Nuclearia delicatula			X	X	X	X				X
Dimastigamoeba gruberi			X	X	X	X				
Amoeba vespertilio			X	X	X	X	X	X		X
" radiosa			X	X	X	X				X
Pelomyxa palustris		X		X	X	X				
Vahlkampfia limax			X	X	X	X				
" albida			X	X	X	X	X	X		X
" guttula			X	X	X	X				
" fragilis		X		X						
Hartmanella hyalina			X	X	X	X	X	X		X
Chlamydophrys stercorea		X		X						
Microgromia sp.			X	X						
Arcella vulgaris	X			X	X	X	X	X		X
Cochliopodium bilimbosum	X			X	X	X				X
Euglypha alveolata	X			X	X	X	X	X		X
Diplophrys archeri			X	X	X	X				
Mastigophora										
Anthophysa vegetans			X		X	X				
Bodo caudatus			X	X	X	X				
" several other species			X	X	X	X	X		X	
Bicoeca sp.			X	X	X	X	X	X		X
Cercobodo spp.			X	X						
Clautriavia parva		X		X						
Hexamitus inflatus				X	X	X	X			
" crassus			X	X	X	X				

Mastigamoeba spp.		X		X	X					
Mastigella spp.		X		X	X					
Helkesimastix faecicola		X		X	X					
Monas spp.			X	X	X	X	X	X		X
Oicomonas spp.			X	X	X	X	X	X	X	X
Pleuromonas jaculans	X			X	X	X	X	X		X
Tetramitus spp.		X		X	X	X				
Spiromonas angusta			X	X	X	X				
Desmarella moniliformis	X				X		X	X		X
Cladospongia elegans			?		X					
Gyromonas ambulans		X		X	X					
Cercomastix parva		X		X	X					
Poteriodendron petiolatum	X			X	X	X	X			
Sterromonas formicina			X	X	X					
Phanerobia pelophila		X		X	X					
Trigonomonas compressa		X		X	X	X				
Trepomonas spp.		X		X	X	X				
Urophagus rostratus		X		X	X	X				
Volvocales										
Carteria spp.			X	X	X	X	X	X		X
Chlamydomonas spp.			X	X	X	X	X	X		X
Chlorogonium spp.			X	X	X	X	X	X		X
Dunaliella salina			X			X				
Eudorina elegans			X		X	X	X	X		
Gonium pectorale			X		X	X	X	X		X
" sociale			X		X	X	X	X		X
Pandorina morum			X		X	X	X			
Polytoma uvella			X	X	X	X				
Polytomella citri			X	X	X	X				
Platymonas spp.			X		X	X	X			
Chlorobrachis (Pyrobotrys gracilis)			X		X	X				
Spondylomorum quarter-narium			X		X	X				
Euglenophyceae - colorless										
Astasia spp.			X	X	X	X				
Entosiphon sulcatum			X	X	X	X				X
Notosolenus spp.			X	X	X	X	X			X
Peranema trichophorum			X	X	X	X				
Euglenophyceae - green										
Cryptoglena pigra	X				X	X	X			X
Euglena acus			X		X	X	X			X
" agilis			X		X	X	X			X
" deses			X		X	X	X			X
" gracilis			X	X	X	X	X			X
" mutabilis	X								X	X
" pisciformis			X	X	X	X	X			X
" polymorpha			X		X	X	X	X		X
" quartana			X	X	X	X				
" sanguinea	X						X			
" tripteris	X				X	X	X	X		X
" viridis	X				X	X	X	X		X

Eutreptia viridis	X				X	X	X	X		X
Lepocinclis butschlii	X				X	X	X	X		X
" ovum	X				X	X	X	X		X
" texta	X				X	X	X	X		X
Phacus parvulus			X	X	X	X				
" triqueter			X		X	X	X	X		X
" pyrum			X	X	X	X				X
Trachelomonas urceolata	X				X	X	X			X
" volvocina	X					X	X	X		X
Chrysophyceae										
Botryococcus braunii	X				X	X				X
Chromulina ovalis	X							X	X	X
" globosa	X				X	X				X
Mallomonas spp.	X					X		X		X
Coccolithophora										
Pontosphaera sp.	X					X		X		X
Dinoflagellata										
Oxyrrhis marina	X					X		X		X
Gymnodinium splendens	X					X				X
Prorocentrum triangulatum	X					X	X	X		X
Cryptophyceae										
Chilomonas paramecium			X	X	X	X	X			
Cryptomonas spp.	X					X	X	X		X
Rhodomonas spp.	X					X	X	X	X	X
Ciliata										
Carchesium sp.	X			X	X	X				X
Chilodonella spp.	X			X	X	X	X			X
Cinetochilum margaritaceum	X			X	X	X	X			X
Colpoda spp.			X	X	X	X				
Colpidium sp.			X	X	X	X				
Glaucoma scintillans			X	X	X	X	X			
Halteria grandinella	X			X	X	X	X			X
Lionotus faeciola	X			X	X	X				X
Metopus sigmoides			X	X	X	X				
Opercularia spp.	X			X	X	X				
Oxytricha spp.	X			X	X	X	X			X
Paramecium spp.			X	X	X	X				
Saprodinium putrinum		X		X	X	X				
Stylonichia spp.	X			X	X	X				X
Trimyema compressa		X		X	X	X				
Urocentrum turbo			X	X	X	X				
Vorticella spp.	X			X	X	X	X			X
Hastatella radians	X					X				
Enchelyomorpha vermicularis		X		X	X	X				

species, pelagic versus neritic species, as Radiolaria and perhaps certain dinoflagellates. Even boreal, termperate and tropic species may occur: *Chrysococcus cingulum* is common at Woods Hole and the Chesapeake but has not been found in three years' examination of Florida Gulf Coast samples. Certain species of ciliates, such as *Irimyema compressa* and *Enchelyomorpha vermicularis* seem restricted to water - or mud - devoid of oxygen, but are widespread if enough such situations are examined.

In this second category, it is always difficult to determine the limiting conditions. *Metopus* (possibly more than one species) is often found along with the two ciliates above but will exist also in the presence of some oxygen. In these instances, is it a lack of oxygen or the presence of H_2S which is effective? In the *Radiolaria* is it the ions of full strength sea water or merely physical osmotic phenomena? The chloromonad *Gonyostomum semen* is common to the brown waters of cedar and cypress swamps but is almost unknown elsewhere. There are many instances of limited distribution but knowledge of the limitations is virtually lacking.

Rare Organisms

Many species are named in the literature which the average observer has never seen. This may mean poor observation, not having sampled the habitat type for the organism, or it may really be rare. The diatom *Attheya zachariasi* has frustules of such transparency that they are seen only with considerable difficulty and its chloroplasts and cytoplasm are usually grouped in a small flattened disc. It is seldom reported in the United States, yet seems common. Another is *Cephalomonas granulosa*, one of the green flagellates. This distinctive organism occurs sparingly in Ohio Valley waters but on one occasion, a slight bloom of them was recorded in Lytle Creek (4); the species was originally described from Maryland (5). *Trachelomonas reticulata* is colorless and Klebs (6) described it from "faulenden" cultures, so it should occur in polysaprobic situations. There are no known records of the species in the United States, except a very dense bloom found by the writer in an Alabama tree hole. A fourth rare organism is the ciliate, *Hastatella radians*. It is large (100 µ) and unmistakable but has been seldom encountered. On April 8, 1956, it was the dominant ciliate, there being 80 per ml, in a deep sink hole in Orange Lake, Florida. The sink hole acted as a concentrating pit for the slowly drying up lake, there being a slow but steady outflow through the 71 foot deep bottom

Undoubtedly then, many organisms which we consider rare, wil attain high numbers if the environment is favorable. The question generally develops into attempts to recognize the optimal factors.

Discussion

Before stating a relationship between organism species or organism numbers and pollution, we must first define the pollutant. There are many kinds of pollution, varying from an excess of organ or putrescible matter to heavy concentrations of inorganic salts. Poisons, even physical conditions, enter the picture. It is hardly worthwhile to list types of pollution here but there are chemical and physical tests which are revealing even when a condition is not apparent to the eye. Conditions having been determined, we ascertain the numbers and kinds of organisms present and the permanence of both the physico-chemical condition and the population.

The mere occurrence of an organism in a sewage treatment plant does not mean it is indicative of pollution - Oxytricha may occur either in a sewage treatment plant or in a hay infusion simply because it finds in each place the bacteria it uses as food, as well as ample oxygen. A long list of organisms occurring in sewage treatment plants could be made and the same organisms would be found in other situations in many cases. Table I is such a list in part. It does not cover the green nonflagellated algae and the diatoms. It is based partly on personal experience and partly on the known literature. No references are given, for there would be too many and in addition, the list does not cover all the references which could be made. It is weakest in regard to the occurrence of ciliates.

This table is intended to show that there are few organisms which are common to only one type of habitat. It does not include those which are common to or which sometimes occur in huge numbers in waters not known to contain substances we would regard as pollutants or fertilizers. Nor does it include laboratory cultures. Undoubtedly, there are enriching substances in the gravel pits around Chillicothe, Ohio, which annually bloom with Uroglena, or the Cedar Swamp at Woods Hole which annually blooms with Gonyostomum. But at present we either do not know what they are, or we do not regard them as pollutants. The same is true of the Gulf of Mexico water in which Gymnodinium brevis blooms so heavily, or the once famous Spirostomum pool at Woods Hole. The vast majority of waters will have many species in common and often one or more of these species will bloom. But the organisms do not classify such waters; hence, fall outside the scope of this discussion.

It cannot be too strongly emphasized that a cause and effect relationship does not necessarily exist simply because of abundance of an organism and occurrence of a defined pollutant. Clark (7) has described mechanical trapping of plankton in certain situations and has also called attention to common orientations and attractions of organisms to others of their own kind. "Trapped" populations may remain in a situation, so we cannot be too sure of even this yardstick. Statements as to the indicator value of an organism in a given situation absolutely demand critical experimental analysis and accumulation of much data. Even then the relationship to pollution may be secondary. The outfall of the Tampa sewage treatment plant apparently has little effect on chlorophyll bearers in its section of Tampa Bay but the number of tintinnid ciliates is considerably higher here than in other sampled sections of the Bay. Counts of the bacterial population have not been made but an increased number there could account for the increased ciliates. The magnitude of the sewage contribution and the size of the Bay are discouraging to attempts at collecting precise and continuous data. It would be necessary not only to secure tidal flow data, but seasonal and vertical biological data here and elsewhere in the Bay, then add chemical analyses. At the end of this costly process, it is questionable where relationships would have been established. In November, there is a bloom of the diatom, Skeletonema, but it varies in density from year to year; it also occurs in other portions of the Bay which receive no sewage. There are repeated dense but local blooms of many dinoflagellates, such as Gymnodinium splendens, Ceratium furca and others in various parts of Tampa Bay, but no explanation of why they occur. Many thousands of dollars have been spent on attempts to determine the cause of the Red Tide organism, Gymnodinium brevis. So far, all it indicates is trouble.

Still there are some hopeful signs. Study of the algae or protozoa in a fairly constant environment reveals some which tend to occur permanently. Yount (8) concluded that no one factor determined the species density in a habitat. He worked in the Silver Springs (Florida) boil area or near it, where many factors are uniform the year round. Even so, light and some other factors fluctuate and since they worked with diatoms this is important. Such seemingly small matters as the surface of a slide soon after immersion, as compared to the same film-covered slide after several days can and do make surprising differences as to what and how many diatoms are found on the slide. There has been too little work of this type but it has been experimental experience that one way to learn what species of colorless Euglenophyceae may be found in a habitat, is to suspend slides for several days.

Studying smaller streams indicates that the more abundant is the oxidizable matter, or the inorganic salts, the more varied and abundant is the suspended population. Lytle Creek (Ohio) studied at five stations and Cowan Creek, nearby, and studied at one station (4) over a period of three months in the summer showed 167 and 89 species respectively. Two of the Lytle Creek stations were in a polluted area, receiving the effluent from a badly overloaded sewage treatment plant. In this area, certain types of Euglenophyceae appeared in large numbers. Others died out, especially most specie of Trachelomonas. Chrysophyceae also failed to pass through this polluted zone, although 12 species were present above. Apparently, these are responses to domestic sewage pollution.

Unhappily, most of the Chrysophyceae are also lacking in the small Santa Fe River of Florida. This stream had a large number (332) of species during the time it was studied and 99 of them were the same as those in Lytle Creek. The Santa Fe is unpolluted and receives little drainage from arable land; total numbers of organism were low. The same story holds for the two forks of the Licking River of Kentucky - that fork draining the steep, wooded areas such as Magoffin County in Eastern Kentucky is fairly rich in species but low in numbers of organisms. The more westerly fork, draining the rich blue grass country, is high in both species and numbers.

After many years, the status of indicator species among the algae and protozoa seems little near clarification. Immediate sewage pollution of a stream, resulting in oxygen depletion, tends to eliminate all but a few species of anaerobic ciliates and colorless flagellates. Settling the sewage in oxidation ponds results in heavy growths of Chlorococcales and Volvocales. Allum (9) says that raw sewage lagoons in South Dakota showed DO values as high as 370% saturation, attributable to algae. Silva and Papenfuss (10) made a systematic study of the algae of sewage oxidation ponds in California and in an extensive comparative review of the literature recorded some 26 genera from at least 10 of their ponds, which list generally agreed with lists of other investigators. A few other algae were recorded and a few Euglenophyceae.

The trouble with observations such as these lies neither in their accuracy nor in the high counts. Rather, it is the tendency we acquire to regard the organisms found as characteristic of sewage pollution. Such is not the case. In the Orange Lake pool referred to above, many of these same species occurred in great numbers and many of them are common in situations where there is no sewage or sewage effluent. In fact, commercial fertilizers will produce heavy growths of some of them.

Partial sewage treatment, resulting in a sharp decrease in bacteria and BOD increases the number of ciliates and colorless flagellates, and in the effluent channels, attached blue green and green algae, the latter usually two genera. Further reduction of BOD tends to produce some eight or ten species of Euglena and Phacus and to increase the species of Volvocales and Euglenophyceae. There is little doubt that certain species of algae and protozoa are readily associated with sewage pollution. But we should distinguish between "characteristic of" and "indicative of," the latter being a more restrictive term.

This last distinction might help make the suggestion of Beck (11) work. In it, he adds the numbers of species found which are known to be associated with clean water to the numbers of species found, which are known to be associated with polluted water. He says it should apply only to macroscopic invertebrates found in Florida. However, a detailed analysis of a stream station usually reveals a large number of species of algae and protozoa. If we can satisfactorily allocate them to one or the other of the above classifications, the result should classify the stream at the station studied. Allocation is a difficult task and few workers are in agreement for more than a very few species.

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ALGAE AS BIOLOGICAL INDICATORS OF POLLUTION

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It appears evident to many workers that particular genera or even species of algae, when considered separately, are not reliable indicators of the presence or absence of organic wastes in water. However, when a number of kinds of algae are considered as a community, that group may be reliable as such an indicator. Such populations may be useful even in the determination of the degree of purification which has occurred; but the data available on this is more limited. Therefore, it has seemed advisable to omit here any attention to intermediate stages in the natural purification process.

Emphasis is placed in this article on the algae which various workers have found to be tolerant to relatively undecomposed sewage or closely related organic wastes. These algae would, in general, be those found to be present and relatively prominent in the polysaprobic and alphameso-saprobic zones in a polluted stream. These terms, however, are used by relatively few workers who have reported sewage tolerant algae. The writer has interpreted their findings as they apply to sewage pollution. Due to the large number of references used, an occasional misinterpretation should not seriously distort the composite record concerning the algae.

The reports of fifty-six workers have been examined to date. The genera and species of algae tolerant to sewage or to related conditions have been recorded, and a total of more than 500 kinds of algae has been compiled. The maximum number of authors listing any one genus was 34, and, one species, 18.

In order to tabulate the information, the writer has allotted arbitrary numerical values to each author's record of an alga. A value of two was given to each alga reported as very highly tolerant and a value of one, to each alga highly tolerant to sewage. Lightly tolerant and non-tolerant algae were not recorded in this compilation. The total points from all of the authors was then determined for each genus and species. The algae were arranged in the order of decreasing emphasis by the authors as a whole. The fifty genera and the fifty species of sewage tolerant algae with the highest total number of points are given in Tables 1 and 2. Plate I. "Polluted Water Algae" illustrates nineteen of the fifty genera listed in Table 1.

Flagellates containing photosynthetic pigments have been included as algae along with the blue-green algae, green algae, and diatoms. The record cannot take into account any inaccurate identifications of the algae which may have been included by each author nor the variations in the many polluted and stagnant waters to which the original records refer.

The lists of algae in Tables 1 and 2 are meant to be aids for individuals engaged in stream pollution surveys or related projects. They give a general consensus of opinion as to the relative significance of the many sewage tolerant algae which have, so far, been reported. Particular care can thus be taken in biological surveys to check for the presence of these genera and species of algae during the microscopic examination of samples.

Samples which are found to contain, in considerable numbers, several of the algae which are high in the lists may be interpreted as indicating water that is polluted or stagnant and with high organic content. The list of species in Table 2 should be more reliable in this respect than the list of genera in Table 1, since almost every genus referred to included certain species which are pollution tolerant and others which are not. This is particularly true with such genera as Navicula, Synedra, and Chlamydomonas. In a few instances this same condition may be true also for a species. For example, Fjerdingstand (2) claims that there are two separate kinds of Ulothrix zonata, the "pollution type" and the "pure water type."

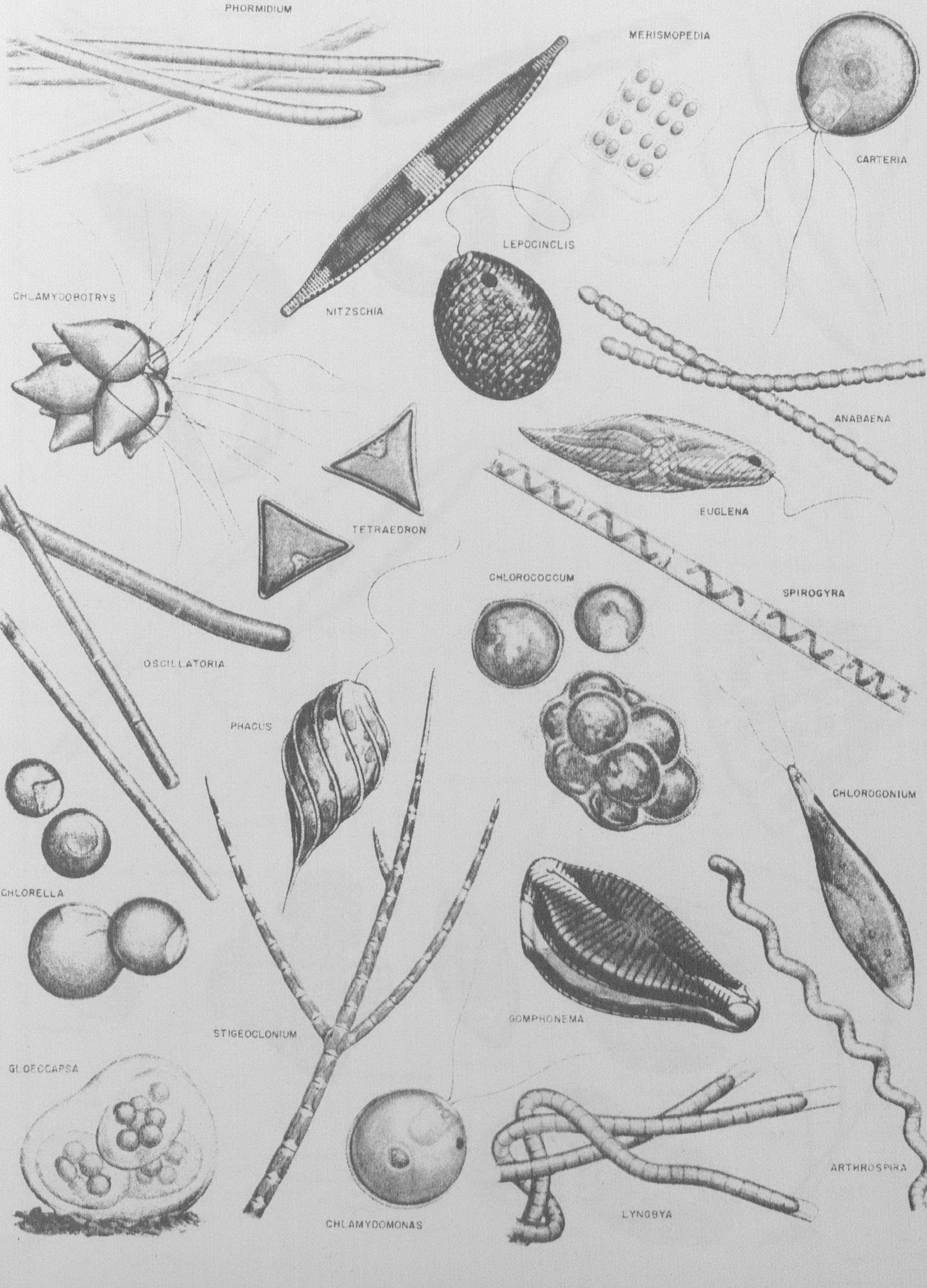
Additional records by other workers would undoubtedly change the relative positions of the algae in both the genus and species lists. This is particularly so for the algae near the ends of the lists where a relatively few reports are responsible for their present positions.

Three algae are included in Table 2 for which no binomial is assigned. These are Chlamydomonas sp., Scenedesmus sp., and Spirogyra sp. For none of these genera was there any one species with a total of five or more points, although the number of points for each genus was high. Relative positions were assigned to these by arbitrarily dividing by three the figures for each of the three genera. Many workers have reported these algae by genus name only and have not referred to the particular species involved. Examples of species which have been reported as sewage tolerant are Chlamydomonas reinhardi, Scenedesmus quadricauda, and Spirogyra communis.

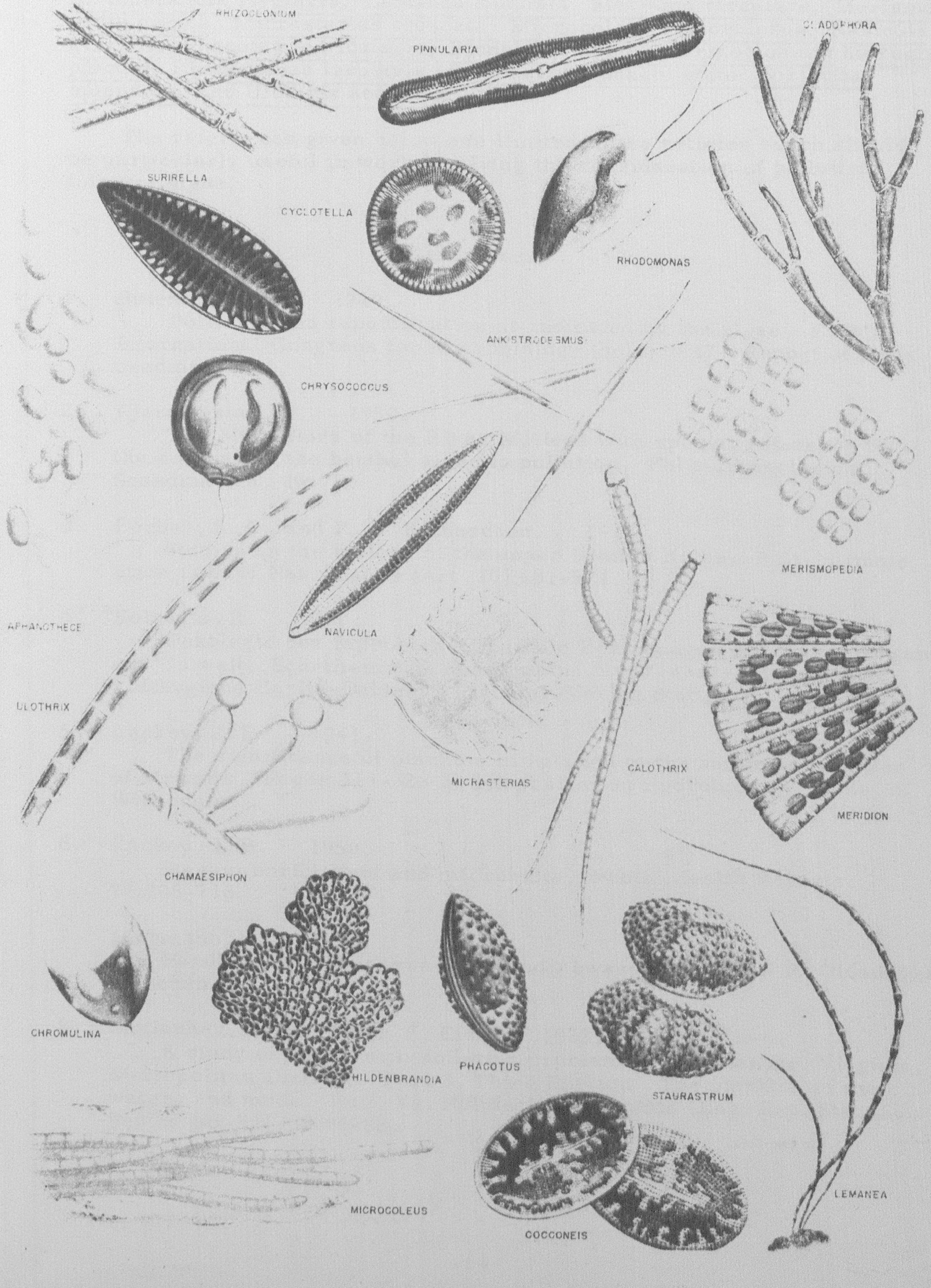
No exhaustive list of the clean water algae has as yet been compiled by the writer, although a smaller representative group of clean water algae has been selected and illustrated in color (10). The same illustrations but in black and white, are included here as Plate II.* The clean water species, listed by genus names only on the plate, are as follows: Ankistrodesmus falcatus var. acicularis, Aphanothece stagnina, Calothrix parietina, Chamaesiphon incrustans, Chromulina rosanoffi, Chrysococcus rufescens, Cladophora glomerata, Cocconeis placentula, Cyclotella bodanica,

*The original illustrations in color, were painted by Mr. Harold J. Walter at the Robert A. Taft Sanitary Engineering Center.

POLLUTED WATER ALGAE



CLEAN WATER ALGAE



Hildenbrandia rivularis, Lemanea annulata, Meridion circulare, Merismopedia glauca, Micrasterias truncata, Microcoleus subtorulosus, Navicula gracilis, Phacotus lenticularis, Pinnularia nobilis, Rhizoclonium hieroglyphicum, Rhodomonas lacustris, Staurastrum punctulatum, Surirella splendida, and Ulothrix aequalis.

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Table 1

Pollution Tolerant Genera of Algae

List of the Fifty Most Tolerant Genera

In order of decreasing emphasis by 56 authorities

	Genera	No. Authors	Total Points *
1.	Oscillatoria	34	57
2.	Euglena	31	53
3.	Navicula	19	32
4.	Chlorella	18	30
5.	Chlamydomonas	17	29
6.	Nitzschia	15	25
7.	Stigeoclonium	18	25
8.	Phormidium	15	22
9.	Scenedesmus	12	18
10.	Synedra	14	17
11.	Arthrospira	9	16
12.	Spirogyra	10	15
13.	Phacus	9	14
14.	Gomphonema	9	14
15.	Melosira	9	12
16.	Pandorina	9	12
17.	Ulothrix	10	12
18.	Lepocinclis	7	11
19.	Lyngbya	7	11
20.	Chlamydobotrys	6	10
21.	Chlorogonium	6	10
22.	Tribonema	6	10
23.	Anabaena	8	10
24.	Spondylomorum	8	10
25.	Carteria	5	9

	Genera	No. Authors	Total Points *
26.	Ankistrodesmus	6	9
27.	Hantzschia	7	9
28.	Pediastrum	7	9
29.	Cladophora	8	9
30.	Anacystis	5	8
31.	Eudorina	6	8
32.	Spirulina	6	8
33.	Cyclotella	7	8
34.	Fragilaria	7	8
35.	Cryptomonas	8	8
36.	Cymbella	4	7
37.	Micractinium	4	7
38.	Closterium	5	7
39.	Stauroneis	7	7
40.	Chlorococcum	4	6
41.	Merismopedia	4	6
42.	Stephanodiscus	4	6
43.	Cocconeis	5	6
44.	Cosmarium	5	6
45.	Cryptoglena	5	6
46.	Gonium	5	6
47.	Oocystis	5	6
48.	Stichococcus	5	6
49.	Surirella	5	6
50.	Trachelomonas	5	6

*Tolerance by author "Very High" - 2 points
Tolerance by author "high" - 1 point

Table 2

Pollution Tolerant Species of Algae

List of the Fifty Most Tolerant Species
In order of decreasing emphasis by 56 authorities

	Species	No. Authors	Total Points*
1.	Euglena viridis	18	33
2.	Nitzschia palea	14	23
3.	Oscillatoria limosa	10	15
4.	Oscillatoria tenuis	11	15
5.	Arthrospira jenneri	8	14
6.	Stigeoclonium tenue	9	14
7.	Euglena gracilis	7	12
8.	Chlorella vulgaris	8	12
9.	Oscillatoria formosa	8	11
10.	Phacus pyrum	5	10
11.	Chlamydomonas sp.	(6)	(10)
12.	Euglena polymorpha	7	10
13.	Oscillatoria chlorina	7	10
14.	Oscillatoria putrida	7	10
15.	Spondylomorum quaternarium	7	10
16.	Oscillatoria chalybea	8	10
17.	Phormidium uncinatum	8	10
18.	Chlorella pyrenoidosa	5	9
19.	Gomphonema parvulum	5	9
20.	Oscillatoria lauterbornii	5	9
21.	Euglena oxyuris	6	9
22.	Lepocinclis texta	6	9
23.	Hantzschia amphioxys	7	9
24.	Euglena deses	6	8
25.	Oscillatoria princeps	6	8

	Species	No. Authors	Total Points*
26.	Pandorina morum	6	8
27.	Phormidium autumnale	6	8
28.	Anabaena constricta	5	7
29.	Chlorogonium euchlorum	5	7
30.	Melosira varians	5	7
31.	Cryptoglena pigra	6	7
32.	Chlamydobotrys gracilis	4	6
33.	Euglena pisciformis	4	6
34.	Lepocinclis ovum	4	6
35.	Merismopedia tenuissima	4	6
36.	Navicula crytocephala	4	6
37.	Nitzschia acicularis	4	6
38.	Scenedesmus sp.	(4)	(6)
39.	Synedra ulna	4	6
40.	Cyclotella meneghiniana	5	6
41.	Euglena intermedia	5	6
42.	Stichococcus bacillaris	5	6
43.	Oscillatoria splendida	6	6
44.	Phormidium foveolarum	6	6
45.	Pediastrum boryanum	3	5
46.	Spirogyra sp.	(3)	(5)
47.	Eudorina elegans	4	5
48.	Euglena fusca	4	5
49.	Surirella ovata	4	5
50.	Ulothrix zonata	4	5

*Tolerance by author "Very High" - 2 points
Tolerance by author "High" - 1 point.

Dr. Ruth Patrick was unable to attend the seminar. However, in order to make our coverage of the subject of indicator organisms more complete she subsequently kindly consented to prepare a paper for inclusion in the Transactions. Her paper on diatoms as indicators of environmental conditions is herewith presented.

DIATOMS AS INDICATORS OF CHANGES IN ENVIRONMENTAL CONDITIONS

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Diatoms, which are one-celled algae belonging to the Chrysophyta, have long been of interest to students of the microscope. The earliest students of these plants were concerned with the structure of the siliceous cell walls and the general morphology. Diatoms were often used as test objects for lenses in order to determine the ability of a lens to define a given structure. One of the first to become interested in the geographical distribution of diatoms was Ehrenberg. In 1829 he published a paper on the geographical distribution of Infusoria (a general grouping which includes diatoms) in North Africa and West Asia. This paper was followed by several others on the distribution of Infusoria in various parts of the world. Other workers such as Greville, Kutzing, W. Smith, Grunow, Van Huerck and Cleve continued to explore this field of interest. They recorded not only the geographical distribution of diatoms but also described the conditions in which diatoms were found.

Cleve (1894) pointed out the importance of a knowledge of the habitats and geographical distribution of diatoms to geological research. Considerable information has been obtained by studies of fossil diatoms as to the extent of the invasion of the sea and the effect of glaciation on the temperature of fresh and marine waters. Much of the knowledge of the paleogeography of Scandinavia has been elucidated in studies of fossil diatoms by Cleve (1899), Cleve-Euler (1940, 1944), Hustedt (1939) and Molder (1943).

In North America the extent of the effect of glaciation on fresh waters has been set forth by studies of diatoms (Hanna, 1933; Patrick, 1946). The succession of changes occurring in lake development has also been determined by diatom studies of Patrick (1936, 1943, 1946, 1954), Pennington (1947) and Ross (1950). It is because many species of diatoms have their best development in water with certain specific chemical characteristics that such correlations are possible.

One of the most important works summarizing what was known as to the ecology of diatoms was published by Kolbe (1927). In this work he clearly sets forth a system for classifying diatoms as to their tolerance to various chloride concentrations in water. Krasske (1927), Hustedt (1953), Legler and Krasske (1940) and Petersen (1943) have also contributed to our understanding of the chloride tolerance of diatoms.

The knowledge of the relationship of the occurrence of given species of diatoms to other chemical characteristics of water, such as pH, iron, nitrates, phosphates, and silicon, has developed greatly during the last 25 years. From time to time this kind of information has been brought together and published by such workers as Hustedt (1938 - 39), Schroeder (1939), Patrick (1948) and Fjerdingstad (1950).

Only very recently has the structure of the diatom population been correlated with the presence of sanitary and industrial wastes. Kolkwitz and Marsson (1908) set forth a system classifying many species of diatoms as to their ability to withstand varying degrees of pollution from sanitary sources. Fjerdingstad (1950) published a very good summary of the more important literature on the effect of varying amounts of pollutants on the occurrence of diatoms. Budde (1930b) found out that the association of species and the relative sizes of populations of species produce the best picture of the effects of waste on the diatom flora. Although many of the studies concerned with the effect of wastes on the aquatic life of a stream have been based on plankton, Jurgensen (1935), Nowak (1940), Butcher (1933, 1940), Patrick (1948) and others have pointed out that it is the attached forms, or those organisms which grow and reproduce in a given area, that give the most reliable indication as to whether the environment of an area is suitable for the support of aquatic life.

Diatoms may float or be carried into habitats where they may survive for a period of several days without dying, although the quality of water is unsuitable for growth and reproduction. This is one of the reasons why diatoms, which in natural or unpolluted rivers indicate so well changes in the environment, may under conditions set forth above persist and thus not appear to indicate the character of the water in which they are found.

Another error which has occurred in the study of diatoms has been pointed out by Fjerdingstad (1950). Workers often fail to examine their collections to see if the diatoms found are living and in good condition. Since diatoms have cell walls of silicon, which persist after they are dead, they may be included as living in a given area unless the above precaution is taken. In our studies of diatoms taken from various rivers in the United States, large numbers of dead frustules of species not living in an area are often found intermixed in the living collections.

Another cause of error in the handling of collections is contamination. Dirty pipettes, beakers and collecting jars may well result in the transfer of species of diatoms from one collection to another and thus produce a false picture of the structure of the diatom flora being studied.

The methods used in collecting diatoms and of studying the slides must be the same if one wishes to compare the diatom flora of various areas. Because these methods are not standardized among diatomists it is often very difficult to compare results. Such considerations are particularly important if one is comparing the number of species and the sizes of the populations.

Another consideration which is often overlooked in discussing indicators of polluted or of deleterious conditions is the number of variable factors and the combination of such factors which may produce the deleterious effects. As pointed out by Fjerdingstad (1950) the classification assigned by Kolkwitz and Marsson (1908) to certain diatom species often does not hold. This undoubtedly is due to several factors but certainly one of the most important is the fact that Kolkwitz and Marsson's system is based on the reaction of organisms to sanitary wastes or wastes with a heavy organic load.

In this system the greatest degree of pollution is characterized by low oxygen content, high bacterial counts, high biochemical oxygen demand and a heavy organic load. However, when one is considering toxic wastes, a stream may have a high dissolved oxygen content, low biochemical oxygen demand and low bacterial counts and yet be inimical to aquatic life because of the presence of toxic substances. Likewise high temperatures may be lethal and only be associated with a lowering of the dissolved oxygen. Whereas organic wastes, toxic wastes and high temperatures in excessive amounts will kill most organisms in sublethal or threshold concentrations they will affect various species in different ways. In most rivers which we have studied in the United States, pollution is rarely of a single type, but rather is a combination of toxic substances and organic wastes often accompanied by high temperatures.

Fjerdingstad (1950) and others have emphasized that various aspects of the diatom flora must be considered, such as the changes in numbers of species, numbers of individuals and kinds of species. As a result of over 100 analyses of diatom floras from natural areas of rivers in eastern and southern United States, our laboratory has found that the numbe of species making up the diatom flora are quite similar. By natural areas are meant areas which so far as we could ascertain from state and federal agencies as well as from information concerning chemical and bacteriological analyses were not adversely effected by effluents entering the river. Indiscriminately selected results from such studies showed the following numbers of species to be present: Marsh Creek, Pa. - 46; Tionesta Creek, Pa. - 42; North Fork of the Holston, Va. - 52, Savannah River, South Carolina and Georgia - 80, 69; Flint River, Ga. - 40; Escambia River, Fla. - 55, 54; Sabine River, Texas - 46, 55; Guadalupe River, Texas - 42; Neches River, Texas - 65.

Part of the variation shown here in the numbers of species present in the various studies is due to the fact that the studies on Marsh Creek, Tionesta Creek, Flint River, Sabine River and Guadalupe River were made in a somewhat different manner than those on the other creeks and rivers.

When one considers the kinds of species a very different picture is presented. In a study of natural river areas soon to be published, 354 species from ten rivers were considered. Of this number only one was found in all ten rivers. Two hundred and two were found in only one river; 74 in two; 30 in three; 20 in four; 9 in five; 5 in six; 9 in seven; 3 in eight and 1 in nine. This clearly shows the difficulty of using single species as indicators. On the other hand, if one considers a group of species as indicating a condition a reliable methodology can be developed. For instance, most of the species of Eunotia and all of the North American species of Actinella are found in soft, usually somewhat acid waters. The presence of an association of any of the species of these genera will indicate these conditions. Likewise, the presence of well developed populations of Synedra affinis or S. pulchella or Navicula pygmea will indicate the presence of brackish water. As a general rule, species which form the largest populations should be selected as indicators. In other words diatoms most truly indicate those physiological conditions which enable them to multiply most rapidly over a period of time.

An analysis of the histograms given by Patrick (1949) shows that the diatom flora responds to the severe effects of deleterious effluents in a manner similar to that of fish and insects. Approximately 200 analyses of sections of rivers and estuaries which have been made by the Limnology Department of the Academy of Natural Sciences of Philadelphia subsequently support these conclusions.

Diatoms are in some cases more sensitive to small changes in the chemistry of the water than are some of the larger forms, as was pointed out by Liebmann according to Fjerdingstad (1950). However, the response of diatoms to lethal concentrations are usually similar to those of fish.

Analyses of results of 50 bioassay tests run on wastes of chemical, steel, gas and electric industries show that the concentrations which produced 50 per cent reduction in growth of diatoms and 50 per cent kill in fish were very similar (Table 1). This work was carried out by Dr. John Cairns, Jr. and Dr. Arthur Scheier. In 75 per cent of the cases one concentration was never more than twice that of the other, and in most cases there was very little difference. Since the concentration recommended as biologically safe is less than a third of the above concentration, it is evident that results from either test will bring about very similar recommendations for safe discharge of wastes. In only eight per cent of the cases was the diatom found to be less sensitive than the fish. These results clearly show

Table 1

Comparison of effects of industrial wastes on fish and diatoms (Figures in column headed "Diatom" represent concentration, in per cent, of effluent causing 50 per cent reduction in division rate in five days; those under "Fish" show concentrations causing death to 50 per cent of the fish in 24 hours and in 48 hours.)

Effluent	Diatom	Fish 24 hours	Fish 48 hours
1	5.3	10	9
2	2.78	2.8	2.8
3	3.65	3.2	2.7
4	0.001	0.18	0.155
5	1.16	3.25	2.87
6	2.3	7.6	6.0
7	no reduction	70.0	60.0
8	49.3	no deaths	90.0
9	2.34	10.0	9.1
10	0.78	0.57	0.52
11	2.15	3.2	3.2
12	18.7	26.5	21.7
13	4.4	75.0	75.0
14	32.8	62.0	52.0
15	65.0	no deaths	no deaths
16	35.0	no deaths	no deaths
17	44.5	1.6	1.6
18	no reduction	12.7	9.0
19	0.74	no deaths	no deaths
20	no reduction	no deaths	no deaths
21	no reduction	no deaths	no deaths
22	56.0	no deaths	no deaths
23	18.0	no deaths	no deaths
24	no reduction	no deaths	no deaths
25	no reduction	no deaths	no deaths
26	no reduction	no deaths	no deaths
27	98.0	no deaths	no deaths
28	no reduction	no deaths	no deaths
29	no reduction	no deaths	
30	no reduction	no deaths	

"No death" or "No reduction" means that 100% concentration of the effluent was not deleterious.

Table 1 (Continued)

Effluent	Diatom	Fish 24 hours	48 hours
31	no reduction	no deaths	
32	no reduction	no deaths	
33	no reduction	no deaths	
34	56.0	no deaths	
35	18.0	no deaths	
36	no reduction	no deaths	
37	no reduction	no deaths	
38	no reduction	no deaths	
39	98.0	no deaths	
40	no reduction	no deaths	
41	no reduction	no deaths	
42	60.0	no deaths	
43	no reduction	no deaths	
44	6.0	6.9	
45	10.0	20.5	
46	0.77	0.44	
47	16.2	42.0	
48	no reduction	no deaths	
49	15.0	29.5	
50	no reduction	no deaths	

value of diatoms as indicators of conditions essential for or inimical to fish life.

Results of work done by the Limnology Department under grants from the U.S. Public Health Service indicate that when pure chemicals are tested singly, responses of fish and diatoms are more varied. More study is needed before an explanation can be given for the more variable responses to pure chemicals than to industrial effluents. The differences were not due to procedure since the same methods were used in both types of tests.

From the many studies that have been made it is apparent that diatoms can be used as a group to indicate the ability of a water to support aquatic life. Collections must be prepared correctly and the studies based only on specimens living at the time collections are made. Furthermore, collections should be taken from populations which are attached or definitely living in the habitat and not from plankton forms.

Diatoms are a desirable group to use for indicating stream conditions for several reasons:

1. They need no special treatment for preservation because the cell wall, on which the identification is based, is composed of silica.

2. The diatom flora of a normal stream is made up of a great many species and a great many specimens. Thus the group lends itself to statistical treatment.

3. Diatoms vary greatly as to their sensitivity to chemical and physical conditions of water. Some species are able to tolerate a wide variety of environments. Therefore, some diatoms can be found in any aquatic habitat inhabitated by plants and animals (excluding bacteria). However, each of these species has a range of conditions in which it achieves best development. Other species of diatoms have a very narrow range of tolerance. Thus we have in the diatoms enough different kinds of species so that they can be used as an indication group in most of the possible types of aquatic environments found in rivers and estuaries.

4. A considerable amount of information is already available as to the type of environment in which many species are found.

In order to use any group of organisms as indicators one must have a method of collecting and studying them which will be comparable for different types of water. A suitable methodology for diatoms has been set forth by Patrick, Hohn and Wallace (1954) by the use of the Catherwood Diatometer.

The Diatometer (Fig. 1) is an instrument which floats in the water and provides a substrate, ordinary glass slides, which seems to be non-selective for diatom growth. These slides are left in the water for a long enough period of time to allow a considerable amount of growth to develop on the slide. In many cases this length of time has been about two weeks. From the studies we have made, it appears that dead diatoms do not remain attached, but slough off the slides. Studies based on these slides thus include only live diatoms which are actually living in a given area.

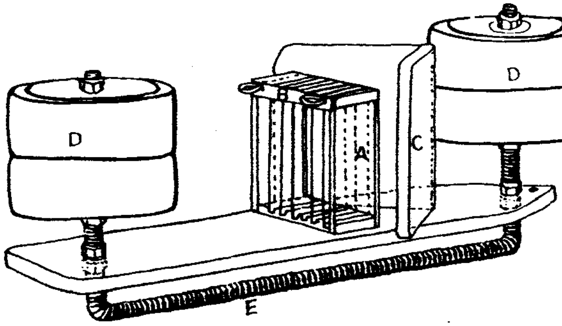
When the slides are prepared for study the diatoms are scraped off and cleaned by the acid method. A small aliquot which is representative of the material is then placed on a slide and mounted in Hyrax.

In these studies all species observed are identified and the number of indivduals of each is recorded. Enough specimens have to be identified in order to construct a truncated normal curve according to the method set forth by Preston (1948). If results are to be comparable the modes of the curves should be in the same interval. Therefore, varying numbers of specimens may be counted. This study usually takes 3 to 4 days to complete.

In such rivers as the Savannah where little change occurs, the structure of the curve remains very similar from season to season and from year to year. However, any serious change in the quality of water is readily seen by a change in the structure of the curve (Figs. 2, 3, 4). In interpreting these curves one must take into consideration the height and position of the mode, the dispersion factor, the number of species found, the total theoretical population, the kinds of species and the number of specimens used in constructing the curve.

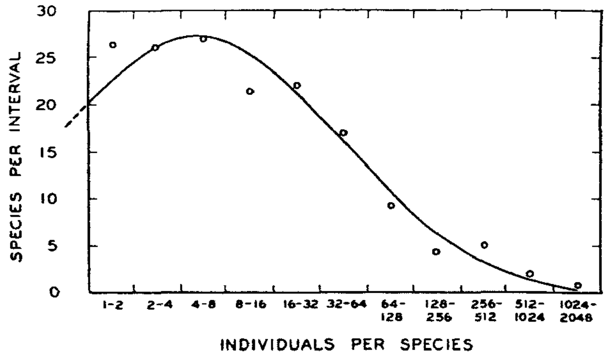
It can be seen that this method avoids many of the pitfalls which have been encountered by trying to use diatoms as indicators of pollution. It provides a uniform method for collecting and studying diatoms. Thus the results are comparable. This method is based on the study of the live species in a given area which are growing and dividing. It is mainly concerned with the number of species and number of individuals of each of the species. It also considers the kinds of species. Thus its basis is a shift in pattern of the whole flora rather than the behavior of a few indicator species.

Of course, it must be remembered that any conclusion based on only one group of organisms or kind of analyses should only be considered as giving an indication of conditions. This applies not only to biological analyses but physical and chemical analyses as well. If one wants a complete picture of river or estuary conditions then all groups of organisms as well as chemical and physical analyses must be included.



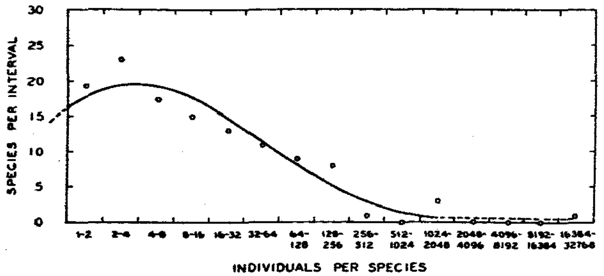
The Diatometer.

Fig. 1



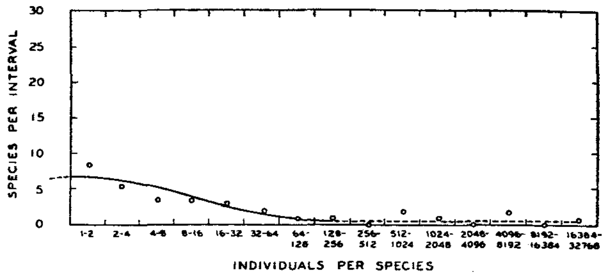
Graph of the Diatom Population from a River Not Adversely Affected by Pollution.

Fig. 2



Graph of a Diatom Population from a River Showing Mild Effects of Pollution.

Fig. 3



Graph of a Diatom Population from a River Showing Severe Effects of Pollution.

Fig. 4

To date this type of study of diatoms has been found to indicate reliably the quality of water as to its ability to support aquatic life. Although in most cases it indicates the condition not only of the water but also of the river bed, two exceptions have been found. These were areas in which the effluents entering a river had recently been greatly curtailed but the condition of the bottom was such that sessile or burrowing forms could not live in it. Thus the quality of water, as verified by bioassay tests on fish and invertebrates, was good even though the bottom was poor.

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USE AND VALUE OF FUNGI AS BIOLOGICAL INDICATORS OF POLLUTION

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A person who has spent more time studying North American forest than the streams which flow through them thinks of indicators in terms of trees. The presence of subalpine fir in Paradise Valley on Mount Rainier indicates that one is in the spurce-fir zone near the upper limit of trees on that type of mountain in that geographical location; the presence of Joshua trees in southeastern California indicates that one is in one phase of the Mojave Desert; the presence of sycamores and American elms in a shallow valley indicates that one is near a stream in the vast eastern deciduous or mixed mesophytic forest.

But these trees all indicate that one is on relatively undisturbed land. Our immediate concern is based on whether or not fungi can be used as indicators of pollution. It has been said (Suter and Moore, 1922) that the Saprolegniaceae can be used as indicators of pollution. It has been said (Suter and Moore, 1922; Butcher, 1932; Cooke, 1954) that Leptomitus lacteus is an indicator of pollution. The filamentous bacterium, Sphaerotilus natans, in its several forms, and the stalked protozoa including Carchesium have bee referred to as fungi which indicate pollution (Butcher, 1932; Cooke, 1954). That little interest has been shown in analysing the components of "sewage fungus" may be seen in Wilson's paper on the microbiota of sewage published in 1944. Here, "fungi indet." is simply one category among the many types of organisms listed according to genus. The number and types of fungi found in polluted water and sewage to date have only indicated that certain strains of a number of common soil fungi have become adapted to or are able to tolerate this different habitat.

Materials from several types of fungus studies will be drawn on in an attempt to determine something of the relationship between fungi and their habitats with special emphasis on pollution.

From the beginning of life, various members of the populations of the world have been leaving their bodies or the remains of their metabolic processes on the land or in the water to continue the process of decay so that the organic materials of which they were composed may be used and reused. One type of intermediate organism in the waters of the world is the group commonly called "sewage fungus". These organisms developed a specialized type of metabolism in which certain forms of carbon and nitrogen compounds can be utilized but not others. As the numbers of man increased, and as his physical requirements increased, the waters of the world have increasingly carried the waste products resulting from this development, and organisms adapted to the special nutrients found therein have thrived and multiplied. In this way, we may suppose, such organisms as Sphaerotilus and Leptomitus have developed, multiplied, and filled their special ecological niches. Leptomitus has developed to the point that it uses fatty acids rather than sugars, and amino acids rather than ammonia (Cantino, 1955).

There are at least three sources of pollution by which food materials usable by fungi are placed in streams. The first may be considered natural pollution. Pieces of decaying vegetable or animal materials may inadvertently reach streams through run-off, passive falling into the water, or other means. We are not concerned with this type of pollution. Many of the true water molds or aquatic fungi - Saprolegniales and related fungi - are found only under these conditions. They require something to attach themselves to during their vegetative phases. Such fragments of materials may serve as food as well as anchoring places for these water molds. Other water molds, belonging to the Chytridiales, do not always live freely in the water but may be parasitic upon various species of algae and in various kinds of pollen which float on the water usually in the spring. When their sources of nutrition become exhausted, these fungi produce resting spores and cells ("gemmae")

The second source of pollution is fecal material and other materials such as ground garbage added to the stream in domestic sewage. Such materials are high in organic content and may furnish large amounts of foods to fungi that have become adapted in one way or another to life in the water or in the stream bed. Under restricted conditions, with certain carbohydrate, nitrogenous, and as yet undetermined additives, and under certain conditions of substrate and bottom materials resulting in special pH and mineral content conditions, certain Saprolegniaceae, Leptomitus lacteus, Sphaerotilus natans, and various soil fungi, such as Fusarium aquaeductuum, Geotrichum candidum, Penicillium lilacinum, and others, may thrive. Under these conditions, such organisms appearing in large numbers will indicate the presence of polluting substances, substances which make the waters of the stream unfit for human and most industrial uses.

The third source of pollution is various types of wastes from industry. Different types of industrial pollutants can produce varying conditions of growth in the vicinity of the point of discharge of the pollutant. Such pollutants will become diluted downstream. It is possible that, at the immediate outfall, oxygen supplies may be too poor and certain substances may be too toxic to support growth, but as oxygen increases and the toxic substances become diluted by increased flow, precipitation, or other factors, the toxic effect will be weakened and growth of various fungi and fungus-like organisms may be encouraged or enhanced. When the pollutant is organic, Leptomitus lacteus and the filamentous bacterium Sphaerotilus natans may thrive under such conditions; and at least Sphaerotilus will present several growth habits, mistaken as species, genera, or varieties in the literature, as the concentrated waste becomes more dilute. At Lytle Creek (Cooke, 1954c), a number of species of fungi have been found in isolations from water, sediment from polls and riffles, and apparently septic bank soil, at the outfall of primary settled sewage.

Both Leptomitus and Sphaerotilus have been cultured in the laboratory with little difficulty after the proper combination of nutrients has been determined. Such combinations indicate only that these organisms have special requirements for sugars and compounds containing nitrogen. In nature such substances are present largely under conditions of pollution by organic wastes from the home or factory; in the wilderness, such substances may be present following death and during decay of plants or animals. It is quite possible that these organisms may be common around watering holes and other places where animals have congregated, where their remains or droppings may have produced local natural pollution.

Another type of fungus may be thought to be more nearly indicative of pollution, especially of the domestic type. In the forest we find these coprophilous fungi associated with dung of wild animals, rarely of man. In many cases these fungi are restricted to specific types of dung: Mucor ramanianus is found on frog droppings; Pilobolus species are usually found on horse droppings. Other types of these fungi are found on the dung of any animal that uses plants as food. The spores of these fungi are so produced and discharged that they become attached to or glued to leaves of plants used as food by grazing or browsing animals. After the spore has passed through the alimentary canal of an animal, it is able to germinate, use the dung as food, and fruit quickly after deposit of the dung. Ephemeral mushrooms like the inky cap and the common mushroom are edible, but since man cooks the mushrooms he eats, their spores are killed before ingestion. Few if any coprophilous fungi are found associated with human feces, especially where this material accumulates as night soil or is carried away in sewers to rivers or sewage treatment plants. While Sordaria humana occurs on other types of dung, the specific name indicates that man also can ingest such fungus spores and that under certain conditions these fungi can be recovered from human wastes.

Three types of fungi which may be placed in this physiological class have been found in sewage polluted water and on trickling filters. A species of Pilobolus (Harvey, 1952) was found at one station on Lytle Creek by use of hemp seed as an isolating medium. It was found again on the Glendale, Ohio, trickling filter. Intensive biochemical nutrition research (Hesseltine, et al., 1953) has shown that this fungus can be propagated in the laboratory without ingestion and alimentation by an animal, but the technique has not been tried at Cincinnati. Ascodesmis microscopica was found only twice (Seaver, 1928) in the world prior to 1955. One of these growths was in Europe on tiger dung, the other at the Bronx Park Zoo on the dung of a raccoon dog. In 1955 the species was found on a trickling filter in the Dayton, Ohio, sewage treatment plant. It requires no special techniques or media but will grow on any medium used in culturing the so-called sugar fungi.

In 1953, Hesseltine, studying the fungi of trickling filters at Pearl River, New York, found a fungus (Subbaromyces splendens) which in pure culture grows only on lima bean agar. This has since been found on trickling filters at Dayton, Ohio, and Pullman, Washington (Becker and Shaw, 1955). It is possible that this fungus may grow in other habitats with similar environments, such as on stones of streams polluted with domestic wastes and on other trickling filter beds.

An organism can become adapted to one set of environmental conditions or another. In some cases the adaptation may be complete, so that the organism cannot tolerate any other conditions, but more often various degrees of adaptation may be attained by an organism. Moser (1949), studying the adaptation of certain fungi, mosses, and seed plants to areas in the tyrolean forests which had been burned by fires ranging from large forest fires to small camp fires, developed the following set of terms to describe the degree of adaptation of the organisms to burned areas: anthrocobiont species were found only on burned soils and are not known to occur elsewhere; anthracophilous species are found more commonly on burned areas; anthracoxenous species occur more commonly on unburned areas but will tolerate burned areas; while anthracophobous species will not grow on burned areas. This set of suffixes can be used to describe other habitat requirements of the same or different species. Since "copro-" has been used commonly in the combination "coprophilous" for a fungus growing on dung, it might be confusing to use in the terms we want. The Greek work "lyma" means "filth". Then, lymabiont species will grow only on or in the presence of fecal materials; lymaphilous species will grow commonly on such material but will also grow on other materials; lymaxenous species will grow commonly on other materials but will tolerate fecal matter; while lymaphobous species will not grow on or in the presence of fecal material. In this sense, a coprophilous species may be either a lymabiont or a lymaphile. A lymaxene could be an organism which on occasion will colonize the substrate material, while a lymaphobe will not grow on such materials. The emphasis here is on nutrient requirements or tolerances, rather than on habitat types or tolerances as is the emphasis in the Kolkwitz (1950) system.

To equate this set of terms with those of Kolkwitz, the following parallels are suggested. Polysaprobes would include both lymabionts and lymaphiles. For use in connection with fungi, polysaprobe would be confusing since it implies the ability to live saprobically on any substrate. Alpha (or strong) mesosaprobes would include lymaphiles primarily, but lymabionts and lymaxenes could be expected to be found. Beta (or weak) mesosaprobes would include lymaphiles, but lymaxenes could be found more frequently than in the preceding categories. Oligosaprobes would include the lymaphobes and probably some lymaxenes and lymaphiles.

Indicators of fecal pollution will thus be found in the first two categories, lymabionts and lymaphiles, rather than restricted to the first category. Coprophilous species connote only those species of fungi or other organisms growing on dung at the time of observation, so the term is not useful for our purposes. Of the lymaphilous species, one must be careful that the habitat is described in more than one way. For instance, the fungus Fusarium aquaeductuum was first described from wooden water pipes which under certain conditions could be plugged with its massive growth. At present, wooden pipes are used infrequently, pure water systems have been rarely checked for the fungus, and it is abundant on trickling filters throughout the country as well as in polluted streams.

In intensively cultivated fields there is strong competition for nutrient materials among the various species, varieties, strains, and clones of fungi present. Such competition has given rise to species that have become adapted to the utilization of special nutrients (Garrett, 1956). A strictly saprobic species will attack almost any dead organic material. Certain species are able to attack young seedlings, produce seedling death, and then use the dead tissues as nutrient sources for primary colonization of new substrata. Other species can penetrate the roots of older plants, grow through their vascular tissues, and produce the type of disease referred to as vascular wilts. Such fungi in the soil may be considered ecological obligate parasites, for although they can be readily cultured on any agar medium on which sugar fungi develop, in nature they cannot compete with those fungi that colonize dead tissue, but must create their own dead tissue by killing the host. As the host plant dies, the fungus can utilize its tissues as food until forced into retirement by competition from other fungi or depletion of nutrient materials.

These vascular wilt fungi belong to the genera *Fusarium* and *Verticillium*, among others. Three species of the wilt-producing fungi, *F. oxysporum*, *F. roseum*, and *F. solani*, have been isolated occasionally from sewage and polluted water. The source of these fungi in the samples that have been studied is unknown, but the species are widely distributed agricultural pests. Nothing is known of their activities away from the soils in which they are of greatest importance as plant disease producing fungi, except that they are capable of causing deterioration of cellulose. In preliminary experiments in dilution water it was apparent that a strain of *F. oxysporum* is capable of using hydrocarbons as its sole source of carbon. That these organisms are isolated occasionally or even commonly from sewage and polluted water indicates that they are capable of competing with other fungi and other organisms for whatever nutrients are offered. In contrast to the soil, where only occasionally does dead organic matter become available to the fungi of a specific unit of soil, or where only occasionally does a plant root become available for colonization even in a field where the crop is relatively densely planted, polluted water presents to the fungus a continuous source of nutrient supply. Such a supply makes it unnecessary for the fungus to assume a resting phase upon the depletion of one source of nutrient while waiting for a new source.

Nutrition requirements of organisms found in industrial types of pollution are more difficult to define. Albritton (1955) is very general on the point of fungus nutrient requirements. Fecal material is at a minimum in such wastes, while sugars and possibly organic nitrogen sources are of greater importance. Here a somewhat different type of organism will be ascendant, although many of the same species will occur commonly in both types of pollution. Again, where a waste contains metallic ions usually inhibitory to the growth of organisms, certain species may become adapted to life in the presence of those substances. For instance, at least one species of *Penicillium*, *P. ochrochloron*, can tolerate high concentrations of copper, another, manganese. Some fungus contaminants can be found commonly in acids thought to be toxic to any growth. In general, such fungi fall into the broad classification recently proposed: "sugar fungi". These fungi are able to utilize simple sugars but in many cases are not able to develop an enzyme system for the degradation of more complex carbohydrates, or other carbon sources.

A further difficulty with the definition of a pollution indicator among the fungi is the inability of the investigator to identify most of the fungus growth in any one sample on sight or on preliminary microscopic examination. Most soil fungi growing in an aqueous habitat do not produce spores but form an extensive mycelial mat. The mat may be formed by the interweaving of a number of mycelia of many species. The orange color produced by Fusarium aquaeductuum is a result of large numbers of spores piled together or produced concurrently. The white color produced by Geotrichum candidum on surface films of trickling filters is quickly masked by algae or other fungi, or the filamentous bacterium Sphaerotilus natans which produces a grey color; or, it may be confused with the occasionally produced white mat of Leptomitus lacteus when that is able to appear. In some cases, species of Penicillium may be observed in fruiting condition, but it is rare that a typical fruiting structure will be produced; the phialides producing the spores may be found singly on the hypha or in atypical clusters, rather than clustered in the typical penicillate brush at the tip of the spore-producing hypha. It is virtually impossible to study the several genera of the white yeasts, the red yeast, and the black yeast by direct observation of scrapings from trickling filter stone or stones in creeks or rivers subjected to continual pollution loads. Such studies must be made by using cultural techniques and studying the colonies that develop directly on properly prepared dilution plates or later in pure culture.

Techniques used most successfully so far in the isolation of fungi from polluted water and sewage have been described by Cooke (1954b). For isolation of aquatic fungi the technique described by Harvey (1952) is adequate. For help in studying the fungi that have been isolated, several books are available. The various media used in the study and identification of yeasts are described by Lodder and Kreger-van Rij (1952). The genera Aspergillus and Penicillium are best studied by techniques defined respectively by Thom and Raper (1945) and by Raper and Thom (1949). When species obtained by baiting or using hemp seed according to Harvey's method are obtained, they can be studied with the help of Coker (1923) or Coker and Matthews (1937). If species of Pythium appear following use of this technique, Middleton (1943) is useful. Parasites of plankton, pollen grains, and similar substrata can be studied with the help of Sparrow (1943). Of other fungi, that have not been studied consistently on one medium or do not belong in the categories mentioned above, some groups cannot be satisfactorily studied at present. These include primitive Ascomycetes, mycelial Basidiomycetes, sterile mycelia, members of the Sphaeropsidales, and such genera as Chaetomium. For none of these groups are there adequate taxonomic treatments, and cultures must be sent to specialists. Of the Sphaeropsidales, most known species are studied with reference to a specific host plant. Cultures from water, sewage, or soil, therefore, cannot be identified satisfactorily without screening the potential host plant population of the region. To aid in the identification of a genus of fungus, the worker can consult Clements and Shear (1932), whose keys are lengthy and cumbersome, or he can study Barnett (1955), who has illustrated nearly one-third of the genera including those that are more common. For more specific information, Gilman's (1945) compilation is useful, and Smith (1954) gives considerable help with the commer species found in industry and the laboratory. Finally, Hughes (1953) points toward newer concepts in developing a workable system of classification of the so-called mold fungi.

A technique for obtaining samples of growth from trickling filters, modified from similar techniques whose origin dates back to 1905, has been developed in which, rather than scraping ths stone, glass slides are placed in mounts in contact with the growth on the stone and continually irrigated by the settled sewage spray applied to the filter beds. Growth on the slides can be removed by scraping with a rubber policeman, the algae counted, the protozoans studied, and the fungi and bacteria plated. If the material to be studied for development of fungi is broken up in a Waring Blendor, an approximation of the importance of each fungal species can be obtained by counting the number of colonies of each species or of all species. Using this technique, it appears that 10 to 15 species of soil fungi form the largest part of the fungal portion of the slimes on trickling filters.

Annotated Bibliography

Albritton, E. C., 1955. Standard Values in Nutrition and Metabolism. xiii, 380. Philadelphia, W. B. Saunders Co.

A tabular presentation of the nutrient requirements of the principal groups of organisms, of certain specific groups and of certain species. Tables also present food and feed values of certain species of organisms, and metabolic products of various organisms used in industry.

Barnett, H. L., 1955. Illustrated Genera of Imperfect Fungi. i, 218. Minneapolis, Burgess Publishing Co.

The commoner genera of mold fungi and plant pathogens are illustrated with line drawings. Habitat descriptions and references are given for each species illustrated.

Becker, J. G. and C. G. Shaw, 1955. Fungi in domestic sewage treatment plants. Applied Microbiology 3: 173-180

A study of fungi found in the sewage treatment plants at Pullman, Washington, and Moscow, Idaho, based on studies of effluents from various process points in the two plants which treat domestic sewage.

Butcher, R. W., 1932. Contribution to our knowledge of sewage fungus. Trans. Brit. Myc. Soc. 17: 112-124

A systematic study of the various organisms which are associated with polluted water and sewage treatment plants and which have been termed "sewage fungus".

Cantino, E. C., 1955. Physiology and phylogeny in the water molds - A reevaluation. Quart. Rev. Biol. 30: 138-149.

A study based on the writer's personal experience with some of the groups of organisms listed as well as on a thorough knowledge of the literature, in which an attempt is made to base a phylogeny of the water molds on what is known of their physiology.

Clements, F. E. and C. L. Shear, 1931. The Genera of Fungi. iv, 496. New York, The H. W. Wilson Co.

A compendium designed to give a key to all genera of fungi known up to 1930 and whose species are listed in Saccardo's Sylloge Fungorum. This manual is unwieldy, has some errors based on increasing knowledge of some of the greater groups, and has many incomplete or uncritical key characters.

Coker, W. C., 1923. The Saprolegniaceae 201. Chapel Hill, The University of North Carolina Press.

A well illustrated monographic study of the commonest or easiest isolated family of water molds, the Saprolegniaceae.

Cooker, W. C. and V. D. Matthews, 1937. Saprolegniales. North American Flora 2: 15-67.

The definitive monograph of the Saprolegniales as known at present. In this work certain concepts presented in the preceding reference are clarified, but it is not illustrated and the two should be used together.

Cooke, W. B., 1954a. Fungi in polluted water and sewage. I. Literature Review. Sewage and Industrial Wastes 26: 539-549.

A review of the papers available at the time on fungi in polluted water and sewage.

Cooke, W. B., 1954b. Fungi in polluted water and sewage. II. Isolation technique. Sewage and Industrial Wastes 26: 661-674.

A description of isolation techniques and a discussion of the various media used in the isolation of fungi from polluted water and sewage.

Cooke, W. B., 1954c. Fungi in polluted water and sewage. III. Fungi in a small polluted stream. Sewage and Industrial Wastes 26:

A quantitative study of the fungi found at eight stations on Lytle Creek.

Garrett, S. D., 1956. Biology of Root-Infecting Fungi. xi, 293. New York, Cambridge University Press.

A number of older concepts are clarified and some new concepts given concerning the biology of root-infecting fungi and their occurrence in the soil.

Gilman, J. C., 1945. A Manual of Soil Fungi. xi, 392. Ames, Iowa State State College Press.

A compilation listing and describing all fungi known to occur in the soil. Since the book is based on available descriptions and not on laboratory experience of the writer, it indicates areas where additional work is required. For instance, in certain larger genera one species may be described as it appears on one medium, another from a different medium.

Harvey, J. V., 1952. Relationship of aquatic fungi to water pollution. Sewage and Industrial Wastes 24: 1159-1164.

A study based on the isolation of water molds from polluted streams in the Little Miami Basin over the period of a year. It appears that few, if any, aquatic fungi are definitely associated with polluted water.

Hesseltine, C. W., 1953. Study of trickling filter fungi. Bull. Torr. Bot. Cl. 80: 507-514.

Geotrichum candidum, Fusarium aquaeductuum and Subbaromyces splendens are considered to be the fungi of most importance on trickling filters treating pharmaceutical and chemical wastes at Lederle Laboratories, Pearl River, New York. Other fungi appearing in isolations were considered contaminants or only incidentally present.

Hesseltine, C. W., et al., 1953. Coprogen, a new growth factor present in dung, required by Pilobolus species. Mycologia 45: 7-19.

A description of the new growth factor coprogen, an indication that it can be produced by other organisms in the habitat, and its effect on Pilobolus

Hughes, S. J., 1953. Conidiophores, conidia and classification. Canadian Journ. Bot. 31: 577-659.

A description of a new system of classification for the Fungi Imperfecti based on methods of spore production.

Kolkwitz, R., 1950. Oekologie der Saprobien. Uber die Beziehungen der Wasserorganismen zur Umwelt. Schrift. ver. Wasser.-, Boden-, und Lufthygiene. 4: 1-64.

An annotated list of species of organisms found in the several zones of a stream identified in the Kolkwitz and Marsson system as: polysaprobic, alpha-mesosaprobic, beta-masosaprobic and oligosaprobic.

Lodder, J. and N. J. W. Kreger-van Rij, 1952. The Yeasts. A Taxonomic Study. xi, 713. New York, Interscience Publishers, Inc.

The definitive manual of yeasts. Classification into genera, families and orders is based on morphology, into species and varieties is based on reactions in culture to a number of standardized morphological and chemical tests.

Middleton, J. T., 1943. The taxonomy, host range and geographic distribution of the genus Pythium. Mem. Torr. Bot. Cl. 20(1): 1-171.

The definitive manual for identification of the difficult genus Pythium which occurs in soils and water. It is isolated occasionally on hemp seed but without knowledge of the species of plant with which the parent strain was associated it is difficult to make an identification.

Moser, M., 1949. Untersuchungen Uber den Einfluss von Waldbranden auf die Pilzvegetation I. Sydowia 3: 336-383.

A study of burned areas in the Austrian Tyrol near Innsbruck in which the concept of four degrees of relationship of a fungus or other organism to its environment is introduced.

Raper, K. B. and C. Thom, 1949. A Manual of the Penicillia. ci, 875. Baltimore, The Williams and Wilkins Co.

The definitive manual for the genus Penicillium. This is most useful in our work since of the 150 species described on standardized media, about half have been isolated during the course of our work.

Seaver, F. J., 1928. The North American Cup Fungi (Operculates). pp. 284. New York, Published by the author.

A manual for the study of the Operculate Discomycetes, cup fungi.

Smith, G., 1954. An Introduction to Industrial Mycology. Fourth Edition. xiv. 378. London, Edward Arnold (Publishers) Ltd.

A manual for workers and students of industrial mycology. In this book the commoner species used in industry and the commoner contaminants of the laboratory and of industry are described. There are valuable chapters on various phases of the biology and use of fungi.

Sparrow, F. K., 1943. Aquatic Phycomycetes, exclusive of the Saprolegniaceae and Pythium. xix, 785. Ann Arbor, The University of Michigan Press.

A manual for the study of various groups of aquatic fungi. This will be of help to one who isolates fungi from waters especially if they are associated with plankton, pollen, and other materials in the water. It does not overlap the work of Coker or Middleton mentioned above.

Suter, R. and E. Moore, 1922. Stream pollution studies. State of New York Conservation Commission, Albany. pp. 1-8, pl. 9.

This little pamphlet indicates that certain aquatic fungi can be used as indicators of pollution but identifications, and therefore groups mentioned appear to be in error.

Thom, C. and K. B. Raper, 1945. A Manual of the Aspergilli. ix, 373. Baltimore, The Williams and Wilkins Co

The definitive manual for the identification of Aspergillus. Species in most of the subgeneric groups have been isolated from polluted water and sewage and use of this manual makes their identification relatively easy.

Wilson, J. N., 1949. Microbiota of sewage treatment plants and polluted streams. pp. 1-15, A.A.A.S. symposium: Limnological Aspects Water Supply and Sewage Disposal.

This paper presents certain basic concepts on the composition of the slimes on trickling filters. While admitting the presence of fungi, no attempt is made to determine the comparative role of any one or another species and of bacteria in the formation of the basic slime.

USE AND VALUE OF BIOLOGICAL INDICATORS OF POLLUTION: FRESH WATER CLAMS AND SNAILS

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I. INTRODUCTION

In discussing fresh water clams and snails (mollusks), not enough is known yet about molluscan ecology to name any species a pollution indicator. There are mollusks tolerant to certain effects of pollutants such as septicity, but even these are not pollution indicators. Species that are found associated with domestic sewage in septic reaches of water, as Musculium transversum, Pisidium idahoensis, Physa integra, and Physa heterostropha, are also found in high dissolved oxygen areas of lakes and streams unpolluted by domestic sewage or putrescible industrial wastes.

On the other hand certain mollusks, such as the Unionidae*, are not associated with near-septic water resulting from pollution. These have an index value in that their presence typically indicates good dissolved oxygen and attendant physical and chemical conditions associated with unpolluted water. Such mollusks can be called clean water index organisms.

Apart from systematic morphological studies, it is not realistic to isolate a single group of organisms such as mollusks from other animals and plants that are associates under similar ecological conditions in clean or polluted water. It is the study of the total biota which tells one most about water conditions.

*Members of the family Unionidae have had various common names applied to them: Mussels, fresh water clams, and naiads.

In this respect, the presence of an assemblage of rat-tailed maggots, Eristalis tenax; sewage mosquitoes, Culex pipiens; sludge worms, Tubifex tubifex; blood worms, Chironomus plumosus; physid snails, Physa integra; and finger-nail clams, Musculium transversum, and an absence of Unionidae, mayflies, caddis worms, stoneflies, and shiners would indicate to investigators stream reaches highly degraded by domestic sewage, for example. Thus, certain associations of organisms that tolerate such pollutional conditions as septicity and the absence of intolerant forms can be looked upon collectively to form pollution tolerant biological assemblages, even though any single mollusk species or other members of the assemblage may not be called a pollution indicator. The presence of intolerant mollusks, with other intolerant animals, lend themselves usefully in sanitary science to establishing parameters around areas of septicity and sludge deposits resulting from domestic dewage.

Information is not available that can be presented to indicate that various species of mollusks can be used to indicate varying degrees of water quality, i.e. from high dissolved oxygen values by gradations to septicity, such as can be measured by chemical tests. Also, various species cannot be used to measure variations in fecal contamination as can certain bacteria.

The majority of studies made in United States waterways dealing with the effects of pollution on mollusks are related to domestic sewage. The principal effect of such pollution on water quality, investigated in relation to mollusk survival, is that of lowered dissolved oxygen. Some attention has also been given to the effects on mollusks of bottom deposits attendant to domestic sewage and silt pollution. Little information dealing specifically with the effects of industrial wastes or their components on fresh water mollusks has been found.

The information presented below can assist those working with biological indices of pollution to group mollusks as either pollution-tolerant or clean-water forms. Consideration is given to the following aspects of this subject: references relating mollusks to pollution; structural and life cycle variations relating to survival in polluted water; natural variations in distribution not related to pollution; and identification sources.

II. DISCUSSION OF SELECTED REFERENCES

Selected references that may be readily available to those working in sanitary sciences are cited here that especially deal with waterways of the United States. No attempt is made to present a complete literature review covering pollution and its effects on mollusks. Many of the included references should point out to those studying bottom organisms the importance of recording chemical and physical data that can be analyzed in relation to tolerances of specific mollusks to pollutants.

Available literature relating mollusks to water chemistry is woefully lacking. When the word "pollution" is used, the general inference is to domestic sewage. Except in a few specific studies of industrial wastes, cognizance often is not taken of the effects of such wastes in association with domestic sewage, even though they may have been related to the presence or absence of mollusks.

In order to make data concerning the effects of pollution on mollusks comparable, pollution should be defined both chemically and physically. It is also necessary to identify mollusks to species, if pollution-tolerant ones are to be exactingly separated from intolerant ones. Specific identification is particularly important to those who hope to find indicators of degrees of pollution.

If work, under field conditions, on the relationship of mollusks to physical and chemical factors is contemplated, Boycott (1936) should be consulted early in the planning stages. Even though relatively few of the species he deals with are found in North America, the information he presents associating mollusks with water chemistry should provide valuable background information for North American studies.

(1) References Relating Mollusks to Pollution in General

The following references relate mollusks to pollution in general without consideration of chemical and physical data. Such papers are valuable, in that they contain references to mollusks already identified to genera or to species by outstanding authorities in Conchology. By being aware of such references aquatic biologists working on water pollution problems have mollusk names available from certain areas, that may give them a lead to identification of current collections.

In relation to water pollution in general with special reference to streams in western Pennsylvania, Ortman (1909) wrote that the Unionidae are the first to be eliminated from polluted waters. Further, he states that the genera Pleurocera, Goniobasis, and Anculosa are usually absent in polluted rivers, but were found surviving when the Unionidae and fishes were, for the greater part, gone from the Allegheny River in Venango County, Pennsylvania. The genera Lymnaea, Physa, and Planorbis are noted to be more resistant because they are air-breathers. Physa is the hardiest and is stated to be a genus "... which represents in certain instances the only remaining life in certain rivers. But there also seems to be a limit to its power of endurance, and in very badly polluted streams also Physa is absent."

Baker (1911), in quoting French investigators, states that Sphaerium, Pisidium, and Planorbis resist the effects of water contaminated with sewage, oil, and chemicals better than Lymnaea. Baker (1911) reports, from his own observations made at Rochester, New York, that the Genessee River into which sewage has been discharged for the past"... ten or fifteen... years is ... at the present time ... of the consistency of dirty, greasy dish water, yet Galba catascopium and Planorbis trivolvis live and thrive by thousands in this seemingly unfavorable environment. The writer's observations have been that chemicals and oil are deadly to molluscan life, while sewage does not materially affect them." In a footnote to this statement, Baker comments that since writing the above, sewage in the Genessee River has become "...of such a highly concentrated form that the mollusks have all disappeared in the river for a mile or two below the point of discharge into the river."

In a second report on the pollution of the Genessee River at Rochester, New York, Baker (1922) states that he has studied its pollution for 27 years, from 1892 to 1919. He mentions that pollutants are "... sewage ... discharged into the river in a crude condition ..," and that "refuse and other waste matter, both liquid and solid, also enter the stream from gas works, tanneries, and manufacturing plants... Mollusk collections that he made in 1892, before pollution became apparent, represented 9 species: Musculium transversum, M. partumeium, Bythinia tentaculata, Planorbis trivolvis, Physa gyrina, P. sayii, P. oneida, Galba caperata, and G. catascopium. In 1907 the above species of Musculium and Bythinia had disappeared, with the air-breathers Planorbis, Physa, and Galba still present but reduced in numbers. In 1910 all mollusks had disappeared and none were found in subsequent collecting trips from 1910 to 1913. Baker (1922) describes studies that G. C. Whipple made on the river in 1912 after molluscan life had disappeared. He reports that Whipple found the dissolved oxygen varying from 5 to 41 per cent of saturation in August.

On one day in this month saturation did not exceed 5 per cent in a 3 mile reach from the surface to bottom in a depth of about 26 feet. He states that in 1917 a large part of the Rochester sewage, 32 million gallons a day, was diverted from the river to a sewage treatment plant, the effluent of which was discharged into deep water of Lake Ontario. In 1919 Baker reports the following mollusks occupying the reach of stream that had become devoid of them before sewage treatment was installed: Musculium transversum, Bythinia tentaculata, Galba catascopium, Planorbis trivolvis, Physa integra, and P. oneida.

According to the study by Wilson and Clark (1912) on the mussel fauna of the Kankakee Basin in relation to destruction by dredging operations, "The most fatal condition is the constant movement of the fine sand and silt along the bottom of dredged channels." They further state, "Portions of the basin which were dredged 15 or 20 years ago show no signs of restocking with mussels, though there are thousands of them close at hand in old channels."

Considering the effects of pollution on the mussel fauna of the Big Vermilion River and its tributaries in Illinois Baker (1922) states that, "sewage pollution has killed all clean water life for a distance of fourteen miles below Urbana and has made the stream an unfavorable environment for a distance of twenty miles. Below this point the fauna is normal and is not affected by sewage pollution." He observed that of that large species of Unionidae, Amblema undulata and Lasmigona complanata, resisted pollution conditions better than others. In a preliminary paper to this report, Baker and Smith (1919) also wrote on the same subject.

Baker (1928), in the pelecypod part of his monograph on Wisconsin fresh water snails and clams, mentions that stream pollution by sewage and manufacturing wastes produces unfavorable conditions for mollusks. In reference to industrial wastes, he writes that coal tars and oils in particular quickly make a stream totally unfit for any kind of animal life.

In his work on the mollusca of Michigan Goodrich (1932) states that Lymnaea stagnalis appressa are being reduced by drainage enterprises and pollution. He adds that this gastropod probably disappeared from great areas in a few years because waterways were used for logging purposes and sawdust disposal.

Van der Schalie (1936) notes that over the years domestic sewage and industrial wastes in general, along with other factors, have had a detrimental effect on the naiad fauna of the St. Joseph River Drainage in southwestern Michigan. Effects of pollution on specific mollusks are not discussed.

Van der Schalie (1936a), when discussing factors contributing to the depletion of naiades in the eastern United States in relation to the fauna of the Mississippi River, states, "Pollution, particularly below the several large cities located on the river and its important tributaries, was responsible for a heavy mortality in glochidia which were attached by bacteria and infusoria." In discussing Ellis' (1931) field investigations relating to the effects of silt on the fisheries of the Mississippi River, he further states that "The river is practically devoid of mussels from the region of St. Louis, Missouri, to its mouth, a condition accounted for by the tons of silt carried to the stream and deposited in it by the waters of the Missouri River which enters the Mississippi near St. Louis. . . . ." Writing about the "South Atlantic Costal drainages" he mentions that mine wastes from coal mines in the headwaters of the James River have damaged the fauna of this stream. With reference to the mussel fauna of the Great Lakes Drainage rivers in Michigan he comments that industrial wastes and sewage are particularly damaging to mussels in the Saginaw drainage, St. Joseph, the Kalamazoo, the Grand, and Rouge Rivers; wastes from beet sugar refineries are felt to be responsible for "unproductive" areas in the Raisin and Pine Rivers. In reference to the above drainage area he states, "In many localities action has been taken by the State to curtail such destructive influences (pollution), though usually much irreparable damage has been done before preventive action has become effective." Thus, in relation to the effects of factors affecting mussels of the eastern United States van der Schalie writes, ". . . silting, pollution by sewage, mine and industrial wastes, power-dam developments and unrestricted mussel gathering for the pearl button industry, have resulted in the critical depletion of the formerly abundant Naiad fauna."

In writing about the depletion of the mussel resources of Michigan van der Schalie (1938a) mentions that among the outstanding factors are pollution in streams by sewage and industrial wastes and the extensive program of power dam developments. He further comments that mussels are among the most sensitive of organisms to pollution and are among the first to perish where pollution is in evidence.

Van der Schalie (1938b) points out that the Cahaba River in northern Alabama, as of 1938, was unusually productive conchologically, but that there are several potential dangers to Cahaba mussels:

the possibility of acid pollution from the Cahaba coal field, industrial waste concentrations and sewage from Birmingham, and dam construction. He writes, "In view of these possible changes, studies of the fauna under natural conditions are highly desirable."

Goodrich (1939) mentions that the lumber industry has affected mollusks by fire, sawdust and rafting. However, he does not assess damages.

From mussel studies of the Grand and Muskegon Rivers in Michigan van der Schalie (1941) writes that many factors are causing the depletion of the indigenous fauna. Thus, it becomes increasingly important to gather ecological and distributional data about them before damage becomes too severe. Factors in addition to dredging, that have damaged mussel populations in the Grand River, are many kinds of industrial wastes and sewage in reaches below Jackson, Lansing, and Grand Rapids. Gathering mussels with apparatus injurious to mussel beds is also mentioned. In relation to the Muskegon River, he lists power dams and pollution as being detrimental to mussels and hindering their distribution through obstructing fish, the "........carrying agents for fresh-water mussels."

Van Horn (1949) associates the air-breathing snails Physa and Planorbis with a zone of recent pollution, and states, "In this zone also, as the oxygen concentration is decreased, one may find sewage fungi ... such as Sphaerotilus, Leptomitus, Thiothrix, and others."

According to van der Schalie and van der Schalie (1950), reporting on the mussels of the Mississippi River, "The several surveys of the Mississippi emphasize that the changes brought about in this drainage through adverse conditions, such as silting, pollution, intensive exploitation of the mussel fauna, power dam construction, etc., are all tending to alter decidedly, as well as to reduce, the original fauna."

Beck (1954), in his ecological classification of the streams of Florida relating organisms to water pollution surveys, refers mollusks of the genera Physa and Ferrissia and the Sphaeriidae to a "Class III" which includes organisms which have been found in heavily polluted areas. He comments that no organisms in this class may be considered indicative of pollution, since organisms contained therein may be found in clean, moderately polluted, or grossly polluted water. Goniobasis spp. is placed in a "Class I" containing organisms that have been found to tolerate no appreciable organic pollution, "...the more sensitive forms." He states that, "The presence of class I organisms is considered to indicate that the water in which they are found is clean."

Carlander (1954) writes about the general effects of pollution, including silt, on the mussels of the upper Mississippi River using material published by the U. S. Fish and Wildlife Service as a basis for her discussion.

In presenting his list of mollusks (Table-1) that can survive "....at least to some degre..." in zones of degradation and recovery, Wurtz (1956) points out that "....we are woefully lacking in knowledge on this subject." He further writes that the exact tolerance limits of the mollusks he lists are not known, and that as far as he can ascertai no mollusks are able to withstand protracted gross pollution. From among the 34 species and subspecies of mollusks he has considered, he states that Physa heterostropha is the most tolerant species that has been found.

TABLE I*

Mollusks Reported to Survive "at least some degree" in Zones of Degradation and Recovery

GILL-BREATHING SNAILS	Family - Physidae	FRESH WATER CLAMS
(Ctenobranchiata)	1. Physa gyrina	Family - Sphaeriidae
Family - Viviparidae	2. P. heterostropha	1. Sphaerium rhomboideum
1. Campeloma integrum	3. P. integra	2. S. corneum
2. C. rufum	4. Aplexa hypnorum	3. S. striatinum
Family Amnicolidae	Family - Planorbidae	4. S. sulcatum
1. Bulimus tentaculatus	1. Helisoma anceps	5. S. (Musculium) securis
	2. H. trivolvis	6. S. (Musculium) tranversum
LUNG-BREATHING	3. Gyralus arcticus	7. Pisidium amnicum
(Pulmonata)	4. Menetus dilatatus	8. P. casertanum
Family - Lymnaeidae	Family - Ancylidae	9. P. compressum
1. Lymnaea caperata	1. Ferrissia fusca	10. P. fallax
2. L. humilis	2. F. tarda	11. P. henslorvanum
3. L. obrussa		12. P. subtruncatum
4. L. polustris		Family - Dresisseniidae
5. L. stagnalis		1. Mytilopsis leucophaeatus[x]
6. L. auricularia		Family - Mactridae[x]
7. Pseudosuccinae columella		1. Rangia cuneata

* Mollusks of Wurtz (1956) that can survive "... at least to some degree ..." in zones of degradation and recovery. Wurtz's data have been organized to form this table by W. M. Ingram.

x Only one species in each family in the United States; the former with a range from Maryland to Florida, and the latter with a range from Alabama to Texas.

(2) References Relating Mollusks to Specific Aspects of Water Quality Associated with Pollution.

The following investigators have presented varying amounts of physical and chemical data that relate mollusks to certain aspects of water quality.

Juday (1908 and 1921) reports a Sphaeriid clam, Corneocyclas [=Pisidum] idahoensis, from septic water in Lake Mendota, Wisconsin; however he does not mention specific pollutional conditions. From laboratory observations he concluded that this clam may remain quiescent with its valves closed for as long as three months each summer in the septic bottom ooze of the lake. He found that clams surviving in septic water with their valves tightly closed became active when placed in aerated water. Shelford (1913) has listed Juday's (1908) work relating to survival of Pisidium idahoensis under anaerobic conditions.

Weston and Turner (1917) found Physa heterostropha, Helisoma trivolvis, Segmentina armigera, and Unio complanata living in a stream below a sewage treatment plant outfall where average monthly dissolved oxygen values in October, June, July, and August were between 1 and 2 p.p.m. These writers do not present D.O. figures other than in monthly averages. The gastropod Campeloma decisum was not taken in any stream reaches where the average monthly D.O. was below 5 p.p.m.

Jewell (1920) discusses mollusks and other organisms associated with clean water and polluted water reaches of the Sangamon River, Illinois. This author mentions that there is some domestic sewage pollution from the effluent of a septic tank in the town of Monticello entering the clean water area, but such pollution only affects the water in a restricted area. Chemical conditions of clean water reaches are stated to be, "...alkalinity to methyl orange of 222 p.p.m. and a pH of 8... transparent to a depth of a foot or more." Bottom materials are said to consist of gravel. Close to shore it was observed that a "...layer of organic deposit covered the bottom... ." Mollusks associated with pools in such water were the Unionidae species, Lampsilis luteola, Quadrula pustulosa, Q. undulata, Q. rubiginosa, Tritigonia tuberculata, Symphynota costata, Strophitus edentulus, and Anodonta grandis, unnamed species of Sphaeriidae, unnamed species of Pleuroceridae, and Campeloma subsolidum.

Below the reach of the Sangamon described above, sewage of Decatur is discharged. Bottom animals were stated to disappear immediately and "...not until Noantic is reached, about 30 miles below

[Decatur], do the first animals appear." No mollusks are listed in Jewell's tables for 75 miles below Decatur. In this distance figures are listed showing variations in chemical and physical conditions: D.O. from 0 to 4.1 p.p.m. alkalinity from 260 to 348 p.p.m., pH 7 to 8, odor from septic and putrid to not pronounced, appearance from sewage to inky to milky to grey to turbid to green-grey. At the 75-mile station where mollusks are first noted to reappear, chemical and physical conditions show: D.O. of 7.8 p.p.m., an alkalinity of 287 p.p.m., a green appearance, and no odor. The mollusks occuring at this station were Unionidae, Quadrula undulata, Q. lachramosa, Lampsilis alata, L. luteola, L. anadontoides, Unio gibbosus, Anodonta grandis, Tritigonia tuberculata, Strophitus edentulus, Alasmodonta costata, unidentified "Sphaerium," and the gastropods Pleurocera elevatum and Campeloma subsolidum. Unionidae and gill-breathing snails at various stations are associated with disolved oxygen conditions that were not less than 7.5 p.p.m.

Richardson published a number of papers relating general pollution of the Illinois River to bottom fauna, among which his 1921, 1925, 1925a, and 1928 papers are cited in the bibliography as being of special interest to those studying mollusks. Richardson's 1925 paper lends itself to an attempt by the writer to generally correlate dissolved oxygen-mollusk relationships at certain stations in the reach from Chillicothe into Spring Bay Narrows, from the few dissolved oxygen figures that he presents under the title, "Bottom Dissolved Oxygen Mid Channel." Three species of Sphaeriidae, Musculium transversum, M. truncatum, and Pisidium complanatum, can be associated with a minimum D.O. of 0.2 p.p.m. The gill-breathing snail, Valvata tricarinata can be associated with a minimum D.O. of 1.4 p.p.m. The gill-breathing snail, Campeloma subsolidum, which Richardson stated as being a survivor under poor dissolved oxygen conditions, can be associated with dissolved oxygen figures between 0.5 to 3.0 p.p.m.

Baker (1926) presents a review of Richardson's 1925 papers on the effects of pollution on bottom organisms in the Illinois River. Data from the review state, "It is shown prior to 1915 there have disappeared from Peoria Lake (a widening of the Illinois River) about forty species of river mussels (only three or four species are left), all of the small snails belonging to the water-breathing [sic. = gill-breathing] family Amnicolidae, all but one species of the large water snails belonging to the Viviparidae, the remaining species being recorded as Campeloma subsolidum, and a varied weed fauna. There remains certain species which appear to be more tolerant of pollutional conditions. These are Musculium transversum, Pisidium compressum, P. pauperculum crystalense, Campeloma subsolidum, and Sphaerium stamineum. Several species less tolerant to sewage conditions were observed in peculiarly favorable conditions, usually in strong current in midchannel or elsewhere where oxygen conditions were good. These included Anodonta imbecillis, an insect Corixa, a caddis-fly larva (Leptocerid), Goniobasis livescens, Pleurocera elevatum lewisii, Quadrula plicata (= peruviana), Hyalella, a sponge, and a Hydropsyche. These species, however, were observed to vary in presence during different years."

Suter and Moore(1922) state that Physa heterostropha may be found in septic regions and list it with Pisidium abditum, Goniobasis virginica, and Campeloma decisum as an organism tolerant of pollution; they do not mention that the latter three species can survive septicity.

Turner (1927) lists Physa heterostropha as found in septic water and Planorbis panus from stagnant water and the vicinity of sewer outlets. Campeloma decisum is described as being more sensitive than the other species since "....it seems to thrive under clean water conditions."

Wiebe (1928), in his paper on the effects of pollution on the upper Mississippi River, collected Helisoma trivolvis, Musculium transversum and Campeloma integrum on August 27 at his Red Wing station with the D. O. at 2.83 p.p.m.; of 22 D. O. measurements made in August the average at this station was 2.25 p.p.m. with a minimum of 1.12 p.p.m. and a high of 4.01 p.p.m. Individuals of Musculium (near) transversum were collected at his Jackson Street station on August 17 with the D. O. at 0.87 p.p.m.; of 22 D. O. measurements made in August the average at this station was 0.87 p.p.m. with a range from 0 to 2.52 p.p.m. His data on tables 4 and 5 indicate that mussels (not identified) were taken in September at the Jackson Street station when the D. O. was 5.73 p.p.m. with the range of 20 readings for September varying from 0.44 to 8.14 p.p.m. He records collections of Anodonta imbecillis on September 17 at the Red Wing Station with the D. O. on this date being 4.39 and a range of 21 readings for September varying from 2.89 to 6.44 p.p.m. Campeloma rufum was taken at two stations where the D. O. was always above 5 p.p.m. for 61 measurements; Wiebe nevertheless comments in relation to this species, "....very likely it is one of the more tolerant forms;" this conclusion is based on the fact that 1,600 specimens per square yard of bottom were taken some 50 yards below a sewage outfall at one station and were associated with 15,120 Tubificidae and 54 Sphaerium notatum per square yard. Pleurocera acuta is reported from two stations where the D. O. was always above 4.30 p.p.m. for 65 measurements.

Purdy (1930), in summarizing pollution data on organisms other than plankton in his Illinois River work, states that the Sphaeriidae are often very numerous in moderately polluted water. He further writes that these mollusks cannot stand "extreme" conditions that Tubificid worms can and that they will die when oxygen becomes largely depleted. He states that, "Apparently their Sphaeriidae large numbers in places where water is polluted is a question of their abundant food supply of microscopic organisms normally found there." Purdy's data for the Illinois River, as correlated by Ingram with those of Hoskins et. al. (1927), show that unidentified Sphaeriidae were collected at Chillicothe where the dissolved oxygen was recorded as low as 1.23 p.p.m. in August. The highest D. O. at this station was 7.79 p.p.m. in February. Unidentified air-breathing snails collected by Purdy (1930) at Lockport, as correlated with Hoskins's et. al. (1927) data, were taken at this station where septicity existed in August and the D.O. was 9.11 in February. The pollution in the Illinois River at the time the above data were collected was from numerous industrial wastes and domestic sewage.

Ellis (1931a) writes that juvenile and young mussels are quite sensitive to oxygen reduction and that adult mussels "... usually become inactive when the oxygen tension of the water is reduced to 20 per cent saturation or less." He emphasizes the detrimental effect of erosion silt on clams. In relation to the general effect of industrial wastes on nussels, he writes, "Whenever concentrated industrial wastes are oured into the streams the fresh-water mussels suffer because of their nability to change location quickly and because of the ease with which he blood of fresh-water mussels takes up the various substances in he surrounding waters...."

Van der Schalie (1938) has presented chemical and physical data ssociated with unpolluted water and water polluted by domestic sewage n relation to the distribution of Unionidae in the Huron River, Michigan. eferring to the effect of pollution on mussels, he states, "Below Ann rbor, sewage has been very detrimental to the fauna. It is true hat sewage may, if not too concentrated, increase the productivity of ections of a stream by increasing the dissolved organic compounds, ut below Ann Arbor and as far as the backwaters of Geddes dam, ollution is so concentrated that it has killed all Naiades. Furthermore, ere is such a heavy deposit of sludge in this zone that it will be many ears before bottom conditions will permit the re-establishment of a una even though the discharge of sewage into the river be discontinued."

Goodrich (1945), in his monograph on the gilled-snail Goniobasis livescens in Michigan, associates water chemistry with its occurrence and with Pleuroceridae in general. He writes that this species has disappeared from the heavily polluted Huron River. He associates massive kills of Pleuroceridae in western Lake Erie with exhaustion of oxygen under ice cover. G. livescens has been found in streams with the alkalinity ranging from 87 to 233 p.p.m. He states that a drop in pH much below 7.0 does not permit survival of pleurocerid life judging by studies made by C.S. Shoup in the Obey River in 1939. Streams with a pH above 7.0 were extensively colonized, while these mollusks were absent in water with a pH of 6.1. Members of the genus Goniobasis in general endure periodic silting of streams that accompany freshets. Certain ones, G. virginica for example, may live in tidal reaches of streams where the salinity is 50 per cent of that of sea water.

Whipple, Fair, and Whipple (1949) present a list of mollusk species that they have associated with the pollution zones of Kolkwitz and Marsson (Table 2). They list no mollusks in the polysaprobic zone that they partially characterize by the presence of black sludge accumulations on the bottom and a lack of oxygen. They note that life in the mesosaprobic zone is commonly tolerant of dilute or imperfectly purified sewage and its products of decomposition, and state that "Many bacteria are still present." Pertaining to the oligosaprobic zone they write, "This is a zone of cleaner water in which mineralization has been completed. ..... The water is practically saturated with oxygen, sometimes even supersaturated." It should be noted from Table 2 that many mollusks associated with the mesosaprobic zone are also associated with the oligosaprobic. One Unionid clam and certain gill-breathing snails of the genera Campeloma, Viviparous, and Valvata that commonly inhabit clean water are associated with the mesosaprobic zone.

Patrick (1949) associated Physa heterostropha with "polluted water" conditions at a station 105 in Lititz Run, Pennsylvania. Chemical data are presented in tables, separated from biological data; such information presented for August, a month indicated to be "low water" for this station, shows a pH of 7.3; D.O., 8.00 p.p.m. turbidity, 10.8 p.p.m.; Nitrogen (N as NH_3^+), 0.015; Nitrogen (N as NO_2), 0.0260; Nitrogen (N as NO_3), 3.120; etc.

TABLE 2

Mollusks Related by Whipple, Fair, and Whipple to Pollution Zones of Kolkwitz and Marsson*

Mollusks	Pollutional Zones of Kolkwitz and Marsson					
	Oligo-saprobic	Poly-saprobic	Mesosaprobic			
			No sub-designation	Alpha	Beta	
FAMILY - SPHAERIIDAE						CLAMS
Pisidium amnicum	x					
" compressum			x			
" fossarinum	x					
" pauperculum			x			
Sphaerium corneum				x	x	
" moenanum	x				x	
" vivicolum	x				x	
" stamineum			x			
" striatinum			x			
Musculium transversum			x			
" truncatum			x			
FAMILY - UNIONIDAE						
Unio batavus	x					
" pictorum	x (indifferent)					
" tumidus					x	
Anodonta mutabilis	x(indifferent)					
FAMILY - MARGARITANIDAE						
Mararitana margaritifera	x					
FAMILY - PHYSIDAE						SNAILS: Lung Breathing Snails
Aplexa hypnorum	X				x	
Physa acuta	x				x	
" fontinalis	x				x	
FAMILY - LYMNAEIDAE						
Lymnaea auricularia					x	
" ovata					x	
" palustris	x				x	
" peregra	x				x	
" stagnalis	x					
FAMILY - HELISOMIDAE						
Planorbis carinatus	x					
" corneus	x				x	
" marginatus	x					
FAMILY - ANCYLIDAE						
Ancylus lacustris	x				x	
" fluviatilis	x				x	
FAMILY - VALVATIDAE						SNAILS: Gill Breath-ing Snails
Valvata piscinalis					x	
" tricarinata	x					
FAMILY - VIVIPARIDAE						
Viviparus contectus				x	x	
" fasciatus					x	
Campeloma subsolidum				x	x	

* Not all species are presented here (arranged by W. M. Ingram)

Gaufin and Tarzwell (1952) reported Physa integra from all stations on Lytle Creek, Ohio, during studies conducted in May and August, and list it as abundant at a station where D.O. was recorded as low as 0.2 p.p.m. Sphaerium solidulum, as reported, was not collected at stations where the D.O. was less than 4.5 p.p.m. as based on diurnal sampling.

On the basis of collections from Lytle Creek Ingram, Ballinger, and Gaufin (1953) report Sphaerium solidulum intolerant of pollution from domestic sewage including septicity and sludge deposits, and intolerant of bottom areas covered with zoogleal organisms. Certain literature relating to the tolerance of the sphaeriidae to pollution is discussed.

Gaufin and Tarzwell (1955) show that during January and February of 1952 Ferrissia rivularis, Sphaerium solidulum, and Pisidium casertanum were not taken in Lytle Creek reaches that had had a septic record in August of 1951, even though the minimum winter D.O. was above 7.0 p.p.m. They present data for October of 1951 showing that Ferrissia rivularis, Musculium transversum, Pisidium casertanum, and Lymnaea humilis modicella also were not collected from reaches that had a septic record in August of 1951. A few Sphaerium solidulum were collected from a station that had had an August septic record.

(3) References Relating Mollusks to Wastes from Specific Industrial Operations

Few available references discuss mollusks in relation to specific wastes from industrial operations in natural waters.

In Culter's (1930) work concerning the blanketing effect of pulp and paper mill wastes in Ticonderoga Creek, New York, he states that Campeloma decisum was abundant where pulp was the thickest. In addition, the following mollusks are listed in a table as being associated with "a pulpy bottom 8 inches thick . . .": Amnicola limosa, Planorbis antrosus, Calyculina hirsutus, Lymnaea decidiosa and Sphaerium fabale. Sphaerium striatinum is listed as occuring on a stream bottom covered by pulp up to one inch thick. Cutler states that at a station in Lake Erie at the mouth of the creek there was no molluscan life; by inference, he attributes this to the drifting action of pulp. At a second station in Lake Erie, also at the mouth of the creek but protected from pulp deposits, he reports the occurrence of the following mollusks: Valvata tricarinata, Amnicola limosa, Bythinia tentaculata, Calyculina securis, Planorbis campanulatus, Planorbis hirsutus, Lymnaea (Acella) haldemanni, Lymnaea decidiosa, Sphaerium stiratinum, and Sphaerium fabale. No consideration was given to possible effects of toxicity on mollusks.

Henderson (1949) has observed that mollusks once killed by wastes from certain viscose operations do not re-inhabit sections of streams rapidly. Through bioassays, he found that quantities of zinc proportional to quantities in the Shenandoah River from a viscose operation proved fatal to snails, daphnia, and bass fry.

Bartsch and Churchill (1949), in studying the effects of waste sulphite liquor on the biota of the Flambeau River, Wisconsin, made obervations relating such a waste to mollusks. They state that Campeloma integrum, with three unnamed snail species, apparently resist high concentrations of waste sulphite liquor, although they are not found immediately below the industrial sewer outfall. The sphaeriid clam, Sphaerium rhomboideum, was likewise noted to be absent from such an area.

Neel (1953), in pollution studies relating to oil refineries of the North Platte River below Casper, Wyoming, writes that absence of larger benthic forms below "...the refinery area may be laid to periodic releases of large quantities of toxic wastes." He comments that a dearth of bottom organisms, including unnamed snails, is probably to be associated with blackish oil rather than to any lethal phenol concentrations. Conducted bioassay tests showed oil to be deadly to benthic snails.

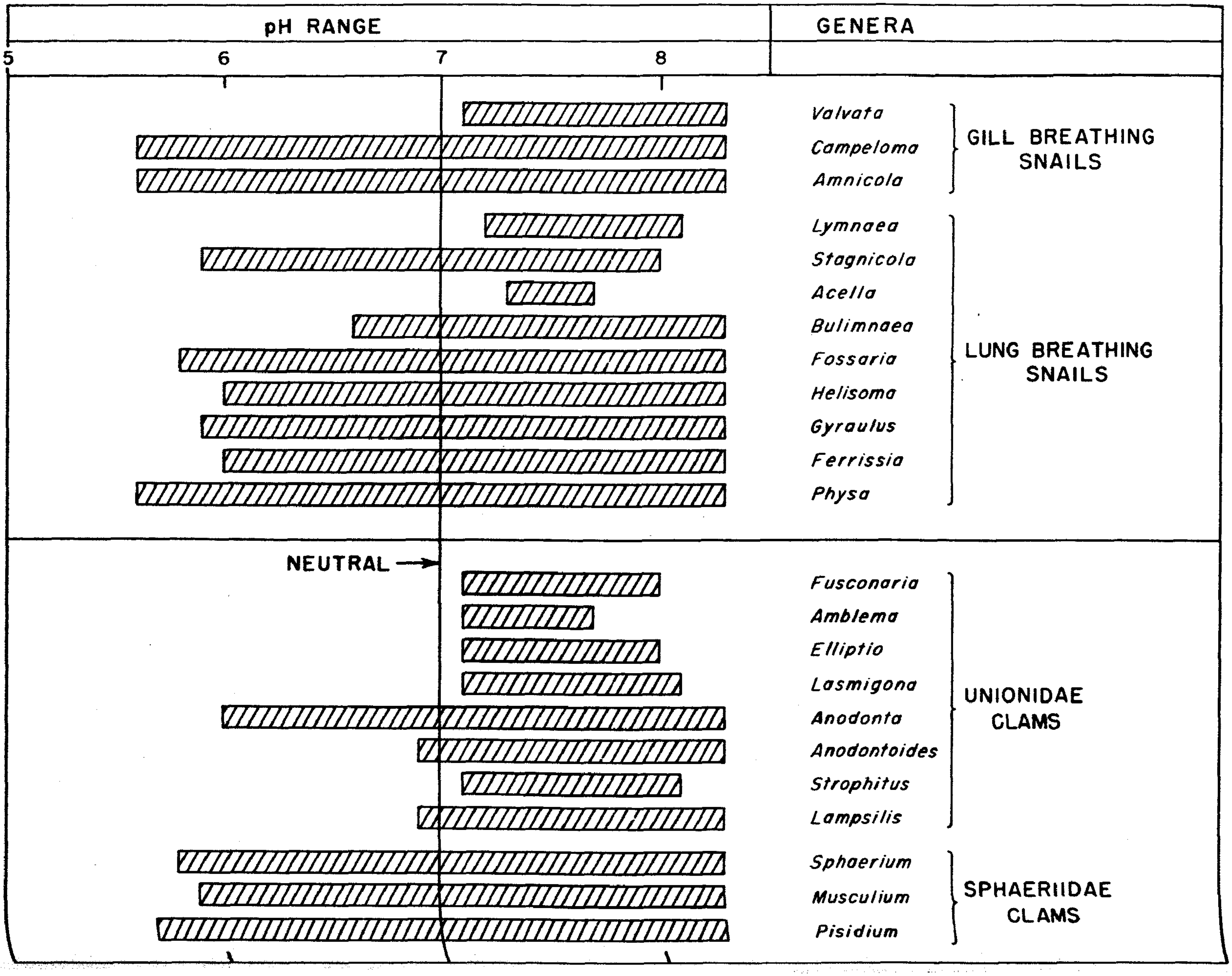
(4) Low Hydrogen Ion Concentration not Associated with Pollution, Under Which Mollusks Have Been Reported Living

Mollusks have been reported living in natural waters where low pH values have not been associated with pollution. Data are presented to illustrate low pH values that many might not suspect mollusks could tolerate.

Based on Morrison's (1932) Wisconsin studies that do not associate with pollution the low pH values recorded therein, various mollusks are shown to live in natural waters with a pH as low as 5.6 (Table 3). Figures indicate that many mollusks live in waters with a pH as high as 8.3; no figure is cited above this pH value. Hydrogen ion figures taken from this work and shown in Table 3, are ones cited under each species in Morrison (1932).

Table 3

pH RANGES ASSOCIATED WITH SELECTED MOLLUSK GENERA (AFTER MORRISON 1932)



Such a range of pH values tolerated by mollusks is within that which is listed by Doudoroff and Katz (1950) tolerable to most fresh water fish without lethal effects. These writers state, "It appears that, under otherwise favorable conditions, pH values above 5.0 and ranging upward to pH 9.0 at least are not lethal for most fully developed fresh-water fishes." In addition, these investigators write, concerning fish survival in relation to pH, "Much more extreme pH values, perhaps below 4.0 and well above 10.0, also can be tolerated indefinitely by resistant species. However, regardless of the nature of acid or alkaline wastes responsible, such extreme conditions, associated with industrial pollution, are evidently undesirable and hazardous for fish life in waters which are naturally so acid." Most species of Unionidae, as reported by Morrison (1932) and dependent upon fish for the completion of their life-cycles, were found in a pH range upward from 6.9. However, one member of the genus Anodonta, A. marginata, was reported from water having a pH of 6.3.

Jewell (1922) has reported mollusks living in the Big Muddy River, Illinois, a stream characterized as a "...naturally acid stream.", with a pH range stated to be from 5.8 to 7.2. Jewell presents species identifications of nine Unionidae from the stream. In relation to snails found in this river she writes, "Abundant as were mussels in the Big Muddy River, only two snails (individuals) were found: one a living Pleurocera elevatum taken opposit Benton..., the other a Campeloma subsolidum taken near Murphysboro. No dead shells were found to indicate that snails had ever been present." This writer states that fish were"...everywhere abundant.", and that dogfish, sunfish and native carp were taken with hook and line, while large numbers of minnows, cat fingerlings (at one time a school of several hundred), and unidentified fry were seen in pools and riffles. There were also numerous swarms of top minnows, while gar, fifteen to eighteen inches in length, were seen in ever-increasing numbers toward the mouth of the river."

The writer has found Physa integra in sections of the Mahoning River, Ohio, where the pH range over several days varied from 4.1 to 7.3.

(5) Mollusks Associated with Sewage Treatment Installations

The information included here deals with species of Physa snails in trickling filter beds of sewage treatment installations treating domestic sewage. A number of persons talking with the writer have stated that they have seen snails in trickling filter beds; however, very little data are available in the literature to describe conditions under which snails live in such situations. Study of snails in trickling filters offers ready access to those who wish to obtain data relating snails to water not completely purified from the effects of pollution. Certain chemical and physical tests, kept routinely at many secondary sewage treatment plants employing trickling filters, can be used to relate snails living in filters to specific ranges of water quality.

Brown (1937) studied Physa anatina living in sprinkling filters in a sewage treatment plant at Urbana-Champaign, Illinois, during parts of the years 1932-35. Brown writes that the plant has Imhoff tanks and sprinkling filters, "... where jets of the sewage are forced into the air for aeration.", and a secondary settling tank. Part of the final effluent is diverted into an experimental lagoon and part into the Saline Drainage Ditch, a tributary of the Big Vermilion River. Except for bacterial numbers no operational data are presented in the paper. In reference to bacteria it states, "At the time crude sewage enters the plant it contains 2, 100, 000 bacteria per cubic centimeter, but when finally treated, the number has been reduced to 700 per cubic centimeter." Brown (1937) believes that snails play a part in the reduction of numbers of bacteria. In addition to collecting Physa anatina in the rock beds of sprinkling filters, individuals were taken from the secondary settling tank and from the Saline Drainage Ditch and experimental lagoon receiving the final effluent. During the course of the study, in addition to Physa, 8 individuals of the snail Fossaria modicella were reported from the secondary settling tank, but from no other structures.

It is mentioned that in maintenance operations from 25 to 30 bushels of empty shells are removed each year in July and November from a conduit of the secondary settling tank. Brown believes that the majority of snails in the sprinkling filter beds die each winter. Reported observations based on shell size present evidence to indicate that life cycles of snails are completed in the sprinkling filter beds. Whether snails occur through the depth of the beds is not indicated.

A 1929 Annual Report of the Urbana-Champaign Sanitary District, as quoted by Brown (1937), mentions that passage of snails from the sprinkling filters into the secondary settling tank, "...proves beyond doubt the presence of a high amount of dissolved oxygen in the lower part of the filters." Associated with this quotation is the statement by Brown that Physa anatina breathes atmospheric oxygen. Referring to other records of snails reported from sewage treatment plants Brown mentions that Physa halei has been reported from a Fort Worth, Texas, installation.

Lohmeyer (1955) has written about the occurrence of an unidentified *Physa* in a high-rate trickling filter of the University of Florida's sewage treatment plant at Gainesville. In March of 1956 snails from this plant were sent to the writer; they were forwarded to W. J. Clench of the Museum of Comparative Zoology of Harvard University, who identified them as *Physa cubensis*, a species of wide distribution in Florida and the West Indies. Lohmeyer does not give operational data relative to the character of water that is applied to the filter. He mentions that dosing the filter for three days with a chlorine residual of approximately 3 p. p. m. resulted in snail control for eight months before operational difficulties were experienced. Mechanical difficulties relating to high-rate filter operation, resulting from *Physa cubensis* snails, are described in detail.

In May of 1955, individuals of varying sizes of *Physa intergra* were collected by the writer from the rock beds of both standard and high-rate trickling filters at the Dayton, Ohio, sewage treatment plant. Egg masses were present on the undersides of stones in the top three inches of the beds. Such information would seem to indicate that this species successfully carries on its life cycle in these trickling filter beds. The following operational data represent extremes that were recorded for water going onto the filters for two weeks preceding snail collections: B. O. D. 59 to 131 p. p. m.; total nitrogen 24. 4 to 24. 8 p. p. m.; ammonia nitrogen 13 to 17. 9 p. p. m., chlorides 122 to 128 p. p. m., and D. O., O. O p. p. m. The dissolved oxygen in water leaving the filters varied from 2. 7 to 4. 9 p. p. m. Hydrogen ion concentrations were not available for the stated period but for the month of April they were about 7. 1.

III. SOME STRUCTURAL AND LIFE CYCLE VARIATIONS RELATING TO MOLLUSCAN SURVIVAL ABILITY IN ASSOCIATION WITH DOMESTIC SEWAGE POLLUTION

Some structural and life cycle variations that relate to differing molluscan abilities to survive septic conditions and a substrate of sludge may consist of differences in: the type of respiratory organs, ability to close the shell for extended periods, weight of shell, and life cycle. Because of such differences among mollusks when associated with domestic sewage pollution, certain of the lung-breathing snails survive better than gill-breathers and certain fingernail clams are more resistant than are the mussels.

(1) Survival of Lung-Breathing Versus Gill-Breathing Snails

Snails possessing lungs can generally be expected to survive under low dissolved oxygen or septic conditions because they typically rely on atmospheric oxygen in breathing. It is important for the aquatic biologist to become familiar with snail morphological characteristics in order to separate readily gill-breathing from air-breathing snails. An obvious character revealing whether the gastropod being dealt with is a gill-breather or a lung-breather is the presence of an operculum in the aperture of the former. Families and common genera of gill-breathing fresh water snails are listed in Table 4 for convenience in separating them from those that typically breathe atmospheric oxygen.

Table 4

FAMILIES AND GENERA OF GILL-BREATHING SNAILS

Family	Genera
Amnicolidae*	Amnicola Pyrgulopsis Hydrobia Somatogyrus Lyogysus Bulimus (Bithynia)
Pleuroceridae	Pleurocera Goniobasis
Valvatidae	Valvata
Viviparidae	Vivipara Campeloma

* Amnicolidae genera listed from Berry, 1943; other families and genera from Baker, 1902.

It is important to point out to those conducting investigations in lakes or deep rivers that the presence of a lung in a snail does not necessarily mean that atmospheric oxygen will serve as the only source for respiration in all seasons. Periodic movement of lung-breathing snail, from shallow to deep water, may be induced by a lowering of water temperature to 10°C. as fall merges into winter and may be an annual occurrence as reported by Cheatum (1934) in Douglas Lake, Michigan. A change in temperature from 10°C. to 21°C is accompanied by an ascent from deeper water to marginal lake areas. Cheatum presents data indicating that when pulmonate snails are submerged, the lung functions as a gill in taking dissolved oxygen from water. Lung-breathing snails may remain submerged for three or four months out of every twelve in areas where winter months are climatically similar to Michigan.

Experimental work conducted by Cheatum indicates that lung snails are not completely dormant when submerged, but rather are in a state of suspended animation, and that they actively respire using dissolved oxygen. Individuals of the following seven species and subspecies of lung-breathing snails are noted by Cheatum to breathe dissolved rather than atmospheric oxygen for a part of each year when submerged in Douglas Lake, Michigan: Lymnaea stagnalis appressa, Lymnaea emarginata angulata, Helisoma campanulatum, Helisoma smithii, Helisoma antrosum percarinatum, Physa sayii crassa, Physa parkeri.

In submerged experimental cages individuals of each of the species named above survived for 65 days with the water temperature varying between 16.8 to 26.6°C. Submergence experiments indicated that mortality was greatest among individuals whose oxygen requirements were the highest. The presented data does not indicate that submerged pulmonate snails can live under conditions of septicity.

In writing about the completion of life cycles, by certain pulmonate snails, when submerged Cheatum (1934) states, "In all probability, many individuals of the species H. campanulatum smithii, H. antrosum percarinatum, L. emarginata angulata, and P. sayii crassa complete their life cycles and reproduce normally without emerging for air."

Shelford (1913) writes that pulmonate snails of the genus Ancylus, "... are said to take water into their lung and thus do not need to come to the surface for air."

It is especially pertinent to point out literature, like the above, concerning lung-breathing snails that can obtain oxygen from either the atmosphere or from water, because in deep water during submerged living conditions in winter certain pulmonates would be killed by oxygen-consuming pollutants that could lower oxygen to asphyxial levels.

Thus, it may not be advisable to always include pulmonate snails with sludge worms, certain blood worms, rat-tailed maggots, and household or sewage mosquitoes as being tolerant to pollutional conditions involving septicity.

Of all snail genera, members of the genus Physa, especially, may occur in great abundance in septic zones of streams. Two species, Physa interga and Physa anatina, are commonly associated with septic zones in shallow streams in the mid-west during summer and fall months.

The writer has not collected any of the gill-breathing snails in polluted water where the dissolved oxygen, as measured during daylight hours, was less than 2 p.p.m. Even though these mollusks possess an operculum which, if tightly sealed, should enable them to close themselves away from low dissolved oxygen waters, the fact that such snails are not reported from septic or near septic water would indicate that low dissolved oxygen may be one of several factors denying such water to them.

(2) Survival Relating to Shell Closure in the Sphaeriidae and Unionidae

The fact that certain of the Sphaeriidae can survive low dissolved oxygen conditions and a shifting bottom of sludge, as related to domestic sewage, and that the Unionidae do not points somewhat speculatively to the ability of certain fingernail clams to close the shell and survive until stream conditions improve.

Allen (1923), in studying reactions of certain Unionidae under low dissolved oxygen conditions writes, "When under conditions of deficient oxygen not only to the siphons widen to bring in more water, but also additional spaces between the mantle edges are thrown open." He does not mention whether all of the Unionidae that he studied opened the valves as indicated under the stated circumstances. The following Unionidae are listed as being used in general experiments: Anodonta grandis, Lampsilis luteolus, L. ligamentinus, L. altus, Quadrula heros, Q. pustulosa, Q. lachrymosa, Q. undulata, Unio crassidens, U. gibbosus, Plagiola elegans, "and others." If the Unionidae, in general, have a response to open their valves under low dissolved oxygen conditions resulting from pollution by domestic sewage and industrial wastes, they are most vulnerable to destruction. If they do open their valves as described by Allen, their bodies are vulnerable to any number of substances in polluted water that may be toxic enough to destroy them. Also, in an open position they could be covered by settable solids.

It is known that the Sphaeriidae can live under septic conditions and on sludge-covered bottoms as discussed earlier. Apparently, when living under conditions of septicity the valves remain tightly closed. Thus, the Sphaeriidae would not be subjected to toxic materials as they would if they lived under such conditions with their valves opened. Juday (1908) has written about the behavior of Corneocyclas [=Pisidium] idahoensis under laboratory conditions in water containing and devoid of dissolved oxygen, and has related such data to field conditions. In water without dissolved oxygen, individuals remained quiescent with their valves tightly closed without activity being observed in the mud of the experimental jars. When individuals were placed in aerated water they became active. He states that his experiments seemingly indicate that this mollusk remains quiescent or dormant in Lake Mendota, Wisconsin, when the muddy ooze at the bottom of the lake contains no dissolved oxygen, a period of about three months each summer. Juday (1921), in further studying Pisidium idahoensis in lake Mendota, writes that there is no free oxygen below a depth of 20 meters from about the middle of July until early October, and again in March for two or three weeks in some years. He mentions that organisms living under such conditions must be "... facultative anaerobes.", and includes in this category, in addition to Pisidium idahoensis, worms of the genera Tubifex and Limnodrilus, and three dipterous larvae: Corethra punctipennis, Chironomus tentans, and Protenthes choreus.

Baker (1928) writes about Sphaeriidae being able to live in the mud bottom of pools where the water has dried up, and Ingram (1941) has reported Pisidium abditum living out of water on the beach of a lake from at least June 15 to September 1.

(3) Survival Relating to Weight of Shell

The entombment effect of heavy sludge or silt pollution may relate to the absence of heavy Unionidae and presence of certain light Sphaeridae, other factors being favorable. In Dawley's (1947) study of the distribution of aquatic mollusks in Minnesota, he comments on the survival of mollusks on varying substrates, "A mussel [Unionidae] is more exacting in its requirements than a snail, being heavier and less motile. The bottom in which it lives may be sand, gravel, or mud, but not rock or soft muck because its foot cannot penetrate rock and it sinks too far into the muck and is smothered." Based upon commonly finding certain Sphaeriidae on a sludge bottom, such a physical substrate may not deter the existence of certain species of this family. General observations, based upon reconnaissance of flocculent bottoms in rivers and streams polluted by domestic sewage, indicate that the Unionidae do not seem to favor such areas.

In studies of erosion silt as a pollutant under laboratory conditions, Ellis (1936) found that certain mussels were unable to maintain themselves, in either sand or gravel bottoms, when a layer of silt from one-fourth to an inch in depth was allowed to accumulate over such, other conditions being favorable to survival. The yellow sand-shell, Lampsilis teres, a sand species, most readily succumbed; the species least readily killed were: Obliquaria reflexa, Quadrula quadrula and Q. metanevra.

Coker et. al. (1922) compiled data of various investigators on types of bottoms on which mussels were reported to be living. From his analysis of such data he concluded, "It appears that the preferred bottom for the majority of species is mud (but not deep, soft mud, to which type of bottom few species are adapted) and gravel, including sand and gravel. Sand ranks next and sandy clay last; but few species of mussels exhibit a preference for sand or sandy clay, and only two are recorded (by one observer) as finding the most favorable environment in a bottom of clay mixed with sand." Baker (1928), in writing about fresh-water clams of Wisconsin, discusses types of bottoms that mussels prefer: gravel, sand, mud, and clay; he says that they are common or abundant in the first three and rare in the latter. A shifting bottom, whether it consists of mud or sand, is stated to be usually devoid of mussels. Fine silt bottoms are always avoided by mussels, and Baker (1928) doubts if mussels could live in such a bottom environment. He states that mussels are usually absent or rare where great quantities of silt are carried into streams. Ingram (1948) reported Anodonta wahlamatensis by the thousands in the soft mud bottom of Stow Lake, San Francisco, California.

Many fresh-water snails are heavy enough to sink into the sludge covering stream bottoms to become buried and suffocate. The writer has observed areas of streams where sludge deposits were two to three feet in thickness, such as reaches of the Mahoning below Warren and Youngstown, Ohio, where Physa intergra used higher aquatic plants as a substrate rather than the flocculent sludge deposits. In sludge filled sections of streams without higher aquatic plants, snails may be found on rock islands protruding from the sludge, and may be absent or rarely occur on sludge. In weight, adult fresh-water snails are much more comparable to the Sphaeriidae than to the Unionidae.

(4) Survival Relating to Type of Life Cycle

Of clams, the Unionidae are especially vulnerable to pollution which may eliminate species by affecting larval stages. It is well known that after being released from the female the immature glochidial stage of the Unionidae must parasitize various fish in order to assure life cycle completion, Lefevre and Curtis (1912), Coker et. al. (1922), van der Schalie (1938), and Jones (1950).

After spending from 10 to 14 days as an external fish parasite, the glochidium drops off the fish and continues its life as a free living form. If a fish is not parashtized, the glochidium dies. No information is available on the direct effects of pollution on the glochidium or on sperm cells which pass freely in water from male to female clam.

Because it is necessary that a part of any Unionid's life cycle be spent as a fish parasite, there is a direct relationship between the effects of pollution on fish and perpetuation of succeeding generations of Unionidae in any stream. If adult Unionidae are more resistant to various pollutional affects than fish, they may survive to die of old age, without succeeding generations developing to replace them. If glochidia-carrying fish are denied areas of streams by pollutants, expanded distribution of the Unionidae is hindered. A number of fish have been reported in the literature as carrying glochidia of various Unionidae, Coker et. al. (1922), Danglade (1922), Murphy (1942), Ingram (1948), Jones (1950). The following fish are examples of some that have been associated with glochidia, and are noted so that those working in water pollution might be aware of them if it is ever desired to correlate mussel-fish relationships relative to pollution: black bullhead, common bullhead, bowfin, eel, sheepshead, gizzard shad, mooneye, pike, spotted catfish, yellow catfish, long and short-nosed gars, red-ear sunfish, orange-spotted sunfish, blue-gill sunfish, small-mouth black bass, largemouth black bass, striped bass, river herring, yellow perch, white crappie, black crappie, sand sturgeon, madtom, sauger, and drum.

The Sphaeriid's sex cells are not subjected to any possible pollutional effect outside of the adult's body. They are hermaphrodites, fertilization is internal, and the young may be carried in the adult for as long as a year, Goodrich and van der Schalie (1944). The growth stage that leaves the parent to fend for itself is a small mirror-image of the adult. Such protected reproduction and shielding of the very young, when compared with the haphazard early life cycle stages of the Unionidae, should enhance survival of fingernail clams over mussels.

Gastropods that one would encounter in water pollution investigations copulate with resulting internal fertilization. Most lay eggs that are attached to submerged objects and, on occasion, to each other's shells; the Viviparidae are ovoviviparous. Thus, the eggs and very young stages of most are exposed to external changing environmental conditions at all times.

IV. NATURAL VARIATIONS IN DISTRIBUTION OF MOLLUSKS NOT RELATED TO POLLUTION

In studying the effects of pollution on bottom organisms, with emphasis on mollusks, cognizance should be taken of natural phenomena affecting distribution not related to pollution. Normal variations in kinds, size, and abundance of mollusks, unrelated to pollution, make inventories of species of little value in pollution studies unless those interested in delineating indicator organisms include chemical, physical, and bacteriological descriptions of water quality so as to establish tolerances of mollusks to pollutants.

It has been shown by Baker (1918) that in lakes the numbers of molluscan species decrease with depth. In further writings about the increase of mollusk abundance in relation to depth, with reference only to mussels, Baker (1928) states that "The great majority of naiades live in comparatively shallow water from a foot to six feet in depth. More rarely they descendto depths as great as 25 feet. Records of fresh water mussels from greater depths than 25 feet are to be viewed with suspicion." Thus, in studying the effects of pollution on benthonic organisms in a lake, one should always be aware that paucity of a variety of mollusks may naturally be related to water depth and not to pollutional effects. In such studies chemical, physical, and bacteriological tests could be most important in presenting data to indicate whether a reduction of molluscan variety was a natural phenomenon of depth or whether it could be attributed to pollution.

In streams, it is known that Unionidae and Gastropods tend to increase in numbers of species from headwaters to the stream mouth, Goodrich and van der Schalie (1944), Baker (1928). For example, Baker (1928) lists on increase of Unionidae from three species upstream to 28 downstream in a 27 mile reach of the Big Vermilion River, Illinois. Certain pollution sources on the headwaters of a stream may be suspect in relation to a dearth of mollusks such as the Unionidae; however, a small number of species may represent a natural condition rather than a relationship to pollution.

There may be a greater number of species and individual gastropods living in stream areas where higher aquatic plants are present and usuable as a substrate in addition to the stream or lake bed. Thus, it is important to select stations to include sampling of higher aquatic plants in studies designed to provide data on indicator organisms. For example, in certain reaches of the Mahoning River, Ohio, in 1952, the writer made collections of bottom organisms in sludge deposits two to three feet in thickness and found no mollusks. In those reaches the river had a water temperature of 96°F., pH of 4.1, and D.O. of 0.2 An initial conclusion from these meager chemical and physical analyses could have been that mollusks were unable to stand such conditions in this stream. However, Physa integra was present by the hundreds in various growth stages as well as in egg masses, using higher aquatic plants as a substrate. Thus, some data were collected to show certain conditions under which *Physa integra* can survive and carry out its life cycle. If higher plants present had not been searched for organisms, one might not have associated this snail at all with such a low pH or high temperature. On the basis of collections limited to the stream bottom, this pulmonate snail would have been associated only with waters having more favorable pH and temperature and with but little sludge.

V. IDENTIFICATION SOURCES FOR FRESH WATER MOLLUSKS

To assist those interested in the relationship of mollusks to water pollution, certain publications which may serve as examples of aids to their identification are cited. Also, certain museums having collections available for comparison of species or personnel that can assist in identification of specimens are presented. Much additional information relative to identification can be obtained by literature searches, or by consulting State and municipal museums and natural history societies.

A great deal of information concerning fresh water mollusks is contained in various numbers of "The Nautilus," a quarterly journal devoted to the interests of conchologists. This journal is edited by Dr. H. B. Baker of the University of Pennsylvania's Zoological laboratories, Philadelphia, Pennsylvania.

The foremost nuseums housing collections of fresh water mollusks are: the United States National Museum, Washington, D. C., with Drs. Harald Rehder as Curator of Mollusks and Dr. J. P. E. Morrison as Associate Curator; the Academy of Natural Sciences of Philadelphia, Pennsylvania, with Dr. Henry A. Pilsbry as Curator of Mollusks and Dr. R. Tucker Abbott as Curator of the Pilsbry Chair of Malacology; Museum of Comparative Zoology of Harvard University, Cambridge, Massachusetts, with William J. Clench as Curator of Mollusks; Chicago Museum of Natural History, Chicago, Illinois, with Dr. Fritz Haas as Curator of Mollusks; Museum of Zoology, University of Michigan, Ann Arbor, Michigan, with Dr. Henry van der Schalie as Curator of Mollusks; California Academy of Sciences, San Francisco, California, with Dr. G. Dallas Hanna as Curator of Mollusks and Dr. Leo George Hertlein as Associate Curator; and the Carnegie Museum, Pittsburgh, Pennsylvania. Smith's (1943) directory of malacologists can be useful to those interested in having mollusks identified, because it lists in alphabetical order, malacologists who are specialists in mollusk identifications.

The following are cited as examples of keys and faunal lists developed from studies limited geographically that can serve to provide species names as a base for specific identification in future studies of fresh water mollusca: Baker (1922) on mollusks of the

Big Vermilion River, Illinois; Eddy and Hodson (1950) on mollusks of the North Central states; Goodrich and van der Schalie (1939) on mollusks of the upper peninsula of Michigan; Morrison (1932) on mollusks of the northeastern Wisconsin lake area; Strecker (1931) on the Unionidae of Texas; and a series of papers by van der Schalie on the Unionidae bearing the following dates from the stated geographical areas: (1936) St. Joseph River, southwest Michigan; (1938b) Cahaba River in northern Alabama and (1938) Huron River in southeastern Michigan; (1940) Chipola River in northwestern Florida; (1948) commercially valuable mussels of the Grand River, Michigan; and (1950) of the Mississippi River.

Examples of regional fresh water mollusk identification guides are: Baker (1898) on clams of the Chicago area and (1902) on gastropods of the same area; Baker (1928) on clams and snails of Wisconsin; Chamberlin and Jones (1929) on the Mollusca of Utah; Goodrich (1932) on mollusca of Michigan; Goodrich and van der Schalie (1944) on mollusca of Indiana; Henderson (1924) (1936) on mollusca of Colorado, Utah, Montana, Idaho and Wyoming; Henderson (1929 and 1936a) on mollusca of Oregon and Washington; Ingram (1948) on the larger fresh water clams of California, Oregon and Washington; and Strecker (1935) on the mollusca of Texas.

The following are monographs, papers, and catalogues of varing degrees of comprehensiveness that deal with fresh water molluscan classification: Baker (1911) on the Lymnaeidae of North and Middle America, Berry (1943) on the Amnicolidae of Michigan; Brooks and Herrington (1944) on a preliminary survey of the Sphaeriidae; Ortman (1919), a monograph of the naiades of Pennsylvania; Pennak (1953), a key to the families and genera of North American fresh water mollusks; Sterki (1916), a catalogue of North American Sphaeriidae; and Walker (1918), a synopsis of fresh water mollusca of North America north of Mexico and (1918), keys to fresh water mollusca of the United States.

VI. SUMMARY

1. Presented literature affirms that little specific information is available relating one species of mollusk to a specific or over-all set of well-defined water quality conditions, and another species to different degrees of water quality.

No information is available to be used in selecting from mollusks any species that may be called a pollution indicator. Several species are tolerant to low dissolved oxygen or septic conditions resulting from pollution of water by domestic sewage. However, such species cannot be considered to indicate pollution, as related to septicity, for they are also found in umpolluted streams and lakes.

2. Most specific data available relating mollusks to certain effects of pollution on water quality deal with survival under septicity. Other such data relate to survival in relation to blanketing bottom deposits and acid water.

a. In relation to survival under conditions of septicity resulting from domestic sewage pollution, general conclusions that have been apparent for many years, can be reaffirmed here: certain Sphaeriidae are collected under such conditions while Unionidae are not; and certain pulmonate snails are commonly found in septic water, while gill-breathers typically are not.

b. In relation to survival on bottoms blanketed by sludge from domestic sewage and by silt, certain Sphaeriidae and Physidae are most often collected.

3. Information on hydrogen ion concentration is presented indicating that a number of genera of mollusks may live naturally in acid water conditions not associated with pollutants.

4. Variations in molluscan structure and types of life cycle are discussed, as such variations may result in enhancing survival under pollutional conditions relating to dissolved oxygen, sludge and silt deposits.

5. Natural variations in the distribution of mollusks in streams and lakes are considered in order to make those working in water pollution aware that variations in numbers of species may be a natural phenomenon not related to pollution.

6. Selected museums and personnel as well as mollusk guides and lists are presented to assist those who are not authorities on molluscan taxonomy with identification.

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THE USE AND VALUE OF AQUATIC INSECTS AS INDICATORS OF ORGANIC ENRICHMENT

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A knowledge of the ecological requirements of aquatic organisms is of outstanding importance in judging the extent of pollution due to organic enrichment in our streams. The species composition of the aquatic population in a given area is determined by the environmental conditions which have prevailed during the developmental period of the organisms involved. If at any time during its development, environmental conditions become lethal for a given organism, that organism will be eliminated even though the unfavorable conditions are of very short duration. The aquatic population which occurs in a given area is, therefore, a representation or indicator of environmental conditions which have prevailed during the life history of the organisms comprising the population.

It is this property of indicating past environmental conditions, especially the extreme conditions of brief duration, that make macro-invertebrates such valuable indicators of pollution. Most representatives of the group have longer life histories than the micro-benthic fauna and are thus better fitted for indicating past conditions than are the latter organisms. In addition, the larger size and the more distinctive morphological characteristics of the macro-invertebrates make them easier to identify under field conditions.

The interpretation of stream conditions based on the biota present has been used for many years. Kolkwitz and Marsson (1908-1909) first proposed the use of aquatic organisms as indicators of the ecological conditions under which they exist. They classified organisms as oligosaprobic, mesosaprobic, and polysaprobic, depending on the concentration of decomposable organic matter in the streams under consideration. Richards (1921, 1928), based upon his studies of the Illinois River, developed a classification of bottom organisms using seven groups of species. These included a pollutional group, three sub-pollutional groups, an air breathing group, current loving species other than pulmonate snails and air breathing insects and clean water species. Patrick (1949) presented a comprehensive method involving the use of histograms to show the response of certain groups of aquatic organisms to given environmental conditions. This method was modified and made more applicable to macro-invertebrates by Wurtz (1955).

A number of writers have published shorter works containing many valuable ideas and suggestions. Bartsch (1948) presented information on the response of the biota to various degrees of pollution. Gaufin and Tarzwell (1952) employed both indicator species and associations in utilizing aquatic invertebrates as indicators of pollution. Schiffman (1953) presented a very useful method of cataloging stream bottom organisms found in Illinois

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with respect to their pollutional tolerance. Beck (1954) contributed a valuable survey method in his simplified ecological classification of aquatic invertebrates found in Florida streams.

In satisfactorily using macro-invertebrates as indicators of organic enrichment and its effects, certain criteria should always be kept in mind. First, several factors other than the presence of a pollutant may limit the distribution of certain species, as for example, the type of bottom, speed of current, lack of certain nutrients, scouring floods, excessive turbidity, and the flight range. Second, the mode of occurrence of the forms considered is just as important as their presence or absence in a given area. For example, most of the macro-invertebrates which characteristically occur in large numbers in heavily enriched water may also be found in limited numbers in cleaner situations. Such forms as the mosquito, *Culex pipiens*, leech, *Macrobdella*, and sludgeworms, *Tubifex* and *Limnodrilus* often occur in the quieter confines of clean water streams in limited numbers, but they reach far greater numbers in waters polluted by organic wastes. When conditions are favorable for those organisms which can adapt to such conditions, they thrive and build large populations. In some instances of organic pollution very often the important factor in determining the occurrence of certain forms is the abundant food supply which favors their growth and numerical increase rather than a deficiency of some material such as dissolved oxygen. Similarly those forms which are most characteristic of clean water conditions, such as mayflies, stoneflies, or caddis flies are occasionally found during winter in stream sections which are highly polluted or septic in summer. When these insects drift into such a stream from nearby tributaries they may live for considerable periods of time because the septic zone of summer often has an adequate dissolved oxygen supply during winter. Thus, before such isolated examples are taken as evidence that the forms involved are tolerant of environmental conditions, an investigation as to their source and abundance in the area is advisable.

In arriving at a better definition of the habitat preference and indicator significance of the various groups of macro-invertebrates consideration of the structural and physiological adaptations of the organisms is very important.

While there are exceptions, in general, an association of mayflies, stoneflies, and caddis flies in a stream is indicative of clean water conditions, and their absence often denotes a super abundance of organic wastes and/or a low oxygen supply if the physical nature of the habitat is otherwise suitable. Usually the presence or absence of representatives of other orders of aquatic insects which breathe by gills, and which are, therefore, dependent upon oxygen dissolved in the water for their respiratory needs, has similar indicator significance. For example, while most aquatic beetles can renew their oxygen supply directly from the atmosphere and are thus unaffected by oxygen-depleting wastes, the larvae, pupae, and adults of those species which are entirely aquatic, are dependent upon dissolved oxygen and are restricted to clean water streams which are well aerated.

In Ohio several species of riffle beetles such as Stenelmis crenata and Stenelmis sexlineata, are found only in the cleanest streams. Their distribution in such a habitat indicates that the family, Elmidae, to which they belong, is a member of the clean water association.

While most gill bearing aquatic insects are limited in their distribution by low dissolved oxygen supplies, some forms which have more than one means of respiration, such as the dragonflies and damselflies, display considerable tolerance to low levels of dissolved oxygen. Their greater adaptability to environments low in dissolved oxygen is made possible by the possession of respiratory structures which are the most highly developed of the various gill systems. These insects can carry on respiration by means of four different structures; namely, (1) caudal tracheal gills; (2) rectal folds; (3) the integument; and (4) spiracles. Since all four organs may function at the same time and many of the stream forms occur in either riffles or shallow marginal areas, the group is remarkably well adapted to withstand the oxygen depleting effects of organic pollution. As a result of this adaptability, the nymphs of both dragonflies and damselflies were often taken by he author in Lytle Creek, Ohio, in sections of the recovery zone where the lissolved oxygen supply in summer was as low as 1.0 p.p.m. for a short ime during the night or in the early morning hours.

In streams receiving large amounts of organic wastes insects of the orders Hemiptera, Coleoptera, and Diptera have the most varied representaion, are the most widespread in their distribution and are least affected by lissolved oxygen concentration. Representatives of these orders may be ound in all stream habitats representing all degrees of environmental nodification and stream recovery. Some species from each group may be ound fairly widely distributed throughout the stream; others while not retricted to either a clean water or "polluted" area, may show by their abunance a strong preference for one or the other type of habitat. Still other pecies, particularly among the Diptera, may be restricted to clean water r to water rich in organic materials.

Of the three orders, the Hemiptera and Coleoptera are the poorest ndicators of organic enrichment and oxygen depletion in the stream. With he exception of the Elmidae, or riffle beetles, other species of beetles nd all of the species of water bugs may be found throughout a stream sually occurring most abundantly in the "polluted" areas where they may nd an abundant food supply. The ability of members of these two orders o withstand the oxygen-depleting effects of organic pollution is due to pecial modifications of their tracheal system. These modifications serve o increase the internal air capacity of the tracheal system, supplement racheal diffusion by ventilation movements when the insects come to the urface for air, and provide supplementary external air stores. Common o all of these forms are the modification of the body surface for breakng the water surface film, and changes in the wings and body surface for apturing and holding stores of air, and in the tracheal system for surace ventilation and connection with the external air stores. In oxygen-deficient waters members of these two groups have only to increase the frequency of their visits to the surface to cope with decreasing oxygen supplies.

The efficiency of these modifications for obtaining and storing atmospheric oxygen is well illustrated by Dytiscus, one of the diving beetles. K. D. Roeder (1953) reports that this beetle can remain submerged for 36 hours without coming to the surface to renew its oxygen supply. Dytiscus, to obtain a supply of oxygen, breaks the surface periodically with hydrofuge hairs, and ventilates violently by means of accessory respiratory muscles. The fore wings, elytra, have a locking mechanism to trap the atmospheric air, and the abdominal and thoracic spiracles are displaced so as to open into this respiratory air store.

Aquatic Diptera may be found in a stream in many different ecological niches in both the clean water and other life zones. However, with the exception of only a few species, representatives of this order are highly selective in their choice of habitat. A number of species such as Diamesa nivoriunda, Cricotopus absurdus, and Calopsectra neoflavella, are found only in the cleanest, most highly aerated sections of a stream, while others such as the mosquito, Culex pipiens, and rattail maggot, Eristalis bastardi, while found in limited numbers in clean water areas, show a decided preference for the organically enriched sections. The variability in their choi of habitat and in their range of distribution is determined largely by the foo getting and respiratory requirements and adaptations of the different individual species. The larvae and pupae of the mosquito and rattail maggot, with their special respiratory tubes, are unaffected by low oxygen supplies as evidenced by the extremely large number of each that may be taken in the most septic areas. Certain redblooded Chironomids, such as Chironom riparius, also demonstrate a remarkable ability to thrive in the septic anc recovery zones. Walshe (1950) has shown that the hemoglobin possessed by midge larvae such as Chironomus riparius, Chironomus plumosus, and clos related species apparently acts in both the transportation and storage of oxygen. Its greatest transport role is during anaerobiosis, when it permits the larva to continue filter feeding in low oxygen tensions and thereby increases the rate of recovery from exposure to such conditions.

As in the case of the insects, the other groups of macro-invertebrates show considerable variation in their distribution and adaptability to varying environmental conditions due to the breakdown of organic matter. Certain groups such as the sludge worms, Tubificidae, may be found in very large numbers in bottom sludges of high organic content in the lower end of the septic zone and the upper end of the recovery zone. Their numbers decrea rapidly as the nature of the bottom sediments change. The ability of two ge of these worms, Tubifex and Limnodrilus, to utilize the rich supply of orga material under practically anaerobic conditions, make them important and conspicuous members of one of the most easily recognized communities characteristic of streams receiving organic wastes.

Summary

In order to utilize macro-invertebrates as indicators of environmental conditions in streams, it is essential to have a knowledge of the species composition and abundance of the various organisms in the populations involved, under the various ecologic conditions which prevail in clean and organically enriched waters. Clean waters, with some exceptions, are characterized by a great variety of invertebrates consisting of herbivores, carnivores, and omnivores, prey and predators, lung, tracheal tube, and gill breathers. In general a population containing numerous gill breathing forms as mayflies, stoneflies, and caddis flies is indicative of clean water conditions and their absence denotes the super abundence of organic materials and/or low oxygen.

By contrast, associations engaged in the utilization of excess organic materials are characterized by few species but large numbers of individuals. The association of organisms normally present under the most septic conditions consists largely of scavengers with few plant and animal eaters. The macro-invertebrates most characteristic of septic zones are those which can exist under conditions of very low oxygen or have adaptations for breathing atmospheric oxygen.

Thus by reference to the qualitative and quantitative composition of an aquatic population as an index of water quality, it is possible to delineate the life zones in a polluted stream.

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BIOLOGICAL INDICES OF WATER POLLUTION, WITH SPECIAL REFERENCE TO FISH POPULATIONS[1]

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A number of investigators have very recently published discussions having to do with biological indices and biological measures of water pollution (1) (2) (7) (13) (14) (15) (16) (26) (27) (28) (29) (30) (36) (38). Fjerdingstad (12) has discussed some of the pertinent European literature. The fundamental concepts presented by these authors are not original, for the idea that aquatic organisms can be useful "indicators" of environmental conditions, and particularly of the degree of pollution of water with organic wastes, has a long history (12). Because of certain novel features and the relatively wide scope of the studies, and the broad implications of some of the conclusions, the work of Patrick (26) (27) (28) (29) (30) has attracted much attention in the United States and seems to deserve the closest scrutiny.

Although much has been written about the various biological indices, there has been no general agreement among the authors as to the meaning of some of the most important terms used in this literature and little effort to clarify the terminology. In view of the variety of backgrounds and dominant interests of individuals concerned with waste disposal and with the effects of wastes on receiving streams, it is not surprising that the term "pollution" does not have exactly the same meaning for all. It is regrettable that a variety of meanings have come to be associated with technical terms such as "biological indicator of pollution". Some of the differences of opinion as to what the biological indices are and what may be their utility doubtless stem from a lack of agreement on the meaning of the word "pollution". Investigators proposing the use of different indicators of pollution should have clarified, it would seem, their ideas as to just what constitutes pollution, or, in other words, exactly what

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[1] Miscellaneous Paper No. 31, Oregon Agricultural Experiment Station.

it is that the indicators can be expected to indicate. Too often this has not been done, or the ideas and definitions presented have not been carefully developed and appear to be unsound from a practical standpoint.

Should the mere change (physical, chemical, or biological) of some aquatic environment resulting from waste disposal be regarded as pollution even when ordinary human use and enjoyment of the water and of associated natural resources have not been affected adversely? When there is evidence of environmental change, is this always reliable evidence of damage to a valuable natural resource? May not certain beneficial uses of water be sometimes seriously interfered with by the introduction of wastes which may cause little or no detectable alteration of biological communities? Have there been any studies which have conclusively demonstrated a useable fixed relation between the biological indices of pollution and the actual fate or change in value of aquatic resources which are subject to damage by pollution? If water pollution can be the result of introduction of any of a great variety of substances, organic and inorganic, is it proper to refer to those biotic responses which are only known to occur in the presence of putrescible organic wastes (i.e. to organic enrichment of water) as "indices of pollution"? Can there be any general biological solution for all problems of detection and measurement of water pollution, or is effort being wasted in a search for such a general solution? Are broad limnological investigations being undertaken where intensive study and appraisal of supposedly damaged natural resources of obvious value to man would be more profitable? Is immediate practical value of research results being claimed improperly in an effort to justify fundamental limnological studies for which no such justification should be necessary? These are questions which all biologists interested in water pollution should perhaps ask themselves. Many of these questions have no categorical answer, but it is hoped that the following discussion will prove thought-provoking. It may not only call attention to certain inconsistencies in claims made and terminology used, but may also indicate the need for revision of objectives or a change of emphasis in pertinent future investigations.

Biological investigation now is an integral part of water pollution detection and control, and biologists have become increasingly aware of their opportunities for contributing to progress in this field of work. Their ideas have been solicited and have been well received by other specialists. In trying to aid the advancement of their science, biologists owe it to their profession to seek thorough understanding of the practical problems of water pollution control. Understanding the complexity of these problems will make apparent the need for thorough and critical testing of new ideas previous to their widespread practical application.

First, it is necessary to consider the meaning of the term "pollution". The introduction of any foreign substance which merely alters the natural quality of water without materially interfering with any likely use of the water cannot be said in a practical sense to constitute pollution. Virtually every stream and lake in any inhabited region receives at least a trace of something which measurably or not measurably alters the natural quality of the water. What is significant or important from a practical standpoint is not the mere presence of the added material, but its influence upon the economic and esthetic value of the water, or on human welfare in a broad sense. It appears that most authorities in the field of water pollution control and abatement agree in defining water pollution as an impairment of the suitability of water for any beneficial human use, actual or potential, by any foreign material added thereto.

This definition agrees with repeatedly expressed judicial opinion, that is, with definitions of "pollution" and of "clean water" established by courts of law. The following legal definition, cited on page 100 of "Water Quality Criteria", a publication of the California State Water Pollution Control Board (4) is typical: "For the purposes of this case, the word 'pollution' means an impairment, with attendant injury, to the use of water that plaintiffs are entitled to make. Unless the introduction of extraneous matter so unfavorably affects such use, the condition created is short of pollution. In reality, the thing forbidden is the injury. The quantity introduced is immaterial." Other definitions cited agree essentially with this one.

In accordance with the above definition of the word pollution, a demonstrable change of some components of the biota of a stream clearly caused by the discharge of some waste into the water is not invariably evidence of pollution, any more than is a demonstrable chemical change. If it cannot be reasonably asserted that a hazard to human health or interference with some beneficial use of the stream, such as fishing, must accompany a particular alteration of the biota, the change cannot correctly be said to indicate pollution. Even the discharge of a waste which eliminates virtually all organisms initially present in a very small or temporary stream capable of supporting no aquatic life of any value to man is not necessarily pollution. Oxygen-depleting organic waste may be thoroughly mineralized in such streams through natural self-purification processes, so that only harmless substances and beneficial plant nutrients may reach larger watercourses to which these streams are tributary.

In agreement with the definition offered above, Beck (1) has defined pollution broadly as "the alteration of any body of water, by man, to such degree that said body of water loses any of its value as a natural resource."

Patrick (28), on the other hand, has proposed a distinctly different, strictly biological definition. This author defines pollution as "any thing which brings about a reduction in the diversity of aquatic life and eventually destroys the balance of life in a stream." By way of explanation, it is further stated that "As conservationists interested in using rivers today but not abusing them so that they are damaged in the future - this is the basis on which pollution should be judged. For it is by preserving the biodynamic cycle that the ability of a river to rejuvenate itself is maintained.

Unfortunately it is not clear just what is to be regarded as pollution according to the definition given by Patrick. Is any reduction in the diversity of aquatic life evidence of pollution which will eventually destroy the "balance of life", or only such a severe reduction of the diversity of life that the ability of the stream to "rejuvenate itself" is indeed destroyed? A reduction of species numbers is not always necessarily followed by the eventual destruction of the "balance of life" in a stream and of the ability of the stream to "rejuvenate itself" (i. e., to undergo natural self-purificatio Patrick (28) has pointed out that the so-called "food chain" in aquatic environments "consists of many series of interlocking links so that if one series is broken another can take over so that the chain is not destroyed." It is well known, also, that in certain "zones" of streams heavily and continually enriched with organic wastes relatively few animal and plant species are present, as a rule, yet natural purification proceeds at a very rapid rate. Here, as in an efficient trickling filter, an ideally adapted and obviously vigorous, healthy, and in certain respects very well balanced biota of limited variety can exist, and the organic waste is mineralized far more rapidly and efficiently than it could possibly be in a previously uncontaminated stream with its original, primitive biota. The ability of the stream to "rejuvenate itself" certainly cannot be said to have been destroyed, or even impaired.

Thus, a stream can be seriously polluted, in any usual sense of the word, without lasting destruction of the "balance of life" and of self-purification capacity (which balance hardly can be permanent anyway, in any unstable environment). On the other hand, mere reduction of the diversity of aquatic life without impairment of any important "food chain" (i. e., the food supply of valuable fishes, etc.), or interference with existing stream uses, does not necessarily have anything to do with the conservation of natural resources. It appears, therefore, that the last-mentioned definition of pollution is unsatisfactory, from a practical standpoint, no matter how it was meant to be interpreted.

Careful consideration of the other pertinent writings of Patrick and of the proposed method of judging stream conditions leads to the conclusion that probably this author regards any marked reduction of the diversity of aquatic life as evidence of pollution.

Beck (1) states that "Patrick's methods suggest that the bio-dynamic cycle should be maintained in the primitive condition," allowing for no equitable stream use, for "any deviation from the primitive bio-dynamic cycle is interpreted by Patrick as evidence of pollution." Actually Patrick has not suggested that an entirely primitive condition of every stream biota should be maintained and has classified as "healthy" certain stream sections which evidently were not in the primitive state. A diversity of organisms approaching that found under undisturbed or primitive conditions does seem to have been regarded, however, as being characteristic of all "healthy", unpolluted waters. This interpretation of Patrick's views may be right or wrong. In any case, the need for clarification thereof, and for better agreement among biologists as to the meaning of terms too often loosely used, is apparent. It is noteworthy that Patrick's definition of pollution, quoted above, implies that an alteration of water quality cannot be pollution if it has no appreciable effect on the diversity of aquatic life, and it can be interpreted as meaning that a marked reduction of the diversity of aquatic life is always associated with pollutional abuse of the aquatic environment. Probably few if any workers directly concerned with water pollution abatement or control can approve such a definition.

One can hardly maintain that the relative worth of any biological environment depends on the number of species that it supports, rather than on the relative abundance of species of some importance or value to man. The presence of many different weeds does not usually contribute to the value of a pasture. Also, it is not always correct to assume that any marked modification of a natural environment and of its original, primitive biota will result in their economic degradation, that is, a reduction in value. The clearing, irrigation, and cultivation of desert and other almost worthless lands, the application of agricultural and other poisons for the control of various pests and weeds, and many other human activities can, indeed, greatly enhance the value of the affected lands while drastically modifying their biotas and reducing the numbers of species present. Not only the production of valuable crops is thus promoted, but sometimes also the production of equally valuable wild game. On the other hand, the destruction of only one or a few animal or plant species of outstanding value (e.g., by some selective poison) obviously can mean great loss. This loss is in no way ameliorated by the fact that most of the organisms in the same environment are not noticeably affected. It is evident that a change of any biota considered as a whole (e.g., the number of species represented, etc.) may not be a direct nor always reliable index and measure of damage to any valuable natural resource. There seems to be no sound basis for a general assumption of their strict or even approximate parallelism.

Although most authors evidently have recognized the economic significance of pollution, it appears that when devising their biological indices and measures of water pollution and its severity some biologists have completely disregarded all economic considerations. They seem to have curiously attached at least as much importance to the elimination of any species of diatom, protozoan, rotifer, or insect as to the disappearance of the most valuable food or game fish species. Yet, some have claimed that their measure of the harmful effects of pollution is a direct measure and therefore is more reliable than any chemical evidence or measure of pollution. Why the fate of harmless algal, protozoan or insect species can be said to indicate directly the extent of damage to a valuable fish population or to any commercial, recreational, or other use of water has not been explained.

If biological indices and measures of the severity of pollution cannot be relied upon always to reveal even the extent of damage to valuable aquatic life, they certainly do not indicate accurately the general pollutional status of any water. Water which is rendered biologically sterile by addition of some substances such as chlorine, or is appreciably enriched with some organic wastes, other than domestic sewage, may be of good sanitary quality and suitable for most ordinary domestic, agricultural, and industrial uses. On the other hand, water in which aquatic life is not markedly and adversely affected can be contaminated with dangerous pathogens or with chemicals which may seriously interfere with one or more of the above-mentioned uses. In view of the great variety of water uses, and the number and complexity of considerations (physical, chemical, biological, psychological, economic, and sociological) which evidently must enter into any reliable determinatic of the degree of interference with these uses by pollution, the evaluation of the over-all pollutional damage cannot be a simple matter. Any contention that some biological observations alone can cut across all of this complexity and show clearly whether the actual and potential uses of a stream have or have not been affected, and the magnitude of the total damage, would appear to be an over-simplification of the problem. It must be admitted that probably nobody has come forth yet with a clear statement of this claim. And yet, unless a different meaning is made perfectly clear, is not this claim implicit in every asseration to the effect that a generally applicable and reliable biological index or measure of the pollutional status or condition of streams has been devised and developed?

Biotic responses to all of the numerous and very different water pollutants are not alike. Early students of water pollution (23) (24) (31) dealt chiefly with pollution by putrescible organic wastes and particularly domestic sewage. In their day, the use of the term "biologic indicators of pollution" when referring to organisms which respond in a certain way to heavy organic enrichment of their medium was perhaps justifiable. Untreated or inadequately treated domestic sewage then was by far the most important and perhaps the only well known and generally recognized water pollutant. Its discharge into public waters in amounts sufficient to bring about appreciable biotic changes being usually a hazard to human health, it was and is almost always pollution in any ordinary sense of the word. Today, the importance of pollutants other than domestic sewage is generally recognized. Yet, many authors still speak of "pollution indicators" when they actually are referring only to indicators of organic enrichment of water with putrescible organic wastes, which may or may not involve demonstrable damage to natural resources. Some readers are known to have been misled by this terminology, believing that the same biological indices are useful in detecting every kind of pollution.

Gaufin and Tarzwell (13), when reporting their studies of stream pollution with domestic sewage, obviously were considering the effects on aquatic life of an oxygen-depleting organic waste only. Nevertheless, such unqualified and seemingly general statements as their conclusion that "Pollutional associations are characterized by few species but large numbers of individuals" can be misleading. As the quoted authors well know, the numbers of many organisms initially present are reduced and the numbers of none are markedly increased in some waters polluted with toxic wastes, suspended solids such as silt, or even oxygen-depleting organic wastes discharged intermittently. These authors undoubtedly did not intend the conclusion in question to be a very broad generalization from their observational results having to do with one kind of pollution only. Their use of the expression "pollutional associations" for designating associations found in waters polluted with domestic sewage, or in waters enriched with putrescible organic matter, can be excused on the ground that no term that is more appropriate than the term "pollutional" has come into general use in the biological literature. Yet, this lack of a more precise terminology is not any less deplorable because the use of inappropriate terms, and terms which are not sufficiently specific, has become prevalent.

Beck (1) (2) explicitly confines his discussion to the subject of "organic pollution". He has proposed the use of a numerical "biotic index", which is said to be "indicative of the cleanliness (with regard to organic pollution) of a portion of a stream or lake" (2). He recognizes that his methods are "confined to fresh waters and encroaching salinity has a marked effect on the fauna of a stream." Inasmuch as many different pollutants, including toxic constituents of some organic wastes, likewise can have a marked effect on the fauna of a stream, it is apparent that Beck's methods may have only very limited applicability. It may be usable only in connection with the investigation and description of waters known in advance to contain no pollutants other than non-toxic putrescible organic matter.

Patrick (26) (27) (28), recognizing the importance of a variety of pollutants, apparently has attempted to devise a general procedure for the reliable biological detection and measurement of the different kinds of pollution. For reasons already indicated, however, this desirable objective appears to be attainable only when one defines pollution as "any thing which brings about a reduction in the diversity of aquatic life", which is not a generally acceptable definition.

Wurtz (38), while evidently realizing the existence and importance of a large variety of pollutants, seems to overlook completely the important differences of biotic responses to the different pollutants. Thus, his Figure 1 suggests that the same pollutional zones, including a "degradation zone" extending from the point of mixing of an effluent with the water of a stream to a "polluted zone" located some distance downstream, can be expected to occur in any heavily polluted stream, regardless of the nature of the pollutant (i.e., whether it be "organic", "toxic", or "physical"). Furthermore, he speaks of "pollution tolerant species" and of "non-tolerant organisms", suggesting that organisms are consistentely tolerant or consistently non-tolerant with respect to all pollutants. Nowhere does he specify that he has in mind resistance to putrescible organic pollutants only, and there is considerable evidence that he has in mind all pollutants. In large degree, Wurtz seems to have adopted methods similar to Patrick's, but one of his innovations seems to require the probably impossible classification of all or nearly all aquatic organisms as "tolerant" and "non-tolerant" to all kinds of pollution, including the various toxicants, etc. Unfortunately, Wurtz does not include in his paper a list of all organisms considered by him to be tolerant and all those thought to be non-tolerant.

There can be no doubt that some of the so-called "pollution-tolerant" organisms, which actually are simply forms known to thrive in waters markedly enriched with organic wastes, are less tolerant with respect to some other water pollutants than a number of the species known as "clean-water" forms. For example, a species of Physa, a genus of snails generally believed to be resistant to organic pollution (1) has been found to be extremely susceptible to dissolved copper. Certain fish (e.g., centrarchids), may fly nymphs, etc., thought to be more susceptible than Physa to the effects of organic pollution, proved much more resistant to copper. An aquatic environment in which "clean-water" organisms are predominant might possibly be more seriously polluted than one with decidedly "pollutional" biota. The biological terminiology evidently needs revision, so that the word pollution would not be used synonymously with organic enrichment.

It appears that, in general, very broad significance of the various biological indices of water quality and the severity of pollution has been only assumed and not actually demonstrated. This is well exemplified by the following quotation from the summary of one of Patrick's papers

(27): "On the premise that the balanced physiological activities of aquatic life in surface waters are essential for the maintenance of healthy water conditions, it may be assumed that the most direct measure of this biodynamic cycle will indicate the condition of the water." It will be noted that we have here an assumption based upon a rather nebulous premise. Most writers have failed to supply entirely satisfactory, clear definitions of terms used (e.g., "pollution", "health", etc.) to show precisely what it is that they believe they can detect or measure biologically. Others have failed to use defined terms in a manner entirely consistent with their own definitions. The need for demonstration of the validity of some of the most fundamental assumptions concerning the reliability of pollution indices designed for general application has not been satisfied. Some authors seem to be of the opinion that the proof is unnecessary. It must be admitted that investigations designed to provide such proof would be extremely complex and difficult, and it is not likely that the search for this proof would be very rewarding, for there can hardly be a simple, general solution for the problem of pollution detection and measurement. Like a panacea, a general test for all kinds of pollutional damage is something for which biologists and engineers alike probably would be wise not to seek.

The value of fish as indicators of environment conditions and the importance of fish population studies in connection with the estimation of the intensity of water pollution now can be considered. Doubtless there is much more published information on the environmental requirements of fish than on the requirements of species of any other group of aquatic organisms excepting perhaps a few invertebrate species of outstanding economic importance. The vast quantity of published data relating to the water quality requirements of fish is partly revealed by a few recently prepared compilations and summaries of some of this information (4) (5) (8) (9) (10) (11) (17) (33). The resistance of many fish species to extreme temperatures, to unusual concentrations of dissolved oxygen and other dissolved gases, to variations of water salinity, and to extremes of pH, their susceptibility to the harmful effects of a great variety of toxic substances and of suspended solids of mportance as water pollutants, the influence of some of these environmental factors upon embryonic development, growth, and activity, and so forth, have all been studied intensively. There exists also a voluminous iterature on the food of fishes, their life history and reproductive requirements, their habitat preferences, movements, avoidance of adverse environmental conditions, and so on.

While it is evident that more is known of the environmental requirements of many fish than is known of the requirements of most, if not all, of the other aquatic organisms often considered as indicators of nvironmental conditions, the use of fish as indicators has received onsiderably less attention than has the use of other major groups, plant nd animal, microscopic and macroscopic. Fisheries workers recognize the difficulty of adequately sampling fish populations even in bodies of water of moderate size, and this, along with the mobility of fishes, has been advanced as a reason for the unsuitability of fish as indicators of environmental conditions. But, other aquatic groups are difficult to sample too, as Needham and Usinger (25) have demonstrated in the case of the invertebrate macrofauna of a riffle. The difficulty of samplin and the mobility of fishes may not be the chief reasons why fish have not been given more consideration as indicators. The taxonomic groups which have received the most attention no doubt have reflected to some extent the special interests of investigators who happened to be working in the field of water pollution. Fish being the usual economic and recreational yield of stream productivity, their study has obvious applied value and so has required no additional justification. Further, the status of a fish population may indicate suitable or unsuitable environmental conditions, but when knowledge of this population is the end or aim of an investigation, the population status is not regarded as an index of anything else. The value of fish as indicators of the suitability of water f uses other than fishing has not been clearly demonstrated. Whatever the reasons may be, the emphasis in most discussions of the "biological indices" has been on groups other than fish, even though very little is known of the environmental requirements of the species of many of these groups.

The value of knowledge of fish populations in connection with the classification of aquatic environments has not been entirely overlooked. Ricker (32) made important use of the brook trout (Salvelinus fontinalis) and the Centrarchidae and Esocidae as a basis for his ecological classification of certain Ontario streams. Fisheries workers frequently use such expressions as "trout waters" or "bass waters", thus conveniently classifying waters according to the fish species for which the waters are well suited. European workers have made more formal use of such a system of stream classification (34) (37). Brinley and Katzin (3) have classified waters and named various pollutional "zones" of streams in the Ohio River drainage basin according to the kinds of fish populations found therein. As has been done with other animals and plants, some species of fish have been classified as to their "saprobic" preferences by a few authors (22) (24) (19) (35). The basis for such classification of fish is highly questionable. Patrick (26) (27) includes fish among the groups considered in her "biological measure" of stream conditions. Doudoroff (7) and Gaufin and Tarzwell (14) have emphasized the need for thorough fish population studies in connection with water pollution investigations and the determination of the pollutional status of waters.

Studies of fish populations in variously polluted waters, which reveal varying susceptibility of different fish species to pollutional conditions in their natural habitats, have been reported by a number of investigators (3) (6) (11) (20). However, sufficiently intensive sampling of fish populations has not often been undertaken in connection with routine polluti surveys and investigations, the sampling of other aquatic life having been probably more often emphasized when the scope of the biological studies has had to be limited. Inasmuch as it is not often possible adequately to study all of the aquatic biota, including the fish, the practical value of information to be obtained by concentrating attention on fish populations must be carefully weighed against that of information to be derived from equally intensive study of some of the other aquatic organisms, and from comparatively superficial study of the entire biota.

The absence or extreme scarcity of some fish in a stream below the oint of entry of a waste, and not above the point of entry, strongly suggests hat the waste is somehow detrimental to these fish, if valuable good and game ish species are among those believed to be adversely affected pollution is ndicated. Neither the presence nor the absence of fish is a reliable indication f suitability or unsuitability of water for domestic, agricultural, and ndustrial uses and for recreational uses other than fishing. Nevertheless, ecause of the great economic and recreational value of many fish species, his information is essential to sound classification of waters according to heir pollutional status.

The presence of fish does not necessarily show that their environment as been suitable for them for a very long time, nor that the species found an survive indefinitely and complete their life cycles under the existing nvironmental conditions. However, the presence of thriving populations f non-migratory species, including numerous representatives of different ge classes whose growth rates have not been subnormal, is significant. suggests strongly that pollution which is highly detrimental to these fishes nd to migratory species whose habitat preferences, natural food, and ater quality requirements are quite similar has not occurred recently. or example, the presence of numerous cottids in Northwestern salmon id trout streams which receive organic wastes is believed to indicate that ssolved oxygen concentrations have been adequate for some time and her environmental conditions probably have been suitable not only for e cottids, but also for migratory salmon and trout. There is now no und reason for believing that the presence of any invertebrate form is more reliable and appropriate biological indicator of the suitability of st environmental conditions for the migratory salmonids than is the esence of cottids.

The value of waters used for fishing, and of the fisheries which they pport, bears no fixed, direct relation to the number of fish species to found therein, just as it bears no such relation to the number of species other organisms present. Some 35 species of fish were collected in the dwestern warm-water stream studied by Katz and Gaufin (20). Because the scarcity of valuable food and game fishes, this small, polluted ream is not regarded as a valuable fishing stream. On the other hand, ny cool, pure streams which are highly valued as trout and salmon eams contain very few fish species other than the salmonids. leed, the invasion of valuable trout waters by other fish species not initially present is generally regarded as evidence of degradation of these waters, for the numbers of trout usually decline when it occurs. Such a change of the fish population can be a result of increasing temperatures and probably also of enrichment (18). Warm, eutrophic waters can support a great variety of fish and other organisms, but trout waters which are approaching this condition can hardly be regarded as "healthy".

Some of the above statements seem to contradict Patrick's (26) (27) conclusion, based on a study of the Conestoga River Basin of Pennsylvania, that "*The results of this study indicate that under healthy conditions a great many species representing the various taxonomic groups should be present.*" It is necessary, therefore, to examine the evidence on which the latter conclusion is based. It appears that, in accordance with Patrick's conception of what a "healthy" stream should be like biologicall only those stations where a variety of organisms judged to be fairly normal or typical was actually found were classed as "healthy". It is not surprising, therefore, that all of the stations classed as "healthy" had indeed this large variety of organisms. Chemical, bacteriological, and other data were collected and considered in selecting and classifying the stations studied. It is clearly indicated, however, that the variety of organisms found (which is the proposed index or measure of stream "health") also was a major consideration. Different conclusions perhaps would have been reached had the initial classification of the stations been based entirely on other criteria of obvious practical import (such as the abundance, condition, and growth rates of valuable native game fish, etc.) and had a greater variety of natural, unpolluted streams been examined. It is noteworthy also that certain stations which evidently were not much affected by waste discharges but lacked the usual variety of organisms (e.g., Station No. 152, in a stream section evidently suited for stocking with trout) were classed as "atypical" stations by reason of certain observed peculiarities, such as low water temperatures, unusual bottom or shore conditions, etc. Other stations which had the expected variety of organisms were classified as "healthy" stations despite noted peculiarities such as marked organic enrichment, unusually high BOD, high CO_2 content, high bacterial content, or great turbidity of the water. Thus, it appears that the rating of the stations was somewhat arbitrary.

When the possibility of certain pollutional damage specifically to fisheries is under consideration, it should be remembered that fishes have varying ecological requirements and habits, differ in their resistance to variations of water quality, and are not all dependent upon all aquatic organisms, nor upon the same organisms, for their food. It has been shown that the growth of some fish species is promoted in certain waters affected by the discharge of organic waste (21), whereas the same waters apparently are rendered unsuitable for some other species (20). A reduction of the number of species of fish-food organisms, with a great increase of abundance of some of the remaining species, which occur often in streams receiving various wastes, doubtless can be harmless or beneficial for some fish species, although this reduction may be detrimental to others. If they are not otherwise adversely affected by environmental changes, those fishes which can well utilize the abundant food organisms will thrive, while others may disappear. Whether the total effect on fisheries will be favorable or unfavorable clearly will depend on the relative commercial and recreational value of those fish populations which are favored and those which are affected adversely. An intensive study of the entire aquatic biota cannot always reveal the extent of pollutional damage to fisheries, unless the relative value of the various forms present (for man, or as food for important fishes) is considered.

To evaluate the effect of environmental changes on fisheries it is necessary to know what fish species were originally present, how highly each is valued, and in what way and to what degree each important species has been affected by waste discharges. The relative abundance and condition of individuals of different species in the waters under investigation and in suitable "control" areas, the growth-rates of different age classes, the palatability of the flesh, and possible interference with normal migratory movements or with other reproductive activities must all be considered. Fish collections taken by carefully planned netting will yield much of this information. Commercial and sport catch records, showing the take per unit of fishing effort, and various field observations (e.g., of spawning areas utilized, etc.) also can be very helpful. Inasmuch as the presence of wastes and other pollutants is by no means the only factor which can directly influence fish populations, the cause of observed differences of fish populations must be determined. In this connection, studies of the food of important fish species and of the relative abundance of available food organisms in waters which are affected and those which are not affected by waste discharges may be essential. However, if detection and evaluation of pollutional damage to fisheries is the only or primary objective of a biological investigation, an enumeration of the species of organisms of all taxonomic groups, or of some single invertebrate group, cannot be deemed a direct approach to the problem at hand. Judged only by its practical utility, it may be a waste of time, effort, and money, which perhaps could be far better expended on more directly pertinent studies. Indeed, it is difficult to imagine pollutional interference with any use or combination of uses of water which could usually be accurately and most efficiently evaluated in such an indirect manner.

A study of the influence of large amounts of organic waste on the ecology of the Tuolumne River of California has recently been completed by Warren (unpublished data). During August and September of 1952, the daily mean discharge rates of this river at the city of Modesto ranged from 293 to 822 cubic feet per second. The daily mean discharge rates of domestic and cannery waste introduced into the Tuolumne at Modesto ranged from 0 to 22.3 cubic feet per second. The 5-day biochemical oxygen demand of samples of this waste ranged from 60 to 575 parts per million. Dissolved oxygen concentrations at stations below the point of waste discharge ranged from zero to supersaturation during this time.

The objective of this study was to determine some of the effects of organic waste discharges on the ecology of the Tuolumne during the different seasons of the year. Some thirty miles of the river were studied, of which only the lower ten were influenced by waste discharges. The phytoplankton, zooplankton, benthic fauna, and fish were studied along with the physical, chemical, and bacteriological conditions in this river. The fishery phase of the investigation represented a small part of the total effort.

The investigation of the Tuolumne River now being complete and its objective more or less realized, it is interesting to consider how well other objectives might have been satisfied by this same study, planned and conducted as it was. For instance, had the objective been to determine the influence of the organic waste specifically on the fisheries of the Tuolumne, could not much of the effort devoted to the bacteriological, phytoplankton, zooplankton, and benthic faunal investigations have been far better expended on a thorough study of the fisheries? One is forced to conclude that were the objective to determine the status of the fisheries, the fish should have received most of the attention. This does not mean that studies of the plankton and of the benthic fauna are not necessary phases of an investigation so oriented. They may be quite necessary, but they should be so planned that the time and effort devoted thereto would not be out of proportion to their contribution to thorough understanding of the status or condition of the valuable fish populations.

The benthic fauna present at stations on the Tuolumne River below the point of waste discharge had many of the recognized "pollutional" characteristics during late summer and early fall. By this time, many of the "clean-water" species present at these stations earlier in the summer, and persisting at stations above the waste outfall, had disappeared. A marked reduction in species numbers had taken place, and at least one species occurred in unusually great numbers. While the bottom fauna showed changes that in accordance with most biological index methods would be regarded as evidence of pollution, rather intensive seining during mid-September resulted in the collection of 10 species of fish at stations above the point of waste discharge and 12 species at stations within the first ten miles below this point. The variety of fish present had certainly not been greatly altered by the introduction of wastes, even though the bottom fauna had been markedly modified.

Collections of young bluegills (Lepomis macrochirus) made in September showed the O-year class to grow faster at stations below the point of waste introduction than at stations above this point. The size difference persisted in the I-year class. The difference in the O-year-class growth rates could probably be attributed to the greater abundance of zooplankton at the downstream stations.

While the above data are interesting, they cannot be taken as evidence that pollution of the Tuolumne damaging to fisheries did not exist. Some evidence indicated interference with a portion of the upstream migration of adult chinook salmon (Oncorhynchus tshawytscha), though the downstream migrant young were presumably unaffected, being apparently absent from the Tuolumne by the time of critical summer river flows and waste discharges. Juvenile shad may perhaps have been affected also. Had the principal objective of the Tuolumne River investigation been an evaluation of damage to fisheries resources by pollution, the study could not have been deemed complete in the absence of conclusive evidence that interference with salmon migrations and other possible damage to valuable fish populations had or had not occurred. None of the proposed "biological measures" of pollution intensity could have revealed the degree of such interference or damage. In order to obtain the crucial evidence required, it would have been necessary to emphasize the fisheries phase of the investigation.

It is not the purpose of this paper to discourage limnological research pertinent to water pollution problems, nor is it intended to deny the value of all biological indicators of pollution. There can be no doubt that a drastic modification of any natural aquatic biota, attributable to a change of water quality, can have highly undesirable aspects or consequences. Such changes presumably are detrimental to human use and enjoyment of natural waters more often than they are not. Many a readily demonstrable effect of wastes upon aquatic life in a valuable stream is suggestive of probable existing or incipient pollution which deserves close attention and investigation. Even before valuable fish populations have been materially affected by some potentially harmful pollutant, an observed detrimental effect upon other organisms which are somewhat more susceptible than fish may give warning of possible future damage to fisheries by continued or additional waste discharges. The nature and the source of existing or incipient pollution also may be revealed by appropriate biological indices. Finally, inasmuch as some of the organisms considered to be indicators of pollution are organisms which can directly interfere with human use or enjoyment of waters (e.g., unsightly slime-forming organisms such as Sphaerotilus, odor-producing algae, etc.), their unusual abundance may not be disregarded in evaluating over-all damage caused by pollution.

CONCLUSIONS

It must be concluded that every change or peculiarity of the flora and fauna of a stream which has been referred to as an index or measure of pollution is in reality only an index of environmental disturbance or environmental anomaly. The disturbance or anomaly indicated may or may not be pollutional in the sense that stream uses are interfered with.

Pollution (i.e., interference with stream uses) can be negligible when the effect on the aquatic biota as a whole is great, and it can be severe when most of the aquatic life is unaffected. Gross pollution often can be demonstrated without any biological investigation. When biological investigation may be necessary, pollutional damage to valuable aquatic organisms can probably best be determined by concentrating attention upon these particular organisms. Yet, since all aquatic life forms are more or less sensitive to changes of water quality, the fate of any of them theoretically can be instructive, revealing something about the nature and magnitude of these changes that may not be obvious nor easily determined otherwise.

A genuine contribution to water pollution science can be made whenever the presence or relative abundance of living organisms of any kind can be shown to be a reliable index of something tangible that one may need to know in order fully to ascertain and understand the pollutional status of an aquatic environment. When proposing and describing the use of such biological indices, one should state specifically what it is that each is believed to indicate, carefully avoiding such general, vague, or abstract terms as "pollution" and "stream health", which may be variously understood. Does it indicate, for example, continual presence of dissolved oxygen in certain concentrations believed to be adequate for sensitive fish species? Does it indicate organic enrichment likely to interfere in some way other than through oxygen depletion with certain specific uses of water? Or does it indicate that particular toxic substances have not recently been present in concentrations likely to be injurious to fish, to man, or to certain crops? No simple biological indicator and no one measure of stream conditions can indicate all of these things. But any species can become a biological indicator of environmental conditions of possible interest as soon as its nutritional and other environmental requirements, its relative resistance to various toxic substances, etc., become known. Widely distributed sessile or sedentary organisms should be the most useful indicators of past conditions. Unfortunately, the water quality requirements of most of the "indicator organisms" have never been thoroughly investigated, so that there is no real knowledge of specific factors which limit their distribution and abundance. Probably nobody now knows just why any of the so-called clean-water organisms begin to disappear from waters subject to progressively increasing organic enrichment. Here is a field for future research which is far more promising than is, for example, the questionable classification of all aquatic organisms as "pollutional", "clean-water", or "facultative". If there are common sedentary organisms whose water quality requirements can be shown to correspond closely with those of valuable fish species, they are potentially useful indicators. At the present time, however, excepting instances of gross pollution, only fish themselves can be said to indicate reliably environmental conditions generally suitable or unsuitable for their own existence.

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BIOLOGICAL CRITERIA FOR THE DETERMINATION OF LAKE POLLUTION

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Many, if not most of our productive warm-water lakes have low-oxygen conditions existing in them through at least a part of the summer. At this time, conditions prevail that closely approach conditions caused artificially by domestic or industrial pollution. In the eutrophic "chironomus" lakes of Connecticut described by Deevy (1941), one part per million of oxygen or less prevailed from late June until early November, but zero oxygen was not recorded.

The fact that aquatic earthworms of the family Tubificidae (*Limnodrilus* and *Tubifex* in this report) and such red midgefly larvae as *Tendipes decorus*, *Tendipes tentans*, *Tendipes plumosus* and *Tendipes riparius* are able to live in water of very low oxygen content is well known. Lindeman (1941) demonstrated that "chironomus" larvae could withstand complete lack of oxygen for four months at 10°C, but Deevy (1941) pointed out that much of the case for existence of these organisms under anerobic conditions rests on analyses made with either the unmodified Winkler method or the Rideal-Stewart modification. Recently, Ruttner (1953) cautioned that Alsterberg's modification of the Winkler method should be used for oxygen determinations otherwise values are lower due to the presence of reducing substances.

This report stemmed from a study of American papers dealing with the quantitative abundance of aquatic earthworms in both lakes and streams. It was concluded last year in a report to the Midwest Benthological Society that in large unpolluted lakes, such as Lake Michigan, Lake Nipigon, Douglas Lake (Michigan), etc., the average number of tubificids per square foot is usually less than 50, but that in lakes in good agricultural areas numbers averaged higher, 100 to about 300 per square foot. At that time, the writer overlooked a paper by Wright and Tidd (1933) which classified the bottom areas of western Lake Erie as clean, lightly-polluted moderately-polluted, and heavily-polluted. Clean bottom was defined as having less than 9.3 (100 per square meter) tubificids and more than 9.3

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Hexagenia (a burrowing mayfly) per square foot, lightly polluted 9.4-93 tubificids per square foot; moderately polluted 94-456 tubificids per square foot; and heavily polluted over 456 tubificids per square foot.

In regular survey work, the writer has been in the habit of looking critically at each sample, identifying the animals present to species if possible, and classifying them as pollution-tolerant, facultative, or clean-water. Each sample, even in a lake where there are usually few species, is capable of revealing conditions in a limited area, especially where the information is correlated with data on oxygen content of the water, biochemical oxygen deman (B.O.D.), other chemical analyses, temperature, etc., collected at the same time.

With the exception of a few samples from White Lake and Saginaw Bay, the bottom samples were collected with a 6 x 6 inch Ekman dredge, and the bottom materials were sieved through a No. 30 sieve.

Main reliance has been placed on bottom samples collected from the deeper waters of lakes outside the littoral zone of vegetation, for it seemed obvious that damage to a lake by pollution would show up there first. While the shoreward zones of vegetation contain a greater variety of organisms, the photosynthetic activity of plants in polluted areas and the circulation of surface waters are likely to create better living conditions in the zone of vegetation than exist in waters deeper than about 15 feet. In the lakes sampled thus far, the deeper-waters comprised most of the lake area. It is believed that conditions must be maintained in a satisfactory condition there if these lakes are to remain productive of fish and fish food organisms.

Since White Lake in Muskegon County, Michigan, had from 8 to 17 species of bottom animals at depths to 60 feet; Muskegon Lake 6 to 8 species at depths to 61 feet; Lake Charlevoix in its less-polluted areas near Boyne City from 3 to 9 species down to depths of 44 feet, it was decided to take a collective look at the lake bottom sampling in the Michigan lake pollution surveys to determine (1) whether more than 100 tubificids per square foot actually represented polluted conditions, (2) what other organisms of the deeper waters might be present and classed as pollution-tolerant or hardy, sensitive or intermediate, (3) any other useful criteria of lake pollution such as comparisons of total numbers of animals, exclusive of the tubificids.

This "collective look" brought regrets that more samples were not taken in many places, but the presentation of the data collected may be of interest and some value in establishing norms and methods of sampling in the future which may lead to quantitative numerical values or norms above or below which damage to bottom animal life is more apparent.

Manistee Lake may be cited as an example of a lake in which even tubificid worms could not live in the deeper waters. In this lake, 17 of 24 samples collected September 22, 1954, were entirely without bottom animals of any kind. This lake has been severely polluted with paper mill and other wastes.

The deeper areas of Fremont Lake, likewise, were apparently severely damaged by organic pollution exceeding the quantity the lake could assimilate, and many of the samples were without tubificid worms and other species normally present. On September 16, 1952, 4 of 14 samples were entirely without animals. A neighboring unpolluted lake, Pickerel Lake, sampled September 15, 1952, of similar depth and without oxygen in its deepest waters had three times as many bottom animals. There were about three times as many Tendipes plumosus and ten times and many Chaoborus in the bottom samples.

In Saginaw Bay, the key area of Lake Huron from a fisheries standpoint, samples were taken at widely scattered points, but it has now developed that these were not collected at the best season; collection apparently followed the emergence of Michigan "caddis" Hexagenia limbata and the "bloodworm" Tendipes plumosus, the young of which occurred in several samples.

Saginaw Bay receives pollution from the Saginaw River which carries industrial and municipal wastes from Midland, Saginaw and Bay City. Minor contributions of pollution are made to the bay by the Kawkawlin and Pinconning rivers. A beet sugar plant at Sebewaing introduces wastes seasonally into the bay. There is also a milk plant located at Sebewaing which discharges treated wastes into Sebewaing Bay. The City of Saginaw began operating a new sewage treatment plant before the 1954 survey. Pollutants peculiar to the operation of a large chemical plant at Midland include brine and phenolic wastes. The latter have been reduced considerably in recent years. Chlorides ranged from about 25-50 parts per million in the bay.

Data on Green Bay, the key area of Lake Michigan, have been included for comparison with Saginaw Bay, although sampling there occurred at a more favorable time, prior to June 1. The area of Green Bay referred to as the "Lower Bay" in Table I is closest to the polluted Fox River which has many paper mills located on its banks between Green Bay and Appleton, Wisconsin.

The series of samples in the Oconto line probably represent relatively unpolluted conditions in spite of the fact that the lower Oconto River is heavily polluted by paper mill wastes.

White Lake, at Whitehall and Montague, Michigan, may be classed as virtually unpolluted. The surveys made of it both preceded and followed the construction of a large chemical plant which provided adequate waste treatment facilities.

Ellsworth Lake, Antrim County, Michigan, a lake in a chain of connected lakes receives canning wastes seasonally. When it was sampled on October 8, 1953, most of the lake had 0.2 part per million or less of dissolved oxygen. Because of the shortage of oxygen, crayfish, which were abundant in the lake at the time, had migrated from the deeper water to the very margin and some even out of the water in an effort to survive low oxygen conditions. Many of them died at the water's edge. Freshly dead midgefly larvae of Tanytarsus nigricans were found in only two bottom stations, but many had risen to the water surface where they were fed upon by gulls. Larvae of the biting midge Palpomyia tibialis[1] were numerous in these bottom samples in spite of the low oxygen, and apparently many of them left the bottom temporarily for only 55 percent of the samples contained them on October 8, 1953. When the lake was revisited February 10, 1954, they were twice as abundant as during the sampling of October 8, 1953, and 91 percent of the samples contained them.

Tendipes plumosus larvae occurred in 64 percent of the Ellsworth Lake samples on October 8, and in 55 percent of the samples collected from under ice cover February 10, 1954, indicating that most of them were hardy enough to survive the temporary period of low oxygen in the lake.

A series of three stations across the lower end of St. Clair Lake located in the Intermediate River chain of lakes immediately above Ellsworth Lake porvided a small amount of data from an unpolluted area to compare with

[1] Identified for the writer by Dr. Willis Wirth, U.S. National Museum.

data from Ellsworth Lake. One of the principal differences was low average number of tubificids, average 9.3 per square foot in St. Clair Lake compared to 52.4 in Ellsworth Lake on October 8, 1953, and an average of 8 tubificids in St. Clair Lake compared with 63 per square foot in Ellsworth Lake on February 10, 1954. Larvae of Calopsectra dives were present in St. Clair Lake samples and absent from Ellsworth Lake.

Paper mill wastes enter Muskegon Lake on the south shore, and in that vicinity tubificid worms were particularly numerous. They were nearly ten times as numerous as they were in the control area on the north side of the lake. Midgefly larvae of Procladius and Tendipes plumosus were present in small numbers in at least half of the samples on both sides of the lake.

Lake Charlevoix at Boyne City is polluted by wastes from a large tannery and by the untreated wastes of the city. An extensive area of the lake bottom near Boyne City contains hair (originating in the tannery wastes). The bottom sampled was roughly divided into two areas. The area of maximum pollution adjacent to the tannery and city had deposits of hair ranging from 2,000-6,950 pounds per acre on a dry weight basis. The area of minimum pollution coincided with hair deposits of less than 2,000 pounds per acre. Tubificids were most abundant in the zone of greatest hair deposits. Midges of Procladius were also most abundant in this zone.

Hart Lake is an artificial impoundment on the South Branch of the Pentwater River. It receives cannery wastes seasonally, and the samples collected here were taken at a time (July 22, 1953) when cherry canning had been in progress for some time. It receives untreated domestic sewage from a city of about 2,200 population. Probably the point of greatest interest in this study was the abundance of Chaoborus punctipennis in the samples collected below the sources of pollution. They ranged in number from 16-504 per square foot, with three samples containing 296, 324, and 504 per square foot. Larvae of Tendipes tentans-plumosus and Procladius were numerous in some samples.

There are many details of description and notes on abundance of species that might have been noted in this report; however, the relatively small number of samples taken in most cases does not justify the drawing of many conclusions at present.

The following quantitative data are presented primarily to show trends of abundance of species in polluted areas. Tables 1 and 2 show average number of species in each area; total number of animals in all samples of each area; average number of animals per square foot, both with and without tubificid worms; average number of tubificids per square foot, abundance of several species of midgefly larvae, phantom midges, and fingernail clams.

Tables 3 and 4 show the frequency of occurrence of the above kinds of bottom animals in all samples in percentages of the number of samples taken.

CONCLUSIONS

A survey of the lake reports showed that an abundance of tubificids in excess of 100 per square foot apparently truly represented polluted habitats. Severe pollutions such as occurred in a depression in Fremont and in virtually all of Manistee Lake precludes all forms of animal life. Such data must be approached with care when statistical analyses are considered because considerable pollution may cause increased abundance in some areas and severe pollution may eliminate or reduce numbers of even the hardy forms in other areas.

The data show that Procladius species are usually present with tubificids in polluted areas, but in severe pollution only the tubificids are able to survive.

Palpomyia tibialis was found to be a very hardy species in one lake where conditions brough about the destruction of Tanytarsus nigricans.

The fingernail clam Pisidium sp. was more prevalent in polluted areas than Sphaerium or Musculium, and judging from the abundance of dead shells in grossly polluted areas, these small mollusks are not able to survive the adverse conditions tolerated by tubificids.

Cryptochironomus digitatus and Calopsectra dives are common associates of Tendipes plumosus or Tendipes tentans, tubificids and Procladius in the deeper waters of the polluted areas sampled. It is presumed that they are hardy, although their hardiness is apparently less than either tubificids or Procladius. The greater average abundance of Procladius in polluted habitats is presumed to be due in part to the fact that they feed upon tubificids.

Chaoborus punctipennis may frequent rather heavily-polluted areas and become abundant there. Although their swimming ability and habit of migrating to the surface at night permits them to leave polluted areas in which tubificids either die or thrive, there is some evidence, as in the comparison of Pickerel Lake (an unpolluted lake) and Fremont Lake (a polluted lake) and the north and south sides of Muskegon Lake, that Chaoborus can not survive in large numbers in a polluted lake where low oxygen conditions have apparently prevailed for an excessive period of time.

When average numbers of bottom animals per square foot including tubificids are compared with average numbers less tubificids, most populations reveal the large contribution of the latter numerically. The difference may also reflect the contribution of organic wastes as a fertilizer in lakes as it did in lower Hart Lake.

Number of species in lakes as well as streams appears to be the most reliable criterion of pollution. Unpolluted Michigan lakes from these limited observations appear capable of supporting a number of species even to depths of 60 feet.

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TABLE 1. AVERAGE NUMBERS OF BOTTOM ANIMALS PER SQUARE FOOT IN POLLUTED MICHIGAN LAKES

	Saginaw Bay July 25-26 1953	Saginaw Bay July 16-17 1954	Green Bay (Wis.) Lower Bay May 27, 1952	Green Bay Oconto line May 26, 1952	White Lake May 26, 27 1954	White Lake Aug 19 1952	Ellsworth Lake Oct. 8, 1953	St. Clair Lake Oct. 8, 1953	Ellsworth Lake Feb. 10, 1954	St. Clair Lake Fel 10, 1954
Number of Samples	8	14	20	7	22	16	11	3	11	3
Average number of species	5.8	9.1	9.7	6.3	12	7.4	6.1	15	5.6	12.7
Total number of animals (all samples)	1,908	3,339	25,332	1,212	10,504	4,163	1,672	824	1,868	629
Average number per square foot	239	239	1,267	173	478	260	152	275	170	210
A. Aquatic earthworms										
Average number of tubificid worms	162	130	1,084	35	98	60	52	9	63	8
Average number less tubificids	77	109	183	138	380	200	100	266	107	202
B. Midges										
Av. number *Procladius sp.*	7.8	5.1	65.2	44.0	53.8	46.0	23.3	44	36	60
Av. number *Cryptochironomus digitatus*	6.0	2.8	7.2	2.9	5.7	5.3	0.4	0.0	0.0	4.0
" " *Tendipes tentans-plumosus*	6.0	22.3	16.0	14.8	46.8	54.3	5.1	0.0	7.6	2.7
" " *Calopsectra dives*	6.3	9.0	16.2	32.6	26.1	1.2	0.0	12.0	0.0	20.0
" " *Pelopia stellatus*	0.0	0.0	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. Phantom midges										
Av. number *Chaoborus punctipennis*	0.0	0.0	0.0	0.0	60.2	10.8	24.4	10.7	19.3	6.7
D. Punkies										
Av. number *Palpomyia sp.*	0.0	0.1	0.0	0.6	4.8	0.0	13.5	8.0	32.0	4.0
E. Fingernail clams										
Av. number *Pisidium*	2.0	3.1	41.3	26.8	14.1	8.9	1.5	2.7	0.7	4.0
" " *Sphaerium-Musculium*	0.0	1.1	8.3	4.0	1.9	0.8	0.7	0.0	0.7	0.0

TABLE 2. AVERAGE NUMBERS OF BOTTOM ANIMALS PER SQUARE FOOT IN POLLUTED MICHIGAN LAKES

		Muskegon Lake. Northside Sept. 15, 1954	Muskegon Lake Polluted Area Sept. 15, 1954	L. Charlevoix. Zone of least pollution May 19, 1954	L. Charlevoix. Zone of max. pollution May 19, 1954	Pickerel Lake Sept. 15 1952	Fremont Lake Sept. 16 1952	Manistee Lake Sept. 15 1953	Manistee Lake Sept. 22 1954	Hart Lake above pollution July 22, 1953	Hart Lal below pollution July 22, 1953
	Number of samples	4	12	11	12	8	14	24	24	2	8
	Average number of species	8.2	5.8	5.7	7.3	3.6	1.5	0.95	0.8	11.5	4.8
	Total number of animals (all samples)	2,396	26,659	1,060	5,839	368	228	720	556	504	5,314
	Average numbers per square foot	599	2,222	96	487	46	16	30	23	252	664
A.	Aquatic earthworms										
	Average number of tubificid worms	227	2,092	19	344	1.0	5.0	18	16	76	373
	Average number less tubificids	372	130	77	143	45.0	11.0	12	7	176	291
B.	Midges										
	Av. number *Procladius sp.*	9.0	10.7	39.3	60.3	2.5	2.0	0.2	1.2	6.0	37.5
	" " *Cryptochironomus digitatus*	0.0	2.3	2.5	1.7	0.0	0.1	0.2	0.2	1.7	12.0
	" " *Tendipes tentans-plumosus*	9.0	8.3	0.7	6.0	9.5	3.4	0.0	0.0	10.0	71.7
	" " *Calopsectra dives*	1.0	0.0	3.3	0.7	0.0	0.0	0.2	0.0	2.0	0.0
	" " *Pelopia stellatus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5
C.	Phantom midges										
	Av. number *Chaoborus punctipennis*	46.0	2.3	0.0	0.0	16.0	0.6	0.0	0.0	2.0	163
D.	Punkies										
	Av. number *Palpomyia sp.*	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	4.0	0.0
E.	Fingernail clams										
	Av. number *Pisidium*	15.0	14.7	6.9	12.6	1.0	0.0	0.5	0.8	40.0	3.5
	" " *Sphaerium-Musculium*	7.0	9.0	0.4	10.0	0.0	0.0	0.2	0.0	22.0	12.5

TABLE 3. FREQUENCY OF OCCURRENCE OF VARIOUS SPECIES IN LAKE SAMPLES EXPRESSED AS THE PERCENTAGE OF THE TOTAL SAMPLES TAKEN

	Saginaw Bay-July 25, 26 1953	Saginaw Bay-July 16. 17 1954	Lower Green Bay, May 27 1952	Green Bay Oconto line May 26, 1952	White L. May 1954	White L. Aug. 19, 1952	Ellsworth Lake Oct. 8, 1953	St. Clair Lake Oct. 8, 1953	Ellsworth Lake Feb. 10, 1954	St. Clair Lake Feb. 10, 1954
Tubificidae	100	100	100	71	95	88	82	67	91	67
Procladius	63	71	95	100	100	94	73	100	82	67
Cryptochironomus digitatus	63	71	80	80	64	50	9.1	0	0	67
Tendipes tentans-plumosus	13	79	70	43	55	69	64	0	55	33
Chaoborus punctipennis	0	0	0	0	64	56	91	33	64	33
Calopsectra dives	50	43	45	86	86	19	0	100	0	67
Pelopia stellatus	0	0	15	0	0	0	0	0	0	0
Palpomyia	0	14	0	14	36	0	55	67	91	67
Pisidium	25	50	65	86	86	44	18	33	9	33
Sphaerium and *Musculium*	0	21	40	43	23	6	9.1	0	18	0

TABLE 4. FREQUENCY OF OCCURRENCE OF VARIOUS SPECIES IN LAKE SAMPLES EXPRESSED AS THE PERCENTAGE OF THE TOTAL SAMPLES TAKEN

	Muskegon Lake North Side Sept. 15, 1954	Muskegon Lake polluted area Sept. 15, 1954	Lake Charlevoix. Zone of least pollution May 19, 1954	L. Charlevoix. Zone of greatest pollution May 19, 1954	Pickerel Lake Sept. 15, 1952	Fremont Lake Sept. 16, 1952	Manistee L. Sept. 15, 1953	Manistee Lake Sept. 22, 1954	Hart Lake above pollution source July 22, 1953	Hart Lake below pollution sources July 22, 1953
Tubificidae	100	92	91	100	13	29	17	21	100	100
Procladius	75	75	100	100	25	21	4	8	100	88
Cryptochironomus digitatus	0	8.3	45	33	0	7	4	4	100	25
Tendipes tentans-plumosus	75	50	18	42	75	36	0	0	100	72
Chaoborus punctipennis	50	25	0	0	75	7	0	0	50	100
Calopsectra dives	25	0	27	17	0	0	4	0	50	0
Pelopia stellatus	0	0	0	0	0	0	0	0	0	25
Palpomyia	0	0	0	0	13	0	0	0	50	0
Pisidium	75	25	18	42	25	0	4	4	100	25
Sphaerium and Musculium	50	33	45	17	0	0	4	0	100	50

THE USE AND ABUSE OF INDICATOR ORGANISMS

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In the foregoing discussions, several methods for the use of indicator organisms for many taxonomic groups have been presented. The discussions have been perhaps more enlightening than the formal papers. As last speaker I have the advantage of having heard all the previous papers and discussions. From all we have heard, it would appear that several points remain to be discussed.

Throughout the foregoing presentations, the term "biological indicators of pollution" has been widely - albeit loosely - used. Most of the speakers agreed that individual species as indicators of pollution do not exist, yet the entire day was devoted to indicator organisms.

The indicator organism program which we use in Florida is based on organisms which are indicative of the absence of organic pollution rather than on its presence. Such a program has served our needs very well. Separate approaches or classifications are needed in the case of chemical or physical pollutants. These are developed as the need arises.

There appear to be many opinions as to just what an indicator organism program is for. Our indicator organism program in Florida is but a part of the overall stream sanitation program. The amount we can learn from the distributions of the stream inhabitants is great. From some of the questions which have been asked here today, it appears obvious that at least a few people came here seeking a simple indicator organism program which would solve all their problems. It should be obvious that no such classification of organisms exists.

The question naturally arises, then, as to just what is to be expected from an indicator organism program. Such a program may prove an excellent tool, with the limitations of a tool in that it may, when handled properly, do some jobs well while proving useless for others.

Perhaps a review of the use of our program in Florida will clarify the generalities stated above. As has been pointed out in literature (Beck, 1954) the more widely distributed stream organisms have been classified with regard to organic pollution in what, historically at least, would appear to be a backward manner. Instead of indicating pollution, our methods are set up with regard to absence of pollution. The former concept was found to be untenable - as most of the previous speakers have indicated.

In general stream surveying the indicator organism method is used in conjunction with the normal chemical, physical and bacteriological methods.

The biological program is thus supplemental to the more widely established methods. From it we obtain the evidence of yet one more discipline. The fauna of a stream is the result of the combined chemical, biochemical, physical, biotic, climatic and geologic factors of the area in which the stream occurs. Chemical or physical damage to a stream may be a rather ephemeral thing, but biotic evidence of such damage may persist for a significant period. Herein lies one of the main values of an indicator organism program, that of, if I may risk approaching the ridiculous, "faunal memory".

The use of indicator organisms in making a quick check of the condition of a stream is of particular value. In the Biotic Index paper (Beck, 1955: 1196) figure 3 was included to show that the results of surveys made one year apart were quite similar when conditions remained constant. Results since then are most interesting. All data are for station 2 which is located about two miles below the source of waste.

Year	Biotic Index
1953	5
1954	2
1955	16
1956	31

It would appear that station 2 was in poor condition in 1953 and in slightly worse condition in 1954. However, a quick check of the stream in 1955 showed evidence of a significant biotic change indicative of less pollutional effect. A visit to the industrial plant revealed that major changes had been made which had already helped the river. Actual work on the stream which revealed this change took less than an hour. The 1956 result reveals that the stream has completely recovered.

The disappearance of a snail (Goniobasis sp.) was the first evidence we had of a new source of pollution in another river. No single classification of organisms will suffice for all aspects of stream pollution. The pollution of two rivers with wastes high in fluoride ion content necessitated a new classification of indicator organisms. Some species which were quite tolerant of organic pollution proved incapable of surviving even moderate concentrations of the fluoride ion. One of the most fluoride-resistant insects is normally a clean water species.

I believe that it has been stated here that indicator organisms seemed to be indicative of dissolved oxygen concentration. We found it impossible to classify stream invertebrates in Florida with regard to dissolved oxygen content of the water. The presence of a great many springs along such rivers as the Suwannee and the St. Johns greatly alters the oxygen picture without damage to the fauna. As a result we find the more sensitive species of invertebrates tolerating surprisingly low oxygen tensions.

It should be stated that the methods we use in Florida are, we feel, useful. They are subject to revision any time better methods are suggested. They are essentially beginning methods and are being studied, subjected to testing and revised almost constantly.

In conclusion it should be stated that nothing has been written or spoken which has in any way shaken my belief in the usefulness of a properly developed indicator organism program as long as the user realizes that any system, no matter how carefully developed, has its limitations. No such system will ever be a substitute for a knowledge of the behavior of streams and of the inhabitants of streams.

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CURRENT INVESTIGATIONS IN WATER POLLUTION BIOLOGY

Current Water Pollution Investigations and Problems in Wisconsin

by

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The Wisconsin statues define the term "pollution" as the "contamination or rendering unclean or impure the waters of the state, or making the same injurious to public health, harmful for commercial or recreational use, or deleterious to fish, bird, animal, or plant life".

The major problems in stream pollution concern themselves principally with domestic wastes and industrial wastes. At the present time, about 94.8% of the population served by sewers is connected to treatment plants. In 1949, 62 communities still discharged untreated sewage. As of January 1, 1956, this number has been reduced to 29, and of these, 5 have plants under construction. There are a total of 319 treatment plants serving 406 communities.

The 35 pulp and paper making firms in Wisconsin operate 45 paper mills, 16 sulphite pulp mills, 5 kraft pulp mills, and 14 groundwood mills. There are 1,766 dairy plants which include some 1,064 cheese factories. In addition, there are 151 canning plants as well as a full complement of other industries. The total sources of pollution in Wisconsin at the present time, number 1,086.

The history of water pollution control in Wisconsin dates back to about 1925. Prior to 1925, there was a limited amount of pollution control through the application of statutes granting certain powers and duties to the State Board of Health, and conservation statutes prohibiting the discharge of certain specified materials to streams.

In 1925, the legislature appropriated $10,000 from conservation funds for water pollution control. This was expended in cooperation with the State Board of Health in a study of major problems.

The legislature in 1927 created the Committee on Water Pollution, consisting of the State Chief Engineer, a member or representative of the Public Service Commission designated by the Commission, a Conservation Commissioner or an employee designated by the Conservation

Commission, the State Health Officer or a member of the Board of Health, and the State Sanitary Engineer or other engineer appointed by the Board of Health. The State Board of Health was designated as the administration agency of the Committee.

The revision of the 1927 act by the 1949 legislature recognized the need for a full-time water pollution control program and provided for a full-time director and authorized an appropriation which made possible the employment of a full-time staff. The present staff consists of the Director, an industrial wastes engineer, an engineer in charge of coordinating stream surveys, a biologist, a chemist, and four field engineers.

The Committee's basic responsibility is to exercise general supervision over the administration and enforcement of all laws relating to the pollution of the surface waters of the state, and to study and investigate all problems connected with the pollution of surface waters of the state and its control, and to make reports and recommendations thereon. It further has the authority:

> "To conduct scientific experiments, investigations, and research to discover economical and practicable methods for the elimination, disposal, or treatment of industrial wastes to control pollution of surface waters of the state. To this end, the Committee may co-operate with any public or private agency when requested by such an agency in the conduct of such experiments, investigations, and research, and may receive on behalf of the state any moneys which any such agency may contribute as its share of the cost under such cooperative arrangements.
>
> "To supervise chemical treatment of waters for the suppression of algae, aquatic weeds, swimmers' itch, and other nuisance-producing plants and organisms.
>
> "To issue general orders and adopt rules and regulations applicable throughout the state for the installation, use, and operation of practicable and available systems, methods, and means for controlling the pollution of the surface waters of the state through industrial wastes, refuse, and other wastes.
>
> "To issue special orders directing particular owners to secure such operating results toward the control of pollution of the surface waters as the Committee may prescribe within a specified time.
>
> "To make investigations and inspections to insure compliance with any general or special orders, rules and regulations which it may issue.

"To enter into agreements with the responsible authorities of other states subject to approval by the Governor relative to methods, means, and measures to be employed to control pollution of any inter-state streams and other waters, and to carry out such agreements by appropriate special and general orders.

"In addition to all other powers and duties of the Committee on Water Pollution, it shall have the power and it shall be its duty to hold a public hearing relative to alleged water pollution upon the verified complaint of six or more citizens filed with the Committee"

During the course of development, cooperative programs of investigation were established with the Sulphite Pulp Manufacturers' Research League, the Pulp and Paper Advisory Committee on Waste Disposal, the National and Wisconsin Canners' Associations, and recently cooperative biological surveys have been conducted with the Institute of Paper Chemistry.

The Sulphite Pulp Manufacturers' Research League has been continuously engaged in research on waste disposal problems of the industry since its formation in 1939. Spent sulphite liquor is produced when wood is cooked for production of the pulp which is used in the manufacture of paper. For each ton of pulp produced, about one ton of solids is contained in the spent liquor which was formerly discharged directly to the streams. One of the first methods developed for the utilization of waste sulphite liquor was that of aerobic fermentation leading to yeast production. Today there are two full-scale yeast plants, one at Rhinelander, Wisconsin producing 14,000 pounds of yeast per day, and one at Green Bay, Wisconsin designed to produce 28,000 pounds of yeast per day. Other processes upon which much work has been done include evaporation and burning of which there are three installations in Wisconsin, the production of vanillin, the use of waste sulphite liquor in roadbinding of which about 60 million gallons is used per year, soil filtration, and others of less potential.

In 1949 when the water pollution control statutes were revised, it was recognized that some of our streams were still being adversely affected by raw sewage and certain industrial wastes. At that time, the state was divided into work areas comprising the 28 major drainage basins, and a field engineer was assigned to each basin and instructed to conduct surveys to determine the condition of the streams and the sources of pollution discharging to the streams. An industrial waste census survey and the data on stream conditions above and below sources of pollution compiled into a comprehensive report, including biological data taken above and below the various sources, then became available for each basin studied. This report, used as an exhibit at a public hearing, provided information of value to the Committee in deciding on the types of orders to be issued in order to eliminate or reduce pollution. The Committee has over a period of years issued 1,086 orders of which 316 have been fully satisfied as of January 1, 1956.

The role of a biologist in the program follows two principal lines or activity. The Committee is charged with the supervision of all chemicals placed in the public waters of the state for the control of algae, weeds, swimmers' itch, or other nuisance-producing organisms. The administration and supervision of the aquatic nuisance control program thus becomes the responsibility of the biologist. During the summer of 1955, for example, some 37,000 pounds of copper sulphate was used for algae control in 15 state lakes. In addition, on an experimental basis, 60 gallons of Cutrine, 90 gallons of Delrad, and 400 pounds of Phygon were used for algae control. The submergent aquatic vegetation control program entailed the use of some 18,800 gallons of commercial sodium arsenite solution in 34 state lakes. In addition, a small quantity of 2,4-D, Dalapon, and methoxone chlorax was used. The control of snails harboring the organism producing swimmers' itch on some of the bathing areas of the state involved the use of 1740 pounds of copper sulphate, 550 pounds of copper carbonate, and 320 pounds of lime.

The other phase of principal biological activity lies in stream survey work. In the past, the stream study program has consisted of biological samples, principally those of bottom organisms, bracketing the sources of pollution in the 28 drainage basins. From 1949 to the present time, some 37 such surveys have been completed. The entire state has now been covered and the initial phase of the pollution investigation program completed.

The current and future biological program is aimed at more intensive studies in more localized areas on the major waterways of the state. Biologists of the Institute of Paper Chemistry at Appleton, Wisconsin have also initiated a program of intensive biological study on some of the rivers affected by the pulp and paper industry.

A recent cooperative biological study with the Institute of Paper Chemistry has been completed on the lower Fox River in the central portion of the state. The river is 39 miles long, has a fall of 4.25

feet per mile, and contains 14 dams. Effluent from 19 paper making processes enters the river at various points and includes the effluent from 5 de-inked mills, 2 rag pulp mills, 1 kraft mill, 4 sulphite mills, and 2 groundwood mills. In addition, there is the effect from algae as tests have indicated an excess of 130 tons per day (dry weight) of algae flowing into the Fox River from Lake Winnebago.

Another biological study of the more intensive type was recently completed on a 200-mile stretch of the Wisconsin River. This section receives effluent from pulping processes of 5 sulphite mills, 3 kraft mills, 2 semi-chemical pulp mills, 1 rag pulp mill, and 3 groundwood mills. The combined pulp production is some 1480 tons of pulp per day, and the combined daily paper production is some 2300 tons. A follow-up partial survey in the late winter of some of the clean-water, summer-time stations indicated quite drastic changes and severe conditions. It is apparent that nearly as much attention must be given to the study of a stream in winter as well as under summer-time conditions.

The Botany Department of the University of Wisconsin has been continuing its efforts in the field of nutritional requirements of blue-green algae. At the same time, a continuous study on the removal of nutrients from sewage effluent by the use of algae is conducted. The study involves nutrient balances, biological examinations, and methods of algal harvesting. Three ponds with a combined area of one-half acre, and with controlled physical factors (flow, depth, and recirculation), are being used in this investigation.

INVESTIGATIONS AND PROBLEMS IN ONTARIO

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In Canada, relatively few biologists are working exclusively in the field of pollution control and water supply. Consequently, it is necessary that the studies include most phases of pollution biology as is evident from the following discussion of "Investigations and problems in Ontario."

Pollution control in Ontario is generally directed by the Pollution Contr Board, which is composed of members from various interested government departments. The actual investigations and control are the responsibility of the Sanitary Engineering Division of the Department of Health and the Fish and Wildlife Division of the Department of Lands and Forests. The Sanitary Engineering Division maintains a laboratory from which both field work and analytical work is done.

Recently, increased emphasis has been placed on education, in order tha excessive pollution caused by a lack of understanding of the harmful effects of municipal and trade wastes to natural waters would be recognized. Cours are now provided for sewage disposal plant operators and sanitary inspector and included in their course of instruction is a section on the life in streams and the effect of pollution. In addition to these courses, the Pollution Contro Board of Ontario has sponsored an Industrial Wastes Conference for the past two years. Technical papers are presented on waste problems and treatment, and each year one or more papers have been devoted to pollution biology.

Probably the most intensive combined biological and chemical investigation made in Ontario concerned the pollution of the Spanish River. This study was prompted by litigation and an injunction obtained by riparian landowners against a Kraft paper mill. The injunction was dissolved by an act of legislature in 1950 pending the investigation. The study was made over the period of one year, during which time continuous observations were mad on biological conditions and samples were regularly taken for chemical anal sis. A quantitative study was made of the bottom organisms from above the mill to the mouth of the river, a distance of about thirty-two miles. The graphic pattern of numbers and species of bottom dwellers clearly demonstrated the deleterious effect of the mill effluent to the river.

Nets were fished more or less continuously over the year, and observations were made on the species and relative abundance of fishes inhabiting the river. It is interesting to note that while fish could traverse the river until stopped by the mill dam, they did not remain in numbers in the section extending for twenty miles below the mill. During the period of investigation, a spill of rosin acid soaps occurred in the mill and passed to the river. As this "slug" of toxic material flowed downstream, it killed all fish in the river and a considerable number for a distance of two miles into the lake. One beneficial result, however, was the killing of an incredible number of lamprey ammoecetes.

The changing of the bottom environment by the deposition of fibre was found to be of prime importance in the elimination of fish food organisms over an extended section of the river.

An interesting observation from this study was the lack of any chemical evidence of serious pollution apart from the time of toxic concentrations of rosin acid soaps.

While this is the only detailed biological study that has been made of pollution from paper mills, it has provided a valuable basis for future surveys.

Mining is a second major industry from which pollution problems arise. Generally, the mines utilize hard rock ores pulverized in many cases to minus 200 mesh. The settling characteristics of these slimes are poor and the productivity of some northern lakes are seriously affected by an artificial turbidity and an unstable bottom. A unique method of determining the rate of deposition of slimes has been devised by using dust-collector cans similar to those used in air pollution studies. These cans are set on the bottom for a specified period of time, then lifted and the contents filtered, ashed and weighed, thus providing a quantitative estimate of the deposition of inorganic solids.

Recently, the mining of uranium ores has added a new problem. The extraction of uranium oxide is made at a pH below one. The highly acid nature of the waste and the leaching of toxic metals which are not recovered make this an especially potent effluent. The problem is further complicated as the natural waters of the region are slightly acid and poorly buffered. Natural populations of Salmonoid species of fish will necessitate a high degree of treatment. This is an example of a knotty problem that may be solved by the use of bio-assay.

While little use has been made of the practical aspect of bio-assay in pollution control, Dr. F. E. J. Fry and his associates of the Ontario Fisheries Research Laboratories have used it extensively in physiological studies on fishes.

A study is presently being completed of the cause and control of excessive blooms of blue-green algae. The field work has been done on the Kawartha Lakes, a chain of eutrophic lakes in Southern Ontario. The interest in this problem began with the death of cattle and other animals after drinking water containing the algal toxin. Records have been collected of at least thirty-six cattle in this region whose death was due to the toxin from blue-green algae.

The study was divided into three phases:

(1) A study of the biological and chemical conditions in the lake.

(2) A study of control measures.

(3) A study of the toxins produced by the algae.

The following are a few of the observations and findings of this experiment.

The plankton were found to grow in quantity at most times of the year, with a definite preponderance of blue-greens during the summer months. The blue-green counts during the four summers that the experiment was conducted reached peaks between 800,000 and 1,200,000 units per litre. A number of chemical determinations were made, including total and soluble phosphorous, various nitrogen analyses, etc. The high productivity of the lake is connected with the fertility, and phosphorous is believed to be of prime importance.

In considering control, the one important source where excess plant nutrients might be removed was the sewage disposal plant for the town of Lindsay. A study was made of the fertilizing quality of the effluent, and a laboratory investigation was begun on methods of removing phosphorous from sewage. Alum, lime, ferric chloride and other chemicals and combinations were tested. Ultimately a choice of alum and activated silica was made because of the strength and settling characteristics of the floc. In addition, the presence of activated silica appeared to enhance the ability of alum to absorb phosphorous. Continuous treatment was started on a trial basis in 1954. In May of 1955, treatment began and extended until the end of September.

The Lindsay disposal plant provides primary clarification only and has a dry weather flow of 1,200,000 Imperial gallons. While there were no special facilities for building floc other than the turbulence within the flow, a reasona good coagulation was obtained and a total of 81% of the total phosphorous was removed. Soluble phosphates were virtually eliminated, and we are reasonably sure that most of the 19% remaining in the effluent was lost with a portion of the alum floc which did not settle. It was estimated that during the five months' treatment last summer, a total of 8487 pounds of phosphorou as "P", was removed. If this figure is converted to tons of fertilizer using superphosphate as an example, it is equivalent to 54 tons. Other improvemer noted were: a 71% reduction in BOD, a 63% removal of suspended solids and a clear effluent.

The average addition of alum was 94 ppm and silica was 3.4 ppm. The total expenditure for chemicals was $5006.00, a cost of $25.75 per million gallons.

It would appear that with proper facilities for mixing and settling, virtually all the phosphorous could be removed and saving would undoubtedly be made on chemicals. This is believed to be the first use of an alum activated silica treatment and one of the first experiments on continuous phosphorous removal from an operating disposal plant.

While a review of the findings has not yet been completed, a considerable drop was noted in the concentration of phosphorous in the river that receives the effluent. The soluble phosphorous in the lake was very low and remained lower than any of the previous years. The total phosphorous was approximately the same as in previous years and while the blue-green algae built up rapidly under favourable weather conditions that occurred all summer, they levelled off at a peak a little less than would have been expected from previous observations and generally remained at that level for the duration of the season. While it is unlikely that a definite correlation will be shown, it is felt that if treatment was continued on a permanent basis, the level of plant nutrients could be lowered sufficiently to maintain a balanced algal population and a reduction of the incidence of nuisance blooms.

The investigation into the nature of the toxin developed at the time of blooms of blue-green algae has been done by the Defence Chemical Laboratories. While not much information is available yet, toxicity to mice has been demonstrated in unialgal cultures. Previous investigations into the nature of the toxin have shown that it does not belong to any of the known poison groups. It is hoped that the development of pure cultures, which is proceeding at present, will lead to the identification of this toxin.

The work done by the Sanitary Engineering Division is primarily routine analysis and regulatory in nature in the fields of water supply and water pollution. There are, however, projects such as have been previously mentioned that the Division is called upon to study or direct. Undoubtedly, this function will increase in the future as the population and industry grows.

The people of Ontario are justly proud of the abundance of desirable species of fish in their waters and care is being taken to conserve this valuable resource.

REPORT ON POLLUTION STUDIES CONDUCTED IN WESTERN CANADA

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I. Industrial Pollution

Emphasis in pollution studies in British Columbia has been on the industrial development of forest product industries. There are at present eight pulp mills on the B. C. coast, four of which are undergoing expansion. One new pulp mill is being constructed, and at least two new pulp mills are in the embryonic stages of planning. Characteristically large water users, pulp mills can be a major source of water pollution. The research work has been closely coordinated with the supervisory branch of the Department of Fisheries, which receives the requests for approval of plans for waste discharge from industry. The policy that "prevention is more effective than abatement" has been continued, and pollution problems are carefully assessed before they occur. In most instances an effort is made to draw on existing data and knowledge in these studies, inasmuch as it is not desirable to hamper industrial development by delays of long-term research. Where additional survey data or bioassay information are required, these are taken as facilities and manpower permit.

From the point of view of economy and lower pollution, new pulp mills are installing equipment for production of Kraft (sulphate) pulp as opposed to the sulphite process. The Kraft process permits reclamation of the salts in the "black liquor" by evaporation and burning of the organic material. ("Black liquor" is the dark brown fluid which results from the cooking of wood chips in an alkaline solution at about 330°F under pressure in a digestor). Any of the liquor which escapes in the wash from the screens is generally too dilute for economical recovery. It is this wash water which constitutes the major pollution hazard from a Kraft pulp mill. Sulphite digestion is predominantly used in the older pulp mills. An economical recovery process for reclamation of the salts in sulphite waste liquors has not yet been perfected for wide use. In general, effluent from a given tonnage of sulphite pulp produces about 10 times as great a pollution hazard as that from an equal tonnage of Kraft pulp.

Newsprint mills contribute a relatively small pollution. The mechanical grinding of wood in groundwood mills results in a discharge of wastes contaminated only by sap, bark extracts, and fibres from the wood. Most of the pollution stems from the oxygen demand of the organic constituents during decomposition.

The major factors which are responsible for the harmful aspects of pulp mill wastes to fish are:

(1) Biochemical oxygen demand

(2) Direct toxicity

(3) Destruction of food organisms

Analysis of pollution problems with respect to Kraft mill effluent has shown that where dilution is sufficient to combat the B. O. D. (biochemical oxygen demand) of the waste, direct toxicity is only secondary. Bioassay studies conducted at this Station show that concentrations greater than 4.0% of Kraft mill effluent are toxic to young sockeye in sea water for periods greater than one week (Brett and Alderdice, 1954). No research has been pursued on the effect of Kraft mill effluent on fish food organisms. There has been no evidence of a severe reduction in plankton and benthicfauna in regions of Bubol Columbia where pulp mill wastes have been discharged.

There have been three basic types of marine systems on the B. C. coast where pulp mill pollution conditions have had to be studied.

A. Inlet-type - Numerous pulp mills in British Columbia are located at the head of an inlet, often adjacent to an estuary. The choice of such a locality stems from the availability of good forests nearby, ease of transportation of logs from inlet logging regions, fresh water supply, hydroelectric power, and favourable harbour facilities. As a hazard to anadromous fishes these pulp mills require the greatest precaution in effluent disposal. Wastes passing into estuarial waters come directly into the path of migrating salmon. A severe case of pollution might completely wipe out a salmon run.

Evaluation of the capacity of an inlet system to receive wastes according to oxygen supply and dilution has been based on the study of Tully (1949) on Alberni Inlet. Waters from runoff undergo a continuous displacement seaward. The use of the fresh water as a tracer to determine the movement and mixing of effluents has permitted prediction of pollution with varying pulp production. Being of about the same density as fresh water, pulp mill effluent mixes only in the surface brackish layer. Thus the most effective control on the extent of pollution in an inlet-type condition is (1) controlled discharge of fresh water above a certain minimum flow, (2) release of the effluent at the surface and (3) maintenance of the effluent in the jet stream of the surface flow.

The problem of pulp mill effluent discharge in volumes above the safe capacity of natural receiving waters was met in the expansion plans of the Port Alberni pulp mill. Present production of 230 tons of unbleached Kraft pulp, established on the basis of Tully's (1949) work, does not impose an excessive pollution load on the inlet. The effluent can be flushed effectively from the inlet by the natural tidal conditions and Somass River discharge. But the expansion to roughly 500 tons of Kraft pulp and about 500 tons of newsprint per day would impose an oxygen demand on the inlet system in excess of the natural supply. This is particularly true with the reduced river flow during the late summer. A stream which normally fluctuates from about

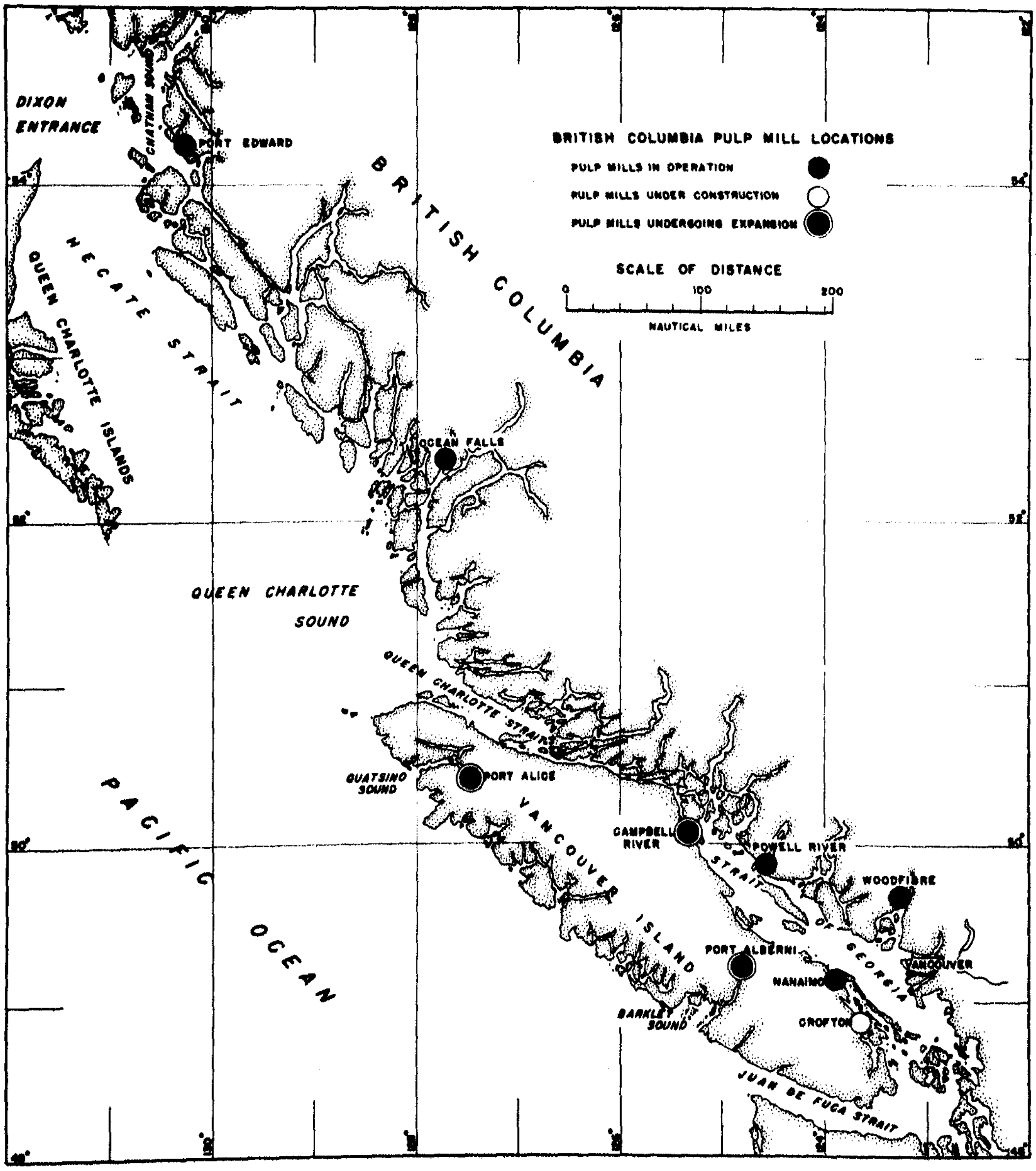

Figure 1. Chart of the British Columbia coast showing existing and proposed pulp mill locations.

300 c.f.s. in September to peaks of 30,000 c.f.s. in November, the Somass River must be maintained at a discharge of 1,000 c.f.s. or greater to overcome any serious pollution from the proposed mill.

B. Coastal Seaway type - In this type of location, effluent is discharged directly into a seaway, use being made of tidal currents and mixing to remove and disperse the wastes rapidly. There is seldom any estuary adjacent to such pulp mill locations so that fisheries of salmon or trout are not likely to be endagered. At the Harmac (Nanaimo) pulp mill, effluent from a daily production of 600 tons of fully bleached Kraft pulp is discharged into Northumberland Channel. Tidal currents here are predominantly southbound (ebb) reaching a knot on certain stages of the tide. The geography is such that little of the effluent reaches the vicinity of Nanaimo Harbour, but is rapidly funnelled into the turbulent waters of Dodds Narrows. With currents commonly over 5 knots strength, pulp mill effluent is completely mixed into the sea water in Dodds Narrows, no traces being detectable below the narrows.

Other examples of this type of effluent discharge can be seen at the Duncan Bay (Campbell River) pulp mill and at Powell River. Duncan Bay, located on Discovery Passage, is relieved of its effluent by the strong tidal currents (up to 7 knots) just south of Seymour Narrows. Powell River discharges effluent into more quiescent waters of Malaspina Strait and Algerine Passage. No evidence of pollution has been reported in either region. Popular sport fishing for salmon is especially renowned at Campbell River.

Any pollution which could arise in these cases in future expansion would probably result from an adverse effect on migrating juvenile salmon. In Discovery Passage large schools of young salmon seek the shelter of the bays and inlets in their journey to the sea. It is hoped that observations can be made on the effect of expansion of the Duncan Bay pulp mill on the young salmon passing through the bay during the summer.

C. Restricted Embayment type - Being intermediate between the inlet-type condition and the coastal seaway, the restricted embayment does permit some flushing by virtue of its openness to an adjoining channel. Generally, however, it possesses a circulation all its own independent of that in adjoining channels. Hence a certain amount of stagnation results.

The pulp mill at Port Edward near Prince Rupert is an example of this type of situation. Shallow, restricted conditions in Wainwright Basin and Porpoise Harbour hinder the flushing of the effluent into Chatham Sound. Large concentrations of mill wastes have been particularly evident during low tides. Mixed reports have been received on the effect of the wastes on the biotic environment. Some observers have reiterated that all marine life in that vicinity is being destroyed. But observations carried out by biologists of the Department of Fisheries (May, 1953) claim that these reports are exaggerated, and that both fish and bottom fauna appear to prevail in normal health. A further investigation of the region is planned in the near future from the Biological Station, Nanaimo.

The proposed pulp mill at Crofton will create another sample of a pulp mill in a restricted embayment. Osborn Bay, into which the effluent would be normally discharged, is relatively sheltered from Stuart Channel; its flushing and circulation are sluggish. The bay is fringed, particularly at the northern end, by oyster-growing leases. Living primarily in the intertidal zone, oysters are vulnerable to any harmful wastes found in the surface waters. In order to prevent the lost of oysters entirely, action would have to be taken to discharge the effluent outside the oyster-growing area or render it innocuous by treatment. The most satisfactory solution to industry economically was the piping of mill wastes into deep water (10 fathoms) in Stuart Channel beyond the Shoal Islands protecting Osborn Bay. Recommendations have been made to this effect and have been met with agreement by both the Provinical Department of Fisheries, Federal Department of Fisheries and Industry.

II. Domestic Pollution

A limited amount of work has been conducted on pollution from domestic wastes in coastal communities of British Columbia.

A. Vancouver Sewage Disposal

Study of the effects of sewage disposal from Vancouver was undertaken as a cooperative effort by the Pacific Oceanographic Group, National Research Council, Institute of Oceanography of the University of British Columbia, Tidal Branch of the Hydrographic Service of Canada, and Air Surveys Branch of the British Columbia Department of Lands and Forests at the request of the Vancouver and Districts Joint Sewerage and Drainage Board. Oceanographic data were collected seasonally in the Fraser River estuary and in Vancouver Harbour over a period from September, 1949 to March, 1951.

Preliminary analyses of these data along with tidal current information and aerial photographic surveys have been used for establishing the circulation in Vancouver Harbour and at the Fraser River estuary. These studies showed how the tides and discharge from the Fraser River combine to produce a characteristic circulation in Burrard Inlet.

Off Point Grey, there is a predominent northward set of the current into English Bay on all stages of the tide. Off Point Atkinson, at the northern shore of the entrance to Burrard Inlet, there is a prevailing westward current out of English Bay. A counterclockwise main eddy system with numerous smaller eddies and stagnation points superimposed on the principal circulation were noted to exist in English Bay.

The results of the oceanographic studies have permitted the evaluation of different outfall points for their suitability in sewage disposal. Final choice of discharge locations was based largely on the effectiveness with which the sewage would be dispersed and removed by the currents.

B. Nanaimo Harbour Sewage Disposal

An oceanographic study of currents in Departure Bay and Nanaimo Harbour was conducted as a cooperative venture between the Pacific Oceanographic Group and community groups of the city of Nanaimo. A large measure of assistance was given by the Nanaimo Yacht Club with their boats, and much volunteer help came from the Nanaimo city employees. Close cooperation was maintained with the local branch (Central Vancouver Island Health Unit) of the Provincial Department of Health and Welfare. The prime concern in the survey was the determination of existing pollution from a health standpoint and further contamination of beaches to be expected from additional sewage discharge.

Results of the survey indicated that Nanaimo Harbour is already polluted by existing sewer outfalls and that beaches within the harbour are not fit to be used for bathing. Departure Bay beach is still free from contamination, but is threatened by any sewage discharged at Brechin Point (located at the northern end of Newcastle Island Channel between Nanaimo Harbour and Departure Bay). Surface currents in both bays are largely wind-driven. The prevailing southeasterly winds would drive any increased sewage output into Departure Bay and gradually wipe out the remaining beach recreational area in the district.

Recommendations were submitted to the City Council of Nanaimo that either the sewage be totally treated before discharge, or it should be piped beyond the barrier islands (Newcastle and Protection Islands). These recommendations are being acted on at present and further survey work is awaited to determine the most appropriate discharge points outside the islands.

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The Relationship of the Polychaetous Annelid Capitella capitata (Fabricius) to Waste Discharges of Biological Origin

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INTRODUCTION

Biologists have long recognized the shortcomings of chemical and physical measurements of water quality, and thus have searched for organisn which could serve as indicators of different degrees of pollution. Organisms particularly those that are attached or bottom inhabitants, are favored by many since they reflect the water conditions not only at the time of sampling but for some time previously.

The use of organisms as indicators of pollution in marine waters has lagged considerably behind that of fresh water studies. Wilhelmi (1916) mentioned that the polychaete Capitella capitata played a similar role in marine waters as Tubifex does in the fresh waters of Germany. Blegvad (1932) studied the bottom fauna in the vicinity of domestic outfall sewers in Copenhagen Harbor, Denmark. He was able to divide the region surrounding one outfall into three zones; an inner region lacking animals and with the substrate characterized by a sulfide odor, an intermediate zone containing a few animal species and with the substrate possessing a sulfide odor, and a third zone showing no measureable effects of the discharge. A second domestic outfall lacked the intermediate or marginal zone. Filice (1954), working in the Castro Creek area of San Francisco Bay, also separated the bottom fauna into three zones: (1) a healthy zone unaffected by waste discharges, (2) a marginal zone characterized by a few tolerant species, notably the polychaetes Capitella capitata, Neanthes succinea (Frey and Leuckart), and Streblospio benedicti Webster, and the molluscans Mya arenaria Linnaeus and Macoma inconspicua (Broderip and Sowerby), and (3) a zone essentially lacking in animals. Recently the author (1955) published the general results of three quantitative bottom surveys conducted in the Los Angeles -- Long Beach Harbors. This area, which receives waste discharges of domestic and industrial origin (Anon, 1952), was divided into five zones on the basis of bottom conditions. Capitella capitata was found to be particularly abundant in regions receiving effluents of biological origin. This organism was the characteristic animal in what was termed the polluted bottom zone. The present report discusses some of the results of bottom surveys made in the Los Angeles-Long Beach Harbor and in some other marine waters of southern California. Emphasis has been placed upon the occurrence of C. capitata and its possible role as an indicator of pollution of biological origin. Biological wastes are herein defined as discharges from fish canneries, domestic sewage, and garbage.

OBSERVATIONS

Los Angeles -- Long Beach Harbors (Figure 1). The bottom of the Los Angeles -- Long Beach Harbor has been sampled five times during the past five years. Three of the samplings were made in 1954. The distribution of C. capitata for the June 1954 Survey is shown in Figure 1. Slip 5 of Los Angeles Inner Harbor receives waste discharges of domestic and fishery origin. The bottom of the inner portion of the slip was covered with fish scales. Capitella capitata was found in the area in large numbers along with a few other species of invertebrates. Domestic, fishery, and non-biological industrial wastes are emptied into Slip 2 of Long Beach Harbor. Here this worm was found with one other species of polychaete. The region of fish harbor in the Los Angeles Harbor receives some of the effluents from the fish canneries and the bottom was covered with fish scales. Capitella capitata was collected either alone or with one or two other species of polychaetes.

Following primary treatment the effluent of the terminal Island sewage treatment plant is discharged into the Los Angeles Outer Harbor. Three outfalls from the fish harbor canneries are located nearby (Figure 2). The samples taken in 1954 at the sewage outfall either contained C. capitata or lacked animals. Additional stations were sampled in December 1955 (Figure 2) to determine whether or not an intermediate assemblage of animals existed between the polluted zone possessing C. capitata and the healthy zones typical of the outer harbors (Reish, 1955; see also Hartman, 1955, Stations 29 and 44b). No animals were taken from the samples near the sewage or fish cannery outfalls. The substrate possessed a strong odor of domestic sewage. Capitella capitata was found in the stations a short distance from these outfalls. The farther the stations were located from the outfall the more varied the fauna became. There was no indication of an intermediate zone between the polluted bottom containing C. capitata and the healthy area.

Alamitos Bay. This bay, which is located at the southern boundary of Los Angeles County, is essentially a clean body of water used primarily for recreation (Reish and Winter, 1954). Capitella capitata was found at three stations in the vicinity of a public dump where garbage was pushed into the bay. This species was present at two other widely separated stations where there was no known pollution. There two stations were the only ones sampled where C. capitata had been collected where there was no pollution of biological origin discharged nearby.

Lower San Gabriel River. The tidal portion of this river is separated from Alamitos Bay by a jetty. This region was sampled in 1952 and again in 1954 (Reish, 1956). No animals were found in the bottom materials in 1952, but animals were encountered at some of the stations in 1954. A portion of the river was diked and dredged about four to five feet deeper in 1952 after the survey of that year. This dredging activity removed the accumulated sludge from the river bottom. A total of 12 different species was collected in 1954, of which C. capitata was the most common. This

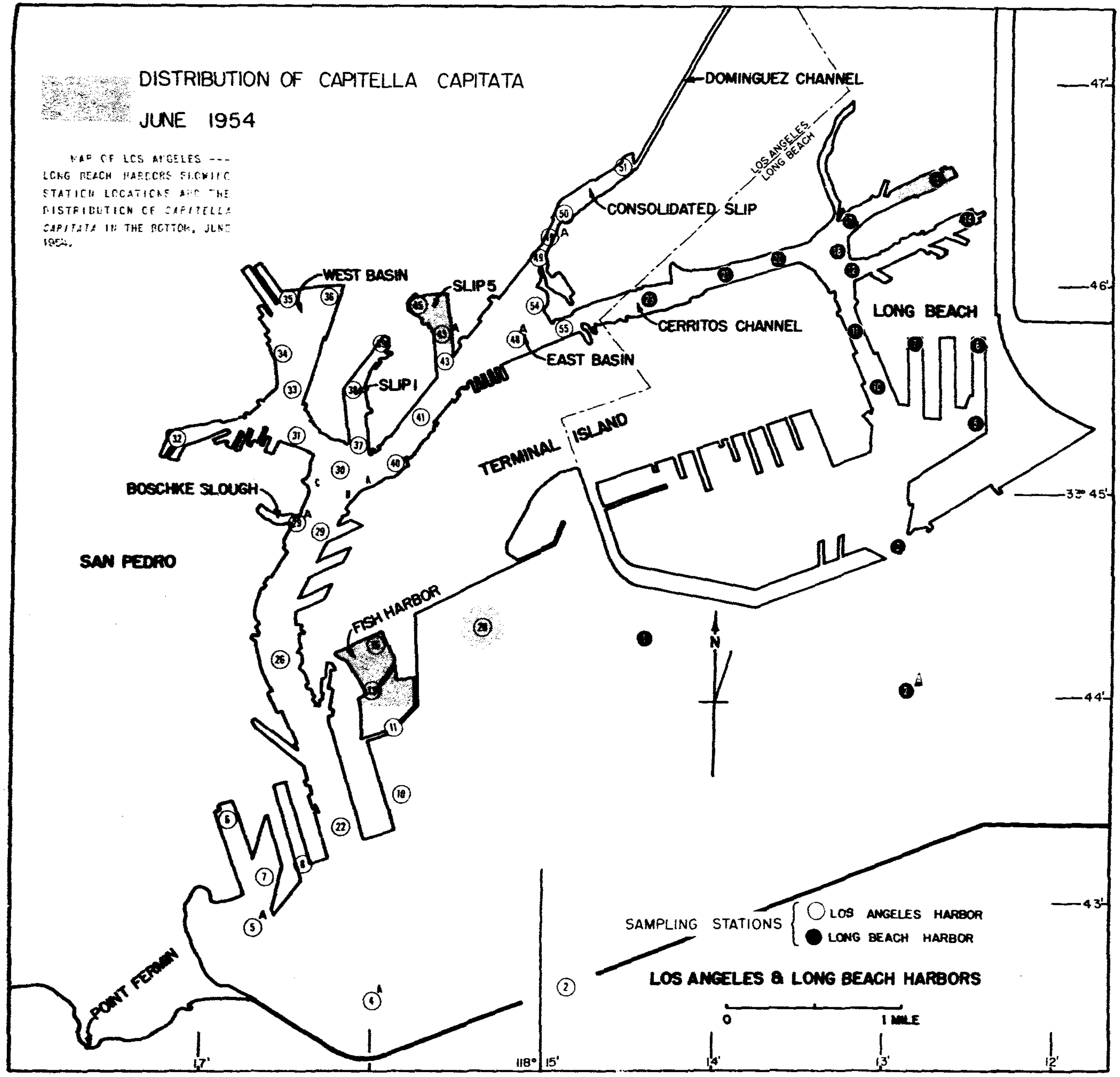
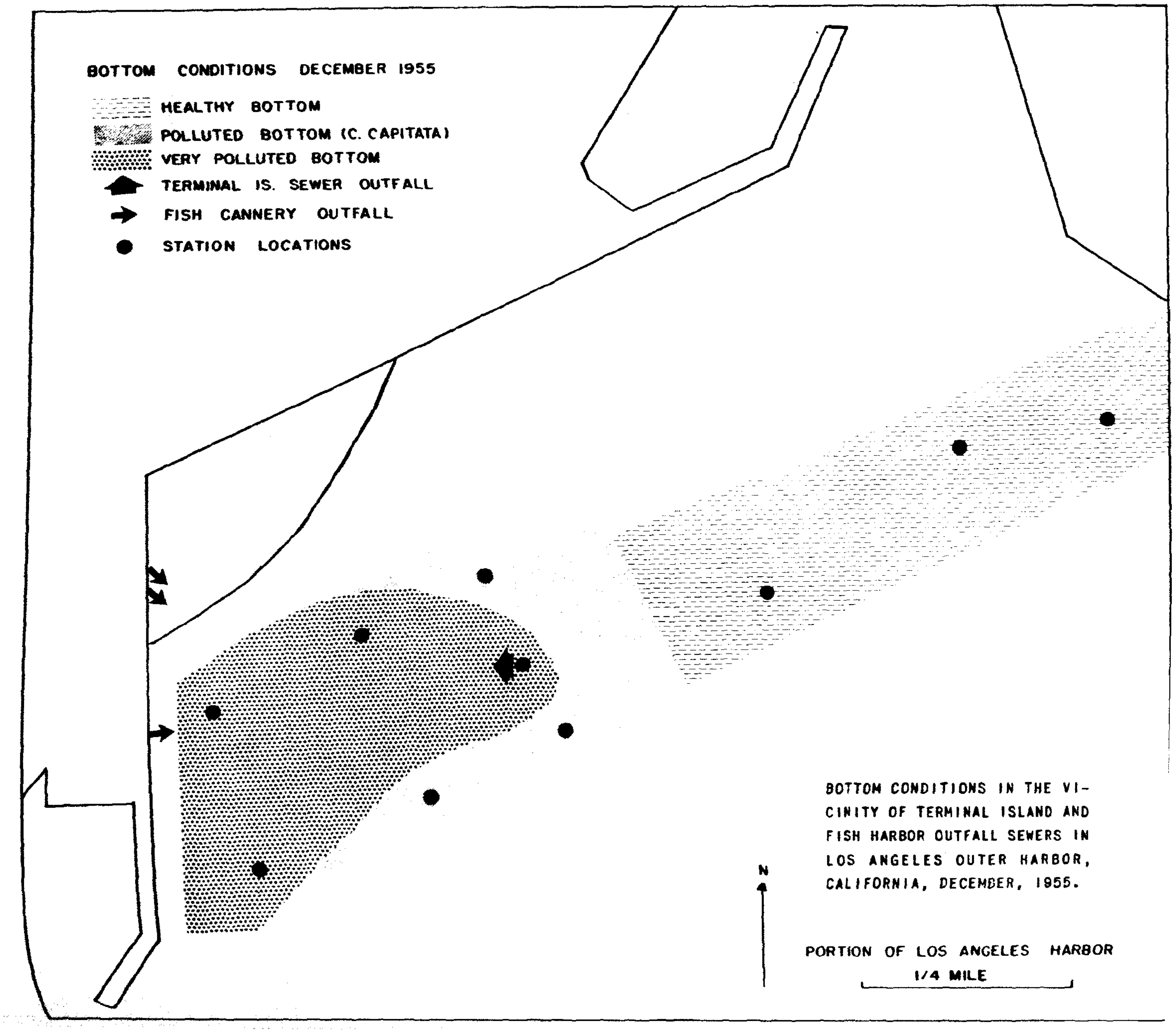

DISTRIBUTION OF CAPITELLA CAPITATA
JUNE 1954
MAP OF LOS ANGELES --- LONG BEACH HARBORS SHOWING STATION LOCATIONS AND THE DISTRIBUTION OF CAPITELLA CAPITATA IN THE BOTTOM, JUNE 1954.
DOMINGUEZ CHANNEL
LOS ANGELES
LONG BEACH
CONSOLIDATED SLIP
WEST BASIN
SLIP 5
SLIP I
EAST BASIN
CERRITOS CHANNEL
LONG BEACH
TERMINAL ISLAND
BOSCHKE SLOUGH
SAN PEDRO
FISH HARBOR
POINT FERMIN
N
47'
46'
33° 45'
44'
43'
17'
118° 15'
14'
13'
12'
SAMPLING STATIONS
LOS ANGELES HARBOR
LONG BEACH HARBOR
LOS ANGELES & LONG BEACH HARBORS
0
1 MILE

FIGURE 1


BOTTOM CONDITIONS DECEMBER 1955
HEALTHY BOTTOM
POLLUTED BOTTOM (C. CAPITATA)
VERY POLLUTED BOTTOM
TERMINAL IS. SEWER OUTFALL
FISH CANNERY OUTFALL
STATION LOCATIONS
BOTTOM CONDITIONS IN THE VICINITY OF TERMINAL ISLAND AND FISH HARBOR OUTFALL SEWERS IN LOS ANGELES OUTER HARBOR, CALIFORNIA, DECEMBER, 1955.
N
PORTION OF LOS ANGELES HARBOR
1/4 MILE

polychaete was found near the outfall sewers of the City of Seal Beach and downstream from the Los Alamitos Naval Air Station.

Newport Bay. This body of water is also essentially clean and used primarily for recreation. However, C. capitata was taken along with fish scales at one station in 1951 and again in 1954. This station was situated at the end of a small arm of the bay in the proximity of fish canneries.

DISCUSSION

Gaufin and Tarzwell (1952), while concerned with fresh water pollution, included four points that should be considered when testing possible indicator of pollution. These criteria are: (1) large number of individuals, (2) few species in the fauna, (3) principally scavenger feeding habits, and (4) either a toleration for low dissolved oxygen or possess some adaptation to a low dissolved oxygen environment. At least some of these qualifications are fulfilled by C. capitata. It has been encountered in large numbers, as many as 400 having been taken in a sample covering a surface area of 100 square inches. Sometimes it was the only species taken. More frequently there were a few additional forms present. In those samples containing organisms only three or four species other than C. capitata were observed at the stations nearest the Terminal Island sewage treatment plant and the fish cannery outfalls. In contrast, there were 15 or more species present at each station throughout much of Los Angeles -- Long Beach Outer Harbors. Capitella capitata burrows into the substrate and engulfs substrate in much the same manner as an earthworm. The oxygen requirements of this worm have not been studied but it has been encountered in bottoms in Los Angeles Inner Harbor where the overlying water lacked oxygen at the time of sampling. However, this was exceptional. A more typical situation was 3.5 ppm oxygen at the stations where C. capitata was taken.

It is not known whether or not a relationship exists between the number of C. capitata present in a sample and the degree of the biological pollution. Field data and laboratory observations indicate that C. capitata has a short life history as it reaches sexual maturity in about a month. It is capable of reproducing throughout the year in southern Californian waters. It is not known whether or not any reproductive peaks occur during the year. This speices is cosmopolitan in its distribution, but, with the exception of the statement by Wilhelmi (1916), the ecology of C. capitata has not been studied in the other geographical areas.

SUMMARY

1. The use of the polychaete Capitella capitata has been discussed as a possible indicator of pollution of biological origin in marine waters.

2. This species has been encountered near outfalls discharging biological wastes in Los Angeles -- Long Beach Harbors, Alamitos Bay, Lower San Gabriel River, and Newport Bay.

3. A special study made in the vicinity of sewage and fish cannery outfalls in Los Angeles Outer Harbor showed no intermediate assemblage of animals between the polluted C. capitata zone and the healthy zone characteristic of much of the outer harbor.

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COOPERATIVE RESEARCH AT OREGON STATE COLLEGE IN THE BIOLOGICAL ASPECTS OF WATER POLLUTION [1]

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Water pollution problems are essentially limnological or oceanographic field problems. Yet, the variability and complex interactions characteristic of real situations make the understanding of that which is observed in the field so difficult that a considerable part of water pollution investigation has had to be carried on in the laboratory. Idealization of the laboratory experiment greatly facilitates the analysis of results, but the idealized experiment rarely approximates reality. Field problems being often too complex for thorough analysis, and laboratory models being usually too simple to be truly representative of natural situations, neither field investigation nor experimentation in the laboratory alone is sufficient for the solution of many water pollution problems. The best information that can be obtained by each approach is needed, and it is highly desirable to bridge the wide gap between ordinary field observations and pertinent idealized experiments. The more nearly laboratory experiments can be designed to model actual situations under study and still retain the advantageous feature of comparative simplicity, and the more nearly a field study can be made to resemble an idealized experiment through the control of variables, the more efficient will be the investigation and the more reliable the interpretation of the findings. Many current problems having to do with the biological aspects of water pollution can be solved only by utilizing these different approaches in an entirely complementary manner.

The cooperative program of investigation in water pollution biology being conducted at Oregon State College by the Department of Fish and Game Management and the R. A. Taft Sanitary Engineering Center of the U. S. Public Health Service includes such complementary field and laboratory research. Personnel of these two organizations, with the aid of a number of graduate students, are participating in a research program designed to provide fundamental information pertaining to present and future biological problems of fresh-water and marine pollution. Close cooperation with various state regulatory agencies and with industry is helpful in keeping this research pertinent to present-day problems and also facilitates anticipation of future problems.

Current field studies and those soon to be initiated at Oregon State College fall into two general categories. The first of these categories includes broad studies of the physics, chemistry and biology of rivers and streams variously affected by pollution. Through these studies it is hoped to increase available information on the influence of domestic and industrial wastes on fresh waters,

1/ Miscellaneous Paper No. 29, Oregon Agricultural Experiment Station

to shed more light on the value of biological indicators, and to furnish ideas and direction for the over-all research program. The second category includes those studies which are more nearly field experiments than they are field surveys. Small streams which can be purposely subjected to controlled experimental pollution and variously modified as necessary, and rivers where appropriate experimental designs of a statistical nature can furnish a mathematical control are to be utilized for these studies.

Plans have been made to undertake research on a small stream in which the desired dilutions of added chemicals or wastes can be maintained. This field study will aid in extending information resulting from laboratory studies in which a system of artificial streams discussed below is employed. Field observations and experiments under the somewhat controlled conditions attainable in a small stream will make possible detailed investigation into the influences of wastes on the productivity of such streams at the plant, herbivore, and carnivore levels of production, the last level including game fish.

The results of short-term laboratory experiments designed to determine what conditions are rapidly lethal for aquatic organisms held in glass vessels, though informative, are of limited practical value. In order to infer from such ordinary bioassay results what changes of water quality can be tolerated for long periods by an organism in its native environment, it must be assumed that the manner of action of the lethal agent under consideration is the same at rapidly fatal and at slowly fatal concentration or intensity levels, and the same in the natural environment as in the aquarium. This assumption is by no means always a valid one. Furthermore, though environmental conditions may not be so adverse as to be demonstrably fatal to aquatic organisms, their effect on a population of these organisms may still be thoroughly destructive if they interfere with the reproduction, development, feeding, growth, normal activity, or migratory movements of individuals of that population. Chronic injury to fish populations, due to pollution, may well be much more common and important than spectacular mortialities of fishes caused by acutely harmful pollutional conditions of relatively brief duration, which may or may not have a serious and lasting adverse effect on fisheries. Therefore, some laboratory experiments should be designed so that their conditions approximate selected features of the natural environment. Some of these experiments should be of prolonged duration and should measure the effects of the tested conditions on some of the essential life processes and over-all well-being of organisms, attention being given to the most susceptible stages of the life-history of the organisms. Knowledge of the concentration of a toxic waste, or of dissolved oxygen, which can be barely tolerated for a short period of time, or when the organisms may be relatively resistant to adverse conditions, may be necessary, but is not sufficient.

Encouraging results have been obtained at Oregon State College by using wooden troughs with various current and bottom conditions as artificial streams for the purpose of evaluating the effects of exposure to relatively low waste concentrations for periods as long as a month on different aquatic organisms. In this way, it has been possible not only to determine long-term lethal concentrations of waste, but also to note the effects of the waste on the feeding and growth of fish, on the development, habits, and emergence of insects and on other life processes of aquatic organisms, such as ecdysis in young crayfish. Changing bottom conditions such as the excessive production of periphyton can be closely observed, as can be the influence of the periphyton on bottom-dwelling forms. These experiments not only make possible a determination of the concentrations of a waste having marked effects on the organisms under artificial stream conditions, but may suggest some of the reasons for these effects.

Thus, when stonefly and caddisfly larvae were held in troughs receiving pulp-mill wastes in various concentrations, it was possible to observe closely the environmental conditions under which mortality occurred and the condition and behavior of the animals before death. The abundant growth of periphyton over the rocks placed on the bottom, and also on some of the experimental animals, the upward movement of the insects from the undersides of rocks where they are usually found (which, in nature, could make them more subject to predation), and the changes of dissolved oxygen concentration among the rocks and beneath the blanket of periphyton all could be readily noted or measured Furthermore, in seeking to determine the causes of distress and mortality of the insects (which may be referable to toxicity of the wastes, to oxygen deficiency, to some mechanical effect of the periphyton, or to their combined influence) it has been possible to evaluate the role of a single environmental factor by modifying the artificial environment with respect to that factor. For example, by introducing oxygen into the water before it is mixed with waste and enters the troughs, relatively high dissolved oxygen concentrations have been maintained, compensating for the oxygen demand of the waste. In this way, and through additional experiments on the influence of dissolved oxygen concentration and current velocity on the survival of insects in cages inserted in glass tubes, which also have been undertaken, the part played by dissolved oxygen reduction in causing the observed pollutional damage to these aquatic animals can be determined.

Long-term experiments with complex industrial wastes inevitably present many problems. One of these is the variability among the several waste samples or batches necessary for completing a single experiment, large amounts of waste being required. The same volumetric dilutions of different waste samples from the same source often differ greatly in toxicity, so that the analysis of test results obtained without standardization of waste toxicity would be difficult, if not impossible. Frequently, the toxic components of complex wastes are unknown, or there are no chemical or physical means for measuring and standardizing the lethal factors. Biological standardization of waste samples has been accomplished with apparently good success by determining for each sample the 24-hour median tolerance limit of one of the test animals (a fish) being used in the long-term experiment. Some constant percentage of this median tolerance limit is then the strength of diluted waste maintained in each experimental trough during the entire course of the experiment, the dilution used thus being adjusted to the relative acute toxicity of the individual samples. This procedure has two distinct advantages. First, the toxicity of the trough dilution, at least for the control species, should remain constant from sample to sample if the short-term and long-term effects of the waste do not vary independently. Secondly, acute lethality data are provided for later comparison with the results of long-term tests of lower concentrations.

Once the relationships between concentrations lethal to a representative fish in a short period of time (median tolerance limits) and concentrations harmful to a variety of stream organisms over a long period are known, it may be possible to predict the long-term harmful concentrations on the basis of short-term test results. Sufficient investigation of the toxicity of a particular industrial waste usually should make possible the determination of dilution factors which, when applied to the short-term median tolerance limit, would yield a reliable estimate of the maximum safe concentration of that waste in the environment of fish and other organisms of importance as fish food. An industry, when supplied with these bioassay application factors, could control waste discharges through routine bioassays of the effluent. Such bioassays would be no more difficult than many chemical and physical determinations now routinely used in the control of industrial effluents. They would, however, provide much greater assurance that the aquatic resources supposedly protected by the waste-control measures are in fact being protected. Only too frequently, ineffective, though complex, chemical tests are being used to evaluate the potential toxicity of industrial wastes to aquatic organisms.

Some of the species which have been used in these experiments for standardization of wastes are believed to vary in their tolerance to certain adverse conditions with such variables as size, age, season, source, time in captivity, and diet. Consequently, the standardization procedure may result sometimes in adjustments to variation in the standard animal rather than adjustments to variability among the waste samples. Needless to say, variations in the tolerance of the fish, as well as variations in the toxicity of the waste, are of considerable interest in connection with practical application of the results In order to make possible their separation and study, as well as to provide a dependable standardization procedure, guppies are now being raised under very constant conditions to furnish an animal more standard than the wild fish. Genetic strain, age and state of sexual development of the guppies at the time of use, as well as the conditions under which they are reared, such as light, temperature, and diet, can be kept fairly constant; and this will result, it is believed, in sufficient uniformity of the test animals. Standardization and control experiments with the guppy will not replace experiments with species of economic importance (e.g., juvenile salmon) but will supplement these experiments. Only in this way can variability in the valuable native fish species be distinguished from variability in the waste.

A rather broad study entitled "The Influence of Dissolved Oxygen upon the Survival, Development, Growth, Activity, and Movements of Fresh-Water Fishes" is now being carried on at Oregon State College. The survival of fish at low concentrations of dissolved oxygen in different waters has been studied intensively, while the temperature, carbon dioxide content, alkalinity, pH and other properties of the water have been varied. In most of these experiments the test water has been renewed continually. The dissolved oxygen content of the flowing water is reduced to the desired level by the controlled bubbling of nitrogen through it while it flows continuously downward through a glass column. Although the duration of most of these experiments has been one to five days, some have been continued for as long as thirty days.

The results of long-term experiments still in progress indicate that the food consumption and growth rate of salmonid fishes can be influenced by reduced dissolved oxygen concentrations which are well above the lethal levels. In these experiments an effort is being made to supply the fish with a diet approximating a natural diet. The rate of food consumption and the relative efficiency of its utilization at each of several different dissolved oxygen levels which are above the lethal level are being determined. It is planned eventually to investigate also the influence of fluctuating dissolved oxygen concentrations upon feeding and growth.

Studies of the influence of dissolved oxygen concentration on the rate of development of salmonid eggs, the percentage of successful hatching, and the survival of hatched larvae have yielded results of considerable interest. It appears that, in almost still water at least, the oxygen concentrations required for successful development and hatching may be quite high in relation to the minimum levels tolerated by fully developed juvenile fish. Inasmuch as current must have an important influence upon the minimum dissolved oxygen requirements of developing eggs, its role needs thorough investigation in connection with further studies of the oxygen requirements of the eggs. Studies in the field may be necessary for determining the range of natural conditions in salmonid redds, so that experimental conditions can be selected accordingly and the results related to circumstances found in nature.

It is strikingly apparent that fish which survive in bottles at barely tolerable dissolved oxygen concentrations are so sluggish or inactive that they cound not maintain themselves and survive indefinitely in their natural environment, where they must actively resist currents, feed, and escape their enemies. With newly developed apparatus, it is possible to study the ability of fish to resist currents of moderate velocity when they are in water with any desired dissolved oxygen concentration. The activity potential of the fish thus can be related to the oxygen concentration. Preliminary results indicate surprising ability of some fish to resist moderate currents for long periods at dissolved oxygen concentrations not very far above the minimum levels tolerated by resting fish. Field studies are needed, in connection with these laboratory studies, for determining the velocity of currents that these fish must normally resist for long and short periods of time in their natural habitats.

The movements of fish, as influenced by variations of the quality of water encountered, often may determine whether or not the fish will be exposed to avoidable injurious environmental conditions, and whether or not they will occur in certain environments where water-quality conditions are tolerable. It is known that not all harmful conditions are readily avoided by fish, and tolerable conditions apparently can be repellent. Avoidance reactions of fishes to reduced dissolved oxygen concentrations, as well as to varying dilutions of industrial wastes, are being investigated at Oregon State College, chiefly through laboratory studies. The application of the results of the laboratory tests to problems encountered in the field presents serious difficulties, the circumstances within the confines of even a large laboratory apparatus being a very poor model of conditions in stream environments of much greater area, but the tests can nevertheless be instructive.

In the Pacific Northwest, as in other parts of the country, suitable industrial sites with adequate process and waste-disposal waters are becoming increasingly scarce. Many industries are now selecting locations adjacent to marine or estuarine waters. The aquatic resources of many of these areas are of tremendous commercial and recreational value. Yet, we now know even less about the basic water-quality requirements of marine organisms and their relative resistance to harmful pollutants than we do about the requirements of fresh-water forms. At the marine laboratory of the Department of Fish and Game Management, studies of the water-quality requirements of a number of marine forms have been initiated and are to be greatly expanded in an effort to fill to some extent this serious gap in our knowledge. These studies are of both short and long duration and include an attempt to reproduce marine environments in the laboratory. Sufficient field work will be carried on to assure pertinence to present and future practical problems.

A research program such as that considered above requires certain facilities and a location where different species are readily available and where the desired field studies are possible. The Department of Fish and Game Management has a fisheries research laboratory on Mary's River at Corvallis, and a marine research laboratory near Newport on Yaquina Bay These laboratories are equipped and operated jointly with the U. S. Public Health Service. The Corvallis laboratory is housed in five buildings, has a supply of river water, and six 250-gallon tanks provided with running water for holding stocks of fish for experimental purposes. Two constant-temperature rooms (one very large and one small) are available at the Corvallis laboratory for standing-water experiments. These have both heating and cooling units. The Yaquina Bay laboratory has both fresh-water and salt-water systems, a spring furnishing a good flow of fresh water for experiments requiring water of considerable purity. Dock and live-box facilities are adequate. There is one large constant-temperature room available at this laboratory. Yaquina Bay probably has a greater variety of commercial and non-commercial fish and shellfish and other marine invertebrates than any other Oregon bay. Corvallis is situated on the Willamette River. This river system, with its varied stream environments, its wide representation of the cold-water and warm-water fish species of North America, and its examples of certain of the effects of domestic and industrial waste disposal, offers excellent opportunities for field study and is a good source of experimental material.

Specialized apparatus has been requisite to much of the work outlined above. Preliminary experiments with artificial streams as a means of studying the influences of wastes on stream ecology have proved very encouraging. The most recently installed apparatus provides a system of six artificial streams. Each stream consists of two 10-foot troughs, one having the water recirculated by a $\frac{1}{2}$-horsepower pump so as to provide riffle-like currents, the other representing a pool-like environment. The water in the troughs is continually renewed, flowing river water. Appropriate bottom materials and lighting result in what is believed to be a rather good model of a stream environment. After the desired plant and animal communities have been established, the wastes to be studied can be introduced by means of chemical pumps in different amounts into the six streams, so as to determine effects of different waste concentrations.

Another apparatus now in use in the laboratory was devised to make possible the study of the effects of low concentrations of dissolved oxygen and other water quality conditions on fish swimming against currents with velocities up to 1 foot per second. This apparatus consists of a glass pipe of 4-inch diameter through which the water is recirculated by means of a centrifugal pump. Water quality in the glass pipe is controlled by exchange at a rate of about 1 liter per minute, the exchange water having its characteristics such as temperature and dissolved gas content adjusted by other appropriate components of the apparatus. An apparatus of this kind now being constructed should make possible the study of the influence of critical water conditions on fish resisting currents of relatively high velocities.

The avoidance reactions of fish to water having various characteristics have been studied in a 2 by 9 foot tank with one-third of its length subdivided by partitions into four channels. Each channel is equipped with an adjustable water input and an adjustable drain which, with proper balancing of flows, result in quite sharp boundaries between waters of different quality at the channel openings. Other apparatus has made possible the study over short or long periods of time of the lethal and other effects of water having temperature, oxygen concentrations, carbon dioxide concentrations, total alkalinity and waste concentration controlled. A number of such constant-flow experimental units, each consisting of about five test vessels (usually of 12-gallon capacity) and such other components as are necessary for adjusting the dissolved gas content or other properties of the water in each container independently, are available at the Corvallis and Yaquina Bay laboratories for studies of the influence of water quality variations upon the survival and growth of fish and the development of their eggs. A laboratory shop and the cooperation of research apparatus specialists have greatly facilitated the development of these and other pieces of equipment necessary for pursuing the problems under investigation.

The provision of these facilities has been possible only through the pooling of resources by the Department of Fish and Game Management and the U. S. Public Health Service in the joint undertaking of all of these investigations. Other departments of Oregon State College and industrial representatives participate in many of these investigations. Considerable support is given these research projects through grants by Federal agencies and by industry, notably The National Council for Stream Improvement.

Much of this research is accomplished with the aid of graduate research assistants and fellows who are employed to work on problems suitable for graduate theses. Master of Science and Doctor of Philosophy degrees may be pursued with majors in fisheries and minors in desired fields. Several of these assistantships and fellowships are available each year. The researc program is complemented by an appropriate instructional program designed to prepare specialists for employment in the water pollution field. College and Public Health Service personnel cooperate in the instructional program.

SOME ASPECTS OF WATER POLLUTION IN THE MISSOURI BASIN

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DESCRIPTION

The Missouri Basin (Figure 1) comprises the larger part of arid and semi-arid regions contributory to the Mississippi Drainage System. Precipitation declines from about 40 inches per year in the eastern part of the basin in Missouri to 10 to 15 inches per year along its western boundary in Montana, Wyoming, and Colorado. The middle basin (the Dakotas, Nebraska, and Kansas) has annual precipitation ranging from 15 to 30 inches, Long dry seasons are prevalent over most of the basin; and the river normally exhibits only two high stages each year-- one coming from melting snow on the praires in March and April, and the other arising from prairie rains and snow melt in mountainous headwater areas in June.

The main stem and many tributaries are undergoing development for irrigation, power production, water supply, navigation, flood control, and waterfowl refuges. The main stem reservoir system, which will consist of three huge one large, and two small impoundments, now lacks only Oahe and Big Bend Reservoirs. Construction of Oahe is in progress. Total storage capacity for this reservoir system exceeds 70,000,000 acre feet. Reservoirs now in operation (Ft. Peck, Garrison, Ft. Randall, and Gavins Point) have permitted significant flow regulation and are retaining a large amount of the suspended sediment carried in the traditionally muddy river.

Tributary developments are, at present, more concerned with irrigation than the main stem impoundments; although the latter will figure in development of new irrigation areas. Irrigation water use may involve natural flow diversion as now practiced in the Yellowstone Valley, reservoir storage as exists on the North Platte River, or ground water pumping from river valleys as is customary along the lower Platte River. Some irrigation reservoirs also furnish hydro-electric power and municipal water supplies. Impoundments used solely as waterfowl refuges compose a very small percentage of impounded waters in the basin. Fish and game interests, however, receive due consideration in the operation of larger multiple purpose reservoirs, some of which also serve as water fowl refuges.

SOURCES OF POLLUTION

The Missouri Basin in primarily an agricultural region, and major industries are concerned with processing agricultural products. Meat packing houses are scattered over the basin but their greatest concentration, and greatest waste contribution is along the Missouri River from Sioux City, Iowa, to Kansas City, Missouri. Wastes from these establishments reach the river in a raw state.

The beet sugar industry, whose refineries or factories discharge a large seasonal waste load, is largely confined to irrigated areas along tributary streams, e. g., the Platte, Big Horn, Belle Fourche, and Yellowstone Rivers. Oil fields and petroleum refineries are located in several areas in the Missouri Basin. The greatest concentration of refineries is in the Kansas City area, but significant contributions of these wastes affect various streams, the North Platte, Yellowstone, and others. Other industrial wastes--steel mill, distillery, and chemical processing discharges--enter the river at the larger main stem municipalities. Salt brines, originating in natural deposits and supplemented by oil well waste flows, are a problem in the Smoky Hill, Solomon, and Saline Rivers in the Kansas Basin.

Missouri Basin streams receive considerable quantities of domestic and municipal wastes, largest concentrations being poured into lower reaches of the main stem. Most municipalities situated on the Missouri lack any form of sewage treatment and raw domestic wastes considerably augment those from industries at the larger cities. Some tributaries are superior to the main stem in this regard. Major municipalities on the James River, for example, all provide wastes reduction equivalent to conventional secondary sewage treatment. Some towns on other tributaries provide treatment for domestic wastes, but allow certain industries to discharge wastes in the rawest form. Billings, Montana, for instance, has a municipal sewage plant, yet major industries--oil refineries, beet sugar plants, and packing houses--provide no waste treatment.

Badlands, regions of easily erodable materials lying above local base levels, are characteristic of more arid regions in the Missouri Basin. Their existence depends upon paucity of rainfall, as those rains that fall bring great quantities of such materials into the Big Horn, Powder, Yellowstone, Little Missouri, and Missouri Rivers. Badlands contribute the largest share of the silt load borne by the Missouri, and were mainly responsible for the river's name of "Big Muddy." Most badlands silt is now caught in main stem reservoirs.

The practice of irrigation also brings much silt into streams with excess water returned to the rivers. It also builds up mineral content, and frequently adds phosphorous, nitrogen, and other algal stimulants.

POLLUTIONAL EFFECTS

Pollutional effects upon aquatic life vary with nature and quantity of waste discharge, stream stage, season, type of stream, and other factors. Rarely does only one kind of waste originate in one locality, and it is usually necessary to consider combined influences of various pollutants.

For example, oil refinery wastes generally eliminate most aquatic animals for varying stream distances; organic wastes normally change the character of the bottom and its biota, and eventually stimulate plankton and other algal growth; and release of both types of waste from one locality frequently results in alteration of the usual effects of each. In several Missouri Basin streams, waste effects are further influenced by irrigation practices.

The North Platte River in Wyoming and Nebraska has been utilized for irrigation since 1850. Development of the reservoir system began in 1909 and has continued to date. Operation of this river consists of storing runoff during seasons of greatest snow melt and later releasing it for irrigation during dry periods. Discharges from upstream reservoirs, frequently used for power generation, are caught in lower reservoirs, whose releases are directly concerned with supply of irrigation demands. Drawdown of lower reservoirs provides capacity for storage of power releases in nonirrigation seasons. The operation envisions maximum power generation consistent with necessary conservation of water for irrigation. With the exception of relatively insignificant amounts of ground water inflow, discharge is wholly controlled by reservoir releases. Reservoirs have been noted to increase ground water discharge in areas just below dams. Dams without power generators are usually cut off completely at the end of the irrigation season; and the stream below must subsist on limited ground water inflow. Below such dams sudden transitions from high-to-low or low-to-high water stages are the rule, and they involve overnight changes from big river to headwater conditions, and vice versa.

Raw municipal sewage and oil refinery wastes enter the North Platte at Casper, Wyoming, about 50 river miles below Alcova Reservoir. Thi reservoir has power generation planned for the future, but at present operates solely to supply irrigation water, and has restricted or no releases at other seasons. In 1950, Alcova discharges varied from 0 to more than 5,000 cfs. During the period of high discharges, roughly May to mid-October, biotic influences of the Casper pollutional load consisted largely of marked plankton suppression by oil refinery discharges, followed at some interval by stimulation from nutrients added in municipa sewage. The plankton population developed in Alcova Reservoir, and its concentration gradually declined in the stream until affected by waste components. When Alcova releases ceased in October, the plankton algae were replaced by benthic growths in shallow areas below Casper.

These algae attained very dense development in the rich medium (discharge was then less than 200 cfs) but were intermittently eradicated for short periods of time by releases of phenols and crude oils. Their photosynthesis promoted supersaturated oxygen concentrations for many miles downstream. The oil refinery discharges, with periodic releases of crude oil and caustics, had eradicated all animal life in 114 miles of stream below Casper, except for certain fishes that led a short-lived existence near the mouths of some tributaries. Organic sludge deposits were completely untenanted by sludge worms and other characteristic organisms. This sludge and that arising from soda-lime water softening in refineries built up concentrations during low flow periods that were scattered downstream for about 100 miles during later flow increases.

Irrigation degradation of the river became progressively greater with downstream distance. Its most obvious influences were accelerated hardness, alkalinity, and turbidity increases. Silt accumulations suppressed benthic organisms, and suspended sediment reduced plankton concentration.

The sugar beet industry discharged processing wastes to the lower North Platte during fall and early winter. Cold water at that time slowed decay of beet particles and other organic matter, and oxygen was not completely exhausted. Flume water containing beet washings added considerably to the river's turbidity. Irrigation ended before the beet processing campaign began; but high river stages were maintained by return of ground water surcharges contributed by irrigation. Except for regions below beet factory discharges, the lower river was generally clear in fall and winter and promoted benthic algal growth. Lime slurry from beet processing added to alkalinity and hardness, and formed unsightly bottom deposits inimical to benthic fauna. Many solids contributed by the beet industry were caught in Kingsley Reservoir. Their decomposition near the bottom of this lake provided nutrients that stimulated algal growth in the river below. Discharges largely consisted of bottom waters that were generally excluded from plankton productivity in the impoundment. Storage in this reservoir reduced hardness and alkalinity in 1950, and it discharged better quality water than it received from upstream.

Prairie streams lacking the benefits of mountain snow melt permit much more limited water use than occurs along the North Platte. The James River, arising in the prairies of central North Dakota and joining the Missouri below Yankton, South Dakota, has been described as the longest non-navigable river on earth.

It is fed by prairie snow melt and limited summer rains, and normally dries or partially dries over long reaches in early autumn. The main flow at such seasons comes from municipal sewage and water discharges in reaches below Huron, South Dakota. The stream bed has a very flat gradient, and tributary inflows have been observed to run upstream during seasons of high runoff. Several low head dams have been constructed to conserve water for municipal usage, stock watering, waterfowl refuges, and other purposes. The river meanders extensively--requiring 710 channel miles to traverse a distance of about 350 miles. Channel restrictions induce extensive inundation of the flood plain in early spring.

The James drains an area of rich soils and has a naturally high biological productivity. Pollution consists largely of municipal sewage treatment plant effluents. Oily discharges from railroad yards and artifical gas plants affect some reaches; and waterfowl have marked effects in and below some impoundments. The usual effect of sewage plant effluents and waterfowl wastes is excessive stimulation of plankton algae, which frequently occasion tastes and odors in water supplies drawn from the river. Effluent from one overloaded sewage plant promotes anaerobiasis in the river--producing an oxygenless zone along one side for a few miles that is followed by a region of dense plankton growth. Tars contributed by a gas plant at that locality have eradicated bottom organisms; and the oxygenless zone is, with the exception of fungi, a lifeless area. Benthic organisms, especially midge larvae and water mites, attain very dense development in areas influenced by concentrations of waterfowl. The Sand Lake Migratory Waterfowl Refuge northeast of Aberdeen, South Dakota, has had transient populations of more than one-half million waterfowl, mostly geese. Due to the number of impoundments, all waste influences are largely localized; and plankton growth usually results in very effective treatment of wastes in each impounded stretch.

The Yellowstone River, although supporting considerable areas of irrigated lands, lacks impoundments. Alleviation of irrigation effects by reimpoundment of water is, therefore, not possible. Thus, water quality deteriorates with downstream distance in lower reaches when irrigation is practiced.

The Yellowstone is also polluted by sugar beet factories, oil refineries, packing houses, and treated and untreated municipal sewage. Oils and tars originating in petroleum refineries have almost completely eradicated bottom fauna in some reaches. Municipal wastes have been noted to lower oxygen and stimulate algal growths.

Periodic taste and odor problems in water supplies withdrawn below Billings result directly and indirectly from waste discharges.

An unusual fish kill affecting long reaches of the Yellowstone was indirectly occasioned by aerial spraying with DDT to control spruce budworm in headwater forest areas. The application was followed by heavy local rains that carried insecticide into the upper Yellowstone system. The DDT was applied in July 1955, and a great mortality of fishes occurred in October and November. Autopsies of fishes indicated starvation as the cause of death. Examination of the streams disclosed a paucity of food organisms, mainly acquatic insects, whose widespread scarcity was then traced back to insecticidal operations in July.

Another unusual case of pollution resulted from practices at a trout hatchery that permitted excess fish food and wastes to reach Rapid Creek in western South Dakota. These materials caused deleterious algal blooms in water supplies taken from the creek, necessitating revision of fish rearing procedures at the hatchery. Fish protection is normally considered an objective of water pollution control, but here fish actively contributed to water pollution.

The above examples are offered to illustrate some pollutional problems related to geography and human practices in the Missouri Basin. Space does not permit reference to various other pollution cases that have been encountered and water quality relationships involved in the main stem reservoir system. The heavy waste load discharged to the lower main stem promotes taste and odor problems in water supplies during low winter discharges, particularly if flow is reduced by ice formation. It also maintains a high concentration of coliform-type bacteria over the reach from Sioux City, Iowa, to below Jefferson City, Missouri. Effects upon the biota in the lower reach are incompletely known. The more desirable fishes, walleyed pike, channel catfish, etc., are most concentrated in the river above Sioux City, while carp form the bulk of the fish catch in polluted reaches below that point.

Water pollution in large areas of the Missouri Basin may appear quantitatively insignificant when compared to that contributed in more heavily industrialized regions. However, limited water supply over such areas results in serious effects from amounts of pollution that would not occasion critical conditions in some areas of great rainfall.

CURRENT INVESTIGATIONS IN WATER POLLUTION BIOLOGY

Investigations and Problems in Ohio

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Prior to white man, it is reported that there was an approximate population of 10,000 Indians whose lives were, generally speaking, based on the factors of self-preservation and actual needs. By 1800, there were some 45,000 whites who introduced the factors of trade and commerce. By 1950, 150 years later, the human population had increased about 176 times the 1800 population to a number of almost 8 million. It is estimated that by 1975, the population will have increased to 10 million.

Prior to the coming of the whites, practically 90 percent of the land was covered by hardwood forests. It may be said that deforestation had begun about the year 1788. By 1880, the entire state was settled, and some land had already been abandoned.

Ohio has turned from an agricultural to an industrial economy. By 1959 or shortly after, the Great Lakes - St. Lawrence waterway is expected to be open, which will have an indeterminable, but probably considerable, impact on the sociological-economic status of the state..

During the early 1900's, water treatment and purification plants were built to safeguard human health from effects of sanitary wastes. By the 1930's, the situation had progressed to the stage at which it was recognized that action was needed to control degradation of the water courses. World War II checked progress temporarily, after which construction of waste treatment plants again began to pick up. In 1951, the Ohio Water Pollution Control Act became a law giving further impetus to corrective steps being taken.

Under this act, now on the statutes as Chapter 6111.01 to 6111.08 inclusive, a Water Pollution Control Board was established in the State Department of Health with the Director of Health as chairman, the other members being the Director of the Department of Commerce, the Director of the Department of Natural Resources, a representative of industry, and a representative of municipal government. The Board administers the pollution abatement program of the State.

This Board is vested with a number of duties and powers. Briefly:

1. To develop programs for prevention and control of new and existing pollution. (Pollution means placing of any noxious of deleterious substances in any waters of the state which renders such water harmful or inimical to the public health, or to animal or aquatic life, or to the use of such waters for domestic water supply, or industrial or agricultural purposes, or for recreation.)

2. To advise and consult with other agencies in furtherance of the program.

3. To encourage and conduct studies concerning pollution.

4. To collect and disseminate information concerning pollution.

5. To prohibit or abate pollution by issued orders dependent on certain specified limitations.

6. To issue permits based on compliance with specifications.

7. To institute legal proceedings to compel compliance with the statute!

Although the Water Pollution Control Board is nominally the pollution control agency, it is still an infant organization which is cautiously developin its program with an eye towards continually improved effectiveness rather than sudden blundering moves which might cause rejection of its purpose. "Industrial wastes and acid mine drainage are exempt from the provisions o: Section 6111.04 of the Revised General Code of Ohio until the Water Pollution Control Board after a hearing determines that a practical means for the removal of the polluting properties of such wastes". "Removal" is interpreted in Administrative Resolution No. 1, of the Water Pollution Control Board, to mean any procedure applied to a waste which will effect a reduction in the polluting quality of that waste. The program operates under a permit system which generally does not specify the amount or characteristics of discharges.

The Board deals with 140 cities (actually 118, since 22 are suburbs), of which 39 do not have treatment works, but construction, planning, or investigations are under way to correct this status.

There are 767 villages, 380 of which have been exempted as having no pollution problems to date; 105 have been postponed because of insignificant pollution or difficulty in financing treatment works; 22 are still to be investi gated. Of the 114 which have been found to need installation of treatment works, all are under construction or planning operations.

The Board deals only with industries which discharge wastes directly into waters of the State. Of roughly 13,000 listed industries in the State, the Board is dealing with only 646. Of this number 283 are "currently acceptabl 181 need improvement, and 182 are now constructing, planning, or making preliminary studies. Those industries which discharge wastes into municip sewers are a primary problem of the city but are also under the supervision of the State Health Department.

At first, the Board began bringing industry into the program by compulsory hearings. Following several such hearings, industrial representatives suggested that this implied that industry was not willing to join in the abatement effort and was as a result receiving unwarranted bad publicity. Date lines were set and industires were allowed to voluntarily apply within that period. All the industries asked into the program under this system are reported to have complied.

The bulk of the Board's information concerning the polluted status of waters is obtained from the Ohio Department of Health. This data generally consists of B. O. D., coliform index, acidity, tastes and odors (phenol tests particularly), suspended and dissolved solids, temperature, and chemical determinations for specific elements. These are generally directed towards human health maintenance. Surveys have been made of the Mahoning, Miami, Maumee, Muskingum and Cuyahoga Rivers.

The Division of Wildlife, Ohio Department of Natural Resources is responsible by statute for the protection and preservation of wild animals. Because of the preponderance of observable effects result in fish kills, the majority of the investigations are carried out by fish managers and game protectors.

The variability of conditions attendant to pollution incidents, of course, necessitates adaptation of procedures to fit the circumstances. The basic procedure is for the county wildlife management agent (known also as the game protector) to notify the district office that a kill has occurred and then to proceed to determine the extent and source until the fisheries technician arrives. Together, an effort is made to pinpoint the source of pollution, to take samples indicative of conditions, such as, pH, dissolved oxygen, temperature, and turbidity tests, and to make counts of the observable dead wild animals.

This report is submitted to the Division's Office of Pollution Abatement which is also notified of the occurrence of the incident at the initiation of the investigation. This office analyzes the information obtained in the field and recommends the action to be taken. A claim for damages is first presented to the offender, and if settlement cannot be reached within 30 days the claim is certified to the Ohio Attorney General for collection.

In 1955, 111 incidents were reported. Sixty-two were reported as harmf and forty-nine as threatening situations. A break-down of the information concerning the sixty-two harmful incidents shows the following data in rounded figures:

Forty percent reported counts or expressed kill figures ranging from 5 to 10, 150 fishes. The highest monetary value was reported as $36.22 for an estimated kill of 2, 289 fish.

Thirty-one percent of the reports showed no counts, because of such matters as too lengthy a period between occurrence and discovery of the kill, non-feasibility of producing creditable estimates because of stream conditions (flooding, natural swiftness, etc.).

Nineteen percent reported damage to habitat, but as yet no basis for monetary value has been developed for collection of these damages.

Five percent of the kills reported were "natural" kills. White bass, Lepibema chrysops, died in great numbers with no determinable cause in Lake Erie. A severe water temperature change is believed to have caused the death of numbers of smelt, Osmerus mordax. A small kill was reported in a lake in which no pollutional discharges could be found.

Three percent reported that the fish had disappeared by the time of the investigation.

Two percent reported no identifiable pollution, although a kill had been reported.

Information obtained from an investigation by the Division is forwarded to the Water Pollution Control Board.

Because it is obvious that present procedures are not entirely adequate, the Division is constantly attempting to improve on its effectiveness. An intensive study of acid mine drainage effects on Raccoon Creek was made to determine the effect of mining operations on a stream and the possibility of reclaiming the watershed; investigation of feasible corrective measures are now under way. An intensive study of the effect of land use improvements is being conducted on the Little Miami River. Statewide pollution investigators whose duties are to supplement and augment the work of regular pollution investigations and to carry out special investigations have been added to the staff of the Office of Pollution Abatement. The Fish Management Section in Wildlife District Two has carried out an investigation showing the improvement on stream fish life after the installation of a sewage treatment plant; data showed a species number increase from one to a more normal population of 18 species in a period of three years. As an initial program an Aquatic Biology Laboratory is being developed in order to provide more adequate information concerning the effects of pollution on fish life.

Other investigators and their investigations of which I am aware are:

Professor Charles Riley, Kent State University, is investigating for the Ohio Reclamation Association, the reclamation of coal mined areas by the formation of ponds in abandonned cuts and plantings on spoil banks.

Dr. W. D. Sheets, Ohio State University, Waste Treatment Laboratory, using a moderate sized pilot plant for the studies, is investigating the effects of plating metals on sewage treatment installations.

Dr. George E. Barnes, Case Institute of Technology, is directing the investigation of the effects of industrial wastes on the biota of sewage systems in an effort to determine the effects of industrial wastes introduced into a municipal system.

The Ohio River Valley Water Sanitation Commission, headquartered in Cincinnati, is an eight state organization of the Ohio River basin. Its role is to abate and prevent pollution of this great river.

Our problems are quite similar to those concerning other states:

1. A rapidly growing population together with ever increasing industrialization. The topography of Ohio is such that, except perhaps for some of the area in the southeastern hilly region, there is little area which can be exempted from development.

2. A need is felt for developing more effective methods of demonstrating harmful effects of pollution on wildlife.

3. A revision of the status has made the interpretation of their meaning and precedence of one section over another more difficult.

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BIOLOGY AND WATER POLLUTION IN GREAT BRITAIN

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In a general way I think it is true to say that the trends of biological studies in problems of water pollution in Britain have been largely influenced by the legislation controlling river pollution. This is not surprising as most of the applied work in this field has been carried out by government agencies. It means, though that a brief review of the legal aspects of pollution is helpful to the understanding of the biological work that has been and is being done in Great Britain in connection with it.

The problem of river pollution, like many other social problems, arose with and out of the industrial revolution. By the middle of the nineteenth century it had become serious in the industrialized central belts of England and southern Scotland. A series of Royal Commissions were set up to study and report on the situation and the last of these remained in being until the early 1920's. These Royal Commissions carried out a great deal of applied research on river pollution and much of the basic work in this field including I believe the development of the five day B. O. D. test - was done by them. As a result of these studies they suggested standards for effluent purity depending on available dilution, but their recommendations were not carried through to legislation to any great extent.

In 1876, before most of the Royal Commissions' studies had been made, River Pollution Prevention Act was passed, but it was really an administrative rather than a technical Act. In effect it made any sort of pollution illegal, but, as the complete prevention of pollution and the existance of industry and urban living were incompatible, the Act provided legal loopholes that were so large that the whole Act proved almost useless as a means of preventing pollution.

This remained the legal situation until 1951 when two River Pollution Prevention Acts, one for Scotland and one for England, became law. These Acts were very similar technically, the differences being chiefly in the bodies charged with the administration of the Acts. For the first time in Great Britain these Acts accepted the principle of allowing a controlled amount of pollution and authorized the setting up of standards of purity to which effluents must comply. As these standards necessarily vary from one river to another and even between different parts of the same river, provision was made for them to be enacted by means of by-laws of limited local operation only.

Apart from the early work carried out by the Royal Commissioners, the Ministry of Agriculture and Fisheries employed a team consisting of Southgate, Pentelow and Butcher to carry out stream surveys in the 1920's and 1930's. These surveys, as one would expect from the later history of the men employed on them, were very thorough and provided a valuable basis for biological survey work in connection with river pollution.

One of the recommendations of the Royal Commission on Sewage Disposal that came to fruition was the setting up of a permanent research laboratory to work on water pollution problems, namely the Water Pollution Research Laboratory (9) of which Dr. Southgate has been Director for many years.

Particularly since the second world war the study of pollution problems in Great Britain has very largely centered on this laboratory and in collaboration with the Fisheries Inspectorate of the Ministry of Agriculture and Fisheries, of which Pentelow is Chief Inspector, a considerable amount of research is at present in progress.

The programes of research have been shaped very largely to meet the requirements of the 1951 legislation already referred to, and particularly to enable standards of purity to be established. The approach to this problem has been four-fold. First, one team which includes Herbert, Merkins, and Kathleen Downing has been carrying out fundamental researches on the toxicity to fish of various chemicals, in particular cyanide, ammonia and carbon dioxide (2) (4) (5) (6) (7) (8). For this work a very fine piece of apparatus for controlled flow experiments has been developed and used.

Another team including Herbert, Alabaster and Allan has been working on the field aspects of the problem by studying rivers in various degrees of pollution and also by conducting field experiments with fish kept under pollutional conditions[1]. Alabasters, in collaboration with other workers has also been engaged in the development of a standard method for measurement of toxicity and although I am not aware that he has yet published his results, he has accumulated a considerable amount of data.

Apart from these purely biological researches, some of the chemists and physical chemists - particularly Downing, Gameson, Knowles and Truesdale - have been working on the problems of re-oxygenation, de-oxygenation, oxygen sag curve and other physical and chemical problems that are very closely related to the biological aspects of river pollution[3].

There has been a great deal of discussion at this seminar on the problem of bioassays and toxicity tests. It may be of interest for me to give, as far as I know it, the British approach to the problem[5].

First there seems to me to be a difference in the goals of the workers in Britain and the United States of America. In Britain it has been assumed that toxicity tests will be carried out by a specialist agency, so that the aim has been to develop a practical and reproduceable test that can be handled by a laboratory specially equipped and staffed for the purpose. In the United States the aim seems to be to develop a test that can be carried out by an industrial control laboratory that is probably not specially equipped or staffed for the purpose.

The British workers have not favoured the method which has come to be known as the Doudoroff, Katz method of retaining fish in solutions of poison through which air is bubbled to maintain oxygen concentrations and measuring 50% tolerance limits, because they felt that the shortcomings of the method, which were of course known and stated by its originators, were as difficult to overcome as the problems posed by some of the more complicated experimental procedures. The shortcomings to which I refer are, of course, the removal of volatile materials by aeration and the difficulty of prescribing safe limits from 50% tolerance limits. Instead the approach of the British workers has been, first by means of the fundamental work of Herbert, Merkins and Downing and the field work already mentioned to try to learn sufficient of the mechanism of toxicity to enable safe limits for toxicity to be calculated from comparatively short term tests, probably measuring median survival times rather than 50% tolerance limits. Secondly, attempts have been made to develop an experimental technique that will enable these measurements to be made without aeration. Controlled flow seems to be the most practical line of enquiry and I believe that current work is taking place along these lines.

Apart from the work being carried out by the Water Pollution Research Laboratory in conjunction with the Ministry of Agriculture and Fisheries, there are other workers in Great Britain interested in freshwater biology who may from time to time carry out work having a bearing on pollution problems but, as far as I know, none of them specialize in this work. The Freshwater Biological Association of the British Empire, which has its headquarters on Lake Windemere does a great deal of valuable research of a rather more fundamental character than the Water Pollution Research Laboratory. The Scottish Home Department has a Brown Trout Research Laboratory at Pitlochry, Perthshire, but it is concerned more with fishery improvement than pollution. Some of the universities have freshwater biology stations; for example: Aberystwyth, where Kathleen Carpenter carried out much of her early pioneer work on freshwater ecology, Liverpool, Glasgow and Wessex - formerly University College Southampton - to mention a few that happen to be known to me personally.

I should like to make it clear that this statement does not purport to be a comprehensive review of the biological work in connection with river pollution in Great Britain. It is merely the observations of one person who has been connected with this work for many years and has tried, often vainly I fear, to keep in touch with the work that is being done in this field. I trust that no one will be hurt by my omissions but will attribute them to my ignorance of their work, not to any disparagement of it.

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THE TRAINING OF AQUATIC SANITARY BIOLOGISTS

THE TRAINING OF AQUATIC SANITARY BIOLOGISTS

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INTRODUCTION

Our subject for discussion this evening is certainly not the least in .mportance among the six panel discussions that comprise this Seminar ›n Biological Problems in Water Pollution. The success of the training ›rograms of today will be reflected in the solutions of tomorrow's problems.

As new problems arise in science and industry, new or revised training ›rograms are born. There is nothing unespected about that, rather it is a ıatural educational trend. There was a time, not too long ago, when a sanitary :ngineer as a separate species, did not exist. Today, many of our larger ıniversities have separate departments of Sanitary Engineering.

But when it comes to sanitary biology the problem is not so simple. ›et us consider for a moment two somewhat analagous training problems rising from the expansion of two fields of study--first, Conservation and econd, Operation Analysis or what the British call Operational Research.

Dwindling national resources accentuated the need for conservation. ıdvocates of one school of thought favored treating conservation as a more r less discrete entity to be presented or taught as a separate subject or eries of subjects. A second viewpoint advocated the teaching of conserva- ion as an integral part of practically all other courses of instruction. After ıuch debate, this latter viewpoint has received widest acceptance.

On a basis of the diversity of subject matter presented during the past our days it is evident that aquatic sanitary biology is indeed a broad field, equiring basic training in mathematics, chemistry, and physics as well as number of branches of biology.

I propose, as a basis for discussion by this panel, that a sanitary biolo- y curriculum be composed of strong basic courses in each of the afore- ıentioned subjects followed by one or possibly two courses designed par- .cularly for the sanitary biologist. Incorporated in these latter courses should e the history of advances in this field, the biological contributions responsible ›r research accomplishments to date, the status of today's research in this eld and the future trends indicated by current investigations. Also, these ourses should set forth the problem needs of the engineer and attempt to ridge any existing gap between these respective disciplines.

What we might term a second reference field is Operation Analysis. is of course a very much newer field than conservation -- an outgrowth military research experience during World War II.

Operations Analysis may be defined as a discipline for planning actions most effectively for some purpose. Optimization of achievement of purpose is stressed. It may involve a problem in reducing traffic casualties or diagnosing the source of pollution in a river. In every case, it involves an action taken for a purpose and involving expenditure of effort.

We may decide, let us say, to allocate a certain amount of effort to accomplish a mission. First, we must know the real specific purpose of the operation. We must establish a problem model. The term model is not used here in the sense of a small scale structure but rather as a thinking device with which to analyse a situation. It is important to devise effective models in approaching a pollution problem or any other kind. Such models are, of course, approximations. Their upper limits or permissible boundaries must be specified.

Thus, from operations research, we learn to construct a problem model, to analyse it, to find out how sensitive our conclusions are to this model.

Presently certain colleges are grouping courses to form special curricula for training students in Operations Analysis.

One highly significant outgrowth of experience to date in Operation Analysis is that investigators representing unrelated disciplines have attacked problems and solved them although these problems have been entirely new and most unique. This is attributed to a fresh and unprejudiced viewpoint provided by the unindoctrinated researcher. To preserve this "take nothing for granted atmosphere", curriculum designers have an understandable hesitancy about prescribing special courses in Operation Analysis.

We are here this evening to consider problems similar to those faced by curriculum makers in the fields of conservation and operation research. Among other things:

(1) Shall we train aquatic sanitary biologists by introducing special courses?

(2) by modifying existing course?

or (3) shall we simply regroup unmodified existing courses? and come up with a cross fertilization that may meet the need.

If we aim to give our undergraduate major and masters degree work as a prerequisite to taking a position in the field, what training courses and fields should be included?

What additions should be made for students continuing for the doctorate?

Are our students to be trained strictly as applied researchers or should we make the program flexible?

What provision can be made for enabling the student to get one or two semesters of actual field experience in sanitary biology before completing his training?

BASIC CONSIDERATIONS

Having dealt with a number of general considerations and alternative approaches to the problem of adequate training of aquatic sanitary biologists, we need now to formulate specifically our problem. Let us consider the objectives of the training program. It has been said that the Case Institute is successful because it turns out what industry wants.

What does the sanitary engineer want from a sanitary biologist? I would like, Mr. Chairman to hear this question discussed.

I'm sure he would, among other things want a biologist capable of recognizing the central problem, of evaluating the techniques most likely to be effective in solving it, and then capable of applying those techniques.

The biologist must recognize alternative courses of action, he must recognize different measures of effectiveness, let us say, in respect to alternative control measures, he must be able to analyse and evaluate the variables involved, he must be capable of careful theoretical analysis and most important be able to subject his data to adequate statistical analysis.

We should perhaps at this point expell the notion that our task here is unique. The correct answer, if there is one, to this training problem may be unique but the problem itself certainly does not stand alone.

In common with chemists, physiologists and teachers of other sciences, we aim to graduate scientists first. Our student product must have those earmarks of a trained mind, one capable of clear and logical thinking for which there is no substitute in the evolution of mankind.

Ernst A. Hauser of M. I. T. states[1] it well "The true purpose of education should not be to make living textbooks, so to speak, rather we must change our curricula so that more emphasis is placed on the disciplines that teach proficiency in doing and thinking."

I believe therefore that the objective in our training program should be to train students to think and do as scientists first and then as sanitary biologists.

Engineers often use public moneys. They need to know what represents the best bet. They will ask what are the best probabilities in selecting an alternative.

I have heard it said that during the war a statistician undertook to evaluate how effective British bombing missions were in helping Russia. Number of casualties was the objective. They started by determining the effect in England of German raids. It was found that 5000 tons of bombs were dropped on England yielding casualties of 0.8 persons per ton. Due to various factors the German equivalent was estimated to be 0.4 persons per ton and later this figure was reduced to 0.2. In other words 400 civilians were killed per month. Then it was realized that, since 40 British bombers each with 5 trained men were lost per month, it therefore required a loss of 200 trained personnel to produce 200 civilian casualties. Not a very encouraging result. Further after the war it was learned that they had been using a wrong measure of effectiveness. Spread-out bombing operations would have been more effective than concentrating on particular centers as was usually done.

This story illustrates, I think, the importance of defining one's problem, evaluating the effectiveness of alternative methods of action or control.

In grouping of training courses to comprise a sanitary biology curriculum, I would attach primary emphasis on the basic sciences prerequisite to aquatic sanitary biology--physics, chemistry, bacteriology, biometry and the calculus accompanied by a number of biology courses which I have elected to leave for discussion by others on this panel better able to present this part of the subject.

Allow me to venture the opinion that too many of our institutions have gone overboard in the offering of specialized courses. Not that I have anything against the refinement of departmental offerings. They, of course, have a place. That definitely is not to serve as a substitute for courses that are more effective in terms of the principal purpose of the program in question. In other words first courses should come first. Thinking capacity--has priority, over textbook mastery.

Again, I would like to hear our panel discuss this thesis: Are we training our students adequately to solve problems? I say emphatically, no. We complain that our current graduates of high schools are steering away from science. If this is true, there doubtless are a number of causative factors. I think the problem approach could well be stressed. Further I believe curriculum makers could well mold their offerings in such a way as to get our graduates to recognize and solve problems. Ability to use the tools of basic science, to focus them on a practical problem, such as how to design a more effective or efficient oxidation lagoon, requires more than routine classroom work in a variety of academic courses. First it presupposes some experience in the practices of thinking or reasoning. A problem such as the lagoon one mentioned, here, is no respecter of courses. Varying disciplines are represented.

In fact, we are in this panel, faced with a problem in operation research. We must define our training problem--our training objective, or purpose, examine alternative means of meeting the objective, undertake to express or state the anticipated effectiveness of the one or more alternatives.

Advances in sanitary biology will depend upon having the best brains and upon having a large corps of dedicated investigators to enlarge the advances. Paraphrasing the words of Paul Weiss (2) of the University of Chicago: "All it takes to join these forces is: some aspirations, to point the goal; some inspiration, to point the way; and a lot of perspiration, to get the job done."

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THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF ENGINEERING

Department of Sanitary Engineering
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April 23, 1956

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Dear Tarzie:

I don't see how I can get to the Thursday seminars and back here to keep appointments that have piled up.

I have a few comments on the training of aquatic sanitary biologists that represent my own prejudices in the matter. They grow from limited local experience and from my total ignorance of the requirements and operations of the official agencies.

1. What are our future requirements for such a specialist group?

It would appear that the demand for sanitary biologists specializing in aquatic biology would be determined by employment in state and federal agencies and by a more limited use in industries that have continuing stream pollution problems. I would hazard the guess that federal demands would not exceed 50 men and that the state demands under most active conditions would be about the same level. It is possible that half this many men would be employed by industry in one capacity or another -- a total of 150 men. This can be quite wrong, of course, and the number might be doubled or halved. Some unexpected legislation might, on the other hand, raise the number to a total of 1,500 or 3,000.

In any case, it would appear that aquatic sanitary biology represents a small profession, far less numerous than sanitary engineering or water works operation. The turnover in this total profession determines the need for special training facilities. If we assume the largest likely number, 3,000, and take an annual turnover of 10%, the yearly requirement would be around 300 to be divided up among the various training facilities in this country. The annual requirement, then, is quite small and well within the scope of a comparatively simple special training unit. If the turnover is larger, say 25%, we have demands of another order, and also evidence that the profession is a fairly unsatisfactory one.

2. Why do we need special training?

Special training seems to be required to permit useful integration of biological observations with other criteria of water quality and changes used by sanitary engineers. The sanitary engineering profession has definite ideas of what biologists are and how they should function in a program. The role of biologist is, however, definitely accessory. The general history of stream pollution investigations in this country has shown a strong movement away from time consuming biological evaluations to the apparently more quantitative evaluations given by sanitary chemistry. In most sanitary engineers' thinking, the biologist's job is to find suitable indicators for water quality. If these correlate well with B.O.D. and other characteristics of the water, he is happy. If they do not, he doesn't know what to do about it and is inclined to drop the matter.

With increase in new industrial wastes, a second major use of biologists has developed -- testing for toxicity and tolerance. Dramatic toxicity results are, of course, big medicine but the ecological evaluation of waste loadings is, as every field biologist knows, much more complicated.

In any case, the special training that is required of biologists appears to be the development of an understanding of sanitary engineering terminology and of the peculiar whims and policies of the "team". This may take some time. Undoubtedly, the best way to establish this rapport is through direct training in the field unit. Classroom training tends to remove itself from current professional uses. Most of our programs that would employ sanitary aquatic biologists grow from special appropriation projects. These are organized from the top down -- the demands on the biologists will be determined by the conditions of the appropriation. Heaven knows what these may be, but they need not be wise or far sighted.

3. What is the role of universities in the training of sanitary aquatic biologists?

The general history of biology departments has shown movement away from natural history toward physiological, biochemical, and genetics type departments. There are few universities with strong natural history divisions. While the natural history courses are by nature intriguing, they have not had the professional potential of other branches -- generally speaking, the professional potential of biology majors has been very low. A few years ago more than half of our "professional biologists" were employed in the Department of Agriculture. These are perhaps the most happily used men in our university grou

Industrial uses have been extremely limited and biological courses have not attracted men who expected to enter industry. This restriction is becoming more apparent and the stature of biology generally has suffered.

The value of any special training offered in a university depends first upon the quality of the men who are attraced to it, and secondly, upon the quality of the men who do the teaching and their understanding of the total problem. It may not be flattering to say that we do not get the most brilliant and promising students in public health, conservation, aquatic biology and other courses that might be conditional to sanitary aquatic biology specialities. It is more realistic to admit that the brilliant students simply don't appear in these courses. Our reservoir of talent is severely limited.

With the decline of natural history training, biology departments have lost contact with the field. Although many members of departments serve on meritorious committees and pass large policy statements, comparatively few work directly with either official agencies or with industries. They are not acquainted with the values involved in any realistic way.

4. How can the training of aquatic sanitary biologists be improved?

It would appear that there is little justification for setting up special courses in universities for training biologists to work in official agencies where the law enforcement component dominates. A better use of limited talent is in the prosecution of special investigations and examination of new phenomena. Here the most resourceful people are demanded. My own feeling is that we can do most by making it possible for biology department members to see what the problems in sanitary biology are really like; to study them in their own way, and to reevaluate them in an original fashion. The official point of view is secondary here. In the development of this relationship industry should take a very active part, especially in those states where conflict of recreational and industrial values is likely. The constitution of a special sanitary biological profession perpetuates the concept of public health losses (as a level to forward conservation interests) and it is doubtful if these men will be happy in the promulgation of this type of compromise. It is very valuable to universities to have men active in industries and biology departments would gain stature considerably by such contact. We have had numerous instances of men initially involved in pollution studies who were able to make real contributions to the solution of production problems in industry. This transfer of ability increases the respect of industry for biologists.

5. What students should be encouraged to enter fields related to aquatic sanitary biology?

It is fairly obvious that men who are to be happy and successful in this field must be capable of growth. Service in a bureau at levels currently reserved for biologists is far from satisfactory. This grows in large part from the limited functions of bureaus. Generally speaking, official agencies lose their most enterprising men to industries and to private organizations because these men prefer the freedom that is possible in private work. At the same time, apprenticeship in bureau service provides an essential background for successful private practice in companies that must maintain contact with official agencies. It is unlikely that the future will differ from the present, although salaries and fringe benefits may make federal and state service more attractive. Two possibilities exist. A man may serve as a highly specialized expert or he may be required to exercise a variety of techniques. The specialist, of course, is subject to limitations of shifting emphasis. A taxonomic entomologist may be in great demand in early phases of an insect borne study, and find himself on the shelf a few years later when practice has been established. It takes a long time to train a good taxonomist. The more generally trained man enters a more varied field of experience. He is usually better adapted to "the team" operation, although personality problems always enter.

It would be most unhappy if sanitary aquatic biology became a catchall for misfit biologists. This could be possible and I am afraid that the possibility would be increased by organizing the field in a series of academic offerings, especially if good beginning salaries and security were offered as additional inducements for entering the field. The problem of finding professional outlets for biologists is not peculiar to the public health and sanitary fields. We must make an effort to analyze the whole employment situation to determine what the best uses and futures may be. Very few biology departments have practical contacts of an order that permit good student placement and student advising.

With all good wishes,

Very sincerely,

sgd/ Charlie

Charles E. Renn
Professor of Sanitary
Engineering

CER:mh

WATER QUALITY CRITERIA FOR AQUATIC LIFE

EFFECTS OF TURBIDITY AND SILT ON AQUATIC LIFE

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Mr. Chairman and Gentlemen of the Seminar, I'm happy to have the opportunity to participate in this panel on water quality criteria and to present to you some of my experiences and those of others dealing with the effects of turbidity and silt on aquatic life. Upon my arrival in the Pacific Northwest as biologist with the Drainage Basin Office, U. S. Public Health Service, several years ago, one of my first tasks was to work with the committee on Water Quality Criteria and Objectives for the Pacific Northwest Pollution Control Council. At that time we sidestepped the question of effects of turbidity and silt on aquatic life. Under the heading of "Floating, Suspended, and Settleable Solids and Sludge Deposits" we stated: "None shall be permissable which are attributable to sewage, industrial wastes or other wastes or which after reasonable dilution and mixture with receiving waters interfere with the best use of these waters for the purpose indicated."

A review of water quality criteria and standards from all over the country discloses a reluctance, if not outright refusal on the part of regulatory agencies to come to grips with this problem. Many consider the control of silt and turbidity to be strictly a matter for agriculture departments -- indeed for the farmer himself -- and are therefore unwilling to touch it as a pollution control problem.

A matter of semantics is involved at this point as many persons such as those connected with the industries of mining and forest products adhere to the strictest connotation of the word "pollution." They consider silt and turbidity as perfectly natural and normal to be carried by water in contrast to pollution which is contended by them to refer only to putrescrible matter and denoting transportation by water of pathogenic bacteria and the like. However, the currently accepted definition as reiterated by most forwardlooking pollution control agencies refers to pollution of water as encompassing the full gamut of substances which adversely affect any of the legitimate uses of the water.

In view of the fact that there is widely differing opinion throughout the country, and the world, in regard to how deleterious silt and turbidity are in natural waters, a committee has recently been organized with the author as chairman to work on a "Critical Review of Representative Literature on Water Pollution by Inert Inorganic Materials." This committee includes Dr. A. F. Bartsch of the Robert A. Taft Sanitary Engineering Center; Dr. I. E. Wallen of the American Association for the Advancement of Science, Washington, D. C.; Dr. Virgil Pratt, University of Idaho, Moscow; and Mr. Philip Pister of the California Fish and Game Department, Garberville, California. We have not made remarkable progress as yet but have set three years as our goal, hoping to have reasonably full coverage by that time, leading toward publication.

My presentation to you should, by rights, be made three years from now when our committee has had an opportunity to complete its work. Nevertheless, we can all profit at this time by a brief discussion of some findings to date. I shall refer to some pertinent activities and publications in the field, including an investigation which I have made with the State of Oregon on effects of gold dredging on an eastern Oregon stream.

Jacob Verduin (1954) at Stone Lab, Put-in-Bay, Ohio, states that turbidity is the major factor in causing poor phytoplankton productivity in Lake Erie.

David Starr Jordan (1889) reported many decades ago on losses of trout and trout spawn owing to turbidity and silt from placer mining operations in Colorado.

Richard Rathbun (1889) in writing of streams in Iowa, tells of change with development of agriculture causing decrease and deterioration of the better food fishes. With the breakup of original sod of the prairies, rivers which formerly had well-defined, deep, narrow channels have widened and become shallow. They tend to overflow their banks in the rainy season and lose most of their water during succeeding months. Sediment and silt, continually loosened by farming, fill pools and riffles in streams, thereby causing rapid disappearance of trout. We are presumably to consider the trout which formerly occupied streams in Iowa as passé just as are the buffalo from the western plains.

Higgins (1931) referred to the enormous quantities of erosion silt entering the Mississippi River along with sewage sludge. Together these agents smothered bottom life, wiped out mussel beds, and the like.

Emmeline Moore (1937) quotes H. K. Townes in saying that siltation dilutes settling organic debris to such an extent that bottom organisms have a poor food supply. There is a continual "snowing under" of bottom organisms. In fact, induced siltation has been used in irrigation canals to inhibit growth of moss and algae.

Tarzwell, (1937) in the course of stream improvement work in Michigan, evaluated the effectiveness of the structures and came up with some enlightening facts on productivity of stream bottom types.

At the lower extreme of the scale is sand with population rating of 1 and silt 10.5; rubble, 30; coarse gravel, 32; but plant beds provide the very highest productivity with ranges up to 452. A normally productive riffle with coarse gravel and rubble will depreciate in productivity stages toward 1 as the riffle fills with sand and silt.

Langlois (1941) pointed out changes in fish fauna in lakes and in streams with ecological changes in these habitats. In lakes, reductioı in species of fish is seen, while in streams there may be a replaceme: of some fish species by others. Those fish that can reproduce under the greatest variety of conditions persist the longest; those requiring the most specific conditions are the first eliminated. Attempts to re-store fish species that have been decimated or eliminated should consist of restoration of the habitat conditions that prevailed when thoı species were thriving.

Bartsch and Schilpp (1953) reported on sand processing wastes fr a glass sand corporation in West Virginia as affecting a small tributar of the Potomac River. They concluded that the differences in producti of plants and animals in affected and unaffected parts of the river are due principally to increased turbidity and solids deposition.

They further concluded that "the physical and biological condition: found to occur in affected parts of the river are ones known to interfer with sport fishing and to affect adversely the production of fish."

The color slides which I have presented show some of the trouble spots in the Pacific Northwest in this matter of siltation and turbidity. Particular reference is made to the situation on the Powder River in eastern Oregon where a gold dredge had been operating for a number of years.

By good fortune I was able, with the help of Messrs. Homer Camı bell of the Oregon State Game Department and Harold M. Patterson of the Oregon State Sanitary Authority to investigate the Powder River in September and October 1953 before the dredge ceased operation and again in November 1955, more than two years after the dredge stoppe Our summary and conclusions at the close of the first phase of the stu were:

1. Turbidity readings ranged from 5 ppm to the control area to 1700 ppm below the dredge. Siltation in pools was very heav

2. A test for rate of sedimentation on a sample of water from be-low the dredge indicated a 60 per cent reduction in suspended solids in 24 hours.

3. Production of fish-food organisms dropped to almost nil in the zone of heaviest pollution.

4. The presence of some bottom fauna at all stations indicates absence of toxic substances in the mining waste. Principal damages are physical smothering action and interference with light penetration necessary for growth of green plants.

5. Results of fish population studies in the various zones of pollution in Powder River indicated a complete alteration of the population from sport fish, rainbow trout and whitefish, found above all sources of pollution to rough fish in the zone of pollution and recovery.

The sedimentation test which was made on waters of the Powder River downstream from the gold dredge indicated a high degree of reduction in suspended matter in a 24-hour period. During stages of normal to low flow a corresponding progressive decrease in suspended matter was observed downstream from the source of pollution. Although the screening of the light was a significant factor in lowered biological productivity, the abrasive or molar action of the larger particles of sediment and the smothering of fish food organisms and fish spawning beds are considered to be of greater importance.

The more recent investigation showed that remarkable recovery had taken place in the Powder River with flushing of silt from the pools and cleansing of riffles by freshets. This signalled the return of a wide variety of bottom fauna in the 15-20 mile reach of the river which had been heavily silted. The Oregon Game Commission has planted trout in this stretch and creel census indicates successful reestablishment of the sport fishery.

This brings us back to our original thesis, namely: The establishme of water quality criteria for silt and turbidity in natural waters. May I be permitted to incorporate here the consensus of opinions expressed by members of the seminar audience? It has been suggested that rather than to propose arbitrary criteria either for turbidity or for settleable solids, some percentage increase above normal low flow concentrations should be established. This would take into consideration differences in watershed and stream or reservoir characteristics.

In conclusion, the problem of siltation and excessive turbidity stemming from activities of man are widespread and difficult to control. Many question the economic feasibility and practicability of control measures, but a few forward-looking pollution control agencies are undertaking measures of control with varying degrees of success. As a guiding principle in establishment of water quality criteria for permissible concentrations of silt and turbidity in streams, certain percentage increases above levels at normal low flow in waters is suggested.

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THE EFFECT OF POLLUTION UPON WILDLIFE

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I am pleased to have this opportunity to join you to learn of the many problems facing those working in the field of pollution and to learn of the progress being made in the technical field toward the alleviation of these problems. It goes without saying that the advance of civilization has brought with it many wonders, but at the same time has left in its wake many things, one of the worst being pollution, which are in contradiction to the many fine recorded accomplishments.

As my portion of this discussion, I shall attempt to summarize for you the effect of pollution upon aquatic life as it relates to our objectives and functions in the field of fish and wildlife conservation.

The programs of the Fish and Wildlife Service have the central objective of insuring the conservation of the Nation's wild birds, mammals, fishes, and other forms of wildlife both for their recreational and economic values. In carrying out this objective, which we share with the States and Territories, it is essential to build up these resources, to prevent their destruction or depletion, and to promote the maximum use and enjoyment in consonance with their perpetuation.

Before discussing the Fish and Wildlife Service's interest in the field of pollution as it relates to wildlife, I shall review briefly the functions of the Service, particularly those which are concerned in one way or another with pollution problems.

The conservation of the North American waterfowl and other migratory birds is undertaken in cooperation with the governments of Canada and Mexico. The Service operates 264 refuges to provide for the needs of waterfowl and other species as a major part of this program. Our investigations and surveys are the principal basis of the hunting regulations, which are enforced by the Service.

The Service is charged with the administration and enforcement of the laws relating to the commercial fisheries of Alaska. It also conducts biological research on marine species of commercial importance off all coasts of the United States and in waters adjacent to territorial possessions. The fishery research program provides information on the size of the resources, rates of decline or increase, and reaction to various intensities of fishing as a basis of conservation programs. The marine fishery program also includes technological, economic, and statistical research as means of promoting trade and commerce in fishery products, improving processing, distribution, and marketing practices, and effecting more complete utilization of resources.

Our programs for the maintenance of the fresh-water fisheries extend to the conservation and development of commercial fisheries of the Great Lakes and other inland waters, and to cooperation with the States in the maintenance of sport fishing throughout the Nation. The maintenance programs for Federal lands and installations depend upon stocking from the Service's hatcheries and upon our programs for the protection and restoration of habitat. Much attention is given to investigation of the effects of water development projects proposed for construction by the Federal Government or under Federal permit or license. In a number of instances the Service maintains and operates fish screens on Federal irrigation projects.

The Service administers the Federal Aid in Fish and Wildlife Restoration Acts, which authorize grants-in-aid to the States and Territories, including investigations, acquisition of land, and development of fish and wildlife habitat. The Service is authorized to provide assistance to, and cooperate with, Federal, State, and public or private agencies and organizations in the development, protection, rearing, and stocking of all species of wildlife and fish.

Among other important programs of the Service are activities relating to international agreements concerning fish and wildlife, including the Whaling Treaty Act, the Sockeye Salmon Fishery Act, and the North Pacific Halibut Fisheries Act; the management of the North Pacific fur seal herd; the administration and enforcement of the various Federal laws relating to wildlife and fisheries, including the restraints upon interstate transportation contained in the Lacey Act, and the Black Bass Act; and the promotion of domestically produced fishery products in commerce, the development of markets for fishery products of domestic origin, and the conduct of research pertaining to American fisheries.

There is seemingly no limit to the varieties of pollution which directly or indirectly are harmful to fish and wildlife resources. Despite this, there is no Federal authority under which the Service enforces regulations governing pollution.

Since we lack the power of enforcement, we must, when the need arises, seek the aid of State and Federal agencies who are empowered with such authority. Our activities in the pollution field are confined largely to research on special problems and to cooperation with State and other Federal agencies. Without the excellent cooperation received from these agencies in working out solutions to our problems, there is no question that our progress in fish and wildlife conservation would be seriously retarded. To keep abreast with pollution problems, the Service is represented on interagency committees which consider water-development planning. We are also represented on a National Research Council Committee which studies and reports on the effects of radioactive wastes on aquatic life, and on the Interdepartmental Committee on Pest Control.

In our investigations of water-development projects proposed for construction by the Federal Government or by private interests under Federal permit or license, we attempt to recognize existing pollution problems and consider the possible influence which the project will have on existing pollution conditions and the consequent effects upon fish and wildlife. After consulting the States, the Public Health Service, and others, recommendations aimed at avoiding future detrimental effects on these resources are made. In many cases it is difficult to identify a water-development project as a direct cause of pollution, although the operation of these projects would clearly worsen the existing problem. The Roanoke River in North Carolina can be cited as an outstanding example of the need for industry and State and Federal Governments to join forces in conquering a severe pollution problem created by wastes from paper mills and municipal sewage, and aggravated by regulation of flow and by the low oxygen content of water released at the Roanoke Rapids Dam. Among other effects, the striped bass fishery is threatened. Here, the forces have joined together to make studies of the situation, and we are hopeful that the many competing uses for water can be satisfied through an abatement program and modification of reservoir operations.

While on the subject of water-development projects, I should like to point out that some projects threaten fish and wildlife resources in certain coastal areas. By changing flow regimen or by re-routing waters, the usefulness of certain marsh areas for waterfowl, other wildlife, and fish may be destroyed as a result of saltwater intrusion into fresh-water areas. In other instances, estuarine environments may become less saline as a result of increased flows of fresh water and seriously affect fish and shellfish production.

A type of pollution that has become one of our most pressing problems is that resulting from the increased use of insecticides, herbicides, and fungicides. It is estimated that farmers, home gardeners, and government organizations charged with control of noxious insects and plants now purchase 700 million pounds of these basic materials each year. This represents about 3 billion pounds of finished pesticides that are sprayed or dusted annually on millions of acres of the Nation's crop, forest, range, and marsh lands.

Aquatic organisms, particularly fish, crabs, and insects, are among the most sensitive of all animal life to poisoning by pesticides. Insecticides, especially the chlorinated hydrocarbon group, offer the greatest hazard. Herbicides are generally less toxic, but in addition to direct poisoning they destroy both aquatic and terrestrial food and cover plants. Other groups of pesticides, such as fungicides and rodenticides, present much less danger under conditions of use.

Our wildlife research on the biological effects of pesticides has been confined principally to the effects of insecticides on wildlife species inhabiting marsh areas. Considerable of our attention in these studies has necessaril been devoted to determining effects of these materials on lower forms of life because of the importance of many invertebrates as food for birds and mammals.

For example, fiddler crabs comprise about 90 percent of the summer diet of clapper rails in New Jersey salt marshes. Cooperative investigations conducted with mosquito control agencies in Atlantic coastal marshes have shown that single applications of 0.4 to 0.5 of a pound of DDT in one-half to one gallon of No. 2 fuel oil per acre produced moderate to severe but temporary losses of fish, crabs, and other invertebrates, while applications of 0.2 to 0.3 of a pound of DDT in one-half to one gallon of No. 2 fuel oil per acre repeated several times a summer for several years caused significant, more lasting damage to most of these forms. There was little direct harm to birds and mammals although long-term routine spraying operations appeared to reduce carrying capacity for these higher animals. The studies also revealed that DDT in granules caused less damage than equivalent dosages in oil solution. Of the newer insecticides, dieldrin, aldrin, and lindane were more toxic than DDT to crabs.

Experimental feeding of these chemicals, including DDT and strobane, to quail and pheasants decreased the number of eggs produced and reduced the hatchability and viability of the eggs. The viability of the young was also reduced. These effects were observed when concentrations in the diet were as low as 1 ppm of aldrin, 5 ppm of dieldrin, 50 ppm of strobane, and 100 ppm of DDT. It has been reported that application of 1 pound of these chemicals per acre under agricultural practices would result in concentration of 50 ppm in seeds and vegetation which may be eaten by wildlife.

The Service has recently been requested to participate in an investigation of fish kills in the Upper Yellowstone River area which possibly resulted from DDT spraying operations for the control of the spruce budworm. In 1955, a high mortality of whitefish and a lesser mortality of trout and suckers occurred in the stream several months after spraying operations in July. Destruction of fish food organisms resulting in fish starvation may be a contributing factor. Although similar spraying in previous years did no apparent damage, this serious incident signals the need for studies which will give us a better understanding of DDT formulations and their effects on fish and wildlife.

Pollution from industrial sources includes a wide variety of substances which are lethal or harmful to fish and wildlife. Among the serious offenders are the wastes from paper and pulp mills, sawmills, chemical manufacturing plants, textile plants, mines, oil fields and processing plants, sugar beet processing plants, meat packing plants, dairy product processing plants, leather processing plants, and many other operations.

The Service has no continuing research program to study the lethal or undesirable characteristics of these wastes upon fish and wildlife, so we look principally to the research studies of others for this type of information. Some of this information is sought by the States under the Federal Aid in Fish Restoration programs which the Service administers. Most of these studies, however, are more in the nature of general pollution surveys.

From about 1941 to 1946, the Service conducted surveys of some of the sources of pollution of this nature and made studies in an effort to determi minimum quantities which fish could withstand. I believe most of you are aware of the difficulties in setting up standards which would apply to a variety of conditions.

This spring, studies by the Service are being initiated in Kentucky in cooperation with the U. S. Public Health Service, the U. S. Geological Surve and the State of Kentucky to determine, under controlled conditions, the effects of coal strip-mining pollution on stream ecology. Since this polluti has a definite limiting effect on the productivity of the aquatic environment these studies will serve as a basis for developing recommendations to protect or improve fish and wildlife habitat which may be, or is, affected by such operations.

There are numerous reports of the deleterious effects of mine wastes upon fish and wildlife. Annually, thousands of waterfowl seeking aquatic food and fish fall victim to poisoning. Such a situation occurs regularly in the Coeur d'Alene River in Idaho. The ore deposits in the area are primarily sulfides, chiefly of lead and zinc associated with deposits of silver, cadmium, bismuth, arsenic, antimony, and iron. Sulfides of various heavy metals become even more dangerous when they change over to other compounds. Little progress has been made during the past 30 years in getting the pollutors to remedy these hazards. In addition, mine slimes have creat less productive aquatic environment in the river and in Coeur d'Alene Lake The pollution situation was studied and reported on by the Service in 1932 and we encourage a study which was inaugurated a year ago by the Coopera tive Wildlife Research Unit at the University of Idaho.

Oddly enough, even the hunter accounts for a significant amount of lead poisoning in waterfowl. Birds feeding in water areas which have been heavily hunted, swallow enough shotgun pellets to result in quick death.

Oil pollution not only destroys our marine and inland fisheries, but results in a heavy mortality of waterfowl. In many cases the oil pollution is from the discharge of barges, ships, and accidental or careless handling of oil transportation, development, and drilling operations. Waterfowl aligh ing on oil sump areas or water areas covered with oil are usually rendered flightless. Oil is also a destroyer of aquatic life including foods of both fish and wildlife. Even in minute quantities oil may impart undesirable tastes to the flesh of fish and shellfish, thus rendering them unpalatable as human food. In cases which come to our attention, the Federal and State authorities are contacted. The Corps of Engineers and Coast Guard are oft contacted in such cases relating to navigation under the Oil Pollution Act of 1924, which is administered by the Department of the Army. Oil operati on certain of our wildlife refuges are kept under close surveillance to avoi damage to waterfowl.

On many areas where serious pollution hazards of various types occur it is necessary for our game management agents to keep the waterfowl driv from these areas.

Silt and washings from mining operations are of much concern to us. Dredging and reworking of stream bottoms for gold and other minerals brings indiscriminate destruction to fishery resources and habitat. The turning over of the stream bottoms destroys important spawning and food producing areas, and silt from such operations quickly snuffs out the lives of incubating fish eggs and aquatic organisms. Salmon and trout on the West Coast and in Alaska come in for their share of punishment from these operations. It seems that solutions to some of these problems could best be solved through the withdrawal of public lands along streams to exclude detrimental mining operation. Such a procedure has been attempted, but has met with considerable opposition from mining interests.

In Alaska, fishery resources are not protected by the Alaska Pollution Act in the case of silt from mining operations. The Act specifically states that "the results of activities connected with gravel washing plants and all phases of Placer Mining Operations shall not be considered pollution within the meaning of this Act."

Service hatchery and planting operations are sometimes handicapped by silt from lumbering and other operations. Certain situations occurring on U. S. Forest lands have been remedied by reporting such matters to the Forest Service which took steps to assure that the lumber operator complie(with the terms of his contract.

Logging and pulp operations have long presented serious problems to fish and wildlife. This industry is relatively new in Alaska, where the effect are now being felt by the salmon fishing industry. A study will be conducted by the Fisheries Research Institute of the University of Washington in coope tion with the Service to determine the effects of these operations.

Municipal pollution, with particular reference to sewage, brings great concern from the standpoint of health, but it also may seriously affect fish and wildlife. In some instances small amounts of organic pollution may be beneficial to fish, but high concentrations may deplete the oxygen supply and result in fish kills or the loss of food organisms. The Service relies heavily upon the States and the Public Health Service to bring about the abatement or elimination of pollution from such sources. There have been occurrences, however, in which the Service's installations such as refuges have been affected, and the Service has encouraged cities to install treatment plants.

As you can readily see, the Service does encounter many pollution problems affecting its interests, few of which can be resolved without assistanc of other agencies. The men who come up with the answers to the technical problems deserve a lot of credit in the total effort aimed at beating pollutio

WATER QUALITY CRITERIA FOR AQUATIC LIFE

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The lakes and rivers of North America have played a very important role in the opening and development of the continent. In our Great Lakes we have a fresh water resource far surpassing any other in the world. Our rivers and hundreds of thousands of inland lakes are outstanding in their many uses and support a diversity of the most valuable fishes to be found anywhere. Perhaps it is because of this great wealth in aquatic resources, which many considered to be inexhaustible, that we have been so remiss in their protection and conservation. Their importance is only now coming to be generally appreciated. The great increases in population since 1900 and the manifold increases in the volume and variety of water uses have shown that in some areas the supply of water is definitely limited. As with other resources we find that value varies directly with demand and inversely with supply. In the western areas which recently suffered from a severe drought it was found that when local drinking water supplies dry up,water will be purchased at any necessary price, however high it may be.

What is true for drinking water is also true in some measure for aquatic life resources. We have already found that as the demand for desirable fishing increases and the supply diminishes, the amount paid for such recreation becomes greater. The development of the country has drastically reduced or eliminated fishing waters in extensive areas. Deforestation, fire, overgrazing, and unwise agricultural practices have increased surface runoff and decreased seepage causing floods, intermittent flow, the drying up of springs, erosion, silting, and the filling of stream beds. Removal of streamside vegetation has promoted bank erosion, the widening of streams, and warming of the water. Industrial and other pollution has blocked fish migration and has rendered many areas unsuitable for fish. These practices which alter or destroy the aquatic habitat are the chief cause of the decline of aquatic life resources. The surest way to eliminate a species or group of species is to destroy their habitat or produce environmental conditions unfavorable for them. The only way to maintain a species is to protect and maintain environmental conditions essential for and conducive to its growth, reproduction and well being.

Protection and conservation of aquatic life is not a simple task. Some have questioned its value and feasibility. Our fisheries are now an important resource. The commercial fisheries of the United States and Alaska have an annual production of almost five billion pounds (1). This crop represents a high protein resource which can be expected to increase in value. While commercial fisheries utilize a great deal of equipment and employ large numbers of people, their economic worth is only a fraction of that of spor fishing. In 1955 there were more than 17 million licensed fisherman in the United States. There are several million additional fisherman who fish in the Great Lakes and in marine waters where a license is not required. According to the National Survey of Fishing and Hunting (2), sport fishing in the United States has an annual value of almost two billion dollars. Within the next 100 years the number of fishermen and the value of the fishery will increase severalfold if the resources can be preserved.

In comparing the beneficial uses of a stream, there has often been a tendency to underestimate the wildlife and recreational values and to take a short rather than a long view. When evaluating our fisheries resources we should consider the returns not just for one year but over the centuries because these resources are renewable. The aesthetic, recreational, and health values of our waters are difficult to measure, but they are great. The recreational industry is a large one and is expanding every year. In a few states it is the first ranking industry and in many others it is of considerable importance. In the industrial state of Michigan it is reputed to rank second. As our population increases there will be an ever growing demand for and use of our forests, parks, preserves, wilderness areas, and streams, where people can engage in water sports and "get back" to nature and relax. It is believed that our aquatic life resources and the aesthetic value of our lakes and streams, which are largely inseparable, are well worth our earnest and sincere efforts to preserve them.

The objective of water quality criteria for the protection of aquatic life is to preserve or restore environmental conditions essential for its growth, reproduction, and well being. If these requirements are known and understood, criteria can be set up which will achieve this objective. If habitat requirements are not fully known, criteria can only be based on the best information available and changed whenever the need is indicated by new information.

Under our present state of knowledge a suitable water cannot be defined in chemical terms alone. There are several reasons for this situation. Different species of fishes and the organisms in their food chain vary widely in their sensitivity to dissolved materials. We do not know the effects on aquatic life of various concentrations of many materials individually or in combination. Mixtures of materials often have effects different from those of the individual components. Further, we do not know minimal lethal levels for many materials or their mixtures, nor do we know the most favorable concentrations of materials essential for the organisms. Perhaps the best definition that can be given of a suitable fish habitat is -- "A suitable fish habitat is one which produces a satisfactory fish crop." The adequacy of a fish crop is judged by its quality and the pounds produced per unit of surface area. Commonly, productivity and suitability are judged by catch per unit effort, growth rate, condition factor, quality of the flesh, and the size and species composition of the catch. In fisheries management an effort is made to manipulate the environment so that conditions are made more favorable for the desired species and less favorable for those not wanted.

While our knowledge of the habitat requirements of fishes is far from complete, enough information is now available so that some criteria and procedures can be set up which will be of value in the maintenance of a satisfactory environment and production of a suitable crop. However, much research is still needed to obtain all the information essential for the solution of this problem.

The environmental requirements of fishes may be roughly grouped under four main headings, (1) a favorable water supply; (2) suitable spawning facilities; (3) an adequate food supply for all age groups; and (4) good pools and shelter. In the abatement and control of pollution we are chiefly concerned with the first requirement, a favorable water supply. Natural waters have widely varying physical and chemical properties. The suitability of any water for fish life depends on its quantity, permanency, and quality; that is, its temperature, the concentration of dissolved atmospheric gases, salts and other minerals, and suspended solids.

As here used water pollution is the addition of any material or waste to a water in such quantities that it interferes with, lessens, or destroys a beneficial use. In this regard perhaps the simplest definition of pollution is "too much." For example, if "too much" is not added, the discharge to a stream of organic matter such as sewage has a fertilizing effect which is beneficial to fish production. However, when the capacity of the stream to utilize organic materials is exceeded so that unfavorable environmental conditions are produced and a beneficial use is damaged, such a discharge becomes pollution. It is evident, therefore, that water quality criteria for the protection of aquatic life must entail some quantitative measurements. Before criteria can be set up we must know or have some measure of how much is "too much" for those species we wish to protect at all stages in their life history and in waters of different quality. These criteria must insure environmental conditions favorable to all life activities and to general well being--mere survival is not enough. These environmental factors will be discussed in some detail.

Environmental Factors

Temperature

As a group, aquatic animals of the temperate zone are adapted to fluctuations in temperature between 39° and 90°F. Not all can withstand this range and some can withstand higher and some lower temperatures for a time. The range of temperature which can be tolerated by different species varies considerably as does their ability to withstand sudden changes or to acclimate themselves to unusual temperatures. Each species has a preferred range of temperature within which it does best and a zone above and below this in which it can survive for short periods. Proper acclimatization enables certain species to survive at temperatures which would be fatal under conditions of sudden exposure.

Fish have a rapid rate of adaptation to high temperatures (3) but adaptation to lower temperatures proceeds at a much slower rate. When suddenly exposed to higher temperatures fish can withstand much higher temperatures in summer than in winter. As the summer season develops, changes in upper lethal temperatures reflect the major changes in water temperature, rising with ascending temperatures and falling as the water cools in the fall. Brett (3) reports that the lethal temperatures for the bullhead rose from 29.1°C. on May 12 to 35.3°C, by July 8. Brett (4) also found that there is a considerable difference in the time required for completion of acclimation with respect to heat tolerance at each level of temperature. It has been shown that some species exhibit geographic differences in their resistance to high temperatures while others do not (5). The writer noted a bass kill in a southern Michigan lake in June 1936 when the water reached 94°F during an unusually hot period. However, a temperature of 96°F. in Wheeler Reservoir in 1938 appeared to have no lethal effects. The difference may have been due to a different acclimation history. It was observed that a temperature of 108°F. killed all the fish in a pond near Savannah, Georgia, in 1945. Allowable peak temperatures brought about by some unnatural cause may, therefore, be somewhat different in different portions of the country, increasing from north to south.

Members of the family Salmonidae are cold water forms. Brook trout seem to do best in streams, the summer temperatures of which range between 52 and 68°F. (6) (7). While trout can survive much higher temperatures for short periods, streams having such temperatures are not first class trout streams. The writer found brown and rainbow trout surviving a peak temperatn of 83°F. in the South Branch of the Pere Marquette River of Michigan in 1930. Brook trout survived peak temperatures of 81° and 82°F. in the East Branch of the Black River on successive days in July 1931. Fry (8) reported the upper lethal temperature for young brook trout (12 to 14 hours exposure) to be about 77.5°F. Such high temperatures are more favorable for minnows and suckers which increase greatly in numbers and compete for food and space with the trout (9). The result is that trout comprise a very small portion of the total fish population of the stream and supply little fishing even though the overall productivity of the stream may be great.

Four fish population studies made in the East Branch of the Black River of Michigan indicated that trout made upon only 9.6 percent of the total number of fish taken in the study areas. In the neighboring Pigeon River, another stream having temperatures above 75°F., trout comprised 15 percent of the total fish population in the areas counted (9). In a nearby cold stream, the West Branch of the Sturgeon River, trout represented 96 percent of the total population. In trout streams having high peak summer temperatures, suckers and minnows comprise the bulk of the fish population. Thus, while temperatures higher than the optimum and high temperatures of short duration (75° to 82°F.) may not kill trout, they produce environmental conditions more favorable for the coarse fishes, which increase at the expense of the trout population. This fact must be taken into consideration in the establishment of temperature criteria for the cold water species. Such criteria must be based on optimum conditions and not on temperatures tolerated by trout. It is believed that for good trout production in streams subject to invasion by coarse species, temperatures should not exceed 68°F.

Since deforestation, overgrazing, unwise land use, removal of streamside shade, and erosion have already caused the warming of streams to such an extent that the amount of trout water has been seriously reduced, and since trout fishing is in highest demand, it is believed that no wastes of significant heat content should be discharged into a trout stream if the stream is to be maintained for trout.

Favorable temperatures are especially important at spawning time for both cold and warm water species. It is well known that bass spawn in the spring when the water temperature exceeds 60°F. If the water is unnaturally warmed to this temperature for a period, spawning may be induced too early in the season. Then if waste discharges are discontinued over a weekend, water temperatures may drop into the 50's with the result that guarding males leave the nests, the eggs are infested with fungi, and no young are produced. Fluctuations of water temperature above and below 60°F. during the spring are detrimental to bass production.

A change in water temperature may affect the aquatic fauna directly or indirectly. While the change may be within the thermal tolerance of the fish, it may so alter environmental conditions that they become unfavorable for essential food organisms and certain life history stages of the fish, or the change may make them more favorable for competitors or predators. Temperature changes will directly affect the metabolic rate, growth, and reproductive processes. They may result in increased or decreased food production, interfere with spawning, or change an important part of the fauna, thereby altering the quantitative makeup of the population.

Although high summer temperatures have been considered of outstanding importance because of their possible lethal effects, it is believed that unnaturally high winter temperatures may be equally important. In the temperate zone the aquatic biota have evolved under conditions of quite wide differences in seasonal temperatures. For example, the eggs of some daphnia have to be chilled or frozen before they hatch. Many other organisms go through resting stages or specific stages of development at certain seasons. Some of the diatoms, for example, are abundant only at temperatures below 50°F. Other forms appear only at certain times of the year and there is a succession of forms with the seasonal changes. At present we have little conception of the changes which might be brought about by permanently elevating stream temperatures. A large portion of the biota might be changed and the whole food chain disrupted. For this reason consideration should be given to upper temperature limits during the winter season. This consideration may require increasing attention as the atomic energy industry develops. Temperature should not be raised to levels that induce spawning at unnatural times if there are periodic drops in temperature, and they should not be such that they interfere with the development of important organisms in the fish food chain. Considerable study is needed before this problem can be approached intelligently.

Waters of significant heat content should not be discharged into a stream in such a manner that they create a temperature block across the stream. Further, an abrupt change of more than 9°F. may affect fishes adversely even if of short duration.

For a well rounded warm water fish population in the Ohio valley area it is believed that peak summer temperatures should not exceed 93°F. at any time or place. In the south such peak temperatures probably should not be above 96°F. This means that in general temperatures will be considerably below these levels. While several species can withstand higher temperatures (100° to 103°F.) for very short periods, 93° and 96°F. represent critical levels for most species in the designated areas. Further, while fish may, through certain adaptations, survive abnormally high temperatures for short periods, they cannot complete their life history at such temperatures. For good producti therefore, temperatures within a favorable range are required.

Settleable Solids and Turbidity

Studies carried out in connection with trout stream improvement investigations in Michigan indicated that sand bottoms are almost barren of benthic organisms and that the addition of sand or silt to rubble or gravel bottom streams greatly decreases stream productivity (9) (10) (11). In fact, shifting sand in quantities so small as to be unnoticed by casual observation can decrease the production of macro-invertebrates by drifting into the spaces between the gravel and thereby decreasing the areas for attachment and cover. It is believed that no inert inorganic, sandy, or other similar wastes should be added to a rubble, gravel bottom stream as such deposition may not only decrease the supply of desirable stream bottom insects but also seriously limit spawning of most nest-building fishes. Studies of the effects of mining wastes in California have shown that salmon select clear water for spawning and that the deposition of silt results in smothering of the eggs (12). Quantitative bottom samples taken in a series of similar streams in California showed that the average number of food organisms was always less in mined areas when inert inorganic materials were discharged to the stream than in nonmined areas (13). On the Scott River, samples from the silted area averaged 36 organisms per square foot, whereas those from the clean stream bottom above the mine averaged 249 per square foot, or 7 times as many. Similar studies (14) have shown that hydraulic mining wastes are detrimental to salmon and trout production. These and wastes from placer mining and from stamp mills and washing operations may completely choke a stream causing it to flow in a shallow sheet over the accumulated deposits. Further, debris dams created by such operations can eliminate the salmon by blocking migration.

From results of studies in various parts of the country, it is apparent that erosion silt is a major stream pollutant and that it produces environmental conditions unfavorable for the reproduction and growth of fishes. Since the character of the stream and its bottom are of prime importance in determining the harmful effects of erosion silt, it is not possible to establish numerical criteria for settleable solids which are universally applicable. In some streams considerable amounts do very little additional harm, while in gravel rubble bottom streams even small amounts, as has been noted, reduce food production and limit spawning. It is believed that criteria on settleable solids should be established to protect environmental conditions in the stream, though they will vary from stream to stream, depending on local conditions.

Turbidity is usually due to solids which settle out slowly or to colloidal materials which may remain in suspension over long periods. The studies of Irwin (15), Wallen (15A), and others at Oklahoma A and M have shown that turbidity must be very high before it exerts a directly harmful effect on fishes. In some tests (15) direct reactions to turbidity did not appear until it reached 20,000 p.p.m. and for one species not until it reached 100,000 p.p.m. Most individuals of all species endured exposure to more than 100,000 p.p.m. for a week or longer but finally died at turbidities of 175,000 to 225,000 p.p.m. Fishes which succumbed to these turbidities had the opercular cavities and gill filaments clogged with silty clay particles.

In Oklahoma, Buck (16) carried on pond studies to determine the effects of turbidity on growth rate. At the end of two growing seasons the average total weight of fish in clear ponds was about 1.7 times that of those in ponds of intermediate turbidities and approximately 5.5 times greater than those in muddy ponds. Of the three species used, large mouth bass were most affected by turbidity. The effect on plankton production was even more striking since the average volume of net plankton in clear ponds during the 1954 growing season was 8 times greater than in ponds having intermediate turbidity and 12.8 times greater than the yield in most turbid ponds. However, catfish survived better in muddy ponds. Game fish feed by sight and in turbid waters they are at a disasvantage when competing with such fish as carp, buffalo, and carp suckers which employ a vacuum cleaner type of feeding. Turbidity can, therefore, bring about a quantitative and qualitative change in the fish fauna. In addition, metallic or sharp particles may kill fishes by causing abrasive injuries to the gills or by clogging the gills and respiratory passages.

Suspended solids and turbidity prevent light penetration, decrease photosynthesis, and thus limit algal production. Since algae are the basic material in the food pyramid, turbidity adversely affects fish production in an indirect manner. In most streams settleable solids and turbidity are largely due to soil erosion. Until erosion is brought under control, little can be done toward clearing up the streams. Reduction of turbidity is a difficult and long time problem which must be carried out in cooperation with soil conservation, agricultural, and forestry interests. In the meantime, however, efforts should be made to control or eliminate other sources of settleable solids and turbidity. Lagooning can be effectively used to remove settleable solids and turbidity from many wastes. Such procedures are essential on all clear streams and they should be initiated in conjunction with efforts to reduce turbidity and settleable solids through control of soil erosion.

Turbidity standards must be somewhat local in their application as they will depend on the area and type of stream. It is possible to set up relatively simple turbidity standards which can be readily checked for compliance by field tests. Turbidity standards might state that a certain percentage of the incident light at the surface shall reach a stated depth betweel 11:00 A.M. and 1:00 P.M. The depth selected would depend on the depth to which the regulatory agency felt the photosynthetic zone should extend. Different types of water differ in their capacities to absorb light. Water transparency is affected by the suspended matter, including the plankton, and by stain or color. In water of the clarity of usual municipal supplies, 9.5 percent of the solar energy present at the surface reaches a depth of 6 feet. Born (39) states that the limit for growth for the higher aquatic plants lies between 2.5 and 3.5 percent of the total surface energy at bottom depth, but that it rapidly declines below 4 percent where severe etiolation occurs in submerged seed plants. There is some evidence that certain algae can grow at levels of 1 percent of the incident light, but it is not definitely known how much light is required for them to produce more oxygen by photosynthesis than they use in their respiration. While criteria will vary with the area they can be kept relatively simple. For example, a criterion for a particular area might state -- under conditions of brilliant sunlight at or near noon 4 percent of surface incident light shall reach a depth of 6 feet. Incident light and light at any given depth can be readily read by means of a photometer fitted for underwater use.

pH

The pH of a water may exert a direct effect on fish if it is very high or very low due to strong bases or mineral acids. It may have an indirect effect through its influence on the toxicity of certain materials such as HCN, H_2S, ammonia, heavy metals, etc. Longwell and Pentelow (17) found that the toxicity of NaS solutions to brown trout was influenced markedly by variations in pH, the toxicity increasing as the pH became lower. The heavy metals are considerably more toxic at lower pH levels probably because they are more soluble. Ammonia becomes rapidly more toxic as the pH is raised above 8.2. The toxicity of a number of weak inorganic and organic acids, including hydrocyanic, hypochlorous, hydrosulfuric, carbonic, and tannic, is increased by lowering the pH.

Extreme pH values of 4 and 10 or slightly above have been tolerated by resistant fishes in certain areas. Some levels at which fish have been killed experimentally are: trout, 9.2; bluegills, 10.5; roach, pike, carp, and tench, 10.4 to 10.8. Fish mortality has been observed within a few hours at pH levels of 3.4 to 4. However, certain fish have been acclimated to live for considerable periods at pH levels as low as 4.5 to 4.2.

Studies of acid bog lakes (18) have shown that yellow perch, brown bullhead, bluegill, and pike can live at a pH of 4.4. Ellis (19) states that the pH of streams generally ranges between 7.4 and 8.5 with an overall range of 6.6 to 9.0, while bog streams and lakes vary from 4.0 to 6.0. He states that in most uncontaminated freshwater streams pH values range from 6.5 to 8.5.

Sudden or wide fluctuations in pH are undesirable. While fish can withstand pH levels as high as 9.5, it is undesirable to have the pH maintained continually between 9 and 9.5 when this level is due to the addition of caustic wastes. Such pH conditions are entirely different from and more harmful than the naturally occurring but brief higher levels which may be as high as 10 or 10.5. These natural high pH levels are produced by photosynthesis due to the removal of CO_2 and they are always accompanied by high D. O. levels. High pH interferes with oxygen uptake of some marine and freshwater fishes and may limit their ability to survive at low oxygen tension (20). At values below 5 and above 9, the pH seriously affects the ability of some fishes to extract oxygen from the water. This ability varies with the species; with bass and crappie the pH can be lowered to almost 4 before their ability is affected (21) (22). In general, fish are able to extract oxygen best at pH levels from 7.0 to 8.5, but such fish as perch, bass, crappie, goldfish, trout, and green sunfish have a wide range of tolerance. The bluntnose minnow and one of the shiners, Notropis whipplii, were found to be very sensitive as they can extract oxygen best at pH 7.0 to 8.0 (20). Some fishes can survive rapid changes in pH. Laboratory studies (21) have indicated that goldfish withstood changes from 7.2 to 9.6, black bass from 6.6 to 9.3, and sunfish from 7.2 to 9.6. The amount of dissolved oxygen is a determining factor as to whether or not these changes can be tolerated (21).

In the range from 5 to 9.5, pH as such has not been shown to be detrimental to fishes. However, changes in this range can drastically affect the toxicity of certain materials, and they also influence the ability of fish to absorb oxygen from the water. Further, it has been noted that in the more productive streams pH usually falls in the range from 6.5 to 8.5. At pH levels above and below these values some of the essential minerals become unavailable. Thus, while pH in the range from 5.0 to 9.5 is not in itself directly harmful to fishes and this range may be used in setting up water quality criteria, from the standpoint of productivity, it is recommended that every effort be made to keep pH values in the range of 6.5 to 8.5.

Dissolved Oxygen

There are a host of environmental and other conditions which influence or determine the solubility of oxygen in water, the amount of dissolved oxygen favorable to fish life, and the minimum amount needed for existence. In fresh waters, temperature is the most important factor affecting the solubility of oxygen. Dissolved solids are rarely present in sufficient amounts to have an appreciable influence. Several environmental conditions may influence the optimum amount of oxygen required by fish or interfere with the obtaining of oxygen by the fish or may change or increase their minimum need for oxygen. Among these are temperature, pH, CO_2, and dissolved solids.

Temperature increases within the range favorable to fish are accompanied by a progressively higher metabolic rate and a continuous increase in the oxygen uptake. Wiebe and Fuller (23) found that at 25°C. the oxygen consumption of largemouth black bass was 282 percent of that at 15°C. At 20°C. it was 177 percent of the consumption at 15°C. This is in accord with the van't Hoff law which states that for any chemical change the rate of reaction is increased between 2- and 3-fold for every 10°C. increase in temperature. Temperature is of outstanding importance in the determination of environmental requirements since the oxygen consumption increases as temperature rises whereas solubility of oxygen decreases. Because the annual range in temperature of streams of the temperate region may be as much as 28°C., oxygen consumption at peak temperatures may be several fold what it is at minimum temperatures, whereas at peak stream or lake temperatures the water will hold only about half as much oxygen as it does at minimum temperatures. Graham (24) found that for speckled trout the rate of oxygen uptake increased with increasing temperature up to the ultimate upper lethal temperature, if sufficient oxygen were available. Water containing less than 75 percent of the air saturation level of oxygen reduced the activity of speckled trout at all temperatures, and above 20°C. (68°F.) fully saturated water is required to allow the full scope of activities. Several other investigators have also found that the oxygen requirements of fishes become greater with increases in temperature (25) (26) (27).

Temperature also markedly affects dissolved oxygen concentrations with are lethal to various species of fish. Burdick (28) found that smallmouth bass died in 5 to 9 hours at oxygen concentrations of 0.7 p.p.m. to 1.17 p.p.m. at temperatures of 52°F. to 72°F. There was also some variation in the turnover time for different species of fishes. At 55°F. and oxygen concentrations of 1 to 2 p.p.m. the turnover times were as follows: brook trout, 1-3/4 hours; brown trout, 2-1/2 hours; and rainbow trout, 3 hours. At 69°F. to 71°F. these fishes turned over in approximately the same time at oxygen concentrations of 2.3 to 3.4 p.p.m.

Several other environmental factors also interfere with oxygen uptake or increase the oxygen requirements of fishes. High and low pH levels interfere with the ability of fishes to absorb oxygen from the water. High CO_2 concentrations interfere with the utilization of dissolved oxygen. Fry and Black (29) found that the common sucker, with its CO_2 sensitive blood, was unable to remove oxygen from water containing CO_2 tension which did not hinder the respiration of bullheads, the latter possessing blood with a very low sensitivity to CO_2. Under pollutional conditions fish generally require more oxygen (45) (46) (20). At low dissolved oxygen levels fish succumb to concentrations of toxic materials which they can tolerate at high dissolved oxygen levels.

Many studies have been made in attempts to determine the lowest D. O. levels tolerated by different species of fish. Gutsell (30) reported that some brook trout could endure, for a short period, an oxygen concentration as low as 1.2 p.p.m.; however, some asphyxiation occurred at a D. O. content of 2.5 p.p.m. Smallmouth black bass lived for a time at 0.4 p.p.m. D.O. Wiebe (22) found that some fish can withstand sudden wide changes in the concentration of oxygen and that they can live in water supersaturated with oxygen. The increase in D.O. was followed by a slowing down of the respiratory movements. Fry (31) states that at 49°F. the ultimate minimal tolerance of brook trout for dissolved oxygen is 0.9 p.p.m. Gardner and King (32) reported the asphyxial level of trout to be 1.1 p.p.m. D.O. at 6.5°C. and 3.4 p.p.m. at 25°C. Thompson (33) on the basis of field studies, reported that carp and buffalo lived in water having 2.2 p.p.m. D.O. However, he found a variety of fishes only when there was over 4 p.p.m. of oxygen and the greatest variety of fishes were present when the D.O. was 9 p.p.m. He found that fish died overnight in water containing less than 2 p.p.m. D.O. Ellis reported (19) that goldfish, perch, catfish, and other species of freshwater fishes when maintained in water of constant flow, composition, and temperature (20° to 25°C.) showed respiratory compensation in both volume and rate when the dissolved oxygen was reduced to slightly below 5 p.p.m.

In addition to those environmental conditions which influence the oxygen requirement, there are several physical, chemical, and physiological conditions which influence the ability of fish to extract oxygen from the water, its need for oxygen, and its ability to resist low oxygen levels. First, it must be realized that ability to extract oxygen from the water and to resist low D.O. levels varies with the species. It is well known that dogfish, carp, and gar can survive at much lower D.O. levels than trout and several other fishes. Some fishes are more efficient in the extraction of oxygen or their blood is not as sensitive to the presence of CO_2.

The amount of oxygen required by fishes is determined in part by activity. It is generally recognized that a man lying in bed does not breathe as deeply or require as much oxygen as one digging a ditch. It has been reported that from two to four times as much oxygen is required by a fish when it is active as when it is quiescent (24) (26) (34). Under actual stream conditions a fish must maintain its position against the current, find, pursue, and catch its food, avoid its enemies, and reproduce. All these activities require oxygen in such amounts that D.O. levels at which the fish can just survive are unsatisfactory. Age, size and season are also of importance. In general, fry and younger fish have a higher metabolic rate and require more oxygen than adults (35) (36). Because of increased activity and their physiological condition fish require more oxygen at the spawning season. Studies carried out in our laboratories indicate that D.O. requirements are different at other times of the year and further, the physical condition of the fish is of outstanding importance in determining requirements and the minimum level tolerated. An actively feeding, rapidly growing fish requires considerable more oxygen than one which feeds very little. Since growth is rapid in the fry to fingerling stage it is expected that for many species D.O. requirements will be higher at this period. Eggs deposited in bottom materials require higher D. O. concentrations than do adult fish. Since the current through the bottom materials is slow, the amount of water flowing by the eggs per unit of time is small and thus it must contain more D. O. to provide needed requirements.

Through acclimation, resistance to low D. O. levels may be increased. Fry (31) reports that through acclimation the lethal dissolved oxygen level can be reduced to about one-half the corresponding value for trout accustomed to air-saturated water. Lower dissolved oxygen levels can be tolerated for considerable periods through an increase in respiration rate and volume of water pumped, reduced activity and food consumption, and an increase in blood haemoglobin (37) (38). By means of such adaptation fishes may live for considerable periods at reduced oxygen concentrations without apparent harm. This does not mean, however, that they can complete their life cycle at such levels. Further, ability to live more or less indefinitely at low oxygen levels does not mean that some of their physiological processes have not been altered so that their well being and growth are adversely affected. It has been reported (4) that the bullhead is unable to become acclimated to increased temperature when D. O. levels are low whereas it becomes rapidly acclimated at normal D. O. levels. Dissolved oxygen levels adequate for growth, reproduction, normal activities, and well being are considerably higher than levels which can be tolerated for extended periods through acclimation and compensation.

Studies of the oxygen requirements of fishes fall into two categories: laboratory investigations, where as many as possible of the variables are controlled, the factor under study is varied, and the effects on fishes directly observed for a relatively short period; and field studies, where the variable in question is measured in different sections of the stream and is related to the fish population in various areas. Both types of study have certain advantages and disadvantages. It is very difficult to relate laboratory results to field conditions, while in the field studies, factors other than the variable in question (dissolved oxygen concentration) might have a bearing upon results. It is believed that the best approach is to carry on both laboratory and field studies so that they supplement each other. In the interpretation of laboratory findings, it must be recognized that fish are usually held under favorable conditions and it is necessary to realize that all findings are not applicable to natural conditions.

The Lytle Creek studies (40) and other field studies have indicated dissolved oxygen concentrations at which fish and their food supply can maintain themselves. Twenty-four hour studies were made on Lytle Creek at all seasons of the year at selected stations to determine D. O., CO_2, pH, and temperature. Such studies or a continuous record of dissolved oxygen are essential for investigations of D. O. requirements as there are great diurnal and seasonal variations in oxygen concentration. Fish populations and growth rate studies were made over a two-year period (41) (42) in order to relate them to environmental conditions and their seasonal variations in in different portions of the stream. Uncontrolled variables encountered in stream studies usually make it difficult to be certain that differences in fish populations are caused by oxygen concentration alone. However, it is believed that variations in oxygen concentration were the important variable in Lytle Creek since fish appeared first in the riffles of the upper zone of recovery and were found first in the pools much farther downstream. Since fish were not present in the pool immediately below the riffles or between them, it is believed this difference is due to D.O. as other limiting factors probably would not change so rapidly. In streams having a considerable biological oxygen demand there are marked differences in D.O. in the pools and riffles. In studying a section of the Scioto River, it was found that the D.O. at the tail of a large pool was 0.1 p.p.m. while about 200 yards downstream, water which had passed over a wide shallow riffle on one side of the stream contained 5.6 p.p.m. oxygen. Some 20 feet from the riffle in the main flow of the river there was 2.5 p.p.m. of oxygen.

In streams polluted with organic wastes, toxic materials such as H_2S, NH_3, and CH_4 may be formed by anaerobic decomposition. The H_2S may escape or be fairly rapidly used by certain bacteria such as Beggiotoa, Thiothrix, and Sphaerotilus (43). Usually much of the NH_3 is converted to NC and both of these materials are rapidly utilized by the dense growths of algae in the recovery zone (44). Most of the CH_4, which is not very toxic, escapes a gas. Thus, while toxic materials may be formed, it is possible that they do not exert a marked effect on the fish.

Determination of the oxygen requirements of fishes and establishment of suitable dissolved oxygen criteria are especially difficult tasks. A great many studies have been made of the oxygen requirements of fishes. Investigat have not always used a uniform approach. In fact, there has been great diversity in the species studied, the conditions under which they were studied, the experimental methods used, the objectives of the study, the caliber of the investigation, and the interpretation of results. Consequently, data obtained have varied widely and have not always been in agreement. Short time studies carried out in aquaria at low temperatures with resistant fishes which are not fed indicate only that certain fishes can survive very low concentrations of dissolved oxygen for limited periods. It should be recognized that these levels are not adequate for normal existence or completion of the life history of all the important fishes. In setting water quality criteria for the protection of aquatic life, it must be recognized that mere survival is not enough and that the minimum dissolved oxygen level should be one suitable for the continuous maintenance of a satisfactory fish crop. Minimum D.O. levels at which some species of fish can, through adaptation, resist death by asphyxiation for a time are not adequate for completion of the normal life cycle. Oxygen levels must be continuously adequate for the general well being of the fish and the maintenance of fish food organisms. Before adequate criteria can be established it is essential, therefore, to know the environmental requirements of the fishes since the objective is to provide suitable conditions for them.

Concentration of dissolved oxygen is often expressed as weekly, monthly, or sometimes daily, averages. Such values are not satisfactory as they do not indicate environmental variations and may actually be misleading from the standpoint of the continued existence of the fish. It is the extreme variations which may become limiting and which are the most important for indicating unfavorable habitats.

Some D. O. criteria have been set up as percentages of saturation. This procedure is deemed undesirable because over the range of temperature observed in our natural waters, 50 percent of saturation may mean 7. 3 p. p. m. oxygen or 3. 5 p. p. m. As temperature increases the amount of oxygen which can be held by the water decreases, whereas the amount required by the fish increases. It is believed that criteria for dissolved oxygen should be expressed in parts per million by weight.

Findings in Lytle Creek have indicated that in a stream section in which the oxygen concentration is usually above 5 p. p. m., the occurrence of concentrations below 5 p. p. m., but not below 3 p. p. m. for a few hours, does not have an adverse affect upon a well rounded warm-water fish population Minnows and other coarse fishes were found in the section where minimum D. O levels dropped to 2 p. p. m. or slightly below. On the basis of these studies and other pertinent data it is believed that for a well rounded warm-water fish population, dissolved oxygen concentrations must not be below 5 p. p. m. for more than 8 hours of any 24-hour period and at no time should they be below 3 p. p. m. For the maintenance of a coarse fish population dissolved oxygen concentrations should not be below 5 p. p. m. for more than 8 hours of any 24-hour period, and at no time should they be below 2 p. p. m.

The salmonoid fishes are not usually found in streams where minimum dissolved oxygen concentrations are lower than 4 to 5 p. p. m. For normal feeding and adequate growth at least 5 p. p. m. dissolved oxygen are required. Successful development of eggs and fry require a minimum of 6 p. p. m., while for the full range of activity for brook trout and perhaps for other members of the family, 7. 6 p. p. m. are required at 15°C. and full air saturation at 20°C. and above (31). It is believed, therefore, that for good salmonoid production dissolved oxygen concentrations should not be less than 6 p. p. m.

Carbon Dioxide

Carbon dioxide may influence the toxicity of other materials or it may in itself be harmful if present in sufficient quantities. Alabaster and Herbert (48) found that CO_2 was not toxic to rainbow trout within a 12-hour exposure at concentrations up to 30 p. p. m. but was toxic at 60 p. p. m. and that period of survival decreased as the concentration increased. The presence of CO_2 in concentrations from 15 to 60 p. p. m. was found to reduce the toxicity of ammonia. Higher concentrations are toxic; 100 to 200 p. p. m. can be rapidly fatal to moderately susceptible fresh water fishes in well oxygenated water. Fifty to 100 p. p. m. can cause distress and may be lethal. Both marine and fresh water fishes vary greatly in their resistance to CO_2.

Wells (49) reports that resistance of fishes to harmful conditions varies with the species, with age or size and weight, with the condition or physiological state of the individual, and with the season. He found that practically all the fishes with which he worked were least resistant just after the breeding season--June, July, and August--and most resistant before it--March, April, May (50). Powers (51) has shown that the ability of marine fishes to extract oxygen at low concentrations was adversely affected by moderate amounts of CO_2 which lowered the pH. The investigation of Black, Fry, and Black (52) demonstrated the influence of CO_2 on the utilization of oxygen by 16 species of fresh water fishes. It was found that oxygen in the respired water at the time of death was higher when the tension of CO_2 was increased. The ability to take up oxygen in the presence of CO_2 varied with the species. Powers and co-workers found (53) that fish are able to absorb oxygen at a low oxygen tension through a wider range of CO_2 tension than is found in the natural waters in which they live. Most workers have found that naturally occurring levels of CO_2 are not detrimental to fishes. It is believed that concentrations under 30 p.p.m. in the absence of other adverse factors will have no harmful effects on most species. The majority of investigations indicate that CO_2 becomes rapidly harmful at concentrations of 100 to 200 p.p.m. Surber (54) found that concentrations between 55 p.p.m. and 78.5 p.p.m. in hard water at pH 6.9 to 7.0 caused a decided increase in the loss of eyed eggs and the number of deformed trout fry. Concentrations up to 43 p.p.m. apparently had no harmful effect.

Dissolved Solids

Natural, unpolluted waters of lakes and streams have in solution small amounts of the anions CO_3^{--}, Cl^-, SO_4^{--}, smaller quantities of NO_3^-, NH_4^+, PO_4^{---}, and NO_2^-, and traces of many others. The metallic cations are Ca^{++}, Mg^{++}, Na^+, K^+, Fe^{+++}, Mn^{++}, and traces of several others. These materials exert a physiological and osmotic effect to which organisms have become adapted. In fact, these dissolved materials are required by the organisms. Rawson (55) found a positive correlation between the total solids in fresh waters and the average standing crop of plankton and bottom fauna. The type of rock formation and soil largely determines the concentration of dissolved solids in a water but erosion may be of considerable importance. Pollution may also be a factor. During the period from 1906-07 to 1934-43, the average amount of dissolved solids in Lake Erie increased from 133 to 165 p.p.m., whereas those in Lake Superior remained unchanged (56) (57).

Criteria for dissolved solids have little meaning if the purpose of the criteria is the protection of aquatic life, unless the materials to be considered as dissolved solids are specified. It is apparent that salts of Hg, Cu, Ag, Zn, Pb, and Cd will have a much different effect on fishes than will equal concentrations of salts of Ca, Na, Mg, and K. In general when total dissolved solids are referred to in relation to water quality criteria, it is the salts of these relatively nontoxic earth metals which are believed to be under consideration. Unnatural concentrations of these salts may affect aquatic life in two ways. If the solution of salts is physiologically unbalanced, one of them may exert a direct toxic effect. If they are physiologically balanced, that is, each is present in quantities sufficient to antagonize any toxic effects of one or more of the others, they may occur in such concentrations that they exert an osmotic effect. Wiebe (58) points out that the osmotic pressure that fish can tolerate depends to a large extent on acclimatization. Fish acclimated to the extremely soft waters of East Texas cannot survive when subjected to salinities to which the fish of the Pecos River are continually exposed. Texas rivers (58) range in total dissolved solids from 45 to 4,810 p.p.m. Wiebe found as much as 28,000 p.p.m. of chloride in a stream where fresh water fish were supposed to live. However, Young (59) indicates that when dissolved solids reach 11,000 p.p.m. only certain fish can tolerate them indefinitely. The ability to resist high concentrations of dissolved solids varies with the species. While some fishes move from marine to fresh water or from fresh to sea water, some species have been reported as being unable to resist concentrations above 3,000 p.p.m. Young reported that Na_2CO_3 in concentrations about 800 p.p.m. was unfavorable for catfish. Huntsman (60) reports that in the Quill lakes of Saskatchewan, which have a total solids content of 16,550 p.p.m., there is a resident fish population of somewhat limited extent. It is believed that total dissolved solids in concentrations up to 3,000 p.p.m. can be tolerated by most fishes if the materials in solution are the nontoxic earth metals and are physiologically balanced.

Chlorides

The amount of chlorides is often considered as a measure of salinity or of total dissolved solids. When dealing with sea water, which is fairly uniform and the composition of which is known, chlorides can be taken as a measure of salinity or dissolved solids. This does not hold, however, for oil field brines and other wastes. Oil field brines differ drastically from one another and from sea water and many wastes contain large quantities of salts other than chlorides.

The chloride ion as such does not have much significance from the standpoint of toxicity to aquatic life. The cation is so much more important that the chloride anion is not generally considered. This is especially true with chlorides of the heavy metals such as mercury, copper, zinc, etc. Even with salts of the relatively nontoxic earth metals the toxicity of their chlorides is evidently attributable to the specific toxicity of the cations present and not to any toxicity of the chloride ions. Since the cations vary greatly in their toxicity to fish and are governing in the determination of toxicity, it is obvious that the chloride ion content of a mixture of salts is not a reliable index of toxicity.

Physiologically balanced mixed salt solutions such as sea water may be harmful to freshwater organisms because of their excessive over-all salt content and osmotic pressure rather than the specific toxicity of any particular ions present. Provided that the salts and other substances dissolved in water are balanced against each other so as to exclude any individual toxic effects, certain hardy freshwater fishes can tolerate waters of osmotic pressures equal to those of their own bloods and even higher for extended periods. Other freshwater fishes, however, have been reported unable to tolerate balanced salt solutions with concentrations of 2,000 to 4,000 p.p.m. It is not known whether a typical freshwater fish can complete a normal life history in water of relatively high osmotic pressure, nor is it known how osmotic pressure affects the life processes of other freshwater organisms. It is, therefore, impossible at present to define the maximal safe osmotic pressure of a freshwater environment. Presumably, the tolerable osmotic pressure entails a salt concentration far higher than the limits imposed by industrial and municipal requirements. When we are dealing only with different concentrations of a particular salt mixture such as sea salt; the composition of which is known and uniform, the chloride content of the solutions (which is easily determined) can be a useful index of osmotic strength. However, when we are dealing with mixed salt solutions of unknown and varying composition (such as oil well brines and other industrial wastes), their chloride ion content is not a reliable index of osmotic strength. For example, an industrial waste brine containing a large amount of sodium sulphate can be much more active osmotically than another brine with a much higher chloride ion content which contains only sodium and calcium chlorides. It is impossible when dealing with mixed wastes to generalize as to the relationship between chloride ion concentration and osmotic, toxic, or over-all pollutional strength. It is believed, therefore, that chloride ion criteria have no practical significance as far as aquatic life is concerned.

Fluorides

Studies at our laboratory in Cincinnati have indicated that fluoride ions do have toxic properties in their own right. Further, they appear to have a cumulative effect. In 10-day tests it was found that the TL_m value for potassium fluoride was 64 p.p.m. It is believed that for good fish production the fluoride content should not exceed 5 p.p.m.

Toxic Materials

There is a great deal of literature dealing with the toxicity of various pure chemicals to fishes. The great majority of investigators have used different approaches and have carried out their studies with a variety of fishes, using different types of water for dilution. Several compilations, reviews, or bibliographies of these studies have been made (38) (61) (62) (63) (64) (67). An examination of these papers clearly indicates that there is great variation in toxic levels reported by various investigators for selected pure chemicals. This variation is especially evident in the California report (61).

The quality of the receiving or dilution water, which is often not reported by some of those investigating toxicity, is of outstanding importance in determining toxicity of a particular material or waste. Several environmental factors may influence toxicity, such as: temperature, CO_2, D. O., pH, alkalinity, hardness, turbidity, and dissolved materials. Certain dissolved materials may significantly affect the toxicity of a waste through their synergistic or antagonistic action or through complexation, precipitation, or other action. In the case of ferro- and ferricyanide solutions, sunlight is of importance as photo decomposition of these materials occurs with the production of toxic HCN (65). The heavy metals are considerably more toxic at low pH since they are more soluble in acidic solutions. In hard waters at higher pH they are precipitated or changed in other ways to become much less toxic. It has been found that beryllium and uranium are 60 to 80 times more toxic in soft water than in hard water (66). Ellis (38) and other investigators have found that ammonia becomes rapidly more toxic at pH values above 8.0. Calcium antagonizes the toxicity of many of the heavy metals whereas some of them are synergistic with each other and become considerably more toxic when mixed; examples are Cu and Zn, Cu and Cd, and Zn and Ni (67).

The toxicity of many of the metal-cyanide complexes is greatly influenced by pH. Doudoroff (68) has reported that fish can withstand 1,000 times as much nickel cyanide complex at pH 8 as at pH 6.5. Among the metabolites, including weak acids and bases, it is the molecule and not the ion which appears to be toxic. Thus weak acids such as HCN and H_2S become more toxic as the pH is lowered and dissociation depressed, whereas weak bases such as NH_4OH become more toxic as the pH is raised.

In general, materials are more toxic at higher temperatures and at low dissolved oxygen levels. Carbon dioxide may, through its effect on pH, render some materials more toxic or it may serve to make others less toxic. Complexation, precipitation, oxidation, dissociation, recombination, or buffering action must also be considered. The influence of water quality and other environmental factors on the toxicity of various materials has been summarized by Tarzwell (69).

The character of the receiving water can cause wide variations in the toxicity of many materials to fishes. Reference to the literature on the toxicity of specific pure chemicals is of little value for determining the toxicity of a complex waste containing these and other materials. Such an approach neglects water quality, which is of particular importance, as well as synergism and antagonism, oxidation, precipitation, complexation, and other actions which may occur in the stream and may greatly influence the toxicity of a waste in a particular stream. Numerical standards for toxicity of specific pure chemicals have little value and can be misleading. From the standpoint of industry they may be very undesirable. If a regulartory agency sets numerical criteria for the heavy metals and other substances which are to be applied over a wide area, they must be set so low that allowable concentrations are not detrimental to aquatic life under those conditions at which they are most toxic. For example, criteria for nickel cyanide wastes discharged into an acid stream would have to be set so low that the HCN formed from the wastes would not be toxic, whereas in

... alkaline stream the criteria could be much higher as an increase of one and one-half pH units from 6.5 to 8.0 decreases the toxicity of this material over 1000 times. Copper is much less toxic in hard water than it is in soft water with a low pH; variation may be as great as 200 times.

It is believed that the best approach to this problem is to make bio-assays with the total waste, using for dilution, water from the receiving stream at the point where the waste is to be discharged. In this way the many variables which influence the toxicity of that particular waste in that stream are taken into consideration and safe disposal or dilution rates can be determined. With certain exceptions (some of the insecticides and other materials whose toxicity is not influenced by water quality), water quality criteria for toxic materials when applied over wide areas should not be expressed as numerical values. It is believed that a tailor-made approach should be used where toxicity of the waste can be determined for the particular receiving stream at the point of discharge. When the toxicity is determined in this way allow-able concentrations for that particular situation can be expressed as p.p.m. or as dilution ratios. Such an approach also permits evaluation and comparison of the toxicity of wastes from different industries along the stream.

Summary and Conclusions

The establishment of water quality criteria for the protection of our valuable aquatic resources is a complicated and difficult problem. Since the basic objective of water quality criteria for the protection of aquatic life is to provide or preserve environmental conditions essential for the survival, normal growth, reporudction, and well being of aquatic organisms a know-ledge of the environmental requirements of these organisms is essential for the establishment of such criteria. Many of the activities of man have modified the aquatic environment. Among these are deforestation, unwise agricultural practices, overgrazing, and pollution. Our aquatic resources which produce millions of dollars yearly in revenues from sport and commercial fishing are a renewable resource worthy of our best efforts for preservation.

Siltation due to erosion has been and is a major pollutant. The solution of this problem by means of erosion control must be a cooperative effort among those agencies dealing with water, soil, and other natural resources.

While all the environmental requirements for aquatic life are not now known, application of the data presently available can be used effectively in the setting up of some criteria. As more data become available existing criteria can be modified and others set up in order to meet the problem adequately.

The quality of the receiving water is particularly important in determining the effects of many wastes. Among the factors influencing the toxicity of pollutants in a particular receiving water are temperature, CO_2, D.O., pH, alkalinity, hardness, and dissolved materials. Other factors which may modify the toxicity of a waste are complexation, precipitation, oxidation, synergy, and antagonism.

In view of the many factors which may influence the effects of pollutants in different streams, it is believed that in most situations numerical criteria when applied over wide areas can be set only for temperature, D. O., and pH. With a few exceptions, such as for insecticides and certain other materials, it is believed a tailor-made approach should be used in setting criteria for toxic substances. This approach would consist of bio-assyas of the waste in question, using for dilution, water from the receiving stream taken from the area into which the waste is to be discharged. Such bio-assays would take into consideration the variables which influence toxicity of that particular waste and can be used to indicate safe concentrations, or the amount of dilution required.

Criteria for settleable solids and turbidity will depend largely on local conditions and will vary with the stream and the area.

When considering large areas pH values should not fall below 5 or exceed 9.5, but for good fish production it is desirable that they be maintained between 6.5 and 8.5.

For a well rounded warm water fish population dissolved oxygen levels should not be below 5 p.p.m. for more than 8 hours in any 24 hour period and at no time should they be below 3 p.p.m. If a coarse fish population only is desired minimum levels may fall to 2 p.p.m. For good production of Salmonoid fishes a minimum of 6 p.p.m. appears to be required. However, trout can live and reproduce in waters where the D.O. content may drop to 4 or 5 p.p.m. but the survival of eggs and fry and the production is usually not so great.

It is suggested that in the northern portion of the country peak temperatures for warm water fishes should not exceed 93°F. In the southern portion of the country fish are better acclimated to higher temperatures and can withstand peak temperatures of 96°F. or higher. While trout can stand peak temperatures of 80°F. to 83°F. for short periods, best production is attained in streams having summer temperatures of 60°F. to 68°F.

Allowable concentrations of toxic complex wastes for each stream or situation should be determined by means of bio-assays and safe dilutions estimated by the use of application factors or by other means.

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