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STREAM SURVEILLANCE AND MONITORING: FIELD AND LABORATORY PROCEDURES

TRAINING MANUAL

U.S. ENVIRONMENTAL PROTECTION AGENCY
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STREAM SURVEILLANCE AND MONITORING: FIELD AND LABORATORY PROCEDURES

This course is designed for personnel engaged in programs concerned with monitoring the quality of surface waters. Interdisciplinary needs and responsibilities in the accumulation and interpretation of data from field and laboratory activities are emphasized.

Upon completion of the course the student will be able to apply suitable methods, techniques, and instrumentation used in field sampling and measurements, and will be familiar with basic laboratory techniques and procedures in the chemical, biological, and bacteriological areas. Advantages and limitations of equipment, methods and techniques will be considered.

ENVIRONMENTAL PROTECTION AGENCY
Office of Water Program Operations
TRAINING PROGRAM

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FLOW MEASUREMENT

I INTRODUCTION

Flow measurements are among the more important data collected during a water quality survey. Such measurements are used to interpret data variations, calculate loadings, and expedite survey planning. If the analysis of survey data involves estimation of loads, the accurate measurement of discharge assumes a level of importance equal to that of laboratory and analytical results.

In the following discussion, procedures for measurement of stream flow and waste discharge are described. Some of these procedures are used in long-term, very detailed water quality and supply studies; others are more suited to short-term pollution surveys.

II PLANNING

A Station Location

Four factors influence location of gauging or flow measurement stations:

1 Survey objectives

2 Physical accessibility

3 Characteristics of the stream bed

4 Hydrologic effects

Survey objectives represent the major influence on station location; depending upon objectives, gauging stations may be located above and/or below confluences and outfalls.

Physical accessibility determines the ease and cost of installation and maintenance of the station. The characteristics of the stream bed may greatly influence the obtainable accuracy of measurement. For instance, rocky bottoms greatly reduce the accuracy of current meters. Sedimentation in pools behind control structures may influence stage-discharge relationships. Hydrologic variations in stream flow may cause washout or bypass of the gauging station. In the Southwest, flash floods have been known to wash out or bypass gauging stations by assuming different channels of flow.

B Methodology

Choice of a specific measurement procedure is dependent upon at least three considerations:

1 The relation between obtainable and desired accuracy

2 Overall cost of measurement

3 The quantity of flow to be measured

Ideally, discharge measurements should be reported to a specific degree of accuracy; the gauging procedure greatly influences this accuracy. The influence of overall cost on the gauging program is readily apparent. Extensive, detailed studies are usually characterized by high costs for automatic instrumentation and low personnel cost; the opposite is usually true for less detailed studies. The range of flows to be measured (within acceptable accuracy) is, of course, not known prior to the survey. However, experienced personnel usually can make reasonable estimates of expected flows from visual observations and other data, and may recommend appropriate gauging procedures. In this regard, experienced personnel always should be consulted.

III MEASUREMENT

A Streams, Rivers, and Open Channels

1 Current Meter

The current meter is a device for measuring the velocity of a flowing body of water. The stream cross section is divided into a number of smaller sections, and the average velocity in each section is determined. The discharge is then found by summing the products of area and velocity of each section.

2 Stage-discharge relationships

Large flows usually are measured by development of and reference to a stage-discharge curve; this procedure has long been used by the U. S. Geological Survey. Such gauging stations are composed of a control structure located downstream of the location of measurement and some type of water level indicator which identifies the height of the water surface above a previously determined datum.

Location of the control structure so that reliable measurements of flow will be obtained at all river stages is particularly important. The water level may be continuously recorded by an automatic recorder located in a wet well or may be indicated directly on a staff gauge located at the bank of the river. Such stations must be calibrated by measurement of flow by velocity-area methods (current meter) at all expected stages of river flow.

3 Weirs

A weir may be defined as a dam or impediment to flow, over which the discharge conforms to an equation. The edge or top surface over which the liquid flows is called the weir crest. The sheet of liquid falling over the weir is called the nappe. The difference in elevation between the crest and the liquid surface at a specified location, usually a point upstream, is called the weir head. Head-discharge equations based on precise installation requirements have been developed for each type of weir. Weirs so installed are called standard weirs. Equations for non-standard installations or unusual types may be derived empirically.

Weirs are simple, reliable measurement devices and have been investigated extensively in controlled experiments. They are usually installed to obtain continuous or semi-continuous records of discharge. Limitations of weirs include difficulty during installation, potential siltation in the weir pond, and a relatively high head requirement, 0.4 - 2.0 feet. Frequent errors in weir installation include insufficient attention to standard installation requirements and failure to assure completely free discharge of the nappe.

a Standard suppressed rectangular weir

This type of weir is essentially a dam placed across a channel. The height of the crest is so controlled that construction of the nappe in the vertical direction is fully developed. Since the ends of the weir are coincident with the sides of the channel lateral contraction is impossible. This weir requires a channel of rectangular cross section, other special installation conditions, and is rarely used in plant survey work. It is more commonly used to measure the discharge of small streams.

The standard equation for discharge of a suppressed rectangular weir (Francis equation) is:

$$Q = 3.33\ LH^{3/2}$$

where

Q = discharge, cfs
L = length of the weir crest, feet
H = weir head, feet

The performance of this type of weir has been experimentally investigated more intensively than that of other weirs. At least six forms of the discharge equation are commonly employed. The standard suppressed weir is sometimes used when data must be unusually reliable.

b Standard contracted rectangular weir

The crest of this type of weir is shaped like a rectangular notch. The sides and level edge of the crest are so removed from the sides and bottom of the channel that contraction of the nappe is fully developed in all directions. This weir is commonly used in both plant surveys and measurement of stream discharge.

The standard equation for discharge of a contracted rectangular weir (corrected Francis equation) is

$$Q = 3.33\ (L - 0.2H)H^{3/2}$$

where

Q = discharge, cfs
L = length of the level crest edge, feet
H = weir head, feet
0.2H = correction for end contractions as proposed by Francis

c Cipolletti weir

The Cipolletti weir is similar to the contracted rectangular weir except that the sides of the weir notch are inclined outward at a slope of 1 horizontal to 4 vertical. Discharge through a Cipolletti weir occurs as though end contractions were absent and the standard equation does not include a corresponding factor for correction.

Figure 1. STANDARD CONTRACTED RECTANGULAR WEIR

The standard equation for discharge through a Cipolletti weir is

$$Q = 3.367\ LH^{3/2}$$

where

Q = discharge, cfs

L = length of the level crest edge, feet

H = weir head, feet

The discharge of a Cipolletti weir exceeds that of a suppressed rectangular weir of equal crest length by approximately 1 percent.

d Triangular weirs

The crest of a triangular weir is shaped like a V-notch with sides equally inclined from the vertical. The central angle of the notch is normally 60 or 90 degrees. Since the triangular weir develops more head at a given discharge than does a rectangular shape, it is especially useful for measurement of small or varying flow. It is preferred for discharges less than 1 cfs, is as accurate as other shapes up to 10 cfs, and is commonly used in plant surveys.

The standard equation for discharge of a 90° triangular weir (Cone formula) is

$$Q = 2.49H^{2.48}$$

where

Q = discharge, cfs
H = weir head, feet

Crest height and head are measured to and from the point of the notch, respectively.

e Accuracy and installation requirements

Quotations of weir accuracy express the difference in performance between two purportedly identical weirs and do not include the effects of random error in measurement of head. Weirs installed according to the following specifications should measure discharge within ± 5% of the values observed when the previously cited standard equations were developed.

1) The upstream face of the bulkhead and/or weir plate shall be smooth and in a vertical plane perpendicular to the axis of the channel.

2) The crest edge shall be level, shall have a square upstream corner, and shall not exceed 0.08 in (2 mm) in thickness. If the weir plate is thicker than the prescribed crest thickness the downstream corner of the crest shall be relieved by a 45° champfer.

3) The pressure under the nappe shall be atmospheric. The maximum water surface in the downstream channel shall be at least 0.2 ft. below the weir crest. Vents shall be provided at the ends of standard suppressed weirs to admit air to the space beneath the nappe.

4) The approach channel shall be straight and of uniform cross section for a distance above the weir of 15 to 20 times the maximum head, or shall be so baffled that a normal distribution of velocities exists in the flow approaching the crest and the water surface at the point of head measurement is free of disturbances. The cross-sectional area of the approach channel shall be at least 6 times the maximum area of the nappe at the crest.

5) The height of the crest above the bottom of the approach channel shall be at least twice, and preferably 3 times, the maximum head and not less than 1 foot. For the standard suppressed weir the crest height shall be 5 times the maximum head. The height of triangular weirs shall be measured from the channel bottom to the point of the notch.

6) There shall be a clearance of at least 3 times the maximum head between the sides of the channel and the intersection of the maximum water surface with the sides of the weir notch.

7) For standard rectangular suppressed, rectangular contracted, and Cipolletti weirs the maximum head shall not exceed 1/3 the length of the level crest edge.

8) The head on the weir shall be taken as the difference in elevation between the crest and the water surface at a point upstream a distance of 4 to 10 times the maximum head or a minimum of 6 feet.

9) The head used to compute discharge shall be the mean of at least 10 separate measurements taken at equal intervals. The head range of the measuring device shall be 0.2 - 1.5 feet.

The capacities of weirs which conform to these specifications are indicated in Table 1.

4 Parshall flume

The Parshall flume is an open constricted channel in which the rate of flow is related to the upstream head or to the difference between upstream and downstream heads. It consists of an entrance section with converging vertical walls and level floor, a throat section with parallel walls and floor declining downstream, and an exit section with diverging walls and floor inclining downstream. Plan and sectional views are shown in Figure 2. Advantages of the Parshall flume include a low head requirement, dependable accuracy, large capacity range, and self cleaning capability. Its primary disadvantage is the high cost of fabrication; this cost may be avoided by use of a prefabricated flume. Use of prefabricated flumes during plant surveys is becoming increasingly popular.

a Standard equations

The dimensions of Parshall flumes are specified to insure agreement with standard equations. Table of dimensions are available from several sources [3, 4]. For flumes of 6 inch to 8 foot throat width the following standard equations have been developed.

1) 6 inch throat width

$$Q = 2.06\ H_a^{1.58}$$

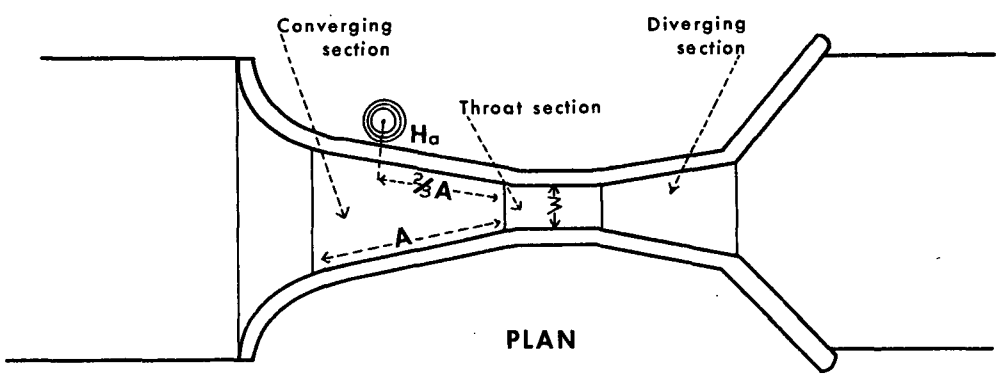
2) 9 inch throat width

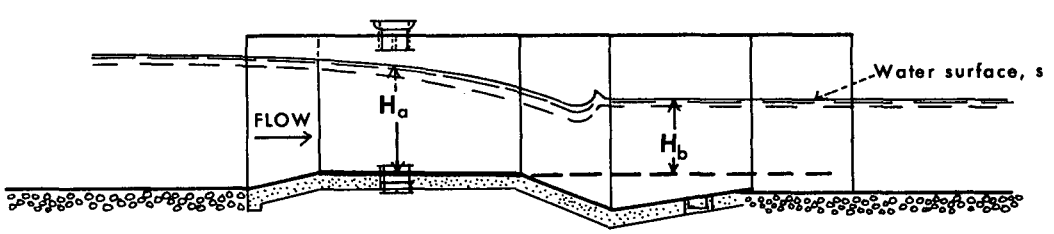
$$Q = 3.07\ H_a^{1.53}$$

TABLE 1 DISCHARGE OF STANDARD WEIRS

Crest Length (Feet)	Contracted Rectangular* Weir (discharge-cfs)		Suppressed Rectangular* Weir (discharge-cfs)		Cipolletti* Weir (discharge-cfs)		90° Triangular* Weir (discharge-cfs)	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
1.0	.590	.286	.631	.298	.638	.301		
1.5	1.65	.435	1.77	.447	1.79	.452		
2.0	3.34	.584	3.65	.596	3.69	.602		
2.5	5.87	.732	6.30	.744	6.37	.753		
3.0	9.32	.881	10.0	.893	10.1	.903		
3.5	13.8	1.03	14.8	1.04	15.0	1.05		
4.0	19.1	1.18	20.4	1.19	20.6	1.20		
4.5	25.6	1.33	27.5	1.34	27.8	1.35		
							6.55	.046
5.0	28.8	1.48	30.6	1.49	30.9	1.51		
6.0	34.9	1.78	36.7	1.79	37.1	1.81		
7.0	41.0	2.07	42.8	2.08	43.3	2.11		
8.0	47.1	2.37	48.9	2.38	49.5	2.41		
9.0	53.2	2.67	55.0	2.68	55.7	2.71		
10.0	59.3	2.97	61.1	2.98	62.0	3.01		

* $H \geq 0.2$ ft, $H \leq 1.5$ ft, $H \leq 1/3$ L





SECTION

FIGURE 2 PARSHALL FLUME

3) 1 to 8 foot throat width

$$Q = 4WH_a^{1.522W^{0.026}}$$

where

Q = free-flow discharge, defined as that condition which exists when the elevation of the downstream water surface above the crest, H_b, does not exceed a prescribed percentage of the upstream depth above the crest, H_a. The prescribed percentage of submergence is 60 percent for 6 and 9 inch flumes and 70 percent for 1 to 8 foot flumes

W = throat width, feet

H_a = upstream head above the flume crest

b Head loss

The head required by a Parshall flume is greater than (H_a - H_b) because H_a is measured at a point in the converging section where the water surface has already begun to decline. Table 2 indicates the total head requirements of standard Parshall flumes. These losses should be added to the normal channel depth to determine the elevation of the water surface at the flume entrance. No head losses are indicated for discharge-throat width combinations for which H_a is less than 0.2 ft. or greater than 2/3 the sidewall depth in the converging section.

c Accuracy and installation requirements

A Parshall flume will measure discharge within ± 5% of the standard value if the following conditions are observed.

1) The dimensions of the flume shall conform to standard specifications.

2) The downstream head, H_b, shall not exceed the recommended percentage of the upstream head, H_a.

TABLE 2 HEAD LOSS IN STANDARD PARSHALL FLUMES UNDER FREE DISCHARGE

Discharge (cfs)	Head Loss, Feet, in Flume of Indicated Width							
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet	7 feet	8 feet
0.5	.08							
1.0	.14	.09	.06					
2.5	.26	.16	.12	.10	.08	.07	.06	.05
5.0	.42	.27	.20	.16	.13	.12	.10	.09
10.0	.70	.45	.34	.27	.22	.19	.17	.15
30.0			.70	.56	.47	.40	.35	.30
50.0					.68	.57	.49	.41

$H_a \geq 0.2$, $H_a \leq 2.0$

3) The upstream head shall be measured in a stilling well connected to the flume by a pipe approximately 1-1/2 inches in diameter.

4) The flume shall be installed in a straight channel with the centerline of the flume parallel to the direction of flow.

5) The flume shall be so chosen, installed, or baffled that a normal distribution of velocities exists at the flume entrance.

5 Tracer materials

Techniques, materials, and instruments are presently being refined to permit accurate measurement of instantaneous or steady flow with several tracer materials. Measurements are made by one of two methods:

a Continuous addition of tracer

b Slug injection

With the first method, tracer is injected into a stream at a continuous and uniform rate; with the second a single dose of tracer material is added. Both methods depend on good transverse mixing and uniform dispersion throughout a stream. The concentration of tracer material is measured downstream from the point of addition. When continuous addition is employed, flow rates are calculated from the equation:

$$q \cdot C = (Q + q)\, c$$

in which q = rate of tracer addition to the stream at concentration, C, Q = the stream flow rate, and c = the resulting concentration of the stream flow combined with the tracer. For the slug injection method

$$Q = \frac{S}{\bar{c}\ \Delta t}$$

in which Q = the stream discharge, S = the quantity of tracer added, $\bar{c}$ = the weighted average concentration of tracer material during its passage past the sampling point, and Δt = the total time of the sampling period. Disadvantages of tracer methods include incomplete mixing, natural adsorption and interference, and high equipment costs.

6 Floats

Floats may be used to estimate the time of travel between two points a known distance apart. The velocity so obtained may be multiplied by 0.85 to give the average velocity in the vertical. Knowing the mean velocity and the area of the flowing stream, the discharge may be estimated. Floats should be employed only when other methods are impractical.

B Pipes and Conduits

1 Weirs and Parshall flumes

Weirs and Parshall flumes are commonly installed in manholes and junction boxes and at outfalls to measure flow in pipes. All conditions required for measurement of open channel flow must be observed.

2 Tracer materials

These methods are popular for measurement of pipe flow because they do not require installation of equipment or modification of the flow. These are especially convenient for measurement of exfiltration and infiltration.

3 Depth-slope

If the depth of the flowing stream and the slope of the sewer invert are known, the discharge may be computed by means of any one of several formulas.

a Manning formula

$$Q = \frac{1.486}{n}\ A\ R^{2/3} S^{1/2}$$

where

Q = discharge, cfs

n = roughness coefficient

A = area of flow, sq. ft.

R = hydraulic radius, ft.

= area divided by wetted perimeter, ft.

S = slope, ft. per ft.

b Chezy formula

$$Q = CA\sqrt{RS}$$

where

Q = discharge, cfs

C = friction coefficient

A = area of flow, sq. ft.

R = hydraulic radius, ft.

= area divided by wetted perimeter, ft.

S = slope, ft. per ft.

4 Open end pipe flow

The following methods can be employed when other more precise means are not practical. They can be employed, however, only when there is free discharge to the air.

a Coordinate method (Figures 3, 4, and 5)

Discharge may be computed by the following formula:

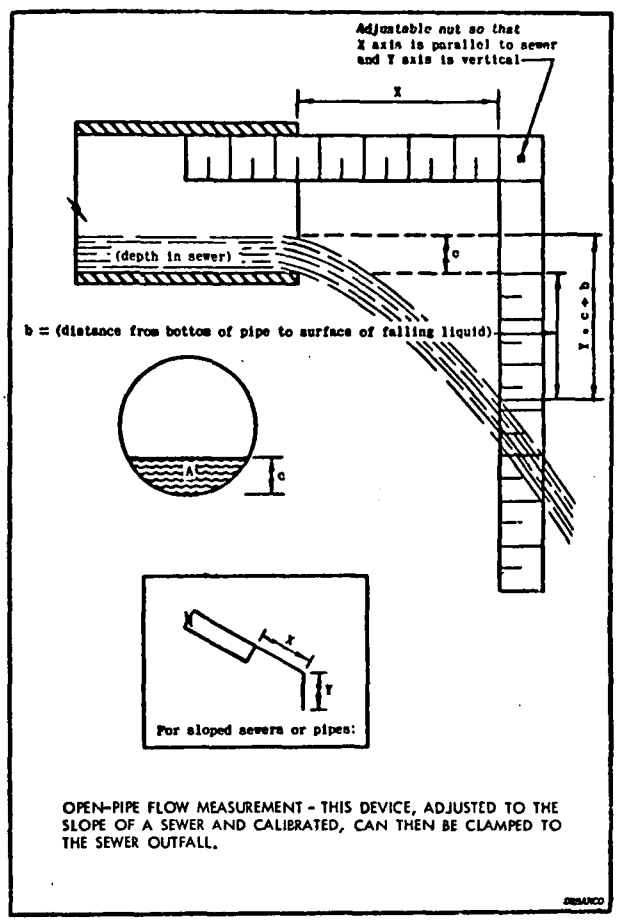
$$Q\ (\text{gpm}) = \frac{1800\ AX}{Y}$$

where

A = cross sectional area of liquid in the pipe (sq. ft.)

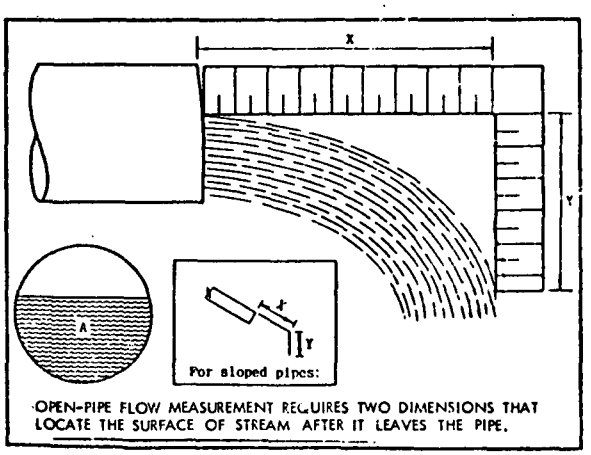
X = distance between the end of the pipe and the vertical gauge in ft., measured parallel to the pipe.

Y = vertical distance from water surface at the end of the pipe to the intersection of the water surface with the vertical gauge, in ft.



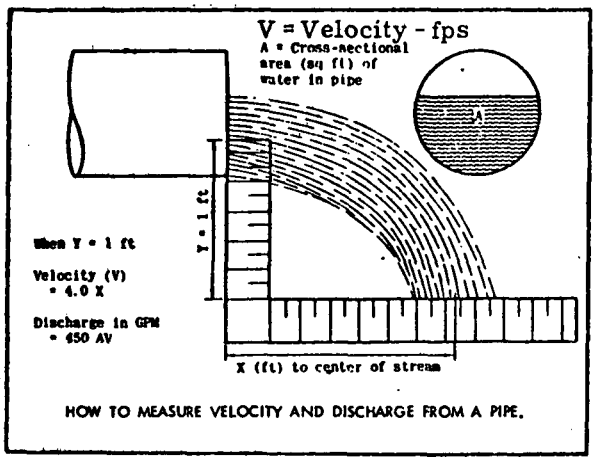
OPEN-PIPE FLOW MEASUREMENT - THIS DEVICE, ADJUSTED TO THE SLOPE OF A SEWER AND CALIBRATED, CAN THEN BE CLAMPED TO THE SEWER OUTFALL.

Figure 3



OPEN-PIPE FLOW MEASUREMENT REQUIRES TWO DIMENSIONS THAT LOCATE THE SURFACE OF STREAM AFTER IT LEAVES THE PIPE.

Figure 4



HOW TO MEASURE VELOCITY AND DISCHARGE FROM A PIPE.

Figure 5

b California pipe flow method (Figure 6)

This method may be used only for horizontal pipes having free discharge. If the pipe is not horizontal, a connection must be made to one that is. The horizontal length must be not less than 6 times the diameter of the pipe. Discharge may be computed by the following formula:

$$Q\ (\text{gpm}) = T \times W$$

$$\text{where } T = 3{,}900\left(1 - \frac{a}{d}\right)^{1.88}$$

$$W = d^{2.48}$$

where a and d are measured in feet

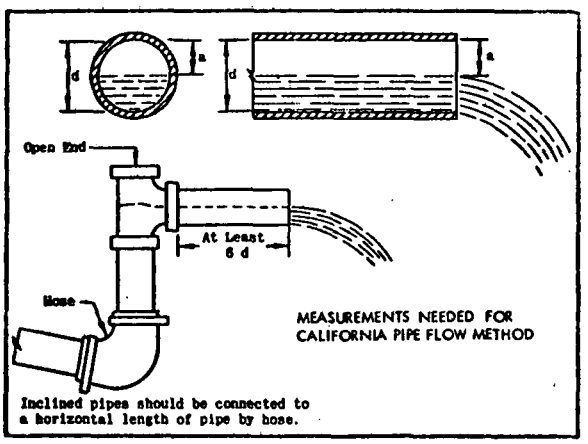


Figure 6

C Head Measuring Devices

Several of the above gauging methods require the measurement of water level in order that discharge may be determined. Any device used for this purpose must be referenced to some zero elevation. For example, the zero elevation for weir measurements is the elevation of the weir crest. The choice of method is dependent upon the degree of accuracy and the type of record desired.

1 Hook gauge

The hook gauge measures water elevation from a fixed point. The hook is dropped below the water surface and then raised until the point of the hook just breaks the surface. This method probably will give the most precise results when properly applied.

2 Staff gauge

The staff gauge is merely a graduated scale placed in the water so that elevation may be read directly.

3 Plumb line

This method involves measurement of the distance from a fixed reference point to the water surface, by dropping a plumb line until it just touches the water surface.

4 Water level recorder

This instrument is used when a continuous record of water level is desired. A float and counterweight are connected by a steel tape which passes over a pulley. The float should be placed in a stilling well. A change in water level causes the pulley to rotate which, through a gearing system, moves a pen. The pen traces water level on a chart which is attached to a drum that is rotated by a clock mechanism. When properly installed and maintained, the water level recorder will provide an accurate, continuous record.

ACKNOWLEDGMENT:

Certain portions of this outline contains training material from prior outlines by P.E. Langdon, A.E. Becher, and P.F. Atkins, Jr.

REFERENCES

1 Planning and Making Industrial Waste Surveys - Ohio River Valley Water Sanitation Commission.

2 Stream Gauging Procedure. U.S. Geological Survey. Water Supply Paper 888. (1943)

3 King, H.W. Handbook of Hydraulics. 4th Edition, McGraw-Hill. (1954)

4 Water Measurement Manual. United States Department of the Interior Bureau of Reclamation. (1967)

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Descriptors: Chezy Equation, Discharge Measurement, Flow, Flow Measurement, Flow Rates, Flumes, Mannings Equation, Open Channel Flow, Pipe Flow, Streamflow, Venturi Flumes, Water Flow, Water Level Recorders, Weirs

WATER LEVEL RECORDERS

A Commonly used instruments produce a graphic record of the rise and fall of a water surface with respect to time.

B Advantages over non-continuous staff gage readings:

1 Continuous record provides most accurate means of determining daily average gage height and flow.

2 Maximum and minimum stages are recorded, and time of occurrence can be noted.

3 Record can be obtained when observer not there, or not available.

C Principal elements

1 Time element: clock mechanism activated by spring, weight, or electric motor.

2 Gage height element activated by a float, cable, or tape, and a counterwieght.

Gear reduction mechanism is provided in the height element so that the gage-height reading on the recorder is changed in proportion to the rise and fall of the water surface.

D Common types of recorders

a <u>Drum Recorders</u>

1 Horizontal drum: clock positions pen along drum axis and gage height element rotates drum.

2 Vertical drum: clock mechanism rotates drum and water stage a function of displacement of pen along drum axis.

b <u>Strip-chart</u>

1 Totalizer - records flow rates with respect to time and automatically totalizes volume. Strip-chart provides graphic record.

E Installation of recorders:

1 Gage tape or cable should be long enough to accomodate the full range of water surface changes.

2 Recorder should be installed over a stilling well and shielded from the weather.

3 Staff gage needed near stilling well to check the recorder.

F Stilling Well, Float Well

1 Prime purpose to prevent oscillations of the float caused by surging water or wave action.

2 Must be anchored to prevent movements that would introduce oscillations within the well.

3 Outside surges can be damped out by restricting area of the water inlet to about 1/1000 of the inside horizontal cross-sectional area of the well.

4 Can be made of galvanized iron culvert pipe, sewer pipe, tongue and groove creosoted lumber, stove pipe, or other suitable material.

5 Sometimes built outside the channel in which the liquid is flowing, and connected to it by an opening or pipe.

6 In sewage applications, provision must be made for frequent cleaning or, water levels in well will not be representative.

7 Inlet to float well must be checked frequently for clogging by sludge and sediment.

G Errors in Float-Operated Devices

1 Float Lag. If a float performs any mechanical work, i.e., turns gears, moves an index hand or a recorder pen, etc., there is always a lag of the index behind the true water level.

2 Line Shift. With every change of stage, a portion of the float line passes from one side of the float pulley to the other. This change of weight changes the depth of flotation of the float, hence the stylus deviates from the true water height by a small amount, depending on the change in stage since the last correct setting, and the weight of the line used between the float and counterpoise.

3 Submergence of Counterpoise. When the counterpoise and any portion of the line becomes submerged, the pull on the float is reduced and its depth of flotation is increased.

4 Corrections for errors due to the above are so small as to be unimportant if instruments are correctly made and properly cared for. All those errors vary inversely as the square of the diameter of the float, and directly as the weight of line and counterpoise. To minimize them, therefore, use a sufficiently large float with light lines and counterweights, and keep the instrument properly oiled and free from dust to render the force required to move the index as low as possible.

5 Errors from humidity effects on charts. All paper is affected by humidity changes. It expands with increasing humidity and contracts as humidity decreases. The result is to introduce errors in the graphs made by recording instruments. Under ordinary conditions those errors are quite negligible. In extreme cases, however, an expansion from excessive humidity of as much as 2% has been noted. The heights, however, may not be in error by that amount. The actual error depends on the height itself and the position of the neutral axis of expansion.

By far the most practicable procedure to prevent paper expansion is to reduce the effect of humidity to a minimum, by keeping excessively humid air from the recorder. The use of a desiccator such as silica gel within the recorder case is quite effective in keeping the interior atmosphere reasonably dry. This turns pink when its capacity is exhausted. Heating in an oven will drive off the moisture and restore it to its normally blue color.

6 The "Human Equation" is the greatest possible source of error, with the following among the more conspicuous reasons for erroneous or incomplete records:

a Incorrect gage reading

b Inaccurate setting of pen

c Failure to wind clock

d Failure to start clock after winding

e Failure to put stylus on paper

f Wrongly attaching float, so that pen moves up when it should go down

g Failure to note gage reading and time on record sheet

h Failure to see that inlet to well is open

i Failure to oil bearings occasionally

j Use of gummy oil

REFERENCES:

1 Hydrographic Data Book. Leupold & Stevens, Inc. Portland, Oregon, 8th ed.

2 Water Measurement Manual. U.S. Dept. of the Interior, Bureau of Reclamation, Denver, CO. 2nd ed.

3 Buchanan, T.J. & Somers, W.P. Stage Measurement at Gaging Stations. Book 3, Chapter A7. Techniques of Water Resources Investigations of the U.S. Geological Survey. U.S. Government Printing Office, Washington, D.C. 1968.

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Descriptors: Flow Measurement, Open Channel Flow, Streamflow, Water Level Recorders

TRACING NATURAL WATERS

I INTRODUCTION

The validity of water pollution studies is dependent upon an ability to describe the actual time and space situation of a pollutant as it blends with the natural receiving water. This understanding is important in order to predict the subsequent effects of the pollutant on the receiving water and subsequent users or to detect the sources of pollution.

The nature of this blending is dependent upon the physical and chemical properties of both the pollutant and the receiving water as well as the mixing and flow characteristics of the receiving channel, basin, or aquifer. Tracing is an attempt to approximate the actual motion and mixing of this blend as it moves through the channel, basin or aquifer.

Although there has been scant reference to tracers in texts there has been rapidly increasing number of technical articles describing their use in recent years. A comprehensive compilation of early radioactive tracer studies may be found in the report of the "Time of Flow Studies, Ottawa River, Lima, Ohio"[1] one of the early attempts to compare various tracer techniques under similar field conditions. Several reports (see Bibliography) have discussed the use of dyes during the early 1960's.

More refined and extensive tracer investigations may be expected as experience is gained and as more complex studies arise. A water quality agency should develop competence in at least one tracer technique and would be wise to be capable in some others as well.

II PURPOSES

Tracer applications are still in a highly developmental stage. One finds many words or phrases to suggest similar field findings. In broad terms these measurements are concerned with indications of mass movement, blend mixing, and flow direction. Basically, tracers are used to determine:

A Flow Rates

1 In freshwater channels or lakes such terms as passage time, time of travel, time of flow, flow quantity or flow volume are used.

2 In estuaries such terms as rate of renewal, flushing rate, drift velocity, mass transfer or net (tidal) drift are used. The tidal fluctuation imposes a need for consideration of flow reversal effects.

3 In subsurface waters such terms as recharge rate, flow rate and residence time are used. Related factors such as basin capacity, porosity and permeability are determined.

B Flow Direction

1 In estuaries

a Direction of flow relative to tidal phases which do not necessarily correspond to slack conditions.

b Obscure current circulation as influenced by the interlated factors of tides, winds, Coriolis forces, topography and density gradients.

2 In ground water

a Aquifer may flow contrary to initial superficial impression.

b Hydrostatic conditions may change and reverse normal flow direction.

C Mixing Patterns

1 Short circuit effects

a Eddying - primarily the result of surface channel and basin configuration.

b Stratification - primarily due to temperature and density differences.

c Inter-connections - between aquifers in ground water as well as solution channels and open fractures.

2 Distribution phenomena

a Dispersion of colloidal, soluble and suspended substances

b Diffusion of temperatures or gaseous substances

III TYPES OF STUDIES

Tracers are used in many situations, including:

A Treatment Plant Units

B Closed Conduits

C Open Channels

D Large Water Bodies

E Hydraulic Models

F Subsurface Aquifers

G Subsurface Basins

IV TYPES OF TRACERS

Materials used for tracers include:

A Floats

1 Surface (wooden and plastic devices as well as fruit). Influenced by wind action and debris.

2 Sub-surface "drogues". Apparent direction must be carefully evaluated.

B Salts

1 Common salt - hard to detect when less than 1 mg/l.

2 Brackish and freshwater mixing in estuaries and coastal aquifers.

3 Ammonium chloride. (See Table 1)

C Dyes, such as:

1 Rhodamine series - See section VI

2 Pontacyl Brilliant Pink B - most stable of fluorescent dyes. Rather expensive dye.

3 Fluorescein - very inexpensive. Fluoresces very near natural stream background level.

4 Uranine - fluoresces near stream background level.

D Radioactive substances, such as: (see Table 2) AEC must approve all experiments. AEC has published tables on permissible concentrations in unrestricted areas.[13]

1 Rubidium-86

2 Iodine-131

3 Tritium (H^3) gives best overall performance in subsurface[6] tracing.

4 Krypton-85 - used recently in gas transfer measurement in laboratory and stream studies.[12]

E Waste return characteristics; significant built-in factors such as:

1 Silt - understandably dependent upon velocity and obstructions.

2 Foam - ABS will foam at levels as low as 1 - 5 mg/l depending on hardness.

Table 1. SUBSURFACE NONRADIOISOTOPE FLUID TRACERS

Tracer	Form	Test conditions	Remarks
Chloride Dextrose Fluorescein Dye	NaCl Sugar	Lab: Columns of sand and sandy loam.	Chloride most rapid. Fluorescein far from satisfactory.
Chloride Dextrose Fluorescein Dye	NaCl Sugar	Field: 4 feet thick aquifer of sand and gravel, 90 feet below surface.	Fluorescein considered best of dyes. Chloride in the large conc. required caused density currents.
Dextrose Ammonia Fluorescein Dye Boron	Sugar Chloride H_3BO_3	Field: Added in Conc. of 10,000 ppm, 500 ppm, 50 ppm, and 200 ppm respectively into a 4 foot thick sand horizon at 2,100 feet below surface in McKean County, Pennsylvania.	Adsorption observed: dextrose - little or none, boron - slight (conc. dropped 30%) ammonia - moderate, fluorescein - strong (moved at rate about 1/10 that of flood water). Greenburg[33] found that borax in Hanford fine sandy loam penetrated only 2 feet.
Boron	$Na_2B_4O_7 \cdot 10H_2O$ H_3BO_3	Field: Injection into input wells. Dilution factor ~5.	Limit of detection is reached after 10^3 dilution.
Thiocyanate	NH_4SCN Alkali-Metal SCN	Field: Water flooding.	Dilution possible 10^5.
Rhodamine B		Field: Karst topography.	Dye not detected after 6×10^6 dilution.
Oxygen-18 Deuterium	H_2O HDO		Variations resulting from seasonal fluctuations are useful to study climate. (Also useful in meteorology and glaciology).

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Table 2. SUBSURFACE RADIOISOTOPE FLUID TRACERS - FIELD TESTS[6]

Tracer	Half-life	Form	Test conditions	Remarks
H^3	12.5 y.	HTO	Karst groundwater	Useful results up to 30 km distance.
			Chalk River soil, undisturbed, but penetrated with driven piezometers.	Fluorescein dye traveled only about 3/4 as fast as tritium.
			Glaciofluvial sand and gravel, various till soils, and fissures and channels.	Chromium complex traveled as fast as tritium.
S^{35}	89.d	Na_2SO_4	Chalk River soil at waste disposal site.	Lags behind tritium indicating some interaction with the soil. In soil containing appreciable amounts of gypsum there would be exchange and loss of S^{35}.
I^{131}	8 d.	Iodine, carrier free	4 feet thick aquifer of sand and gravel, 90 feet below surface.	Should be used with several ppm stable iodine carrier.
I^{131} Co^{60}	8 d. 5.2 y.	KI EDTA - (Co) and $K_3Co(CN)_6$	Tracer compounds used with carriers KI, EDTA-(Na) and $K_3Co(CN)_6$. Limestone aquifer consisted of some argillaceous material and marly dolomite. Single-well pulse technique.	Co^{60} complex unsuitable in this aquifer. $K_3Co^{60}(CN)$ appeared to be most suitable of tracers tested in limestone aquifer.
Br^{82}	36 h.	NH_4Br	Aquifers in alluvium and permeable strata in calcareous marl series.	Br^{82} satisfactory under these conditions.
Rb^{86}	19 d.	RbCI	Groundwater direction at reservoir site in Egypt.	Tracer detectable 7 miles from injection after 5 d.
I^{131} P^{32}	8 d. 14 d.	NaI Na_2HPO_4	Single pulse tracing at dam site for Jesenitz Reservoir on Odrava River in Czechoslovakia.	Detected very high subterranean water velocity necessitating sealing of porous layer.
I^{131} Br^{82} Na^{24}	8 d. 36 h. 15 h.	NaI NaBr NaCI	Single pulse tracing at dam site on arges River in Roumania.	Determined direction and velocity of flow of subsurface water.

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3 Acid - severe effects upon total stream ecology.

4 Temperature - infra-red filming techniques reveal 1°C temperature differences.

5 Lignins - "Orzan" is a commercial product based upon these non-degrading materials.

6 Dyes - prevalent in textile wastes.

F Biota, various techniques possible with:

1 Mammals

2 Fish

3 Shellfish

V DESIRABLE CHARACTERISTICS

Ideally a tracer should be:

A Biologically innocuous to human and aquatic life, with special reference to

1 Acute toxicity

2 Long-term toxicity

3 Carcinogenic effects

4 Genetic effects

B Stable or persistant despite the effects of

1 Stream chemical constituents

2 Bacteria

3 Sunlight

4 Adsorption

5 Temperature

6 Wind action

7 Inherent decay

8 Channel obstructions

9 Stratification

C Readily detectable either

1 Visually and/or

2 Instrumentally despite dilution or background effects;

D Representative: coincide with the real waste and stream blend under study: this involves miscibility and specific gravity characteristics similar to the blend.

E Economic: this involves careful evaluation of the costs for

1 Materials

2 Material preparation, and release - ease of handling

3 Detection equipment

4 Detection technique and recording - convenience in operation

5 Stream deterioration

F Esthetically agreeable: inoffensive to:

1 Taste

2 Sight - conflict with easy detection

VI ELEMENTS OF A TRACER STUDY - USING RHODAMINE DYES (Currently in widespread use for surface studies.)

A Rhodamine Dyes

1 Available from American Cyanamid, DuPont, Allied Chemical and General Aniline.

2 Price $4.95 per pound except Rhodamine WT which is $10.00 per pound.

3 Specific gravity of 0.99 to 1.12 depending on the proportions of Alcohol, Ethylene Glycol, Acetic Acid, and Water used as solvents in preparing the solution.

4 Solutions normally 15%, 20%, 30%, or 40% dye by weight.

5 pH 1.0 to 9.0.

6 Peak wavelength of adsorption at 525-556μ and of fluorescence at 548-585μ are not normally found in natural waters.

7 Detectable to 0.5 μg/l with continuously sampling and recording equipment.

8 Visibly red to approximately 1.0 mg/l.

9 Rapid dispersion when dropped in water.

10 Subject to some destruction by light, but much less than experienced with fluorescein.

11 Destroyed by agents such as hypochlorite. Rhodamine WT is more resistant.

12 Essentially non-toxic.

B Measuring Equipment

1 Available from Turner Associates, Beckman Instruments, Inc., American Instrument Co., Inc.

2 Price as low as $1,000 for photofluorometer complete or $500 for unit to modify a spectrophotometer.

3 Available for individual grab sample analysis or continuous analysis of sample pumped through instrument.

4 Automatic recorders available.

5 Will measure several dyes (fluorescein, rhodamine, etc.)

C Fluorometer Calibration

The instrument should be calibrated before and after a study. The calibration is a function of temperature (about 2.3%/°C) so two or more runs with temperatures covering the expected range of the study water are desirable. Also it is advisable to make a calibration with the study water. This will check for background and any difference in fluorescent characteristics. A few helpful hints are:

1 Pumping a known concentration sample, from a plastic bag immersed in a constant temperature water bath, through the meter is a convenient calibration procedure. Continuous dousing with a concentrated solution into the bag will give various instrument readings through the detectable ranges.

2 Flush the system with methyl or butyl alcohol solution before each calibration to remove traces of dye.

3 Bubbles in the flow cuvette will cause erratic readings.

4 The fluorometer door is not light tight, so the instrument should be draped and used out of intense light to prevent erroneous high readings.

5 The sample lines on a continuous flow system will transmit light through the instrument door causing erroneous high readings. Taping these tubes with black electrical tape for about one foot will alleviate problem.

D Study Procedures

1 Select objectives which then dictate:

a Length of time to be studied

b Release point

c Monitoring locations and schedules

2 Determine physical properties of affected area.

a Probable net flows

b Total water volumes

c Probable water temperature

d Natural fluorescent background

e Salinity

f Suspended solids

3 Estimate required quantities of dye

4 Calibrate fluorometers

5 Conduct mock run to test equipment

6 Public relations

7 Release and monitor dye

8 Process and interpret data

9 Report

VIII DISPERSION STUDY COMPUTATIONS

A Most Dispersion Equations are Normalized Solutions of Ficks Second Law

$$\frac{\partial c}{\partial t} = D_x \frac{\partial^2 c}{\partial x^2}$$

c = concentration, x = distance,
t = time, D_x = diffusion coefficient

B Hydraulic Model Studies of the Fate of Pollution

From instantaneous release compute steady state nonconservative pollutant distribution

$$C_{(xy)} = \int f(t) + g(t)$$

where f(t) represents concentration function and g(t) = decay function

C Coastal Water Dispersion Study, Hilo Bay, Hawaii[9]

1 From instantaneous release measure concentration profile during slack water

2 Using these measurements and a two dimensional dispersion model compute D_x and D_y.

3 Two dimensional model

$$\frac{\partial c}{\partial t} = D_x \frac{\partial^2 c}{\partial x^2} + D_y \frac{\partial^2 c}{\partial y^2} - \bar{U}\frac{\partial c}{\partial x} - \bar{V}\frac{\partial c}{\partial y}$$

$\bar{U}$ = velocity in x direction
$\bar{V}$ = velocity in y direction

Normalized solution:

$$C = \frac{W_D}{2\pi\sigma_x\sigma_y} \exp - \frac{1}{2}\left[\left(x - \frac{ut^2}{\sigma_x}\right) + \left(y - \frac{vt^2}{\sigma_y}\right)\right]$$

D Channel Studies

1 From instantaneous release compute longitudinal dispersion coefficient

2 Measure time-concentration profile at given station

3 This C vs. t curve fits equation

$$C = \frac{M}{A(4\pi Dt)^{\frac{1}{2}}} \exp - \frac{(x - ut)^2}{4\,Dt}$$

4 Take log of both sides

$$Ct^{\frac{1}{2}} = \log\left(\frac{M}{A4\pi D}\right) - \frac{(x - ut)^2}{4\,Dt} \log e$$

5 D can be determined from semi-log

plot of $Ct^{\frac{1}{2}}$ vs. $\frac{(x - ut)^2}{t}$

REFERENCES

1 Straub, C.P., Ludzack, F.J., Hagee, G.R., and Goldin, A.S. Time of Flow Studies. Ottawa River, Lima, Ohio. Transactions, Amer. Geophysical Union, Vol. 39. pp 420-426. 1958

2 Feuerstein, D.L., and Selleck, R.E. Flourescent Tracers for Dispersion Measurements. Journal of the Sanitary Engineering Division, ASCE, Vol. 89, No. SA4, Proc. Paper 3586, pp 1-21. August, 1963.

3 Buchanan, Thomas J. Time of Travel of Soluble Contaminants in Streams. Journal of the Sanitary Engineering Division, ASCE, Vol. 90, No. SA3, Proc. Paper 3932, pp 1-12. June, 1964.

4 Wright, R.R. and Collings, M.R. Application of Fluorescent Tracing Techniques to Hydrologic Studies. Hydraulic Engrs., USGS, Atlanta, GA., Jour. AWWA, Vol. 56:748. June, 1964.

5 Pritchard, D.W. and Carpenter, J.H. Measurements of Turbulent Diffusion in Estuarine and Inshore Waters. Chesapeake Bay Institute, Johns Hopkins University. Contribution No. 53. 1960.

6 Ault, W.U., and Hardeway, J.E. Surface Tracing with Radioisotopes. Isotopics, Isotodes, Inc. Vol. 2 #1. January, 1965.

7 Diachishin, A.N. Dye Dispersion Studies. Jour. San. Engr. Div., ASCE, Vol. 89, No. SA1, Proc. Paper 3386, p. 29. January, 1963.

8 O'Connell, R.L., and Walter, C.M. Hydraulic Model Tests of Estuarial Waste Dispersion. Jour. San. Engr. Div., ASCE, Vol. 89, No. SA1, Proc. Paper 3394, January, 1963. p. 51.

9 O'Connell, R.L., and Walter, C.M. A Study of Dispersion in Hilo Bay, Hawaii. Report prepared for the U.S. Army Engineer District, Honolulu, Hawaii. September, 1963.

10 Tsivoglou, E.C., et al. Tracer Measurements of Atmospheric Reaeration. I. Laboratory Studies. Presented at the Water Pollution Control Federation Conference, Bal Harbour, Florida. September, 1964.

11 Cawley, W.A. and Rutledge, W. C. Application of Radioactive Tracer Techniques to Stream Improvement. Journal, San. Eng. Div., ASCE, Vol. 92, No. SA1, Proc. Paper 4640, p. 1, February, 1966.

12 Tsivoglou, E.C., O'Connell, R.L., Walter, C.M., Godsil, P.J., and Logsdon, G.S. Tracer Measurements of Atmospheric Reaeration-1. Laboratory Studies. Jour. WPCF, Vol, 37, p. 1343.

13 U.S. Atomic Energy Commission, Reprint from Federal Register, 17915, Part 30.70, Dec. 17, 1964, p. 5.

14 American Cyanamid Company, A Discussion of Techniques and Tracer Dyes used in Water Tracing, Dyes and Textile Chemicals Department - Bound Brook, NJ 08805

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Descriptors: Fluorescence, Fluorescent Dye, Path of Pollutants, Radioisotopes, Tracers

SAMPLING IN WATER QUALITY STUDIES

I INTRODUCTION

A Objective of Sampling

1 Water quality characteristics are not uniform from one body of water to another, from place to place in a given body of water, or even from time to time at a fixed location in a given body of water. A sampling program should recognize such variations and provide a basis for interpretation of their effects.

2 The purpose of collection of samples is the accumulation of data which can be used to interpret the quality or condition of the water under investigation. Ideally, the sampling program should be so designed that a statistical confidence limit may be associated with each element of data.

3 Water quality surveys are undertaken for a great variety of reasons. The overall objectives of each survey greatly influence the location of sampling stations, sample type, scheduling of sample collections, and other factors. This influence should always be kept in mind during planning of the survey.

4 The sampling and testing program should be established in accordance with principles which will permit valid interpretation.

a The collection, handling, and testing of each sample should be scheduled and conducted in such a manner as to assure that the results will be truly representative of the sources of the individual samples at the time and place of collection;

b The locations of sampling stations and the schedule of sample collections for the total sampling program should be established in such a manner that the stated investigational objectives will be met; and

c Sampling should be sufficiently repetitive over a period of time to provide valid data about the condition or quality of the water.

B Sample Variations

Interpretation of survey data is based on recognition that variations will occur in results from individual samples. While it is beyond the scope of this discussion to consider the implications of each in detail, the following can be identified as factors producing variations in data and should be considered in planning the sampling program.

1 Apparent Variations

a Variations of a statistical nature, due to collection of samples from the whole body of water, as contrasted with examination of all the water in the system.

b Variations due to inherent precision of the analytical procedures.

c Apparent variations are usually amenable to statistical analysis.

2 True Differences

a Variations of a cyclic nature

Diurnal variations, related to alternating periods of sunlight and darkness.

Dirunal variations related to waste discharges from communities.

Seasonal variations, related to temperature and its subsequent effects on chemical and biological processes and interrelationships.

Variations due to tidal influences, in coastal and estuarine waters.

b Intermittent variations

Dilution by rainfall and runoff.

Effects of irregular or intermittent discharges of wastewater, such as "slugs" of industrial wastes.

Irregular release of water from impoundments, as from power plants.

c Continuing changes in water quality

Effects downstream from points of continuous release of wastewater.

Effects of confluence with other bodies of water.

Effects of passage of the water through or over geological formations of such chemical or physical nature as to alter the characteristics of the water.

Continuing interactions of biological, physical, and chemical factors in the water, such as in the process of natural self-purification following introduction of organic contaminants in a body of water.

II LOCATION OF SAMPLING STATIONS

A The Influence of Survey Objectives

Much of the sampling design will be governed by the stated purpose of the water investigation. As an example of how different objectives might influence sampling design, consider a watercourse with points A and B located as indicated in Figure 1.

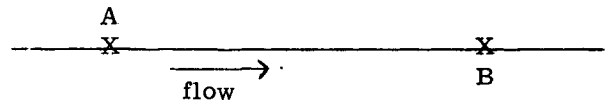


Figure 1

Point A can be the point of discharge of wastes from Community A. Point B can be any of several things, such as an intake of water treatment plant supplying Community B, or it might be the place where the river crosses a political boundary, or it may be the place where the water is subject to some legitimate use, such as for fisheries or for recreational use.

1 Assume that the objective of a water quality investigation is to determine whether designated standards of water quality are met at a water plant intake at Point B. In this case, the objective only is concerned with the quality of the water as it is available at Point B. Sampling will be conducted only at Point B.

2 Alternately, consider that there is a recognized unsatisfactory water quality at Point B, and it is alleged that this is due to discharges of inadequately treated wastes, originating at Point A. Assume that the charge is to investigate this allegation.

In this case the selected sampling sites will include at least three elements:

a At least one sampling site will be located upstream from Point A, to establish base levels of water quality, and to check the possibility that the observed conditions actually originated at some point upstream from Point A.

b A site or sites must be located downstream from Point A. Such a site should be downstream a sufficient distance to permit adequate mixing in the receiving water.

c Sampling would be necessary at Point B in order to demonstrate that the water quality is in fact impaired, and that the impairment is due to influences traced from Point A.

B Hydraulic Factors

1 Flow rate and direction

a In a survey of an extended body of water it is necessary to determine the rate and direction of water movement influences selection of sampling sites. Many workers plan sampling stations representing not less than the distance water flows in a 24-hour period. Thus, in Figure 1, intervening sampling stations would be selected at points representing the distance water would flow in about 24 hours.

b In a lake or impoundment direction of flow is the major problem influencing selection of sampling stations. Frequently it is necessary to establish some sort of grid network of stations in the vicinity of the suspected sources of pollution.

c In a tidal estuary, the oscillating nature of water movement will require establishment of sampling stations in both directions from suspected sources of pollution.

2 Introduction of other water

a In situations in which a stream being studied is joined by another stream of significant size and character, sampling stations will be located immediately above the extraneous stream, in the extraneous stream above its point of juncture with the main stream, and in the main stream below the point of juncture.

b Similar stations will be needed with respect to other water discharges, such as from industrial outfalls, other communities, or other installations in which water is introduced into the main stream.

3 Mixing

a Wherever possible, one sampling point at a sample collection site is used in stream surveys. This usually is near the surface of the water, in the main channel of flow.

b In some streams mixing does not occur quickly, and introduced water moves downstream for considerable distances below the point of confluence with the main streams. Example: Susquehanna River at Harrisburg, where 3 such streams are recognizable in the main river. Preliminary survey operations should identify such situations.

When necessary, collect separate samples at two or more points across the body of water.

c Similarly, vertical mixing may not be rapid. This is noted particularly in tidal estuaries, where it may be necessary to make collections both from near the bottom and near the surface of the water.

d Collection of multiple samples from a station requires close coordination with the laboratory, in terms of the number of samples that can be examined. Some types of samples may be composited. The decision must be reached separately for each type of sample.

C Types of Analytical Procedure

1 Samples collected for physical, chemical, and bacteriological tests and measurements may be collected from the same series of sampling stations.

2 Sampling stations selected for biological (ecological) investigation require selection of a series of similar aquatic habitats (a series of riffle areas, or a series of pool areas, or both). The sites used by the aquatic biologist may or may not be compatible with those used for the rest of the survey. Accordingly, in a given stream survey, the stations used by the aquatic biologist usually are somewhat different from the stations used for other examinations.

D Access to Sampling Stations

For practical reasons, the sampling site should be easily reached by automobile if a stream survey, or by boat if the survey is on a large body of water. Highway bridges are particularly useful, if the sample collector can operate in safety.

III FACTORS IN SCHEDULING OF SAMPLING PROGRAMS

A Survey Objectives

B Time of Year

1 In short-term water quality investigations, particularly in pollution investigations, there often is need to demonstrate the extremes of pollution effects on the aquatic environment. For this reason, many short-term surveys are conducted during the warmer season of the year, at such times as the water flow rate and volume is at a minimum and there is minimum likelihood of extensive rainfall.

2 In a long-term investigation, sampling typically is conducted at all seasons of the year.

C Daily Schedules

As shown in an introductory paragraph, water quality is subject to numerous cyclic or intermittent variations. Scheduling of sample collections should be designed to reveal such variations.

1 In short-term surveys it is common practice to collect samples from each sampling site at stated intervals through the 24-hour day, continuing the program for 1 - 3 weeks. Sampling at 3-hour intervals is preferred by many workers, though practical considerations may require extension to 4- or even 6-hour intervals.

2 In an extended survey there is a tendency to collect samples from each site at not more than daily intervals, or even longer. In such cases the hour of the day should be varied through the entire program, in order that the final survey show cyclic or intermittent variations if they exist.

3 In addition, sampling in tidal waters requires consideration of tidal flows. If samples are collected but once daily, many workers prefer to make the collections at low slack tide.

4 In long-term or any other survey in which only once-daily samples are collected, it is desirable to have an occasional period of around-the-clock sampling.

IV IDENTIFICATION OF SAMPLING SITES

A *River Mile System*

The FWPCA method of identifying points on a water course is by counting river miles from the mouth (or junction with a larger stream) back to the source. This should not be confused with other systems, such as those in which the river mile is started at the source of the stream and proceeds to the mouth of the stream or confluence with another body of water.

B STORET System

The STORET System is a computer-oriented data processing system used by FWPCA for storage, retrieval, and analysis of water quality data collected by federal, state, local, and private agencies.

The system includes a complex system - based on the river mile system - for identifying sampling locations on all rivers and streams in the United States. A recent addition to the system introduces a location procedure based on geographic coordinates; this procedure is especially adapted to location of sampling stations in large bodies of water such as lakes and impoundments.

Not all locations have been coded at this time, although the coding systems have been established. The interested worker should consult Public Health Service Publication No. 1263, "The Storage and Retrieval of Data for Water Quality Control," 1963.

IV SAMPLE COLLECTION

A Types of Samples

1 "Grab" sample - a grab sample is usually a manually collected single portion of the wastewater or stream water. An analysis of a grab sample shows the concentration of the constituents in the water at the time the sample was taken.

2 "Continuous" sample - when several points are to be sampled at frequent intervals or when a continuous record of quality at a given sampling station is required, an automatic or continuous sampler may be employed.

a Some automatic samplers collect a given volume of sample at definite time intervals; this is satisfactory when the volume of flow is constant.

b Other automatic samplers take samples at variable rates in proportion to changing rates of flow. This type of sampler requires some type of flow measuring device.

3 "Composite" sample - a composite sample is the collection and mixing together of various individual samples based upon the ratio of the volume of flow at the time the individual samples were taken to the total cumulative volume of flow. The desired composite period will dictate the magnitude of the cumulative volume of flow. The more frequently the samples are collected, the more representative will be the composite sample to the actual situation. Composite samples may be obtained by:

a Manual sampling and volume of flow determination made when each sample is taken.

b Constant automatic sampling (equal volumes of sample taken each time) with flow determinations made as each sample is taken.

c Automatic sampling which takes samples at pre-determined time intervals and the volume of sample taken is proportional to the volume of flow at any given time.

B Type of Sampling Equipment

1 Manual sampling

a Equipment is specially designed for collection of samples from the bottom muds, at various depths, or at water surfaces. Special designs are related to protection of sample integrity in terms of the water characteristic or component being measured.

b For details of typical sampling equipment used in water quality surveys. see outlines dealing with biological, bacteriological, and chemical tests in this manual.

c Manual sampling equipment has very broad application in field work, as great mobility of operation is possible, at lower cost than may be possible with automatic sampling equipment.

2 Automatic sampling equipment

Automatic sampling equipment has several important advantages over manual methods. Probably the most important consideration is the reduction in personnel requirements resulting from the use of this equipment. It also allows more frequent sampling than is practical manually, and eliminates many of the human errors inherent in manual sampling.

Automatic sampling equipment has some disadvantages. Probably the most important of these is the tendency of many automatic devices to become

clogged when liquids high in solids are being sampled. Individual portions of composite samples are usually quite small which may in some cases be disadvantageous. In using automatic samplers, sampling points are fixed, which results in a certain loss of mobility as compared to manual methods.

Automatic sampling equipment should not be used indiscriminately; some types of samples - notably bacteriological, biological, and DO samples - should not be composited. In cases of doubt, the appropriate analyst should be consulted.

a Compositing samplers

1) Jar and tube sampler - this type samples effectively when flow is nearly constant. As water drains from the upper carboy, the vacuum created syphons waste into the lower one. The rate-of-flow is regulated by the pinch clamp to fill the lower carboy during the sampling period. (See Figure 2)

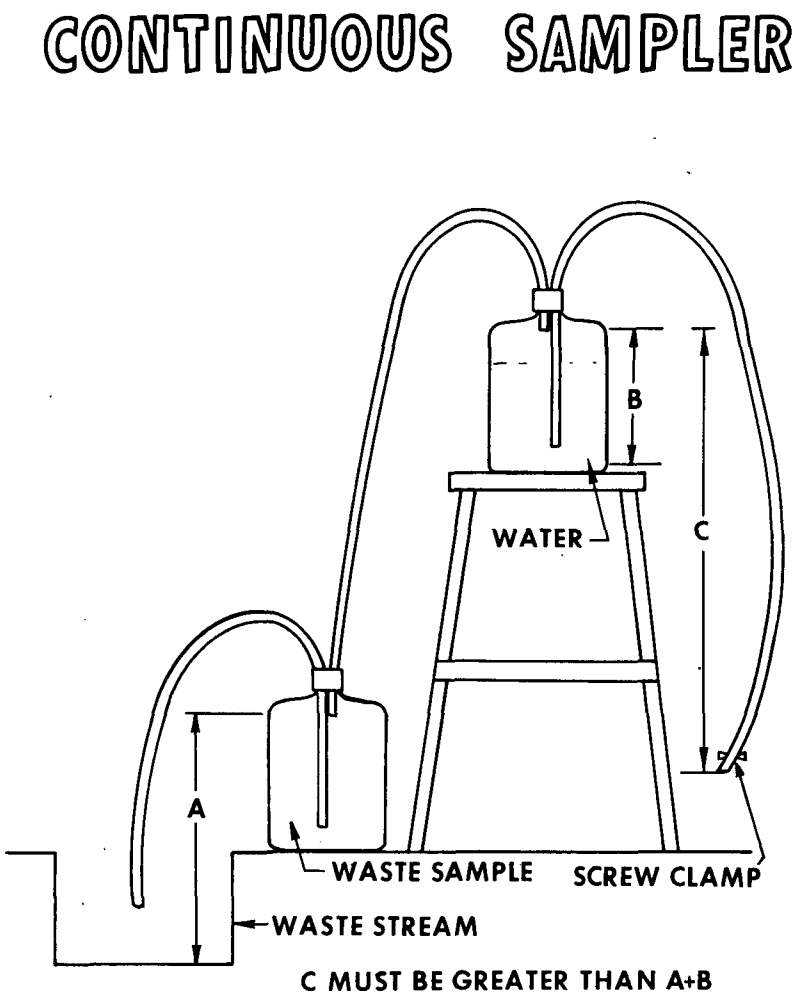


Figure 2

2) Scoop type

a) Rotating scoop

This device consists of a power driven scoop mounted upstream from a weir. The scoop is so designed and mounted that the sample volume grabbed on each rotation of the scoop is proportional to the flow, as governed by the head on the weir. The scoop may be rotated at a constant speed or timed to sample at fixed time intervals.

b) Revolving wheel with cups (Figure 3)

This device consists of a power driven wheel or disc mounted upstream from a weir. A number of freely suspended buckets are mounted at varying distances from the axis so that increased flow will cause more buckets to be filled, thereby giving a sample proportionate to flow. Both this device and the rotating scoop sampler can, of course, be used for non-proportionate sampling.

c) Bucket elevators

This device may consist of a single bucket alternately lowered into and raised out of the waste stream, or it may consist of a series of buckets on an endless chain passing through the waste stream. In either case, it will include a tripping mechanism to cause the bucket or buckets to spill into a sampling funnel. Both types may be operated continuously or timed for intermittent operation. This method is not well adapted to proportionate sampling.

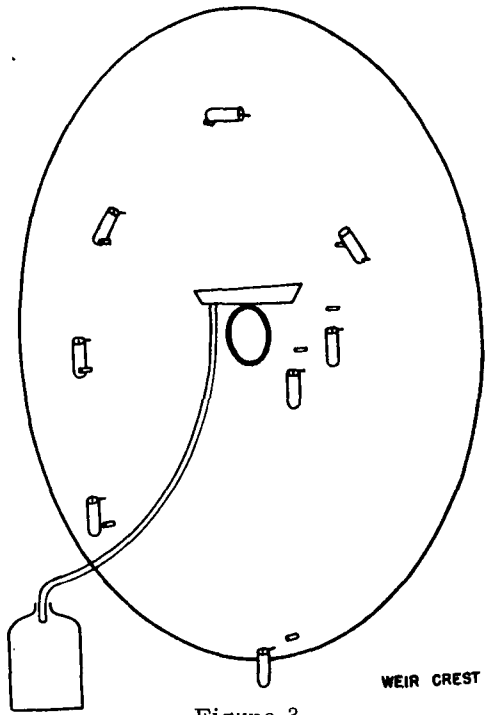


Figure 3
WHEEL WITH BUCKETS

3 Pumps

a) Chemical feed pumps have been found useful for sampling, because of their ability to meter out small doses of liquids. A timing mechanism may be used to make the pump run for longer periods during heavy flow, thereby allowing collection of the sample in proportion to flow. These pumps are usually provided with adjustable stroke and variable speed features which allow variation of the volume of sample being pumped. Figure 4 illustrates a battery operated pump.

b) Automatic shift sampler (Figure 5)

Figure 5 shows the automatic shift sampler. It consists first of a Randolph or other "squeegee-" type pump unit.

The 2-rpm gear motor drives the pump at between 1 and 2 rpm through the spring-loaded adjustable-pitch pulley and adjustable motor-base arrangement.

We use 1/8-in. (.32-cm) ID or 1/4-in. (.64-cm) ID polyethylene tubing for sample intake from the waste stream. The sample flow is delivered to the distributor via a 3/16-in. (.48-cm) ID Tygon tube which is supported loosely by a wire attached to the framework.

Operation of the distributor is very simple. The 1-rpm clock motor powers the chain-and-sprocket drive which turns a threaded bolt. Rotation of the bolt moves the discharge tube down the plastic trough at a rate equal to one division every eight hours. With the 10 sample-jar receivers the timer can be set on Friday, and the 9 week-end shift samples can be picked up on Monday.

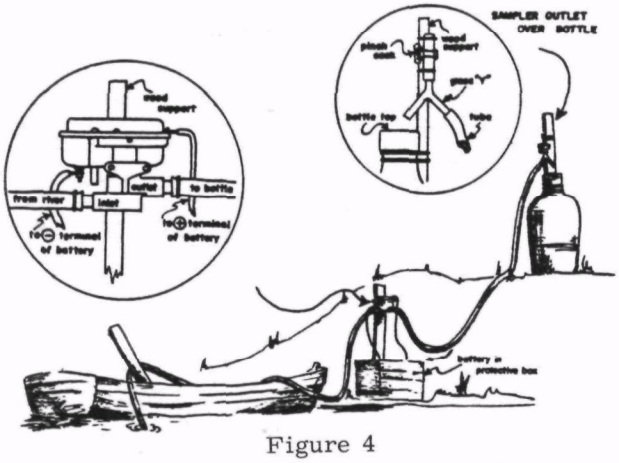
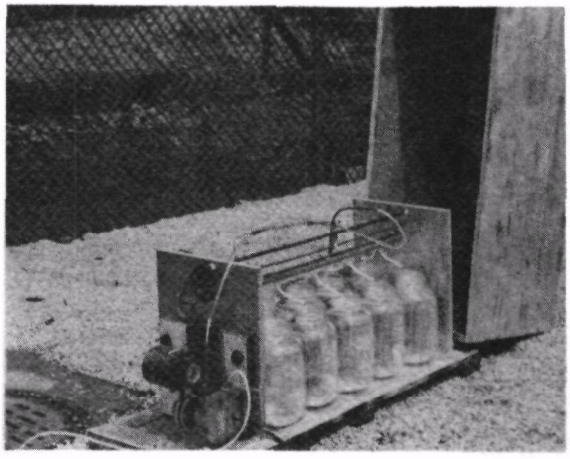


Figure 4
BATTERY OPERATED PUMP



4) Solenoid-valve arrangements

A solenoid valve employed in connection with a timing device may be used for withdrawing waste from a pipe under pressure. Used in connection with a pump such devices may be employed in sampling free flowing streams. (See Figures 6 and 7.)

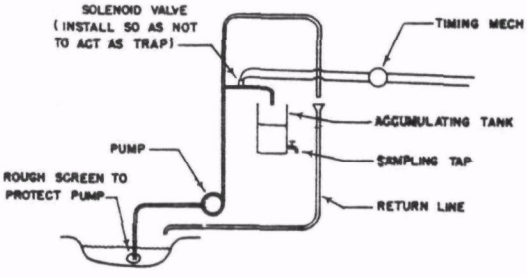


Figure 6
PUMP - SOLENOID VALVE - TIMER TYPE SAMPLER

5) Vacuum operated

In its simplest form, the vacuum is created by a suitably mounted siphon. It collects the sample at a uniform rate and is not suitable for use when proportional sampling is required.

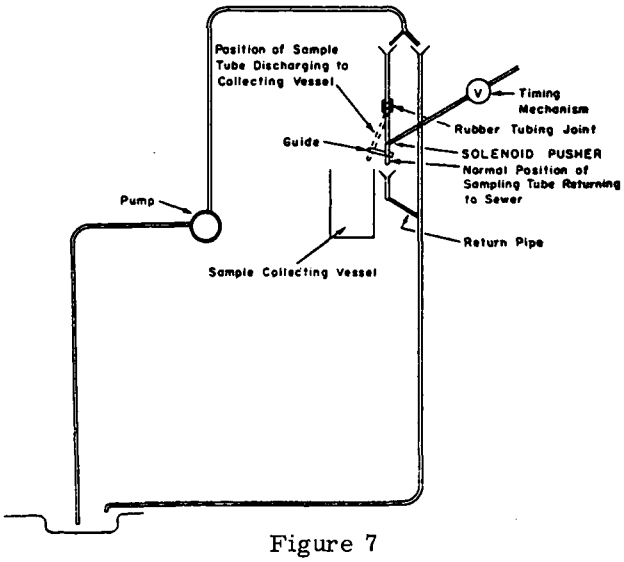


Figure 7
PUMP - SOLENOID-DIVERTER - TIMER TYPE SAMPLER

6) Air vent control

This type of device is illustrated in Figure 8. The rate of sample collection is controlled by the bubbling mechanism. It is not suitable for use when proportionate sampling is required.

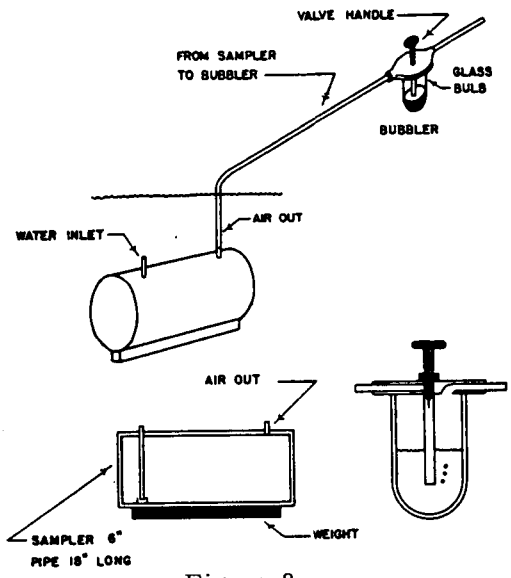


Figure 8
AIR RELEASE TYPE SAMPLER

7) Drip sampler

Two types of this device are illustrated in Figures 9 and 10. Both devices are simple methods of obtaining a composite sample at a fairly constant rate.

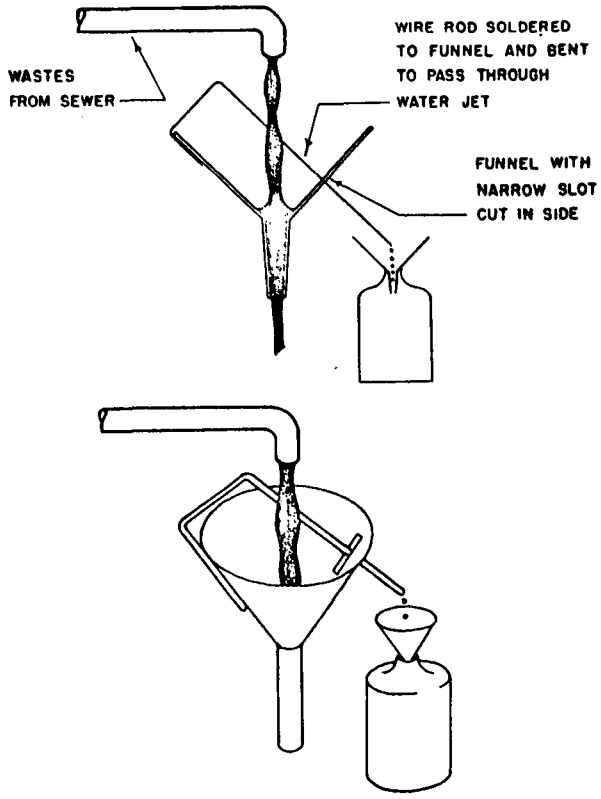


Figure 9
FUNNEL AND ROD DIVERTER

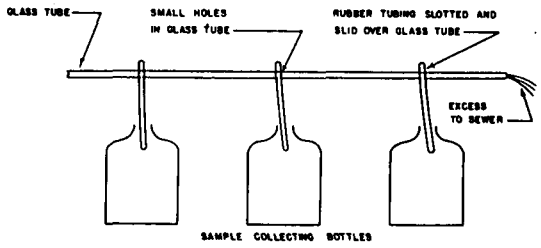


Figure 10
DRIP TUBE SAMPLERS

b Continuous recording equipment

Instruments have been developed which provide direct measurement of temperature, pH, conductivity, color, and dissolved oxygen. Such instruments may be equipped for continuous recording. Instruments of this type are quite expensive and their installation is often difficult. They are best adapted to permanent installations, although good portable non-recording instruments are available for the measurement of temperature, pH, and conductivity.

VI SOME CONSIDERATIONS IN SAMPLING OPERATIONS

All procedures in care and handling of samples between collection and the performance of observations and tests are directed toward maintaining the reliability of the sample as an indication of the characteristics of the sample source.

A Sample Quantity

1 Samples for a series of chemical analyses require determination of the total sample volume required for all the tests, and should include enough sample in addition to provide a safety factor for laboratory errors or accidents. Many workers collect about twice the amount of sample actually required for the chemical tests. As a rule of thumb, this is on the order or 2 liters.

2 Bacteriological samples, in general, are collected in 250 - 300 ml sterile bottles; approximately 150 - 200 ml of samples is adequate in practically all cases.

B Sample Identification

1 Sample identification must be maintained throughout any survey. It is vital, therefore, that adequate records be made of all information relative to the source of the sample and conditions under which the collection was made. All information must be clearly understandable and legible.

2 Every sample should be identified by means of a tag or bottle marking, firmly affixed to the sample bottle. Any written material should be with indelible marking material.

3 Minimum information on the sample label should include identification of the sample site, date and time of collection, and identification of the individual collecting the sample.

4 Supplemental identification of samples is strongly recommended, through maintenance of a sample collection logbook. If not included on the sample tag (some prefer to duplicate such information) the logbook can show not only the sample site and date and hour of collection, but also the results of any tests made on site (such as temperature, pH, dissolved oxygen). In addition, the logbook should provide for notation of any unusual observations made at the sampling site, such as rainfall, direction and strength of unusual winds, or evidence of disturbance of the collection site by human or other animal activity.

C Care and Handling of Samples

1 As a general policy, all observations and tests should be made as soon as possible after sample collection.

a Some measurements require performance at the sampling site, such as temperature, pH, dissolved oxygen, chlorine, flow rate, etc.

b Some tests are best made at the sampling site because the procedures are simple, rapid, and of acceptable accuracy. These may include such determinations as conductivity.

c Some additional determinations, such as alkalinity, hardness, and turbidity may be made in the field, provided

that ease, convenience, and reliability of results are acceptable for the purposes of the study.

2 Samples to be analyzed in the laboratory require special protection to assure that the quality measured in the sample represents the condition of the source. Many samples, especially those subjected to biological analysis, require special preservation, protection, and handling procedures. In case of doubt, the appropriate analyst should be consulted. Most common procedures for sample protection include:

a Examination within brief time after collection.

b Temperature control.

c Protection from light.

d Addition of preservative chemicals.

Applications of these sample protective procedures are along the following lines:

3 Early examination of sample

Applicable to all types of samples.

4 Temperature control

a All biological materials for examination in a living state should be iced between collection and examination.

b Bacteriological samples should be iced during a maximum transport time of 6 hours. Such samples should be refrigerated upon receipt in the laboratory and processed within 2 hours.

c Chemical samples often require icing

Preservation by refrigeration at $4^{o}C$ is recommended for acidity, alkalinity, BOD, color, sulfate, threshold odor, and other samples. Holding times vary. Quick freezing will permit retention of many samples for up to several months prior to laboratory examination.

5 Protection from light

a Any constituent of water which may be influenced by physiochemical reactions involving light should be protected. DO samples brought to the iodine stage, for example, should be protected from light prior to titration.

b In addition, any water constituent (such as dissolved oxygen) which may be influenced by algal activity should be protected from light.

6 Addition of chemical preservatives

a Bacteriological samples <u>never</u> should be "protected" by addition of preservative agents. The only permissible chemical additive is sodium thiosulfate, which is used to neutralize free residual chlorine, if present.

b Samples for biological examination should be protected by chemical additives only under specific direction of the principal biologist in a water quality study.

c For chemical tests, preservatives are useful for a number of water components. The following examples are cited:

<u>Nitrogen and phosphorus analyses:</u>

The addition of 40 mg $HgCl_2$ per liter of sample and refrigeration at $4^{o}C$ will retard biological activity which might otherwise alter the concentration of these constituents

Metals: The addition of 5 ml of HNO_3 acid per liter of sample prevents precipitation of the metal in the container.

COD and Organic Carbon: Addition of 2 ml sulfuric acid per liter of sample is suggested.

In general, samples requiring retardation of biological activity can be temporarily preserved by addition of chloroform; tests should be run as soon as possible, however.

Cyanide determinations may be delayed temporarily through addition of alkali to the sample. Addition of sodium hydroxide to pH 10 is recommended.

Sulfide analysis may be delayed up to 7 days by addition of 2 ml/liter of sample of 2N solution of zinc acetate.

Phenol analysis can be delayed temporarily by acidification to pH 4.0 with phosphoric acid and preservation with 1 gram $CuSO_4 \cdot 5H_2O$ per liter of sample.

REFERENCES

1 Standard Methods for the Examination Water, Sewage and Industrial Wastes. 13th Ed. A.P.H.A. 1971

2 Planning and Making Industrial Waste Surveys. Ohio River Valley Water Sanitation Commission.

3 Industry's Idea Clinic. Journal of the Water Pollution Control Federation. April, 1965.

This outline was prepared by H. L. Jeter, Director, National Training Center, EPA, WPO, Cincinnati, OH 45268 and P.F. Atkins, Jr., formerly Sanitary Engineer, Training Activities.

Descriptors: Instrumentation, On-Site Investigations, Preservation, Samplers, Sampling, Water Sampling, Handling, Sample, Surface Waters, Wastewater

SAMPLE HANDLING - FIELD THROUGH LABORATORY

I PLANNING A SAMPLING PROGRAM

A Factors to Consider:

1 Locating sampling sites

2 Sampling equipment

3 Type of sample required

a grab
b composite

4 Amount of sample required

5 Frequency of collection

6 Preservation measures, if any

B Decisive Criteria

1 Nature of the sample source

2 Stability of constituent(s) to be measured

3 Ultimate use of data

II REPRESENTATIVE SAMPLES

If a sample is to provide meaningful and valid data about the parent population, it must be representative of the conditions existing in that parent source at the sampling location.

A The container should be rinsed two or three times with the water to be collected.

B Compositing Samples

1 For some sources, a composite of samples is made which will represent the average situation for stable constituents.

2 The nature of the constituent to be determined may require a series of separate samples.

C The equipment used to collect the sample is an important factor to consider. ASTM[1] has a detailed section on various sampling devices and techniques.

D Great care must be exercised when collecting samples in sludge or mud areas and near benthic deposits. No definite procedure can be given, but careful effort should be made to obtain a representative sample.

III SAMPLE IDENTIFICATION

A Each sample must be unmistakably identified, preferably with a tag or label. The required information should be planned in advance.

B An information form preprinted on the tags or labels provides uniformity of sample records, assists the sampler, and helps ensure that vital information will not be omitted.

C Useful Identification Information includes:

1 sample identity code
2 signature of sampler
3 signature of witness
4 description of sampling location detailed enough to accommodate reproducible sampling. (It may be more convenient to record the details in the field record book).
5 sampling equipment used
6 date of collection
7 time of collection
8 type of sample (grab or composite)
9 water temperature
10 sampling conditions such as weather, water level, flow rate of source, etc.
11 any preservative additions or techniques
12 record of any determinations done in the field
13 type of analyses to be done in laboratory

IV SAMPLE CONTAINERS

A Available Materials

1 glass
2 plastic
3 hard rubber

B Considerations

1 Nature of the sample - Organics attack polyethylene.

2 Nature of constituent(s) to be determined - Cations can adsorb readily on some plastics and on certain glassware. Metal or aluminum foil cap liners can interfere with metal analyses.

3 Preservatives to be used - Mineral acids attack some plastics.

4 Mailing Requirements - Containers should be large enough to allow extra volume for effects of temperature changes during transit. All caps should be securely in place. Glass containers must be protected against breakage. Styrofoam linings are useful for protecting glassware.

C Preliminary Check

Any question of possible interferences related to the sample container should be resolved before the study begins. A preliminary check should be made using corresponding sample materials, containers, preservatives and analysis.

D Cleaning

If new containers are to be used, preliminary cleaning is usually not necessary.

If the sample containers have been used previously, they should be carefully cleaned before use.

There are several cleaning methods available. Choosing the best method involves careful consideration of the nature of the sample and of the constituent(s) to be determined.

1 Phosphate detergents should not be used to clean containers for phosphorus samples.

2 Traces of dichromate cleaning solution will interfere with metal analyses.

E Storage

Sample containers should be stored and transported in a manner to assure their readiness for use.

V SAMPLE PRESERVATION

Every effort should be made to achieve the shortest possible interval between sample collection and analyses. If there must be a delay and it is long enough to produce significant changes in the sample, preservation measures are required.

At best, however, preservation efforts can only retard changes that inevitably continue after the sample is removed from the parent population.

A Functions

Methods of preservation are relatively limited. The primary functions of those employed are:

1 to retard biological action
2 to retard precipitation or the hydrolysis of chemical compounds and complexes
3 to reduce volatility of constituents

B General Methods

1 pH control - This affects precipitation of metals, salt formation and can inhibit bacterial action.

2 Chemical Addition - The choice of chemical depends on the change to be controlled.

Mercuric chloride is commonly used as a bacterial inhibitor. Disposal of the mercury-containing samples is a problem and efforts to find a substitute for this toxicant are underway.

To dispose of solutions of inorganic mercury salts, a recommended procedure is to capture and retain the mercury salts as the sulfide at a high pH. Several firms have tentatively agreed to accept the mercury sulfide for re-processing after preliminary conditions are met.[4]

3 Refrigeration and Freezing - This is the best preservation technique available, but it is not applicable to all types of samples. It is not always a practical technique for field operations.

C Specific Methods

The EPA Methods Manual[2] includes a table summarizing the holding times and preservation techniques for several analytical procedures. This information also can be found in the standard references[1, 2, 3] as part of the presentation of the individual procedures.

VI METHODS OF ANALYSIS

Standard reference books of analytical procedures to determine the physical and chemical characteristics of various types of water samples are available.

A EPA Methods Manual

The Methods Development and Quality Assurance Research Laboratory of the Environmental Protection Agency, has published a manual of analytical procedures to provide methodology for monitoring the quality of our Nation's Waters and to determine the impact of waste discharges. The title of this manual is "Methods for Chemical Analysis of Water and Wastes."[2]

For some procedures, the analyst is referred to Standard Methods and/or to ASTM Standards.

B Standard Methods

The American Public Health Association, the American Water Works Association and the Water Pollution Control Federation prepare and publish a volume describing methods of water analysis. These include physical and chemical procedures. The title of this book is "Standard Methods for the Examination of Water and Wastewater."[3]

C ASTM Standards

The American Society for Testing and Materials publishes an annual "book" of specifications and methods for testing materials. The "book" currently consists of 33 parts. The part applicable to water is a book titled, "Annual Book of ASTM Standards, Part 23, Water; Atmospheric Analysis".[1]

D Other References

Current literature and other books of analytical procedures with related information are available to the analyst.

E NPDES Methodology

When gathering data for National Pollutant Discharge Elimination System report purposes, the analyst must consult the Federal Register for a listing of approved analytical methodology. There he will be directed to pages in the above cited reference books where acceptable procedures can be found. The Federal Register also provides information concerning the protocol for obtaining approval to use analytical procedures other than those listed.

VII ORDER OF ANALYSES

The ideal situation is to perform all analyses shortly after sample collection. In the practical order, this is rarely possible. The allowable holding time for preserved samples is the basis for scheduling analyses.

A The allowable holding time for samples depends on the nature of the sample, the stability of the constituent(s) to be determined and the conditions of storage.

1 For some constituents and physical values, immediate determination is required, e.g. dissolved oxygen, pH.

2 Using preservation techniques, the holding times for other determinations range from 6 hours (BOD) to 7 days (COD). Metals may be held up to 6 months.[2]

3 The EPA Methods Manual[2] includes a table summarizing holding times and preservation techniques for several analytical procedures. This information can also be found in the standard references[1, 2, 3] as part of the presentation of the individual procedures.

4 If dissolved concentrations are sought, filtration should be done in the field if at all possible. Otherwise, the sample is filtered as soon as it is received in the laboratory. A 0.45 micron membrane filter is recommended for reproducible filtration.

B The time interval between collection and analysis is important and should be recorded in the laboratory record book.

VIII RECORD KEEPING

The importance of maintaining a bound, legible record of pertinent information on samples cannot be over-emphasized.

A Field Operations

A bound notebook should be used. Information that should be recorded includes:

1 Sample identification records (See Part III)

2 Any information requested by the analyst as significant

3 Details of sample preservation

4 A complete record of data on any determinations done in the field. (See B, next)

5 Shipping details and records

B Laboratory Operations

Samples should be logged in as soon as received and the analyses performed as soon as possible.

A bound notebook should be used. Preprinted data forms provide uniformity of records and help ensure that required information will be recorded. Such sheets should be permanently bound.

Items in the laboratory notebook would include:

1 sample identifying code
2 date and time of collection
3 date and time of analysis
4 the analytical method used
5 any deviations from the analytical method used and why this was done
6 data obtained during analysis
7 results of quality control checks on the analysis
8 any information useful to those who interpret and use the data
9 signature of the analyst

IX SUMMARY

Valid data can be obtained only from a representative sample, unmistakably identified, carefully collected and stored. A skilled analyst, using approved methods of analyses and performing the determinations within the prescribed time limits, can produce data for the sample. This data will be of value only if a written record exists to verify sample history from the field through the laboratory.

REFERENCES

1 ASTM Standards, Part 23, Water; Atmospheric Analysis.

2 Methods for Chemical Analysis of Water and Wastes, EPA-MDQARL, Cincinnati, OH 45268, 1974.

3 Standard Methods for the Examination of Water and Wastewater, 13th edition, APHA-AWWA-WPCF, 1971.

4 Dean, R., Williams, R. and Wise, R., Disposal of Mercury Wastes from Water Laboratories, Environmental Science and Technology, October, 1971.

This outline was prepared by A. Donahue, Chemist, National Training Center, MPQD EPA, WPO, Cincinnati, Ohio 45268.

Descriptors: On-Site Data Collections, On-Site Investigations, Planning, Handling, Sample, Sampling, Water Sampling, Surface Waters, Preservation, Wastewater

SAMPLE PRESERVATION

Complete and unequivocal preservation of samples, either domestic sewage, industrial wastes or natural waters, is a practical impossibility. Regardless of the nature of the sample, complete stability for every constituent can never be achieved. At best, preservation techniques can only retard the chemical and biological changes that inevitably continue after the sample is removed from the parent source.

The changes that take place in a sample are either chemical or biological. In the former case, certain changes occur in the chemical structure of the constituents that are a function of physical conditions. Metal cations may precipitate as hydroxides or form complexes with other constituents; cations or anions may change valance states under certain reducing or oxidizing conditions; other constituents may dissolve or volatilize with the passage of time. Metal cations may also adsorb onto surfaces (glass, plastic, quartz, etc.), such as, iron and lead. Biological changes taking place in a sample may change the state of an element or a radical to a different state. Soluble constituents may be converted to organically bound material in cell structures, or cell lysis may result in release of cellular materials into solution. The well known nitrogen and phosphorus cycles are examples of biological influence on sample composition.

Methods of preservation are relatively limited and are intended generally to (1) retard biological action, (2) retard hydrolysis of chemical compounds and complexes and (3) reduce volatility of constituents.

Preservation methods are generally limited to pH control, chemical addition, refrigeration, and freezing. Table 1 shows the various preservatives that may be used to retard changes in samples.

Table 1

Preservative	Action	Applicable to:
$HgCl_2$	Bacterial Inhibitor	Nitrogen forms, Phosphorus forms
Acid (HNO_3)	Metals solvent, prevents precipitation	Metals
Acid (H_2SO_4)	Bacterial Inhibitor	Organic samples (COD, oil and grease, organic carbon)
	Salt formation with organic bases	Ammonia, amines
Alkali (NaOH)	Salt formation with volatile compounds	Cyanides, organic acids
Refrigeration	Bacterial Inhibitor	Acidity - alkalinity organic materials, BOD, color, odor, organic P, organic N, carbon, etc., biological organisms (coliform, etc.)

In summary, refrigeration at temperatures near freezing or below is the best preservation technique available, but is not applicable to all types of samples.

The recommended choice of preservatives for various constituents is given in Table 2. These choices are based on the accompanying references and on information supplied by various Regional Analytical Quality Control Coordinators.

Table 2

RECOMMENDATION FOR SAMPLING AND PRESERVATION OF SAMPLES ACCORDING TO MEASUREMENT[1]

Measurement	Vol. Req. (ml)	Container	Preservative	Holding Time[6]
Acidity	100	P, G[2]	Cool, 4°C	24 Hrs.
Alkalinity	100	P, G	Cool, 4°C	24 Hrs.
Arsenic	100	P, G	HNO_3 to pH< 2	6 Mos.
BOD	1000	P, G	Cool, 4°C	6 Hrs.[3]
Bromide	100	P, G	Cool, 4°C	24 Hrs.
COD	50	P, G	H_2SO_4 to pH< 2	7 Days
Chloride	50	P, G	None Req.	7 Days
Chlorine Req.	50	P, G	Cool, 4°C	24 Hrs.
Color	50	P, G	Cool, 4°C	24 Hrs.
Cyanides	500	P, G	Cool, 4°C NaOH to pH 12	24 Hrs.
Dissolved Oxygen				
Probe	300	G only	Det. on site	No Holding
Winkler	300	G only	Fix on site	No Holding

Table 2 (Continued)

RECOMMENDATION FOR SAMPLING AND PRESERVATION OF SAMPLES ACCORDING TO MEASUREMENT (1)

Measurement	Vol. Req. (ml)	Container	Preservative	Holding Time (6)
Fluoride	300	P, G	Cool, 4°C	7 Days
Hardness	100	P, G	Cool, 4°C	7 Days
Iodide	100	P, G	Cool, 4°C	24 Hrs.
MBAS	250	P, G	Cool, 4°C	24 Hrs.
Metals				
Dissolved	200	P, G	Filter on site HNO_3 to pH < 2	6 Mos.
Suspended			Filter on site	6 Mos.
Total	100		HNO_3 to pH < 2	6 Mos.
Mercury				
Dissolved	100	P, G	Filter HNO_3 to pH < 2	38 Days (Glass) 13 Days (Hard Plastic)
Total	100	P, G	HNO_3 to pH < 2	38 Days (Glass) 13 Days (Hard Plastic)

Table 2 (Continued)

RECOMMENDATION FOR SAMPLING AND PRESERVATION OF SAMPLES ACCORDING TO MEASUREMENT (1)

Measurement	Vol. Req. (ml)	Container	Preservative	Holding Time (6)
Nitrogen				
Ammonia	400	P, G	Cool, 4°C H_2SO_4 to pH < 2	24 hrs. (4)
Kjeldahl	500	P, G	Cool, 4°C H_2SO_4 to pH < 2	24 Hrs. (4)
Nitrate	100	P, G	Cool, 4°C H_2SO_4 to pH < 2	24 Hrs. (4)
Nitrite	50	P, G	Cool, 4°C	24 Hrs. (4)
NTA	50	P, G	Cool, 4°C	24 Hrs.
Oil and Grease	1000	G only	Cool, 4°C H_2SO_4 to pH < 2	24 Hrs.
Organic Carbon	25	P, G	Cool, 4°C H_2SO_4 to pH < 2	24 Hrs.
pH	25	P, G	Cool, 4°C Det. on site	6 Hrs. (3)
Phenolics	500	G only	Cool, 4°C H_3PO_4 to pH < 4 1.0g $CuSO_4$/1	24 Hrs.
Phosphorus				
Ortho-phosphate Dissolved	50	P, G	Filter on site Cool, 4°C	24 Hrs. (4)

Table 2 (Continued)

RECOMMENDATION FOR SAMPLING AND PRESERVATION OF SAMPLES ACCORDING TO MEASUREMENT[1]

Measurement	Vol. Req. (ml)	Container	Preservation	Holding Time[6]
Hydrolyzable	50	P, G	Cool, 4°C H_2SO_4 to pH< 2	24 Hrs.[4]
Total	50	P, G	Cool, 4°C	24 Hrs.[4]
Total, Dissolved	50	P, G	Filter on site Cool, 4°C	24 Hrs.[4]
Residue				
Filterable	100	P, G	Cool, 4°C	7 Days
Non-Filterable	100	P, G	Cool, 4°C	7 Days
Total	100	P, G	Cool, 4°C	7 Days
Volatile	100	P, G	Cool, 4°C	7 Days
Settleable Matter	1000	P, G	None Req.	24 Hrs.
Selenium	50	P, G	HNO_3 to pH< 2	6 Mos.
Silica	50	P only	Cool, 4°C	7 Days
Specific Conductance	100	P, G	Cool, 4°C	24 Hrs.[5]
Sulfate	50	P, G	Cool, 4°C	7 Days

Table 2 (Continued)

RECOMMENDATION FOR SAMPLING AND PRESERVATION OF SAMPLES ACCORDING TO MEASUREMENT[1]

Measurement	Vol. Req. (ml)	Container	Preservation	Holding Time[6]
Sulfide	50	P, G	2 ml zinc acetate	24 Hrs.
Sulfite	50	P, G	Cool, 4°C	24 Hrs.
Temperatue	1000	P, G	Det. on site	No Holding
Threshold Odor	200	G only	Cool, 4°C	24 Hrs.
Turbidity	100	P, G	Cool, 4°C	7 Days

1 More specific instructions for preservation and sampling are found with each procedure as detailed in this manual. A general discussion on sampling water and industrial wastewater may be found in ASTM, Part 23, p. 72-91 (1973).

2 Plastic or Glass

3 If samples cannot be returned to the laboratory in less than 6 hours and holding time exceeds this limit, the final reported data should indicate the actual holding time.

4 Mercuric chloride may be used as an alternate preservative at a concentration of 40 mg/l, especially if a longer holding time is required. However, the use of mercuric chloride is discouraged whenever possible.

5 If the sample is stabilized by cooling, it should be warmed to 25°C for reading, or temperature correction made and results reported at 25°C.

6 It has been shown that samples properly preserved may be held for extended periods beyond the recommended holding time.

An excerpt from Manual of Chemical Analysis of Water and Wastes, 1974, EPA, Methods Development and Quality Assurance Research Laboratory, NERC-Cincinnati, Ohio 45268.

Descriptors: Preservation, Sampling, Wastewater(Pollution), Water.

BACTERIOLOGICAL SAMPLING IN THE FIELD

I INTRODUCTION

The first step in the examination of a water supply for bacteriological examination is careful collection and handling of samples. Selection of sample sites, frequency of sampling, and numbers of samples are all directly related to the survey objectives.

II SELECTION OF SAMPLING LOCATIONS

The basis for locating sampling points is collection of representative samples.

A Grab samples from streams are frequently collected for control data or application of regulatory requirements. A grab sample can be taken in the stream near the surface.

B For intensive stream studies on source and extent of pollution, representative samples are taken by considering site, method, and time of sampling. The sampling sites may be a compromise between physical limitations of the laboratory, detection of pollution peaks, and frequency of sample collection in certain types of surveys. Decisions must be made as to the total number of samples that can be processed by laboratory personnel and the types of samples desired, as, for instance, those measuring immediate water quality or those measuring an averaging collection method.

A site designed to measure average conditions should be far enough downstream to ensure a complete mixing after the source entry of pollutants. Averaging does not eliminate variations but tends to minimize sharp fluctuations. Since these stations are removed from the point source, a sampling program should not be instituted with excessive sampling.

C Samples from lakes, ponds, or reservoirs usually are collected at the same depth and on frequent occasions may be collected over the entire surface.

D Oceanic or estuarine sample sites can often cover large areas and may, depending upon the survey objectives, necessitate vehicular and/or boat transporation. Frequently the need for surface and depth sampling programs are necessary especially if tidal or current patterns create a stratified environment.

III BOTTLES FOR WATER SAMPLES

A The sample bottles should have capacity for at least 100 ml of sample, plus an air space. The bottle and cap must be of bacteriological inert materials. Resistant glass or heat resistant plastic are acceptable. Wide mouth ground-glass stoppered bottles (Figure 1), plastic bottles of heat-resistant polyethylene, and 8 ounce glass jars with rubber lined screw caps are popular types in many laboratories.

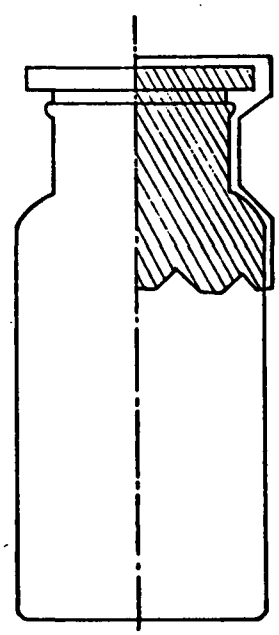


Figure 1

All bottles must be properly washed and sterilized. Protect the top of the bottles and cap from contamination by paper or metal foil hoods. Both glass and heat resistant plastic bottles may be sterilized in an autoclave. Hold plastic at 121°C for at least 10 minutes. Hot air sterilization, 1 hour at 170°C, may be used for glass bottles.

B Add sodium thiosulfate to bottles intended for halogenated water samples. A quantity of 0.1 ml of a 10% solution provides 100 mg/liter in a 100 ml sample. This level shows no effect upon viability or growth.

C Supply catalogs list wide mouth ground-glass stoppered bottles of borosilicate resistance glass, specially for water samples.

IV TECHNIQUE OF SAMPLE COLLECTION

Follow aseptic technique as nearly as possible. Nothing but sample water must touch the inside of the bottle or cap. To avoid loss of sodium thiosulfate, fill the bottle directly and do not rinse. Always remember to leave an air space.

A In sampling from a distribution system, first run the faucet wide open until the service line is cleared. A time of 3-5 minutes generally is sufficient. Reduce the flow and fill the sample bottle without splashing. Some authorities stress flaming the tap before collection. A chlorine determination is often made on the site.

B The bottle may be dipped into some waters by hand. Avoid introduction of bacteria from the human hand and from surface debris. Some suggestions follow: Hold the bottle near the base with one hand and with the other remove the hood and cap. Push the bottle rapidly into the water mouth down and tilt up towards the current to fill. A depth of about 6 inches is satisfactory. When there is no current move the bottle through the water horizontally and away from the hand. Lift the bottle from the water, spill a small amount of sample to provide an air space, and return the uncontaminated cap.

C Samples may be dipped from swimming pools. Determine residual chlorine on the pool water at the site. Test the sample at the laboratory to check chlorine neutralization by the thiosulfate.

D Sample bathing beach water by wading out to the two foot depth and dipping the sample up from about 6 inches below the surface. Use the procedure described in III.

E Wells with pumps are similar to distribution systems. With a hand pumped well, waste water for about five minutes before taking the sample. Sample a well without a pump by lowering a sterile bottle attached to a weight. A device which opened the bottle underneath the water would avoid contamination by surface debris.

F Various types of sampling devices are available where the sample point is inaccessible or depth samples are desired. The general problem is to put a sample bottle in place, open it, close it, and return it to the surface. No bacteria but those in the sample must enter the bottle.

1 The J - Z sampler described by Zobell in 1941, was designed for deep sea sampling but is useful elsewhere (Figure 2). It has a metal frame, breaking device for a glass tube, and sample bottle. The heavy metal messenger strikes the lever arm which breaks the glass tubing at a file mark. A bent rubber tube straightens and the water is drawn in several inches from the apparatus. Either glass or collapsible rubber bottles are sample containers.

Commercial adaptations are available.

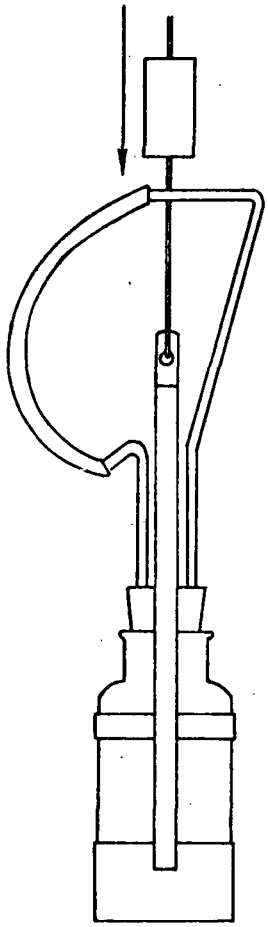


Figure 2

Reproduced with permission of the Journal of Marine Research 4:3, 173-188 (1941) by the Department of Health, Education and Welfare.

2 Note the vane and lever mechanism on the New York State Conservation Department sampler in Figure 3. When the apparatus is at proper depth the suspending line is given a sharp pull. Water inertia against the vane raises the stopper and water pours into the bottle. Sufficient sample is collected prior to the detachment of the stopper from the vane arm allowing a closure of the sample bottle.

3 A commercial sampler is available which is an evacuated sealed tube with a capillary tip. When a lever on the support rack breaks the tip, the tube fills. Other samplers exist with a lever for pulling the stopper, while another uses an electromagnet.

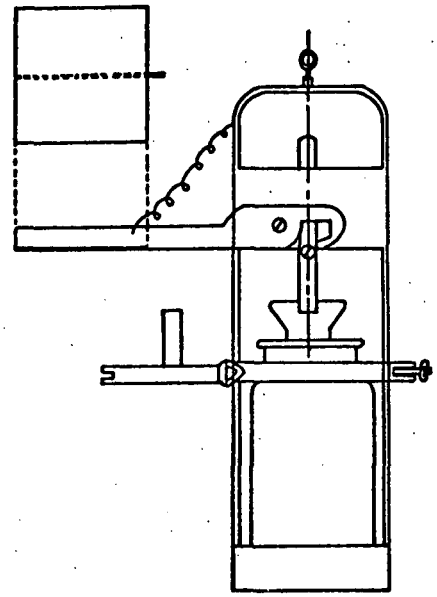


Figure 3

V DATA RECORDING

A Information generally includes: date, time of collection, temperature of water, location of sampling point, and name of the sample collector. Codes are often used. The location description must be exact enough to guide another person to the site. Reference to bridges, roads, distance to the nearest town may help. Use of the surveyors' description and maps are recommended. Mark identification on the bottles or on securely fastened tags. Gummed tags may soak off and are inadvisable.

B While a sanitary survey is an indispensable part of the evaluation of a water supply, its discussion is not within the scope of this lecture. The sample collector could supply much information if desired.

VI SHIPPING CONDITIONS

A The examination should commence as soon as possible, preferably within one hour. A maximum elapsed time between collection and examination is 30 hours for potable water samples and 8 hours for other water samples (collection 6 hours and laboratory procedures 2 hours). Standard Methods (13th Edition) recommends icing of samples between collection and testing.

VII PHOTOGRAPHS

A photograph is a sample in that it is evidence representing water quality. Sample collectors and field engineers may carry cameras to record what they see. Pictures help the general public and legal courts to better understand laboratory data.

REFERENCES

1 APHA, AWWA, WPCF, Standard Methods for the Examination of Water and Wastewater. (12 Ed.) 1965.

2 Prescott, S. C., Winslow, C.E.A., and McCrady, M. H. Water Bacteriology. 6th Ed., 368 pp. John Wiley and Sons, Inc., New York. 1946.

3 Haney, P.D., and Schmidt, J. Representative Sampling and Analytical Methods in Stream Studies. Oxygen Relationships in Streams, Technical Report W58-2 pp. 133-42. U.S. Department of Health, Education and Welfare, Public Health Service, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. 1958.

4 Velz, C. J. Sampling for Effective Evaluation of Stream Pollution. Sewage and Industrial Wastes, 22:666-84. 1950.

5 Bathing Water Quality and Health III Coastal Water. 134 pp. U.S. Department of Health, Education, and Welfare, Public Health Service, Robert A. Taft Sanitary Engineering, Cincinnati, Ohio. 1961.

6 Zobell, C. E. Apparatus for Collecting Water Samples from Different Depths for Bacteriological Analysis. Journal of Marine Research, 4:3:173-88. 1941.

This outline was originally prepared by A. G. Jose, former Microbiologist, FWPCA Training Activities, SEC and updated by the Training Staff, National Training Center, MPOD, WPO, EPA, Cincinnati, Ohio 45268.

Descriptors: Equipment, Microbiology, Sampling, Water Sampling

BIOLOGICAL FIELD METHODS

I INTRODUCTION

A Due to the variability in habitats and modes of living, methods for the collection of different types of aquatic organisms differ. In general we can recognize those that swim or float and them that crawl, those that are big and those that are little. Each comprises a part of "the life" at any given survey station and consequently a "complete" collection at any given station would include all types of organisms that live there.

B Field collecting methods in this outline are grouped under 4 general categories.

1 Bottom (or benthic organisms)

2 All types of plankton will be considered. Benthic forms which break off or loose their hold to a substrate may become temporarily a part of the plankton.

3 The periphyton or "aufwuchs" community, which resembles both the plankton and the benthics.

4 Fishes represent the fourth major group and need no further qualification.

C Aquatic mammals and birds, in most cases, require still other approaches and are not included here.

D A definite policy should be established as to the size range of organisms to be collected and counted, i.e.: microscopic only, microscopic and macroscopic, those retained by "30 mesh" screens only or #10 nets, invertebrates and/or vertebrates, etc.

II Certain Standard Supplementary Procedures are a Part of All Collecting Techniques

A In order to be interpreted and used, every collection must be associated with a record of environmental conditions at the time of collection.

1 Data recorded should include the following as far as practicable:

Location

Station number (particular location of which a full description should be on record)

Date

Time

Air temperature

Water temperature (at various depths, if applicable)

Salinity (at various depths, if applicable)

Tidal flow (ebb or flood)

Turbidity (or light penetration, etc.)

Weather

Wind direction and velocity

Sky or cloud cover

Water color

Depth

Type of bottom

Type of collecting device and accessories

Method of collecting

Type of sample (quantitative or qualitative)

Number of samples at each station

Chemical data, e.g., dissolved oxygen, nutrients, etc.

Collector

Miscellaneous observations (often very important!)

2 All collecting containers should be identified at least with location, station number, sample number, and date. Spares are very handy.

3 Much transcription of data can be eliminated by using sheets or cards with a uniform arrangement for including the above data. The same field data sheet may include field or laboratory analysis.

B Compact kits of field collecting equipment and materials greatly increase collecting efficiency, especially if collection site is remote from transportation.

III COLLECTION OF BOTTOM OR BENTHIC ORGANISMS

A Shoreline or Wading Depth Collecting

1 Rakes, shovels, dipnets, or dredges may be used here depending upon type of bottom, area to be sampled, or qualitative or quantitative samples.

2 Ekman dredges are best used on soft bottoms. This dredge is essentially a completely closing clamshell type dredge with spring operated jaws. Size of dredge is usually 6" X 6" or 9" X 9".

3 For samples of hard packed clay or sand, or gravelly bottoms, or where the current is moderately swift a Petersen dredge may be used.

a This consists of two heavily constructed half cylinders hinged together, and closed by a strong lever action. The area sampled is commonly 0.1m^2.

b To be effective in cutting into hard bottoms they are built to weigh 30 - 40 lbs., and may have additional weights added to bring the total weight up to 70 lbs. or more.

c Due to their weight, especially when loaded with bottom material they are best handled by a small winch or crane. However, they can be hand operated in shallow waters using a stout line of large enough diameter to be handled by wet hands.

4 Ooze suckers and core samplers are other devices for sampling mud bottom organisms.

5 After bottom samples to obtain marine organisms are collected, they are best placed into some type of container and then washed and grossly screened and picked in the field, conditions permitting.

a This removes large stones, etc., and gives one a quick idea of what the sample consists of.

b With many bottom samples, weight becomes a problem. Washing, therefore, reduces this weight making it easier to transport the samples. Also space is saved with smaller samples.

6 Hand picking of readily visible plants and animals is valuable for immediate qualitative description.

a A frame of known dimensions may be placed over an area to be sampled and the material within cropped out. This is especially good for larger plants. This method yields quantitative data.

b Underwater swimming or use of SCUBA is quite valuable for direct observation and collecting.

B Deep Water Benthic Collecting

1 When sampling from vessels, a crane and winch, either hand or power operated, is used. The Petersen dredge is commonly employed. SCUBA diving is used also. The general ideas described for shallow waters when applicable, apply to deeper waters.

2 Since most biological populations are not normally distributed, but rather

some form of log normal distribution occurs, it is most always best to take 2 but preferably 3 or more bottom samples at a station in order to get a true estimate of the population.

C Besides the types of bottom collecting gear mentioned here, there are many more types available, such as the orange-peel bucket, the Shipdek sampler, the plow dredge, scallop type dredge, etc. Each of these has its own advantages and disadvantages and it is up to the worker and his operation to decide what is best for his particular needs. The Petersen and Eckman dredges are perhaps the most commonly used.

IV THE COLLECTION OR SAMPLING OF PLANKTON

A Phytoplankton - Planned Program

1 A planned program of analysis should involve periodic sampling at biweekly, weekly or even more frequent intervals.

2 A well-planned study and analysis of the growth pattern of phytoplankton in one year can provide a basis for predicting conditions for the following year since seasonal patterns tend to be repeated from year to year.

a Since the seasons and years differ, the more records accumulated, the more useful they become.

b As the time for an anticipated bloom approaches, the frequency of collecting and analyses may be increased.

B Field Equipment

1 Two general aspects of plankton analysis are commonly recognized: quantitative and qualitative. Either approach is useful, a combination is best.

2 Equipment for collecting samples in the field is varied.

a A Kemmerer type sampler, a Nansen Bottle, or some similar vessel of known volume is suggested for quantitative sampling.

b Plankton nets selectively concentrate (depending upon mesh size) the sample in the act of collecting and capturing certain larger forms which escape the bottles. Only the more elaborate nets are quantitative, however.

c Other instruments such as the Clark-Bumpus, or the continuous plankton recorder may be employed for special purposes.

d For phytoplankton, #20 or #25 size nets are commonly used. Usually a net diameter of 5 - 10 inches is sufficient.

C Zooplankton Collecting

1 Since zooplankton have the ability to swim away from water bottles, etc. nets towed at moderately slow speed are used for their capture.

a Number 12 nets (aperature size 0.119 mm, 125 meshes 1 inch) or smaller numbered net sizes are commonly used. A net diameter greater than 5" is preferred. Frequently half meter nets or larger are employed. These may be equipped with flow measuring devices for measuring the amount of water entering the net.

b Other instruments such as the Clark-Bumpus, Gulf-Stream, Hardy continuous plankton recorder, and high-speed instruments are used for collecting zooplankton, also.

c The devices used for collecting plankton capture both the plant and animal types. The mesh size (net no.) is a method for selecting which category of plankton is to be collected.

2 Plankton is subjected to the force of the winds and currents. As a result, the plankton is often in patches or "wind rows." For this reason when using a net, it is often desirable to collect at right angles to the wind or current.

3 Not only do plankton occur horizontally in patches, but vertically as well. Zooplankton, especially are very numerous near the bottom in daylight. Therefore, a series of tows at different depths is necessary to obtain a complete sampling. One technique often employed is to take an oblique tow from the bottom to the top of the water column.

D The Location of Sampling Points is Important

1 Both shallow and deep samples are suggested. Sometimes, the surface film itself is significant.

2 The number of sampling stations established is limited only by the ability of the laboratory to analyze the samples.

a There should be at least one sample station near an outfall.

b Every major natural division of the bay or estuary should be sampled.

c Additional sampling points should be established on the basis of experience and resources.

d Continuous sampling, or the compositing of periodic samples over a 24-hour period, for example, is also very useful.

e For plankton, it is necessary to sample different periods in the stage of the tide, otherwise samples would be biased to a given time, or type of water carried by the tidal currents.

f Pilot studies to indicate sampling locations and intervals are often mandatory.

g Some studies require random sampling points.

V COLLECTING PERIPHYTON, ETC.

A Hand-picking of forms attached to rocks, pilining, etc. is very valuable.

B Microscopic attached forms or microbenthos may be sampled by scraping or otherwise removing all organisms from a measured surface area.

C Another technique used widely is to suspend artificial substrates (slides, cement blocks, wood, rope, plastic, etc.) with clean surfaces in the water. After a designated time interval organisms become attached which are subsequently removed and studied.

VI FISH COLLECTING

A Fish must be sought in the obscure and unlikely areas as well as the obvious locations in order for the collection to be complete. Several techniques should be employed wherever possible (this is appropriate for all biota). It is advisable to check with local authorities to inform them of the reasons for sampling, because many of the techniques are not legal for the layman. In this area, perhaps more than any other, professionally trained workers are important. Also, there must be at least one helper, as a single individual always has difficulty in pulling both ends of a 20 foot seine simultaneously. The more common techniques are listed below.

B Seines

1 Straight seines range from 4 - 6 feet and upwards in length. "Common

sense" minnow seines with approximately 1/4 inch mesh are widely used along shore for collecting the smaller fishes.

2 Bag seines have an extra trap or bag tied in the middle which helps trap and hold fish when seining in difficult situations.

C Gill nets are of use in offshore and/or deep waters. They range in length from approximately 30 yards upward. A mesh size is designed to catch a specified size of fish. The trammel net is a variation of the gill net.

D Traps range from small wire boxes or cylinders with inverted cone entrances to semi permanent weirs a half mile or more in length. All tend to induce fish to swim into an inner chamber protected by an inverted cone or V-shaped notch to prevent escape. Current operated rotating fish traps are also very effective (and equally illegal) in suitable situations.

E Trawls are submarine nets, usually of considerable size, towed by vessels at speeds sufficient to overtake and scoop in fish, etc. The mouth of the net must be held open by some device such as a long beam (beam trawl) or two or more vanes or "otter boards" (otter trawl).

1 Beam and otter trawls are usually fished on the bottom, but otter trawls when suitably rigged are now being used to fish mid-depths.

2 The Isaacs-Kidd midwater trawl resembles a plankton net 10 - 15 feet in diameter. It is proving very effective for collecting at mid-depths.

F Electric seines and screens are widely employed by fishery workers in small and difficult streams. They may also be used in shallow water areas with certain reservations.

G Poisoning is much used in fishery studies and management. Most widely used and generally satisfactory is rotenone in varying formulations, although many others have been employed from time to time. Under suitable circumstances, fish may even be killed selectively according to species.

H Personal observation by competent personnel, and also informal inquiries and discussions with local residents will often yield information of real use. Many laymen are keen observers, although they do not always understand what they are seeing. The organized creel census technique yields data on what and how many fish are being caught.

I Angling remains in its own right a very good technique in the hands of the skilled practitioner, for determining what fish are present. Spear-fishing also is now being used in some studies.

J Fish are often tagged to trace their movements during migration and at other times. Miniature radio transmitters can now be attached or fed to fish (and other organisms) which enable them to be tracked over considerable distances. Physiological information is often obtained in this way. This is known as telemetry.

VII DISPOSITION AND PRESERVATION OF COLLECTIONS

A The organisms may be picked out on the spot, or the entire mess taken into the laboratory for further analysis.

B Organisms may be simply counted, weighed, or measured volumetrically; usually a combination is best. They may be separated and recorded in groups or species.

C Specimens may be simply observed and recorded or they may be preserved as a permanent record.

D Preservation - provisions should be made for the field stabilization of the sample until the laboratory examination can be

made if more than an hour or so (especially in warm weather) is to elapse.

1 Refrigeration or icing is very helpful.

2 Ninety-five per cent ethanol (ethyl alcohol) is highly satisfactory. A final strength of 70% is necessary for prolonged storage.

3 Formaldehyde is more widely available and is effective in concentrations of 3 - 10% of the commercial formulation. However, it shrinks and hardens specimens, collector, and laboratory analyst without favor! In order to minimize bad effects from formalin, commercial neutral formaldehyde is recommended.

   a Phytoplankton is best preserved with a 3% formalin solution.

   b Zooplankton, benthos, and fish are usually preserved in a 5 - 10% solution.

4 Lugol's solution is good for phyloplankton.

5 Other formulations are available.

VIII SOURCE OF COLLECTING EQUIPMENT

Many specialized items of biological collecting equipment are not available from the usual laboratory supply houses. Consequently, the American Society of Limnology and Oceanography has compiled a list of companies handling such items and released it as: "Special Publication No. 1, Sources of Limnological Apparatus and Supplies." This may be obtained from the Secretary of the Society for $1.00.

REFERENCES

1 Barnes, H. (ed.). Symposium on New Advances in Underwater Observations. Brit. Assoc. Adv. Sci., Liverpool. pp. 49-64. 1953.

2 Hedgepeth, Joel W. Obtaining Ecological Data in the Sea Chapter 4 in "Treatise on Marine Ecology and Paleoecology." Memoir 67. Geol. Soc. Am. 1963.

3 Isaacs, John D. and Columbus, O.D. Oceanographic Instrumentation NCR Div. Phys. Sci. Publ. 309, 1954. 233 pp.

4 Sverdrup, H.U. et al. Observations and Collections at Sea. Chapter X in: The Oceans, Their Physics, Chemistry, and Biology. Prentice-Hall, Inc., N.Y. 1942. 1087 pp.

5 Welsh, Paul S. Limnological Methods. The Blakiston Co. Pa. 1948.

THE AQUATIC ENVIRONMENT

Part 1: The Nature and Behavior of Water

I INTRODUCTION

The earth is physically divisible into the lithosphere or land masses, and the hydrosphere which includes the oceans, lakes, streams, and subterranean waters.

A Upon the hydrospere are based a number of sciences which represent different approaches. Hydrology is the general science of water itself with its various special fields such as hydrography, hydraulics, etc. These in turn merge into physical chemistry and chemistry.

B Limnology and oceanography combine aspects of all of these, and deal not only with the physical liquid water and its various naturally occurring solutions and forms, but also with living organisms and the infinite interactions that occur between them and their environment.

C Water quality management, including pollution control, thus looks to all branches of aquatic science in efforts to coordinate and improve man's relationship with his aquatic environment.

II SOME FACTS ABOUT WATER

A Water is the only abundant liquid on our planet. It has many properties most unusual for liquids, upon which depend most of the familiar aspects of the world about us as we know it. (See Table 1)

TABLE 1

UNIQUE PROPERTIES OF WATER

Property	Significance
Highest heat capacity (specific heat) of any solid or liquid (except NH_3)	Stabilizes temperatures of organisms and geographical regions
Highest latent heat of fusion (except NH_3)	Thermostatic effect at freezing point
Highest heat of evaporation of any substance	Important in heat and water transfer of atmosphere
The only substance that has its maximum density as a liquid (4°C)	Fresh and brackish waters have maximum density above freezing point. This is important in vertical circulation pattern in lakes.
Highest surface tension of any liquid	Controls surface and drop phenomena, important in cellular physiology
Dissolves more substances in greater quantity than any other liquid	Makes complex biological system possible. Important for transportation of materials in solution.
Pure water has the highest di-electric constant of any liquid	Leads to high dissociation of inorganic substances in solution
Very little electrolytic dissociation	Neutral, yet contains both H^+ and OH^- ions
Relatively transparent	Absorbs much energy in infra red and ultra violet ranges, but little in visible range. Hence "colorless"

B Physical Factors of Significance

1 Water substance

Water is not simply "H_2O" but in reality is a mixture of some 33 different substances involving three isotopes each of hydrogen and oxygen (ordinary hydrogen H^1, deuterium H^2, and tritium H^3; ordinary oxygen O^{16}, oxygen 17, and oxygen 18) plus 15 known types of ions. The molecules of a water mass tend to associate themselves as polymers rather than to remain as discrete units. (See Figure 1)

SUBSTANCE OF PURE WATER

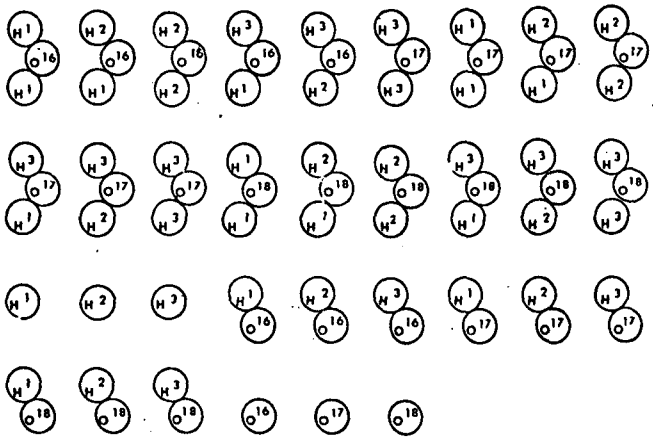


Figure 1

2 Density

a Temperature and density: Ice. Water is the only known substance in which the solid state will float on the liquid state. (See Table 2)

TABLE 2

EFFECTS OF TEMPERATURE ON DENSITY OF PURE WATER AND ICE*

Temperature (°C)	Density	
	Water	Ice**
-10	.99815	.9397
- 8	.99869	.9360
- 6	.99912	.9020
- 4	.99945	.9277
- 2	.99970	.9229
0	.99987----	.9168
2	.99997	
4	1.00000	
6	.99997	
8	.99988	
10	.99973	
20	.99823	
40	.99225	
60	.98324	
80	.97183	
100	.95838	

* Tabular values for density, etc., represent estimates by various workers rather than absolute values, due to the variability of water.

** Regular ice is known as "ice I". Four or more other "forms" of ice are known to exist (ice II, ice III, etc.), having densities at 1 atm. pressure ranging from 1.1595 to 1.67. These are of extremely restricted occurrence and may be ignored in most routine operations.

This ensures that ice usually forms on top of a body of water and tends to insulate the remaining water mass from further loss of heat. Did ice sink, there could be little or no carryover of aquatic life from season to season in the higher latitudes. Frazil or needle ice forms colloidally at a few thousandths of a degree below 0° C. It is adhesive and may build up on submerged objects as "anchor ice", but it is still typical ice (ice I).

1) Seasonal increase in solar radiation annually warms surface waters in summer while other factors result in winter cooling. The density differences resulting establish two classic layers: the epilimnion or surface layer, and the hypolimnion or lower layer, and in between is the thermocline or shear-plane.

2) While for certain theoretical purposes a "thermocline" is defined as a zone in which the temperature changes one degree centigrade for each meter of depth, in practice, any transitional layer between two relatively stable masses of water of different temperatures may be regarded as a thermocline.

3) Obviously the greater the temperature differences between epilimnion and hypolimnion and the sharper the gradient in the thermocline, the more stable will the situation be.

4) From information given above, it should be evident that while the temperature of the hypolimnion rarely drops much below 4° C, the epilimnion may range from 0° C upward.

5) When epilimnion and hypolimnion achieve the same temperature, stratification no longer exists. The entire body of water behaves hydrologically as a unit, and tends to assume uniform chemical and physical characteristics. Even a light breeze may then cause the entire body of water to circulate. Such events are called overturns, and usually result in water quality changes of considerable physical, chemical, and biological significance. Mineral-rich water from the hypolimnion, for example, is mixed with oxygenated water from the epilimnion. This usually triggers a sudden growth or "bloom" of plankton organisms.

6) When stratification is present, however, each layer behaves relatively independently, and significant quality differences may develop.

7) Thermal stratification as described above has no reference to the size of the water mass; it is found in oceans and puddles.

b The relative densities of the various isotopes of water influence its molecular composition. For example, the lighter O_{16} tends to go off first in the process of evaporation, leading to the relative enrichment of air by O_{16} and the enrichment of water by O_{17} and O_{18}. This can lead to a measurably higher O_{18} content in warmer climates. Also, the temperature of water in past geologic ages can be closely estimated from the ratio of O_{18} in the carbonate of mollusc shells.

c Dissolved and/or suspended solids may also affect the density of natural water masses (see Table 3)

TABLE 3
EFFECTS OF DISSOLVED SOLIDS ON DENSITY

Dissolved Solids (Grams per liter)	Density (at 4° C)
0	1.00000
1	1.00085
2	1.00169
3	1.00251
10	1.00818
35 (mean for sea water)	1.02822

d Types of density stratification

1) Density differences produce stratification which may be permanent, transient, or seasonal.

2) Permanent stratification exists for example where there is a heavy mass of brine in the deeper areas of a basin which does not respond to seasonal or other changing conditions.

3) Transient stratification may occur with the recurrent influx of tidal water in an estuary for example, or the occasional influx of cold muddy water into a deep lake or reservoir.

4) Seasonal stratification is typically thermal in nature, and involves the annual establishment of the epilimnion, hypolimnion, and thermocline as described above.

5) Density stratification is not limited to two-layered systems; three, four, or even more layers may be encountered in larger bodies of water.

e A "plunge line" (sometimes called "thermal line") may develop at the mouth of a stream. Heavier water flowing into a lake or reservoir plunges below the lighter water mass of the epiliminium to flow along at a lower level. Such a line is usually marked by an accumulation of floating debris.

f Stratification may be modified or entirely suppressed in some cases when deemed expedient, by means of a simple air lift.

3 The viscosity of water is greater at lower temperatures (see Table 4). This is important not only in situations involving the control of flowing water as in a sand filter, but also since overcoming resistance to flow generates heat, it is significant in the heating of water by internal friction from wave and current action. Living organisms more easily support themselves in the more viscous (and also denser) cold waters of the arctic than in the less viscous warm waters of the tropics. (See Table 4).

TABLE 4

VISCOSITY OF WATER (In millipoises at 1 atm)

	Dissolved solids in g/L			
Temp. ° C	0	5	10	30
-10	26.0	----	----	----
- 5	21.4	----	----	----
0	17.94	18.1	18.24	18.7
5	15.19	15.3	15.5	16.0
10	13.10	13.2	13.4	13.8
30	8.00	8.1	8.2	8.6
100	2.84	----	----	----

4 Surface tension has biological as well as physical significance. Organisms whose body surfaces cannot be wet by water can either ride on the surface film or in some instances may be "trapped" on the surface film and be unable to re-enter the water.

5 Heat or energy

Incident solar radiation is the prime source of energy for virtually all organic and most inorganic processes on earth. For the earth as a whole, the total amount (of energy) received annually must exactly balance that lost by reflection and radiation into space if climatic and related conditions are to remain relatively constant over geologic time.

a For a given body of water, immediate sources of energy include in addition to solar irradiation: terrestrial heat, transformation of kinetic energy (wave and current action) to heat, chemical and biochemical reactions, convection from the atmosphere, and condensation of water vapor.

b The proportion of light reflected depends on the angle of incidence, the temperature, color, and other qualities of the water; and the presence or absence of films of lighter liquids such as oil. In general, as the depth increases arithmetically, the light tends to decrease geometrically. Blues, greens, and yellows tend to penetrate most deeply while ultra violet, violets, and orange-reds are most quickly absorbed. On the order of 90% of the total illumination which penetrates the surface film is absorbed in the first 10 meters of even the clearest water, thus tending to warm the upper layers.

6 Water movements

a Waves or rhythmic movement

1) The best known are traveling waves caused by wind. These are effective only against objects near the surface. They have little effect on the movement of large masses of water.

2) Seiches

Standing waves or seiches occur in lakes, estuaries, and other enclosed bodies of water, but are seldom large enough to be observed. An "internal wave or seich" is an oscillation in a submersed mass of water such as a hypolimnion, accompanied by compensating oscillation in the overlying water so that no significant change in surface level is detected. Shifts in submerged water masses of this type can have severe effects on the biota and also on human water uses where withdrawals are confined to a given depth. Descriptions and analyses of many other types and sub-types of waves and wave-like movements may be found in the literature.

b Tides

1) Tides are the longest waves known, and are responsible for the once or twice a day rythmic rise and fall of the ocean level on most shores around the world.

2) While part and parcel of the same phenomenon, it is often convenient to refer to the rise and fall of the water level as "tide," and to the resulting currents as "tidal currents."

3) Tides are basically caused by the attraction of the sun and moon on water masses, large and small; however, it is only in the oceans and possibly certain of the larger lakes that true tidal action has been demonstrated. The patterns of tidal action are enormously complicated by local topography, interaction with seiches, and other factors. The literature on tides is very large.

c Currents (except tidal currents) are steady arythmic water movements which have had major study only in oceanography although they are most often observed in rivers and streams. They are primarily concerned with the translocation of water masses. They may be generated internally by virtue of density changes, or externally by wind or terrestrial topography. Turbulence phenomena or eddy currents are largely responsible for lateral mixing in a current. These are of far more importance in the economy of a body of water than mere laminar flow.

d Coriolis force is a result of interaction between the rotation of the earth, and the movement of masses or bodies on the earth. The net result is a slight tendency for moving objects to veer to the right in the northern hemisphere, and to the left in the southern hemisphere. While the result in fresh waters is usually negligible, it may be considerable in marine waters. For example, other factors permitting, there is a tendency in estuaries for fresh waters to move toward the ocean faster along the right bank, while salt tidal waters tend to intrude farther inland along the left bank. Effects are even more dramatic in the open oceans.

e Langmuir circulation (or L. spirals) is the interlocking rotation of somewhat cylindrical masses of surface water under the influence of wind action. The axes of the cylinders are parallel to the direction of the wind.

To somewhat oversimplify the concept, a series of adjoining cells might be thought of as chains of interlocking gears in which at every other contact the teeth are rising while at the alternate contacts, they are sinking (Figure 2).

The result is elongated masses of water rising or sinking together. This produces the familiar "wind rows" of foam, flotsam and jetsam, or plankton often seen streaking windblown lakes or oceans. Certain zoo-plankton struggling to maintain a position near the surface tend to collect in the down current between two Langmuir cells, causing such an area to be called the "red dance", while the clear upwelling water between is the "blue dance".

This phenomenon may be important in water or plankton sampling on a windy day.

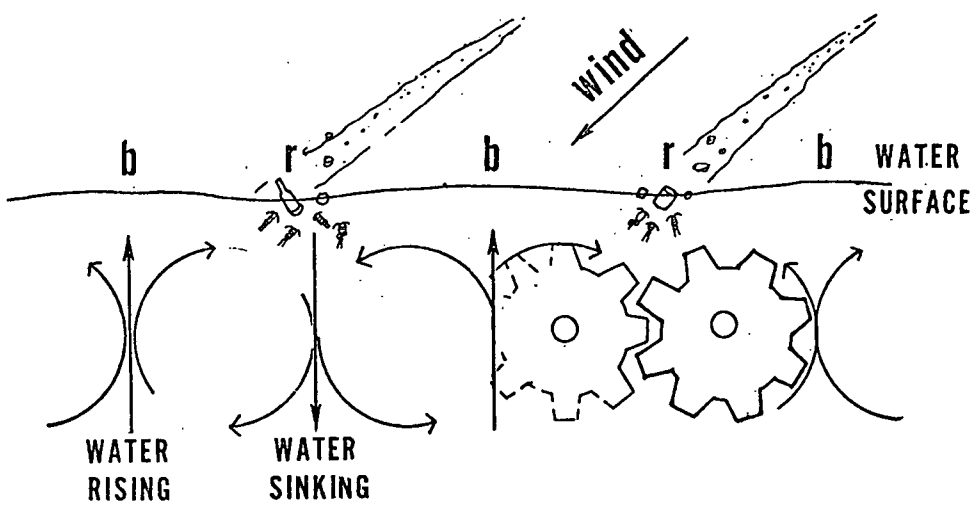


Figure 2. Langmuire Spirals
b. Blue dance, water rising. r. Red dance, water sinking, floating or swimming objects concentrated.

6 The pH of pure water has been determined between 5.7 and 7.01 by various workers. The latter value is most widely accepted at the present time. Natural waters of course vary widely according to circumstances.

C The elements of hydrology mentioned above represent a selection of some of the more conspicuous physical factors involved in working with water quality. Other items not specifically mentioned include: molecular structure of waters, interaction of water and radiation, internal pressure, acoustical characteristics, pressure-volume-temperature relationships, refractivity, luminescence, color, dielectrical characteristics and phenomena, solubility, action and interactions of gases, liquids and solids, water vapor, phenomena of hydrostatics and hydrodynamics in general.

REFERENCES

1 Buswell, A. M. and Rodebush, W. H. Water. Sci. Am. April 1956.

2 Dorsey, N. Ernest. Properties of Ordinary Water - Substance. Reinhold Publ. Corp. New York. pp. 1-673. 1940.

3 Fowle, Frederick E. Smithsonian Physical Tables. Smithsonian Miscellaneous Collection, 71(1), 7th revised ed., 1929.

4 Hutcheson, George E. A Treatise on Limnology. John Wiley Company. 1957.

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Descriptors:
Aquatic Environment, Estuarine Environment, Lentic Environment, Lotic Environment, Currents, Marshes, Limnology, Water Properties

THE AQUATIC ENVIRONMENT

Part 2: The Aquatic Environment as an Ecosystem

I INTRODUCTION

Part 1 introduced the lithosphere and the hydrosphere. Part 2 will deal with certain general aspects of the biosphere, or the sphere of life on this earth, which photographs from space have shown is a finite globe in infinite space.

This is the habitat of man and the other organisms. His relationships with the aquatic biosphere are our common concern.

II THE BIOLOGICAL NATURE OF THE WORLD WE LIVE IN

A We can only imagine what this world must have been like before there was life.

B The world as we know it is largely shaped by the forces of life.

1 Primitive forms of life created organic matter and established soil.

2 Plants cover the lands and enormously influence the forces of erosion.

3 The nature and rate of erosion affect the redistribution of materials (and mass) on the surface of the earth (topographic changes).

4 Organisms tie up vast quantities of certain chemicals, such as carbon and oxygen.

5 Respiration of plants and animals releases carbon dioxide to the atmosphere in influential quantities.

6 CO_2 affects the heat transmission of the atmosphere.

C Organisms respond to and in turn affect their environment. Man is one of the most influential.

III ECOLOGY IS THE STUDY OF THE INTERRELATIONSHIPS BETWEEN ORGANISMS, AND BETWEEN ORGANISMS AND THEIR ENVIRONMENT.

A The ecosystem is the basic functional unit of ecology. Any area of nature that includes living organisms and nonliving substances interacting to produce an exchange of materials between the living and nonliving parts constitutes an ecosystem. (Odum, 1959)

1 From a structural standpoint, it is convenient to recognize four constituents as composing an ecosystem (Figure 1).

a Abiotic NUTRIENT MINERALS which are the physical stuff of which living protoplasm will be synthesized.

b Autotrophic (self-nourishing) or PRODUCER organisms. These are largely the green plants (holophytes), but other minor groups must also be included (See Figure 2). They assimilate the nutrient minerals, by the use of considerable energy, and combine them into living organic substance.

c Heterotrophic (other-nourishing) CONSUMERS (holozoic), are chiefly the animals. They ingest (or eat) and digest organic matter, releasing considerable energy in the process.

d Heterotrophic REDUCERS are chiefly bacteria and fungi that return complex organic compounds back to the original abiotic mineral condition, thereby releasing the remaining chemical energy.

2 From a functional standpoint, an ecosystem has two parts (Figure 2)

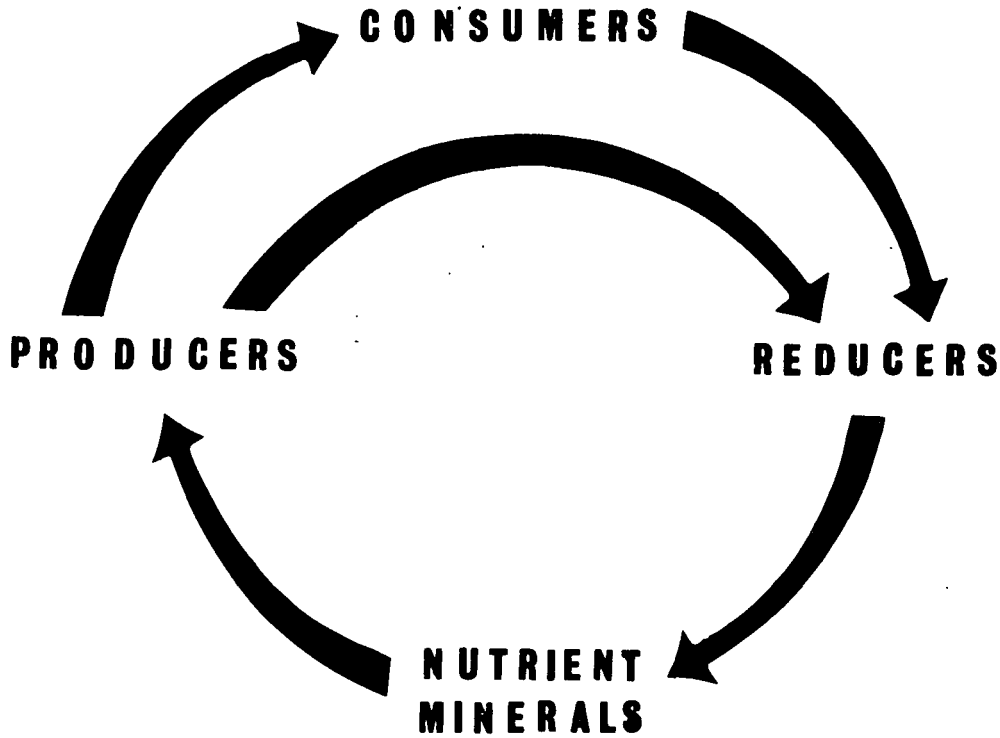


FIGURE 1

a The autotrophic or producer organisms, which construct organic substance.

b The heterotrophic or consumer and reducer organisms which destroy organic substance.

3 Unless the autotrophic and heterotrophic phases of the cycle approximate a dynamic equilibrium, the ecosystem and the environment will change.

B Each of these groups includes simple, single-celled representatives, persisting at lower levels on the evolutionary stems of the higher organisms. (Figure 2)

1 These groups span the gaps between the higher kingdoms with a multitude of transitional forms. They are collectively called the PROTISTA.

2 Within the protista, two principal subgroups can be defined on the basis of relative complexity of structure.

a The bacteria and blue-green algae, lacking a nuclear membrane may be considered as the lower protista (or Monera).

b The single-celled algae and protozoa are best referred to as the Higher Protista.

C Distributed throughout these groups will be found most of the traditional "phyla" of classic biology.

IV FUNCTIONING OF THE ECOSYSTEM

A A food chain is the transfer of food energy from plants through a series of organisms with repeated eating and being eaten. Food chains are not isolated sequences but are interconnected.

RELATIONSHIPS BETWEEN FREE LIVING AQUATIC ORGANISMS

Energy Flows from Left to Right, General Evolutionary Sequence is Upward

PRODUCERS	CONSUMERS	REDUCERS
Organic Material Produced, Usually by Photosynthesis	Organic Material Ingested or Consumed Digested Internally	Organic Material Reduced by Extracellular Digestion and Intracellular Metabolism to Mineral Condition
ENERGY STORED	ENERGY RELEASED	ENERGY RELEASED
Flowering Plants and Gymnosperms	Arachnids, Mammals, Insects, Birds	Basidiomycetes
Club Mosses, Ferns	Crustaceans, Reptiles	
Liverworts, Mosses	Segmented Worms, Amphibians, Molluscs, Fishes	Fungi Imperfecti
Multicellular Green Algae	Bryozoa, Primitive Chordates, Rotifers	
Red Algae	Roundworms, Echinoderms, Flatworms	Ascomycetes
Brown Algae	Coelenterates, Sponges	Higher Phycomycetes
DEVELOPMENT OF MULTICELLULAR OR COENOCYTIC STRUCTURE		
HIGHER PROTISTA		
Unicellular Green Algae, Diatoms, Pigmented Flagellates	Protozoa: Amoeboid, Cilliated, Flagellated, (non-pigmented), Suctoria	Lower Phycomycetes (Chytridiales, et. al.)
DEVELOPMENT OF A NUCLEAR MEMBRANE		
LOWER PROTISTA (or: Monera)		
Blue Green Algae, Phototropic Bacteria, Chemotropic Bacteria	Saprophytic Bacterial Types	Actinomycetes, Spirochaetes

FIGURE 2

BI. ECO. pl. 2a. 1. 69

B A food web is the interlocking pattern of food chains in an ecosystem. (Figures 3, 4) In complex natural communities, organisms whose food is obtained by the same number of steps are said to belong to the same trophic (feeding) level.

C Trophic Levels

1 First - Green plants (producers) (Figure 5) fix biochemical energy and synthesize basic organic substances. This is "primary production".

2 Second - Plant eating animals (herbivores) depend on the producer organisms for food.

3 Third - Primary carnivores, animals which feed on herbivores.

4 Fourth - Secondary carnivores feed on primary carnivores.

5 Last - Ultimate carnivores are the last or ultimate level of consumers.

D Total Assimilation

The amount of energy which flows through a trophic level is distributed between the production of biomass (living substance), and the demands of respiration (internal energy use by living organisms) in a ratio of approximately 1:10.

E Trophic Structure of the Ecosystem

The interaction of the food chain phenomena (with energy loss at each transfer) results in various communities having definite trophic structure or energy levels. Trophic structure may be measured and described either in terms of the standing crop per unit area or in terms of energy fixed per unit area per unit time at successive trophic levels. Trophic structure and function can be shown graphically by means of ecological pyramids (Figure 5).

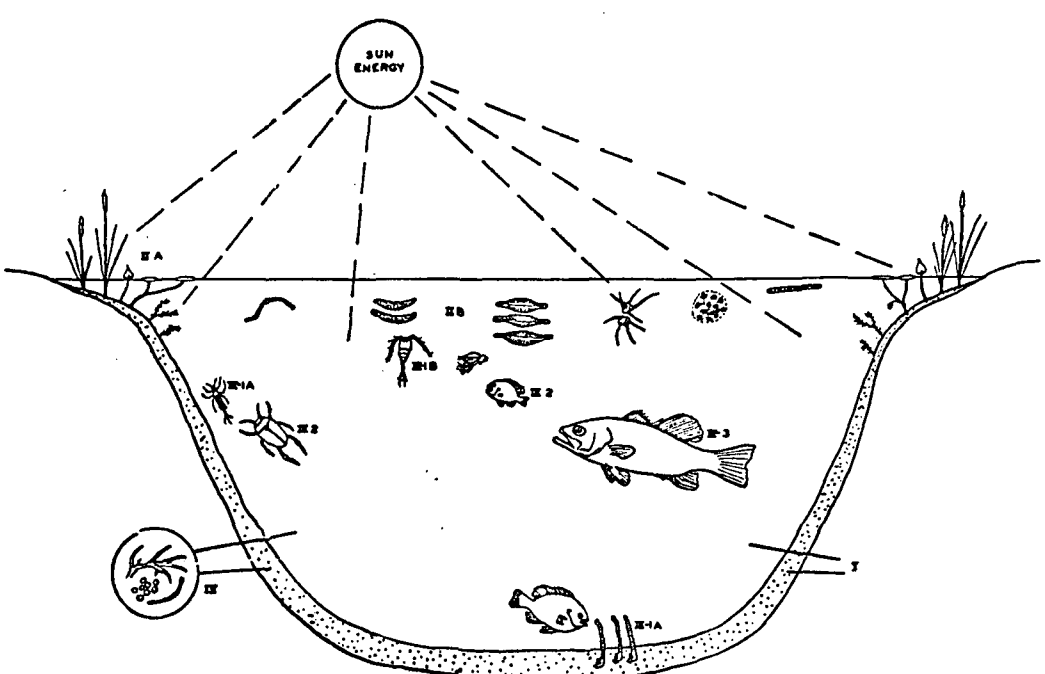
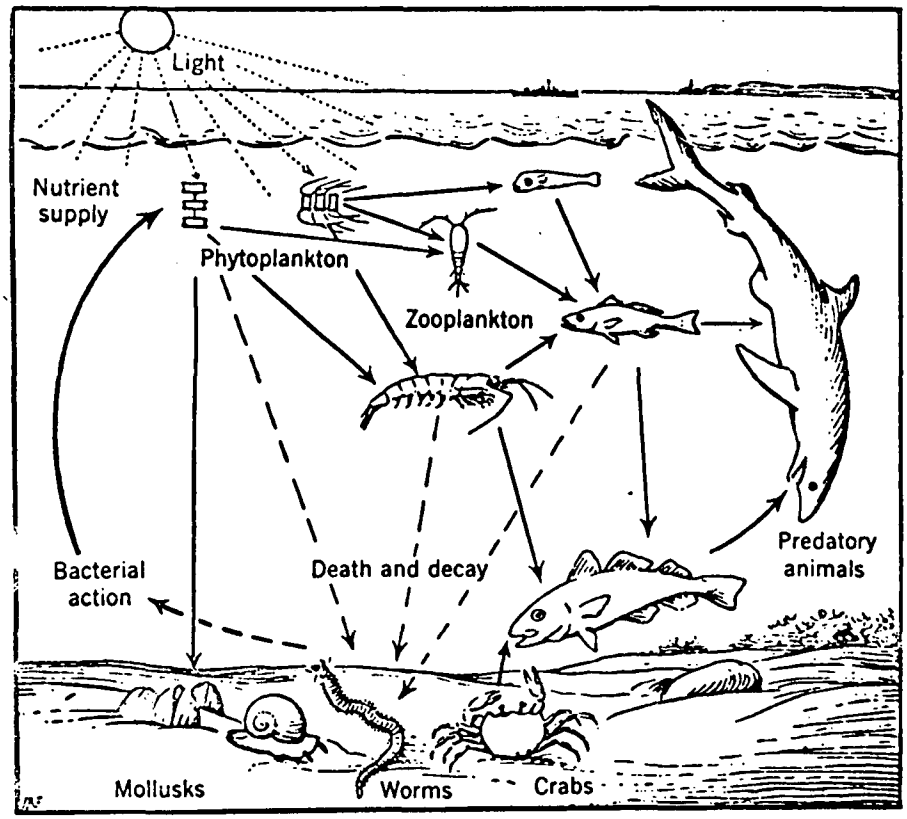


Figure 3. Diagram of the pond ecosystem. Basic units are as follows: I, abiotic substances–basic inorganic and organic compounds; IIA, producers–rooted vegetation; IIB, producers–phytoplankton; III-1A, primary consumers (herbivores)–bottom forms; III-1B, primary consumers (herbivores)–zooplankton; III-2, secondary consumers (carnivores); III-3, tertiary consumers (secondary carnivores); IV, decomposers–bacteria and fungi of decay.



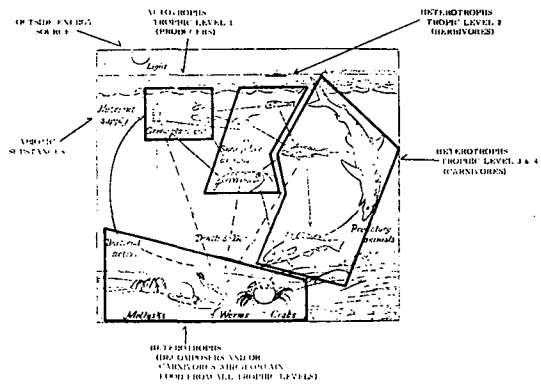


Figure 4. A MARINE ECOSYSTEM (After Clark, 1954 and Patten, 1966)

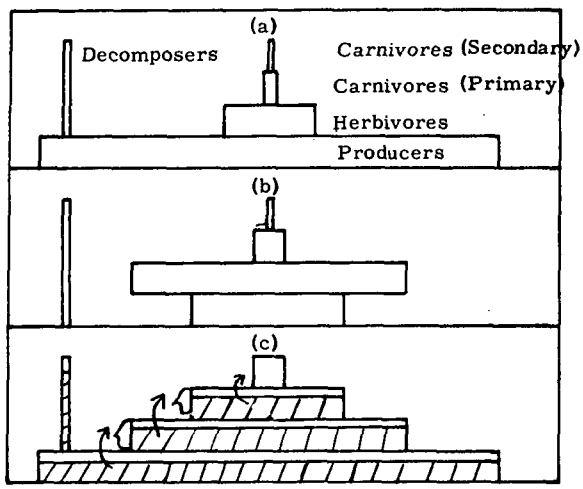


Figure 5. HYPOTHETICAL PYRAMIDS of (a) Numbers of individuals, (b) Biomass, and (c) Energy (Shading Indicates Energy Loss).

V BIOTIC COMMUNITIES

A Plankton are the macroscopic and microscopic animals, plants, bacteria, etc., floating free in the open water. Many clog filters, cause tastes, odors, and other troubles in water supplies. Eggs and larvae of larger forms are often present.

1 Phytoplankton are plant-like. These are the dominant producers of the waters, fresh and salt, "the grass of the seas".

2 Zooplankton are animal-like. Includes many different animal types, range in size from minute protozoa to gigantic marine jellyfishes.

B Periphyton (or Aufwuchs) - The communities of microscopic organisms associated with submerged surfaces of any type or depth. Includes bacteria, algae, protozoa, and other microscopic animals, and often the young or embryonic stages of algae and other organisms that normally grow up to become a part of the benthos (see below). Many planktonic types will also adhere to surfaces as periphyton, and some typical periphyton may break off and be collected as plankters.

C Benthos are the plants and animals living on, in, or closely associated with the bottom. They include plants and invertebrates.

D Nekton are the community of strong aggressive swimmers of the open waters, often called pellagic. Certain fishes, whales, and invertebrates such as shrimps and squids are included here.

E The marsh community is based on larger "higher" plants, floating and emergent. Both marine and freshwater marshes are areas of enormous biological production. Collectively known as "wetlands", they bridge the gap between the waters and the dry lands.

VI PRODUCTIVITY

A The biological resultant of all physical and chemical factors in the quantity of life that may actually be present. The ability to produce this "biomass" is often referred to as the "productivity" of a body of water. This is neither good nor bad per se. A water of low productivity is a "poor" water biologically, and also a relatively "pure" or "clean" water; hence desirable as a water supply or a bathing beach. A productive water on the other hand may be a nuisance to man or highly desirable. It is a nuisance if foul odors and/or weed-chocked waterways result, it is desirable if bumper crops of bass, catfish, or oysters are produced. Open oceans have a low level of productivity in general.

REFERENCES

1 Clarke, G. L. Elements of Ecology. John Wiley & Sons, New York. 1954.

2 Cooke, W. B. Trickling Filter Ecology. Ecology 40(2):273-291. 1959.

3 Hanson, E. D. Animal Diversity. Prentice-Hall, Inc., New Jersey. 1964.

4 Hedgpeth, J. W. Aspects of the Estuarine Ecosystem Amer. Fish. Soc., Spec. Publ. No. 3. 1966.

5 Odum, E. P. Fundamentals of Ecology. W. B. Saunders Company, Philadelphia and London. 1959.

6 Patten, B. C. Systems Ecology. Bio-Science. 16(9). 1966.

7 Whittaker, R. H. New Concepts of Kingdoms. Science 163:150-160. 1969.

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Descriptors:
Aquatic Environment, Estuarine Environment, Lentic Environment, Lotic Environment, Currents, Marshes, Limnology, Water Properties

THE AQUATIC ENVIRONMENT

Part 3. The Freshwater Environment

I INTRODUCTION

The freshwater environment as considered herein refers to those inland waters not detectably diluted by ocean waters, although the lower portions of rivers are subject to certain tidal flow effects.

Certain atypical inland waters such as saline or alkaline lakes, springs, etc., are not treated, as the main objective here in typical inland water.

All waters have certain basic biological cycles and types of interactions most of which have already been presented, hence this outline will concentrate on aspects essentially peculiar to fresh inland waters.

II PRESENT WATER QUALITY AS A FUNCTION OF THE EVOLUTION OF FRESH WATERS

A The history of a body of water determines its present condition. Natural waters have evolved in the course of geologic time into what we know today.

B Streams

In the course of their evolution, streams in general pass through four stages of development which may be called: birth, youth, maturity, and old age.

These terms or conditions may be employed or considered in two contexts: temporal, or spatial. In terms of geologic time, a given point in a stream may pass through each of the stages described below or: at any given time, these various stages of development can be loosely identified in successive reaches of a stream traveling from its headwaters to base level in ocean or major lake.

1 Establishment or birth. This might be a "dry run" or headwater stream-bed, before it had eroded down to the level of ground water.

During periods of run-off after a rain or snow-melt, such a gulley would have a flow of water which might range from torrential to a mere trickle. Erosion may proceed rapidly as there is no permanent aquatic flora or fauna to stabilize streambed materials. On the other hand, terrestrial grass or forest growth may retard erosion. When the run-off has passed, however, the "streambed" is dry.

2 Youthful streams. When the streambed is eroded below the ground water level, spring or seepage water enters, and the stream becomes permanent. An aquatic flora and fauna develops and water flows the year round. Youthful streams typically have a relatively steep gradient, rocky beds, with rapids, falls, and small pools.

3 Mature streams. Mature streams have wide valleys, a developed flood plain, are deeper, more turbid, and usually have warmer water, sand, mud, silt, or clay bottom materials which shift with increase in flow. In their more favorable reaches, streams in this condition are at a peak of biological productivity. Gradients are moderate, riffles or rapids are often separated by long pools.

4 In old age, streams have approached geologic base level, usually the ocean. During flood stage they scour their beds and deposit materials on the flood plain which may be very broad and flat. During normal flow the channel is refilled and many shifting bars are developed.

(Under the influence of man this pattern may be broken up, or temporarily interrupted. Thus an essentially "youthful" stream might take on some of the characteristics of a "mature" stream following soil erosion, organic enrichment, and increased surface runoff. Correction of these conditions might likewise be followed by at least a partial reversion to the "original" condition).

C Lakes and Reservoirs

Geological factors which significantly affect the nature of either a stream or lake include the following:

1 The geographical location of the drainage basin or watershed.

2 The size and shape of the drainage basin.

3 The general topography, i.e., mountainous or plains.

4 The character of the bedrocks and soils.

5 The character, amount, annual distribution, and rate of precipitation.

6 The natural vegetative cover of the land is, of course, responsive to and responsible for many of the above factors and is also severely subject to the whims of civilization. This is one of the major factors determining run-off versus soil absorption, etc.

D Lakes have a developmental history which somewhat parallels that of streams. This process is often referred to as natural eutrophication.

1 The methods of formation vary greatly, but all influence the character and subsequent history of the lake.

In glaciated areas, for example, a huge block of ice may have been covered with till. The glacier retreated, the ice melted, and the resulting hole became a lake. Or, the glacier may actually scoop out a hole. Landslides may dam valleys, extinct volcanoes may collapse, etc., etc.

2 Maturing or natural eutrophication of lakes.

a If not already present shoal areas are developed through erosion and deposition of the shore material by wave action and undertow.

b Currents produce bars across bays and thus cut off irregular areas.

c Silt brought in by tributary streams settles out in the quiet lake water

d Algae grow attached to surfaces, and floating free as plankton. Dead organic matter begins to accumulate on the bottom.

e Rooted aquatic plants grow on shoals and bars, and in doing so cut off bays and contribute to the filling of the lake.

f Dissolved carbonates and other materials are precipitated in the deeper portions of the lake in part through the action of plants.

g When filling is well advanced, mats of sphagnum moss may extend outward from the shore. These mats are followed by sedges and grasses which finally convert the lake into a marsh.

3 Extinction of lakes. After lakes reach maturity, their progress toward filling up is accelerated. They become extinct through:

a The downcutting of the outlet.

b Filling with detritus eroded from the shores or brought in by tributary streams.

c Filling by the accumulation of the remains of vegetable materials growing in the lake itself. (Often two or three processes may act concurrently)

III PRODUCTIVITY IN FRESH WATERS

A Fresh waters in general and under natural conditions by definition have a lesser supply of dissolved substances than marine waters, and thus a lesser basic potential for the growth of aquatic organisms. By the same token, they may be said to be more sensitive to the addition of extraneous materials (pollutants, nutrients, etc.) The following notes are directed toward natural geological and other environmental factors as they affect the productivity of fresh waters.

B Factors Affecting Stream Productivity (See Table 1)

TABLE 1

EFFECT OF SUBSTRATE ON STREAM PRODUCTIVITY*

(The productivity of sand bottoms is taken as 1)

Bottom Material	Relative Productivity
Sand	1
Marl	6
Fine Gravel	9
Gravel and silt	14
Coarse gravel	32
Moss on fine gravel	89
Fissidens (moss) on coarse gravel	111
Ranunculus (water buttercup)	194
Watercress	301
Anacharis (waterweed)	452

*Selected from Tarzwell 1937

To be productive of aquatic life, a stream must provide adequate nutrients, light, a suitable temperature, and time for growth to take place.

1 Youthful streams, especially on rock or sand substrates are low in essential nutrients. Temperatures in mountainous regions are usually low, and due to the steep gradient, time for growth is short. Although ample light is available, growth of true plankton is thus greatly limited.

2 As the stream flows toward a more "mature" condition, nutrients tend to accumulate, and gradient diminishes and so time of flow increases, temperature tends to increase, and plankton flourish.

Should a heavy load of inert silt develop on the other hand, the turbidity would reduce the light penetration and consequently the general plankton production would diminish.

3 As the stream approaches base level (old age) and the time available for plankton growth increases, the balance between turbidity, nutrient levels, and temperature and other seasonal conditions, determines the overall productivity.

C Factors Affecting the Productivity of lakes (See Table 2)

1 The size, shape, and depth of the lake basin. Shallow water is more productive than deeper water since more light will reach the bottom to stimulate rooted plant growth. As a corollary, lakes with more shoreline, having more shallow water, are in general more productive. Broad shallow lakes and reservoirs have the greatest production potential (and hence should be avoided for water supplies).

TABLE 2

EFFECT OF SUBSTRATE ON LAKE PRODUCTIVITY *

(The productivity of sand bottoms is taken as 1)

Bottom Material	Relative Productivity
Sand	1
Pebbles	4
Clay	8
Flat rubble	9
Block rubble	11
Shelving rock	77

* Selected from Tarzwell 1937

2 Hard waters are generally more productive than soft waters as there are more plant nutrient minerals available. This is often greatly influenced by the character of the soil and rocks in the watershed and the quality and quantity of ground water entering the lake. In general, pH ranges of 6.8 to 8.2 appear to be most productive.

3 Turbidity reduces productivity as light penetration is reduced.

4 The presence or absence of thermal stratification with its semi-annual turnovers affects productivity by distributing nutrients throughout the water mass.

5 Climate, temperature, prevalence of ice and snow, are also of course important.

D Factors Affecting the Productivity of Reservoirs

1 The productivity of reservoirs is governed by much the same principles as that of lakes, with the difference that the water level is much more under the control of man. Fluctuations in water level can be used to deliberately increase or decrease productivity. This can be demonstrated by a comparison of the TVA reservoirs which practice a summer drawdown with some of those in the west where a winter drawdown is the rule.

2 The level at which water is removed from a reservoir is important to the productivity of the stream below. The hypolimnion may be anaerobic while the epilimnion is aerobic, for example, or the epilimnion is poor in nutrients while the hypolimnion is relatively rich.

3 Reservoir discharges also profoundly affect the DO, temperature, and turbidity in the stream below a dam. Too much fluctuation in flow may permit sections of the stream to dry, or provide inadequate dilution for toxic waste.

IV CULTURAL EUTROPHICATION

A The general processes of natural eutrophication, or natural enrichment and productivity have been briefly outlined above.

B When the activities of man speed up these enrichment processes by introducing unnatural quantities of nutrients (sewage, etc.) the result is often called cultural eutrophication. This term is often extended beyond its original usage to include the enrichment (pollution) of streams, estuaries, and even oceans, as well as lakes.

V CLASSIFICATION OF LAKES AND RESERVOIRS

A The productivity of lakes and impoundments is such a conspicuous feature that it is often used as a convenient means of classification.

1 Oligotrophic lakes are the younger, less productive lakes, which are deep, have clear water, and usually support Salmonoid fishes in their deeper waters.

2 Eutrophic lakes are more mature, more turbid, and richer. They are usually shallower. They are richer in dissolved solids; N, P, and Ca are abundant. Plankton is abundant and there is often a rich bottom fauna.

3 Dystrophic lakes, such as bog lakes, are low in Ph, water yellow to brown, dissolved solids, N, P, and Ca scanty but humic materials abundant, bottom fauna and plankton poor, and fish species are limited.

B Reservoirs may also be classified as storage, and run of the river.

1 Storage reservoirs have a large volume in relation to their inflow.

2 Run of the river reservoirs have a large flow-through in relation to their storage value.

C According to location, lakes and reservoirs may be classified as polar, temperate, or tropical. Differences in climatic and geographic conditions result in differences in their biology.

VI SUMMARY

A A body of water such as a lake, stream, or estuary represents an intricately balanced system in a state of dynamic equilibrium. Modification imposed at one point in the system automatically results in compensatory adjustments at associated points.

B The more thorough our knowledge of the entire system, the better we can judge where to impose control measures to achieve a desired result.

REFERENCES

1 Chamberlin, Thomas C. and Salisburg, Rollin P. Geological Processes and Their Results. Geology 1: pp i-xix, and 1-654. Henry Holt and Company. New York. 1904.

2 Frey, David G. Limnology in North America. Univ. Wisc. Press. 1963.

3 Hutcheson, George E. A Treatise on Limnology Vol. I Geography, Physics and Chemistry. 1957. Vol. II. Introduction to Lake Biology and the Limnoplankton. 1115 pp. 1967. John Wiley Co.

4 Hynes, H.B.N. The Ecology of Running Waters. Univ. Toronto Press. 555 pp. 1970.

5 Ruttner, Franz. Fundamentals of Limnology. University of Toronto Press. pp. 1-242. 1953.

6 Tarzwell, Clarence M. Experimental Evidence on the Value of Trout 1937 Stream Improvement in Michigan. American Fisheries Society Trans. 66:177-187. 1936.

7 U. S. Dept. of Health, Education, and Welfare. Public Health Service. Algae and Metropolitan Wastes. Transactions of a seminar held April 27-29, 1960 at the Robert A. Taft Sanitary Engineering Center. Cincinnati, OH. No. SEC TR W61-3.

8 Ward and Whipple. Fresh Water Biology. (Introduction). John Wiley Company. 1918.

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Descriptors:
Aquatic Environment, Estuarine Environment, Lentic Environment, Lotic Environment, Currents, Marshes, Limnology, Water Properties

THE AQUATIC ENVIRONMENT

Part 4. The Marine Environment and its Role in the Total Aquatic Environment

I INTRODUCTION

A The marine environment is arbitrarily defined as the water mass extending beyond the continental land masses, including the plants and animals harbored therein. This water mass is large and deep, covering about 70 percent of the earth's surface and being as deep as 7 miles. The salt content averages about 35 parts per thousand. Life extends to all depths.

B The general nature of the water cycle on earth is well known. Because the largest portion of the surface area of the earth is covered with water, roughly 70 percent of the earth's rainfall is on the seas. (Figure 1)

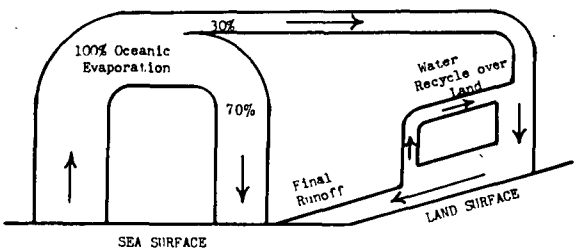

Figure 1. THE WATER CYCLE

Since roughly one third of the rain which falls on the land is again recycled through the atmosphere (see Figure 1 again), the total amount of water washing over the earth's surface is significantly greater than one third of the total world rainfall. It is thus not surprising to note that the rivers which finally empty into the sea carry a disproportionate burden of dissolved and suspended solids picked up from the land. The chemical composition of this burden depends on the composition of the rocks and soils through which the river flows, the proximity of an ocean, the direction of prevailing winds, and other factors. This is the substance of geological erosion. (Table 1)

TABLE 1

PERCENTAGE COMPOSITION OF THE MAJOR IONS OF TWO STREAMS AND SEA WATER

(Data from Clark, F.W., 1924, "The Composition of River and Lake Waters of the United States", U.S. Geol. Surv., Prof. Paper No. 135; Harvey, H.W., 1957, "The Chemistry and Fertility of Sea Waters", Cambridge University Press, Cambridge)

Ion	Delaware River at Lambertville, N.J.	Rio Grande at Laredo, Texas	Sea Water
Na	6.70	14.78	30.4
K	1.46	.85	1.1
Ca	17.49	13.73	1.16
Mg	4.81	3.03	3.7
Cl	4.23	21.65	55.2
SO_4	17.49	30.10	7.7
CO_3	32.95	11.55	$+HCO_3$ 0.35

C For this presentation, the marine environment will be (1) described using an ecological approach, (2) characterized ecologically by comparing it with freshwater and estuarine environments, and (3) considered as a functional ecological system (ecosystem).

II FRESHWATER, ESTUARINE, AND MARINE ENVIRONMENTS

Distinct differences are found in physical, chemical, and biotic factors in going from a freshwater to an oceanic environment. In general, environmental factors are more constant in freshwater (rivers) and oceanic environments than in the highly variable and harsh environments of estuarine and coastal waters. (Figure 2)

A Physical and Chemical Factors

Rivers, estuaries, and oceans are compared in Figure 2 with reference to the relative instability (or variation) of several important parameters. In the oceans, it will be noted, very little change occurs in any parameter. In rivers, while "salinity" (usually referred to as "dissolved solids") and temperature (accepting normal seasonal variations) change little, the other four parameters vary considerably. In estuaries, they all change.

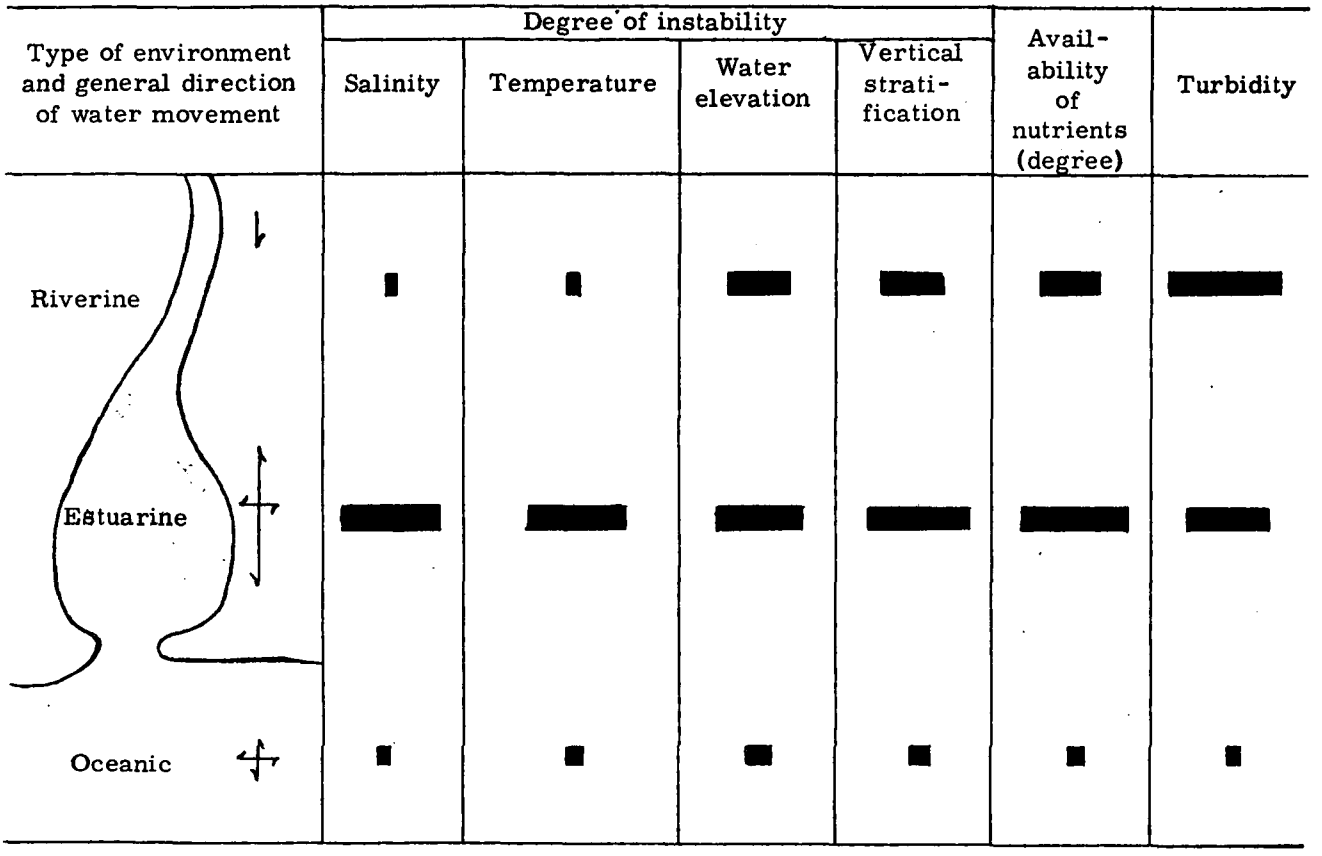

Figure 2. RELATIVE VALUES OF VARIOUS PHYSICAL AND CHEMICAL FACTORS FOR RIVER, ESTUARINE, AND OCEANIC ENVIRONMENTS

B Biotic Factors

1 A complex of physical and chemical factors determine the biotic composition of an environment. In general, the number of species in a rigorous, highly variable environment tends to be less than the number in a more stable environment (Hedgpeth, 1966).

2 The dominant animal species (in terms of total biomass) which occur in estuaries are often transient, spending only a part of their lives in the estuaries. This results in better utilization of a rich environment.

C Zones of the Sea

The nearshore environment is often classified in relation to tide level and water depth. The nearshore and offshore oceanic regions together, are often classified with reference to light penetration and water depth. (Figure 3)

1 Neritic - Relatively shallow-water zone which extends from the high-tide mark to the edge of the continental shelf.

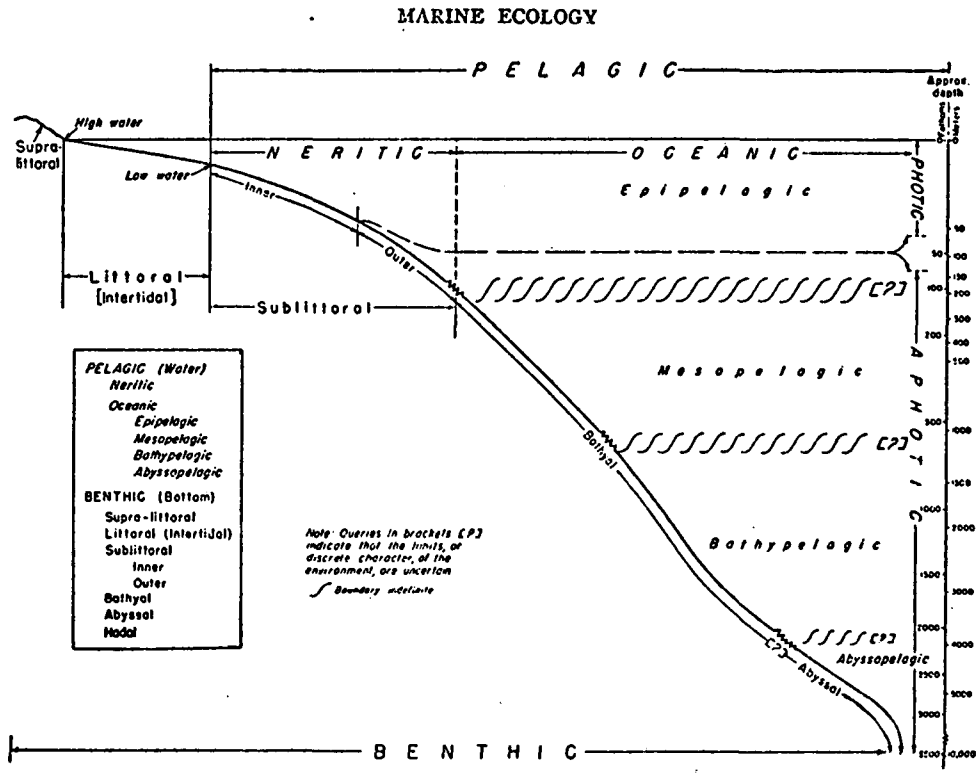

FIGURE 3—*Classification of marine environments*

a Stability of physical factors is intermediate between estuarine and oceanic environments.

b Phytoplankters are the dominant producers but in some locations attached algae are also important as producers.

c The animal consumers are zooplankton, nekton, and benthic forms.

2 Oceanic - The region of the ocean beyond the continental shelf. Divided into three parts, all relatively poorly populated compared to the neritic zone.

a Euphotic zone - Waters into which sunlight penetrates (often to the bottom in the neritic zone). The zone of primary productivity often extends to 600 feet below the surface.

1) Physical factors fluctuate less than in the neritic zone.

2) Producers are the phytoplankton and consumers are the zooplankton and nekton.

b Bathyal zone - From the bottom of the euphotic zone to about 2000 meters.

1) Physical factors relatively constant but light is absent.

2) Producers are absent and consumers are scarce.

c Abyssal zone - All the sea below the bathyal zone.

1) Physical factors more constant than in bathyal zone.

2) Producers absent and consumers even less abundant than in the bathyal zone.

III SEA WATER AND THE BODY FLUIDS

A Sea water is a remarkably suitable environment for living cells, as it contains all of the chemical elements essential to the growth and maintenance of plants and animals. The ratio and often the concentration of the major salts of sea water are strikingly similar in the cytoplasm and body fluids of marine organisms. This similarity is also evident, although modified somewhat in the body fluids of fresh water and terrestrial animals. For example, sterile sea water may be used in emergencies as a substitute for blood plasma in man.

B Since marine organisms have an internal salt content similar to that of their surrounding medium (isotonic condition) osmoregulation poses no problem. On the other hand, fresh water organisms are hypertonic (osmotic pressure of body fluids is higher than that of the surrounding water). Hence, fresh water animals must constantly expend more energy to keep water out (i.e., high osmotic pressure fluids contain more salts, the action being then to dilute this concentration with more water).

1 Generally, marine invertebrates are narrowly poikilosmotic, i.e., the salt concentration of the body fluids changes with that of the external medium. This has special significance in estuarine situations where salt concentrations of the water often vary considerably in short periods of time.

2 Marine bony fish (teleosts) have lower salt content internally than the external environment (hypotonic). In order to prevent dehydration, water is ingested and salts are excreted through special cells in the gills.

IV FACTORS AFFECTING THE DISTRIBUTION OF MARINE AND ESTUARINE ORGANISMS

A Salinity. Salinity is the single most constant and controlling factor in the marine environment, probably followed by temperature. It ranges around 35,000 mg. per liter, or "35 parts per thousand" (symbol: 35‰) in the language of the oceanographer. While variations in the open ocean are relatively small, salinity decreases rapidly as one approaches shore and proceeds through the estuary and up into fresh water with a salinity of "0 ‰ (see Figure 2)

B Salinity and temperature as limiting factors in ecological distribution.

1 Organisms differ in the salinities and temperatures in which they prefer to live, and in the variabilities of these parameters which they can tolerate. These preferences and tolerances often change with successive life history stages, and in turn often dictate where the organisms live: their "distribution."

2 These requirements or preferences often lead to extensive migrations of various species for breeding, feeding, and growing stages. One very important result of this is that an estuarine environment is an absolute necessity for over half of all coastal commercial and sport related species of fishes and invertebrates, for either all or certain portions of their life histories. (Part V, figure 8)

3 The Greek word roots "eury" (meaning wide) and "steno" (meaning narrow) are customarily combined with such words as "haline" for salt, and "thermal" for temperature, to give us "euryhaline" as an adjective to characterize an organism able to tolerate a wide range of salinity, for example; or "stenothermal" meaning one which cannot stand much change in temperature. "Meso-" is a prefix indicating an intermediate capacity.

C Marine, estuarine, and fresh water organisms. (See Figure 4)

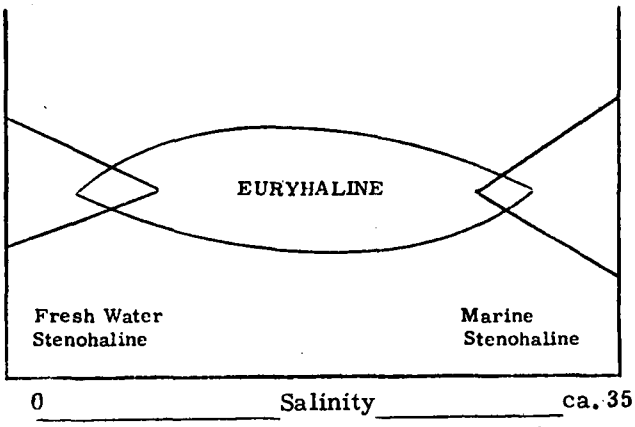

Figure 4. Salinity Tolerance of Organisms

1 Offshore marine organisms are, in general, both stenohaline and stenothermal unless, as noted above, they have certain life history requirements for estuarine conditions.

2 Fresh water organisms are also stenohaline, and (except for seasonal adaptation) meso- or stenothermal. (Figure 2)

3 Indigenous or native estuarine species that normally spend their entire lives in the estuary are relatively few in number. (See Figure 5). They are generally meso- or euryhaline and meso- or eurythermal.

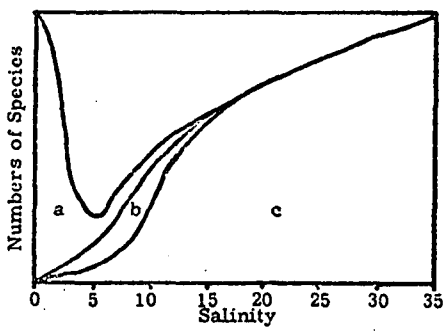

Figure 5. DISTRIBUTION OF ORGANISMS IN AN ESTUARY

a Euryhaline, freshwater
b Indigenous, estuarine, (mesohaline)
c Euryhaline, marine

4 Some well known and interesting examples of migratory species which change their environmental preferences with the life history stage include the shrimp (mentioned above), striped bass, many herrings and relatives, the salmons, and many others. None are more dramatic than the salmon hordes which lay their eggs in freshwater streams, migrate far out to sea to feed and grow, then return to the stream where they hatched to lay their own eggs before dying.

5 Among euryhaline animals landlocked (trapped), populations living in lowered salinities often have a smaller maximum size than individuals of the same species living in more saline waters. For example, the lamprey (Petromyzon marinus) attains a length of 30 - 36" in the sea, while in the Great Lakes the length is 18 - 24".

Usually the larvae of aquatic organisms are more sensitive to changes in salinity than are the adults. This characteristic both limits and dictates the distribution and size of populations.

D The effects of tides on organisms.

1 Tidal fluctuations probably subject the benthic or intertidal populations to the most extreme and rapid variations of environmental stress encountered in any aquatic habitat. Highly specialized communities have developed in this zone, some adapted to the rocky surf zones of the open coast, others to the muddy inlets of protected estuaries. Tidal reaches of fresh water rivers, sandy beaches, coral reefs and mangrove swamps in the tropics; all have their own floras and faunas. All must emerge and flourish when whatever water there is rises and covers or tears at them, all must collapse or retract to endure drying, blazing tropical sun, or freezing arctic ice during the low tide interval. Such a community is depicted in Figure 6.

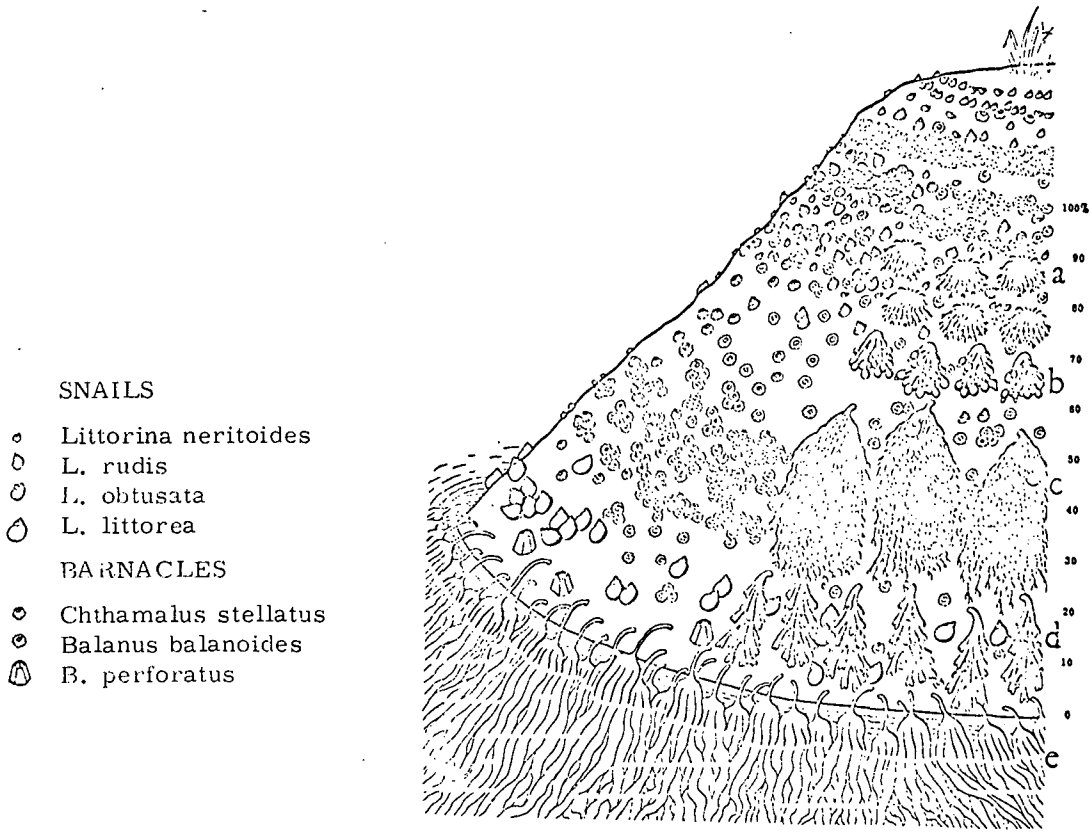

Figure 6

Zonation of plants, snails, and barnacles on a rocky shore. While this diagram is based on the situation on the southwest coast of England, the general idea of zonation may be applied to any temperate rocky ocean shore, though the species will differ. The gray zone consists largely of lichens. At the left is the zonation of rocks with exposure too extreme to support algae; at the right, on a less exposed situation, the animals are mostly obscured by the algae. Figures at the right hand margin refer to the percent of time that the zone is exposed to the air, i.e., the time that the tide is out. Three major zones can be recognized: the Littorina zone (above the gray zone); the Balanoid zone (between the gray zone and the laminarias); and the Laminaria zone. a. Pelvetia canaliculata; b. Fucus spiralis; c. Ascophyllum nodosum; d. Fucus serratus; e. Laminaria digitata. (Based on Stephenson)

V FACTORS AFFECTING THE PRODUCTIVITY OF THE MARINE ENVIRONMENT

A The sea is in continuous circulation. Without circulation, nutrients of the ocean would eventually become a part of the bottom and biological production would cease. Generally, in all oceans there exists a warm surface layer which overlies the colder water and forms a two-layer system of persistent stability. Nutrient concentration is usually greatest in the lower zone. Wherever a mixing or disturbance of these two layers occurs biological production is greatest.

B The estuaries are also a mixing zone of enormous importance. Here the fertility washed off the land is mingled with the nutrient capacity of seawater, and many of the would's most productive waters result.

C When man adds his cultural contributions of sewage, fertilizer, silt or toxic waste, it is no wonder that the dynamic equilibrium of the ages is rudely upset, and the environmentalist cries, "See what man hath wrought"!

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REFERENCES

1 Harvey, H. W. The Chemistry and Fertility of Sea Water (2nd Ed.). Cambridge Univ. Press, New York. 234 pp. 1957.

2 Hedgpeth, J. W. (Ed.). Treatise on Marine Ecology and Paleoecology. Vol. I. Ecology Mem. 67 Geol. Soc. Amer., New York. 1296 pp. 1957.

3 Hill, M. N. (Ed.). The Sea. Vol. II. The Composition of Sea Water Comparative and Descriptive Oceanography. Interscience Publs. John Wiley & Sons, New York. 554 pp. 1963.

4 Moore, H. B. Marine Ecology. John Wiley & Sons, Inc., New York. 493 pp. 1958.

5 Reid, G. K. Ecology of Inland Waters and Estuaries. Reinhold Publ. Corp. New York. 375 pp. 1961.

6 Sverdrup, Johnson, and Fleming. The Oceans. Prentice-Hall, Inc., New York. 1087 pp. 1942.

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Descriptors:
Aquatic Environment, Estuarine Environment, Lentic Environment, Lotic Environment, Currents, Marshes, Limnology, Water Properties

THE AQUATIC ENVIRONMENT

Part 5: Wetlands

I INTRODUCTION

A Broadly defined, wetlands are areas which are "to wet to plough but too thick to flow." The soil tends to be saturated with water, salt or fresh, and numerous channels or ponds of shallow or open water are common. Due to ecological features too numerous and variable to list here, they comprise in general a rigorous (highly stressed) habitat, occupied by a small relatively specialized indigenous (native) flora and fauna.

B They are prodigiously productive however, and many constitute an absolutely essential habitat for some portion of the life history of animal forms generally recognized as residents of other habitats (Figure 8). This is particularly true of tidal marshes as mentioned below.

C Wetlands in toto comprise a remarkably large proportion of the earth's surface, and the total organic carbon bound in their mass constitutes an enormous sink of energy.

D Since our main concern here is with the "aquatic" environment, primary emphasis will be directed toward a description of wetlands as the transitional zone between the waters and the land, and how their desecration by human culture spreads degradation in both directions.

II TIDAL MARSHES AND THE ESTUARY

A "There is no other case in nature, save in the coral reefs, where the adjustment of organic relations to physical condition is seen in such a beautiful way as the balance between the growing marshes and the tidal streams by which they are at once nourished and worn away." (Shaler, 1886)

B Estuarine pollution studies are usually devoted to the dynamics of the circulating water, its chemical, physical, and biological parameters, bottom deposits, etc.

C It is easy to overlook the intimate relationships which exist between the bordering marshland, the moving waters, the tidal flats, subtidal deposition, and seston whether of local, oceanic, or riverine origin.

D The tidal marsh (some inland areas also have salt marshes) is generally considered to be the marginal areas of estuaries and coasts in the intertidal zone, which are dominated by emergent vegetation. They generally extend inland to the farthest point reached by the spring tides, where they merge into freshwater swamps and marshes (Figure 1). They may range in width from nonexistent on rocky coasts to many kilometers.

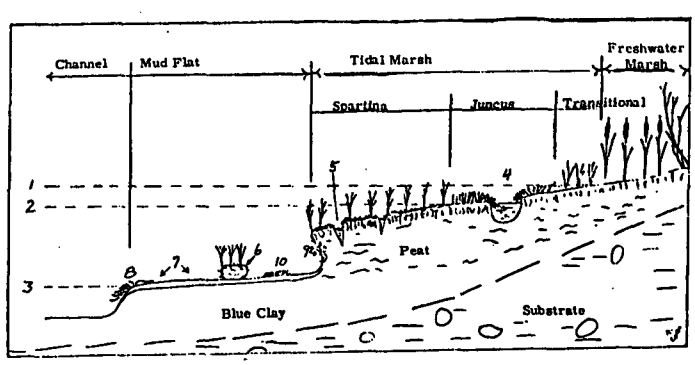

Figure 1. Zonation in a positive New England estuary. 1. Spring tide level, 2. Mean high tide, 3. Mean low tide, 4. Bog hole, 5. Ice cleavage pool, 6. Chunk of Spartina turf deposited by ice, 7. Organic ooze with associated community, 8. eelgrass (Zostera), 9. Ribbed mussels (modiolus)-clam (mya) - mud snail (Nassa) community. 10. Sea lettuce (Ulva)

III MARSH ORIGINS AND STRUCTURES

A In general, marsh substrates are high in organic content, relatively low in minerals and trace elements. The upper layers bound together with living roots called turf, underlaid by more compacted peat type material.

1 Rising or eroding coastlines may expose peat from ancient marsh growth to wave action which cuts into the soft peat rapidly (Figure 2).

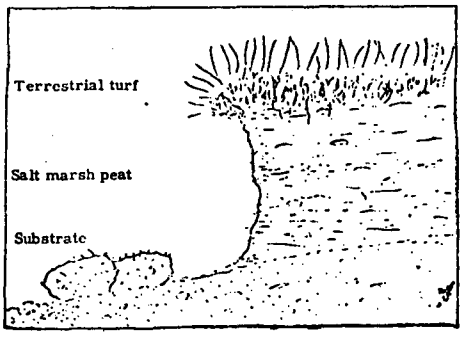

Figure 2. Diagrammatic section of eroding peat cliff

Such banks are likely to be cliff-like, and are often undercut. Chunks of peat are often found lying about on harder substrate below high tide line. If face of cliff is well above high water, overlying vegetation is likely to be typically terrestrial of the area. Marsh type vegetation is probably absent.

2 Low lying deltaic, or sinking coastlines, or those with low energy wave action are likely to have active marsh formation in progress. Sand dunes are also common in such areas (Figure 3). General coastal configuration is a factor.

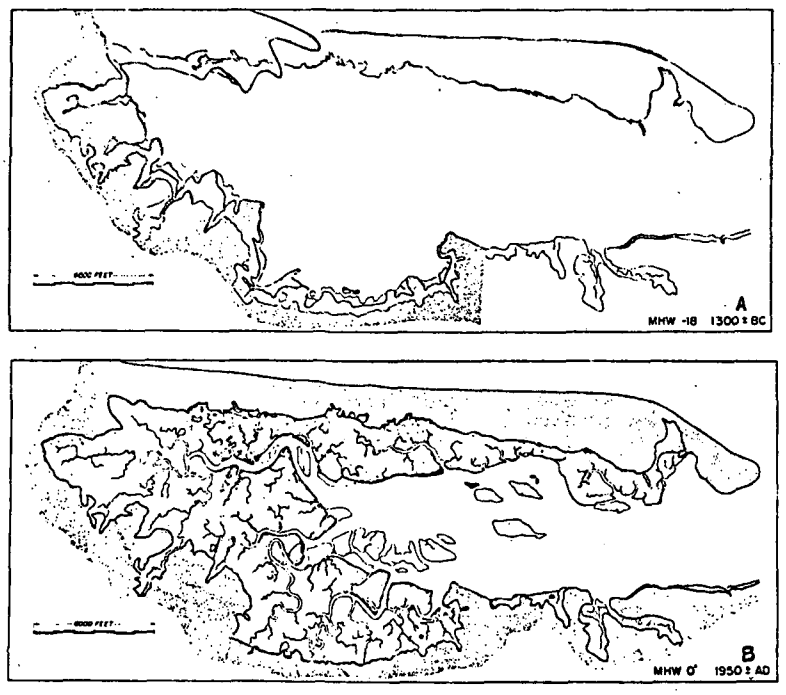

Figure 3

Development of a Massachusetts Marsh since 1300 BC, involving an 18 foot rise in water level. Shaded area indicates sand dunes. Note meandering marsh tidal drainage. A: 1300 BC, B: 1950 AD.

a Rugged or precipitous coasts or slowly rising coasts, typically exhibit narrow shelves, sea cliffs, fjords, massive beaches, and relatively less marsh area (Figure 4). An Alaskan fjord subject to recent catastrophic subsidence and rapid deposition of glacial flour shows evidence of the recent encroachment of saline waters in the presence of recently buried trees and other terrestrial vegetation, exposure of layers of salt marsh peat along the edges of channels, and a poorly compacted young marsh turf developing at the new high water level (Figure 5).

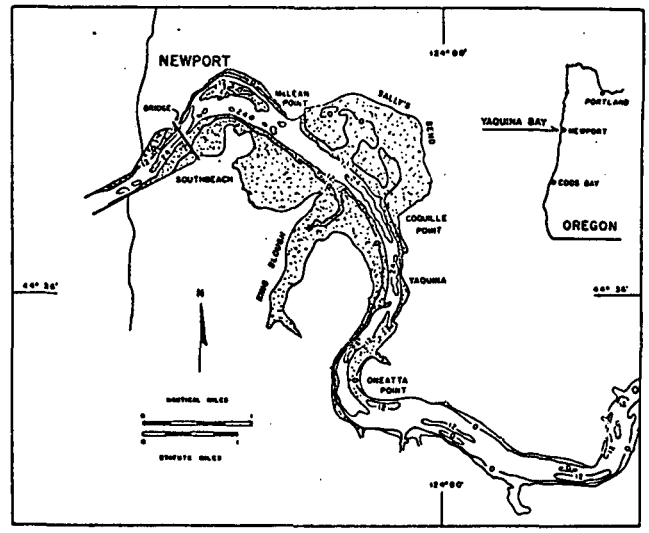

Figure 4 A River Mouth on a Slowly Rising Coast. Note absence of deltaic development and relatively little marshland, although mud flats stippled are extensive.

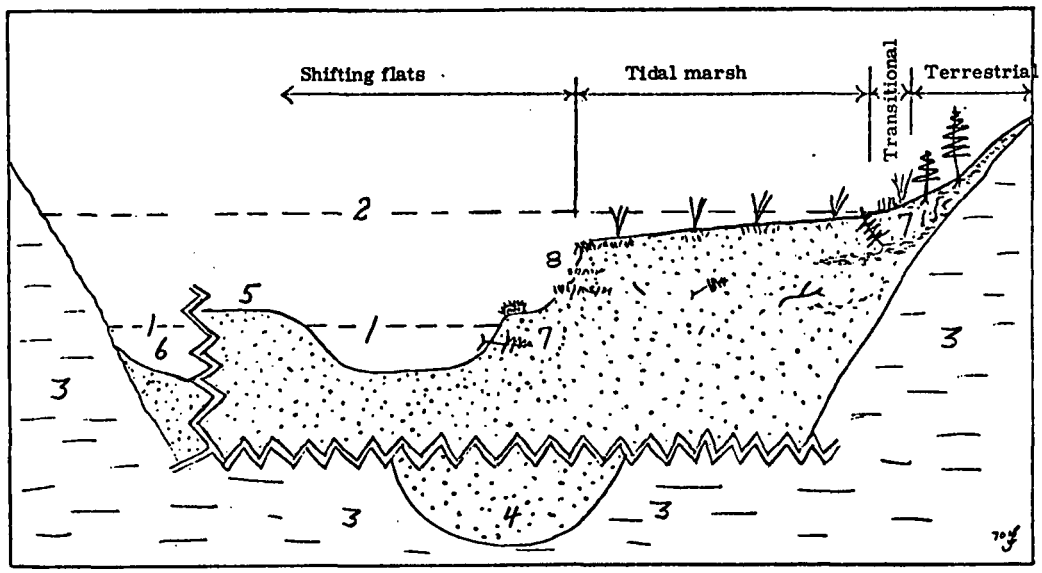

Figure 5 Some general relationships in a northern fjord with a rising water level. 1. mean low water, 2. maximum high tide, 3. Bedrock, 4. Glacial flour to depths in excess of 400 meters, 5. Shifting flats and channels, 6. Channel against bedrock, 7. Buried terrestrial vegetation, 8. Outcroppings of salt marsh peat.

b Low lying coastal plains tend to be fringed by barrier islands, broad estuaries and deltas, and broad associated marshlands (Figure 3). Deep tidal channels fan out through innumerable branching and often interconnecting rivulets. The intervening grassy plains are essentially at mean high tide level.

c Tropical and subtropical regions such as Florida, the Gulf Coast, and Central America, are frequented by mangrove swamps. This unique type of growth is able to establish itself in shallow water and move out into progressively deeper areas (Figure 6). The strong deeply embedded roots enable the mangrove to resist considerable wave action at times, and the tangle of roots quickly accumulates a deep layer of organic sediment. Mangroves in the south may be considered to be roughly the equivalent of the Spartina marsh grass in the north as a land builder. When fully developed, a mangrove swamp is an impenetrable thicket of roots over the tidal flat affording shelter to an assortment of semi-aquatic organisms such as various molluscs and crustaceans, and providing access from the nearby land to predaceous birds, reptiles, and mammals. Mangroves are not restricted to estuaries, but may develop out into shallow oceanic lagoons, or upstream into relatively fresh waters.

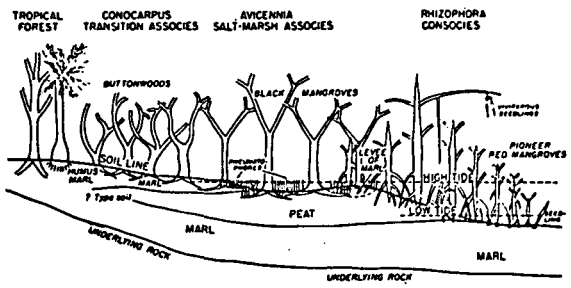

Figure 6 Diagrammatic transect of a mangrove swamp showing transition from marine to terrestrial habitat.

IV PRODUCTIVITY OF WETLANDS

A Measuring the productivity of grasslands is not easy, because today grass is seldom used directly as such by man. It is thus usually expressed as production of meat, milk, or in the case of salt marshes, the total crop of animals that obtain food per unit of area. The primary producer in a tidal marsh is the marsh grass, but very little of it is used by man as grass. (Table 1)

The nutritional analysis of several marsh grasses as compared to dry land hay is shown in Table 2.

TABLE 1. General Orders of Magnitude of Gross Primary Productivity in Terms of Dry Weight of Organic Matter Fixed Annually

Ecosystem	$gms/M^2/year$ (grams/square meters/year)	lbs/acre/year
Land deserts, deep oceans	Tens	Hundreds
Grasslands, forests, eutrophic lakes, ordinary agriculture	Hundreds	Thousands
Estuaries, deltas, coral reefs, intensive agriculture (sugar cane, rice)	Thousands	Ten-thousands

TABLE 2. Analyses of Some Tidal Marsh Grasses

T/A	Percentage Composition					
Dry Wt.	Protein	Fat	Fiber	Water	Ash	N-free Extract
Distichlis spicata (pure stand, dry)						
2.8	5.3	1.7	32.4	8.2	6.7	45.5
Short *Spartina alterniflora* and *Salicornia europaea* (in standing water)						
1.2	7.7	2.5	31.1	8.8	12.0	37.7
Spartina alterniflora (tall, pure stand in standing water)						
3.5	7.6	2.0	29.0	8.3	15.5	37.3
Spartina patens (pure stand, dry)						
3.2	6.0	2.2	30.0	8.1	9.0	44.5
Spartina alterniflora and *Spartina patens* (mixed stand, wet)						
3.4	6.8	1.9	29.0	8.1	10.4	42.8
Spartina alterniflora (short, wet)						
2.2	8.8	2.4	30.4	8.7	13.3	36.3
Comparable Analyses for Hay						
1st cut	6.0	2.0	36.2	6.7	4.2	44.9
2nd cut	13.0	3.7	28.5	10.4	5.9	38.5

Analyses performed by Roland W. Gilbert, Department of Agricultural Chemistry, U.R.I.

B The actual utilization of marsh grass is accomplished primarily by its decomposition and ingestion by micro organisms. (Figure 7) A small quantity of seeds and solids is consumed directly by birds.

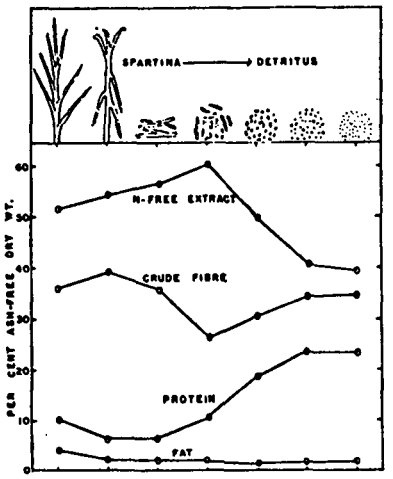

Figure 7 The nutritive composition of successive stages of decomposition of Spartina marsh grass, showing increase in protein and decrease in carbohydrate with increasing age and decreasing size of detritus particles.

1 The quantity of micro invertebrates which thrive on this wealth of decaying marsh has not been estimated, nor has the actual production of small indigenous fishes and invertebrates such as the top minnows (Fundulus), or the mud snails (Nassa), and others.

2 Many forms of oceanic life migrate into the estuaries, especially the marsh areas, for important portions of their life histories as is mentioned elsewhere (Figure 8). It has been estimated that in excess of 60% of the marine commercial and sport fisheries are estuarine or marsh dependent in some way.

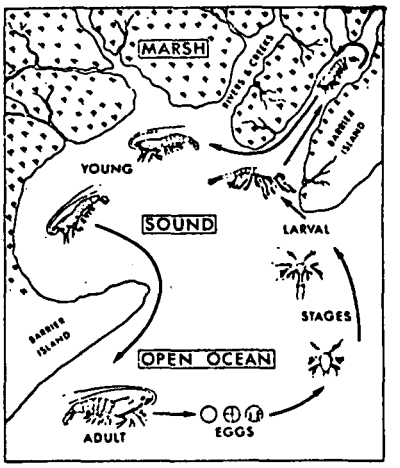

Figure 8 Diagram of the life cycle of white shrimp (after Anderson and Lunz 1965).

3 An effort to make an indirect estimate of productivity in a Rhode Island marsh was made on a single August day by recording the numbers and kinds of birds that fed on a relatively small area (Figure 9). Between 700 and 1000 wild birds of 12 species, ranging from 100 least sandpipers to uncountable numbers of seagulls were counted. One food requirement estimate for three-pound poultry in the confined inactivity of a poultry yard is approximately one ounce per pound of bird per day.

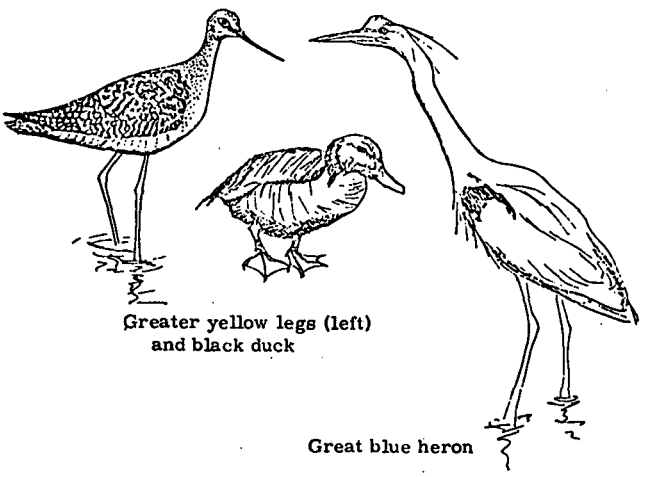

Figure 9 Some Common Marsh Birds

One-hundred black bellied plovers at approximately ten ounces each would weigh on the order of sixty pounds. At the same rate of food consumption, this would indicate nearly four pounds of food required for this species alone. The much greater activity of the wild birds would obviously greatly increase their food requirements, as would their relatively smaller size.

Considering the range of foods consumed, the sizes of the birds, and the fact that at certain seasons, thousands of migrating ducks and others pause to feed here, the enormous productivity of such a marsh can be better understood.

V INLAND BOGS AND MARSHES

A Much of what has been said of tidal marshes also applies to inland wetlands. As was mentioned earlier, not all inland swamps are salt-free, any more than all marshes affected by tidal rythms are saline.

B The specificity of specialized floras to particular types of wetlands is perhaps more spectacular in freshwater wetlands than in the marine, where Juncus, Spartina, and Mangroves tend to dominate.

1 Sphagnum, or peat moss, is probably one of the most widespead and abundant wetland plants on earth. Deevey (1958) quotes an estimate that there is probably upwards of 223 billions (dry weight) of tons of peat in the world today, derived during recent geologic time from Sphagnum bogs. Particularly in the northern regions, peat moss tends to overgrow ponds and shallow depressions, eventually forming the vast tundra plains and moores of the north.

2 Long lists of other bog and marsh plants might be cited, each with its own special requirements, topographical, and geographic distribution, etc. Included would be the familiar cattails, spike rushes, cotton grasses, sedges, trefoils, alders, and many, many others.

C Types of inland wetlands.

1 As noted above (Cf: Figure 1) tidal marshes often merge into freshwater marshes and bayous. Deltaic tidal swamps and marshes are often saline in the seaward portion, and fresh in the landward areas.

2 River bottom wetlands differ from those formed from lakes, since wide flood plains subject to periodic inundation are the final stages of the erosion of river valleys, whereas lakes in general tend to be eliminated by the geologic processes of natural eutrophication often involving Sphagnum and peat formation. Riverbottom marshes in the southern United States, with favorable climates, have luxurient growths such as the canebrake of the lower Mississippi, or a characteristic timber growth such as cypress.

3 Although bird life is the most conspicuous animal element in the fauna (Cf: Figure 9), many mammals, such as muskrats, beavers, otters, and others are also marsh-oriented. (Figure 12)

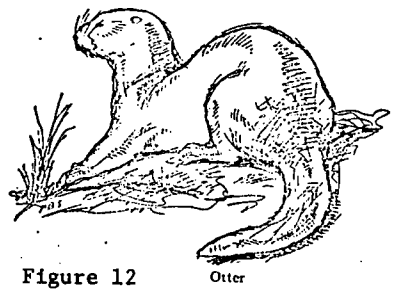

Figure 12 Otter

VI POLLUTION

A No single statement can summarize the effects of pollution on marshlands as distinct from effects noted elsewhere on other habitats.

B Reduction of Primary Productivity

The primary producers in most wetlands are the grasses and peat mosses. Production may be reduced or eliminated by:

1 Changes in the water level brought about by flooding or drainage.

a Marshland areas are sometimes diked and flooded to produce fresh-water ponds. This may be for aesthetic reasons, to suppress the growth of noxious marsh inhabitating insects such as mosquitoes or biting midges, to construct an industrial waste holding pond, a thermal or a sewage stabilization pond, a "convenient" result of highway causeway construction, or other reason. The result is the elimination of an area of marsh. A small compensating border of marsh may or may not develop.

b High tidal marshes were often ditched and drained in former days to stabilize the sod for salt hay or "thatch" harvesting which was highly sought after in colonial days. This inevitably changed the character of the marsh, but it remained as essentially marshland. Conversion to outright agricultural land has been less widespread because of the necessity of diking to exclude the periodic floods or tidal incursions, and carefully timed drainage to eliminate excess precipitation. Mechanical pumping of tidal marshes has not been economical in this country, although the success of the Dutch and others in this regard is well known.

2 Marsh grasses may also be eliminated by smothering as, for example, by deposition of dredge spoils, or the spill or discharge of sewage sludge.

3 Considerable marsh area has been eliminated by industrial construction activity such as wharf and dock construction, oil well construction and operation, and the discharge of toxic brines and other chemicals.

C Consumer production (animal life) has been drastically reduced by the deliberate distribution of pesticides. In some cases, this has been aimed at nearby agricultural lands for economic crop pest control, in other cases the marshes have been sprayed or dusted directly to control noxious insects.

1 The results have been universally disastrous for the marshes, and the benefits to the human community often questionable.

2 Pesticides designed to kill nuisance insects, are also toxic to other arthropods so that in addition to the target species, such forage staples as the various scuds (amphipods), fiddler crabs, and other macroinvertebrates have either been drastically reduced or entirely eliminated in many places. For example, one familiar with fiddler crabs can traverse miles of marsh margins, still riddled with their burrows, without seeing a single live crab.

3 DDT and related compounds have been "eaten up the food chain" (biological magnification effect) until fish eating and other predatory birds such as herons and egrets (Figure 9), have been virtually eliminated from vast areas, and the accumulation of DDT in man himself is only too well known.

D Most serious of the marsh enemies is man himself. In his quest for "lebensraum" near the water, he has all but killed the water he strives to approach. Thus up to twenty percent of the marsh--estuarine area in various parts of the country has already been utterly destroyed by cut and fill real estate developments (Figures 10, 11).

E Swimming birds such as ducks, loons, cormorants, pelicans, and many others are severely jeopardized by floating pollutants such as oil.

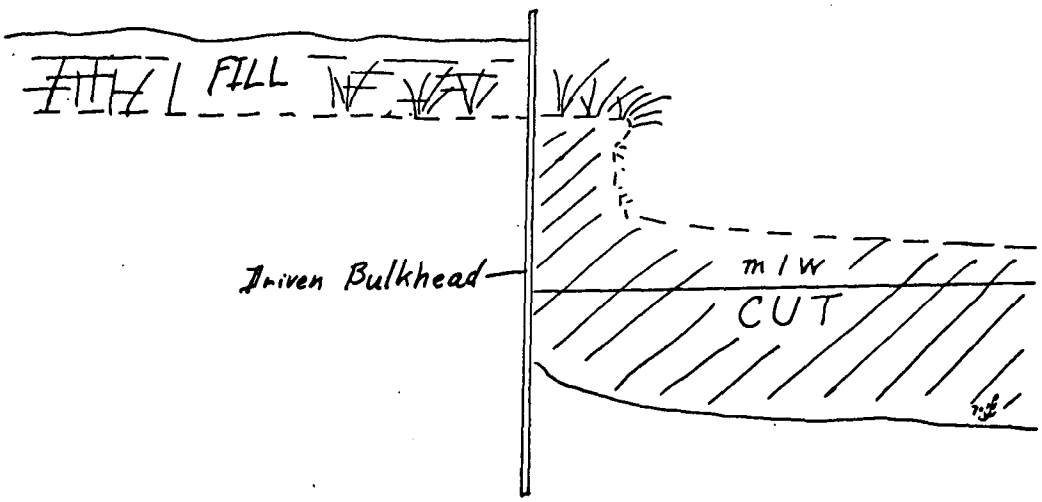

Figure 10. Diagrammatic representation of cut-and-fill for real estate development. mlw = mean low water

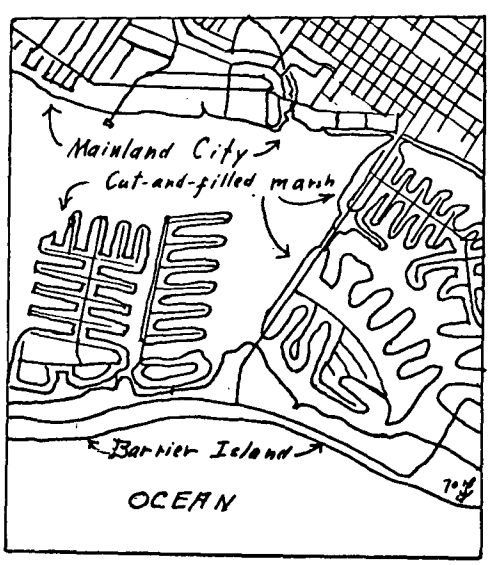

Figure 11. Tracing of portion of map of a southern city showing extent of cut-and-fill real estate development.

VII SUMMARY

A Wetlands comprise the marshes, swamps, bogs, and tundra areas of the world. They are essential to the well-being of our surface waters and ground waters. They are essential to aquatic life of all types living in the open waters. They are essential as habitat for all forms of wildlife.

B The tidal marsh is the area of emergent vegetation bordering the ocean or an estuary.

C Marshes are highly productive areas, essential to the maintenance of a well rounded community of aquatic life.

D Wetlands may be destroyed by:

1 Degradation of the life forms of which it is composed in the name of nuisance control.

2 Physical destruction by cut-and-fill to create more land area.

REFERENCES

1 Anderson, W.W. The Shrimp and the Shrimp Fishery of the Southern United States. USDI, FWS, BCF. Fishery Leaflet 589. 1966.

2 Deevey, E.S., Jr. Bogs. Sci. Am. Vol. 199(4):115-122. October 1958.

3 Emery, K. O. and Stevenson. Estuaries and Lagoons. Part II, Biological Aspects by J.W. Hedgepeth, pp. 693-728. in: Treatise on Marine Ecology and Paleoecology. Geol. Soc. Am. Mem. 67. Washington, DC. 1957.

4 Hesse, R., W. C. Allee, and K. P. Schmidt. Ecological Animal Geography. John Wiley & Sons. 1937.

5 Morgan, J.P. Ephemeral Estuaries of the Deltaic Environment in: Estuaries, pp. 115-120. Publ. No. 83, Am. Assoc. Adv. Sci. Washington, DC. 1967.

6 Odum, E.P. and Dela Crug, A.A. Particulate Organic Detritus in a Georgia Salt Marsh - Estuarine Ecosystem. in: Estuaries, pp. 383-388, Publ. No. 83, Am.Assoc. Adv. Sci. Washington, DC. 1967.

7 Redfield, A.C. The Ontogeny of a Salt Marsh Estuary. in: Estuaries, pp. 108-114. Publ. No. 83, Am. Assoc. Adv. Sci. Washington, DC. 1967.

8 Stuckey, O.H. Measuring the Productivity of Salt Marshes. Maritimes (Grad School of Ocean., U.R.I.) Vol. 14(1): 9-11. February 1970.

9 Williams, R.B. Compartmental Analysis of Production and Decay of Juncus reomerianus. Prog. Report, Radiobiol. Lab., Beaufort, NC, Fiscal Year 1968, USDI, BCF, pp. 10-12.

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Descriptors: Aquatic Environment, Estuarine Environment, Lentic Environment Lotic Environment, Currents, Marshes, Limnology

THE IDENTIFICATION OF AQUATIC ORGANISMS

I INTRODUCTION

A There are few major groups of organisms that are either exclusively terrestrial or aquatic. The following remarks will therefore apply in large measure to both, but primary attention will be directed to aquatic types.

B One of the first questions usually posed about an organism is: "What is it," usually meaning "What is it's name?" The naming or classification of biological organisms is a science in itself (taxonomy). Some of the principles involved need to be understood by anyone working with organisms.

1 Names are the "key number," "code designation," or "file references" which we must have to find information about an unknown organism.

2 Why are they so long and why must they be in Latin and Greek? File references in large systems have to be long in order to designate the many divisions and subdivisions. There are over a million and a half items (or species) included in the system of biological nomenclature (very few libraries have a million books).

3 The system of biological nomenclature is regulated by international congresses.

a It is based on a system of groups and super groups, of which the foundation (which actually exists in nature) is the species. Everything else has been devised by man and is subject to change and revision as man's knowledge and understanding increase.

b The categories employed are as follows:

Similar species are grouped into genera (genus)

Similar genera are grouped into families

Similar families are grouped into orders

Similar orders are grouped into classes

Similar classes are grouped into phyla (phylum)

Similar phyla are grouped into kingdoms

4 The scientific name of an organism is its genus name plus its species name. This is analogous to our system of surnames (family names) and given names (Christian names).

a The generic (genus) name is always capitalized and the species name written with a small letter. They should also be underlined or printed in italics when used in a technical sense. For example:

Homo sapiens - modern man

Homo neanderthalis - neanderthal man

Esox niger - chain pickerel

Esox lucius - northern pike

Esox masquinongy - muskellunge

b Common names do not exist for most of the smaller and less familiar organisms. For example, if we wish to refer to members of the genus Anabaena (an alga), we must simply use the generic name, and:

Anabaena planctonica,

Anabaena constricta, and

Anabaena flos-aquae

are three distinct species which have different significances to water treatment plant operations.

5 A complete list of the various categories to which an organism belongs is known as its "classification." This may be written as follows for Phacus pyrum a green flagellate, for example:

Kingdom Plantae

Phylum Thallophyta

Class Algae

Order Volvocales

Family Phacotaceae

Genus Phacus

Species pyrum

a It should be emphasized that since all categories of above species are essentially human concepts, there is often divergence of opinion in regard to how certain organisms should be grouped. Changes result.

b The most appropriate or correct name for a given species is also sometimes disputed, and so species names too are changed. The species itself, as an entity in nature, however, is relatively timeless and so does not change to man's eye.

II KEY TO SELECTED LARGER GROUPS OF AQUATIC ANIMALS

The following key is intended to provide an introductory acquaintance with some of the more common aquatic organisms. While most of the groups mentioned are animals, a few other groups often confused with them are also included. Technical nomenclature is kept to a minimum.

In the use of the key, each couplet should be considered by itself, without reference to any other couplet. Select the alternative that seems most reasonable and either proceed to the couplet indicated, or else accept the name of the group as given if the selection is a terminal statement. "Cf: --" means to compare with the statement indicated. "POL" means that members of the group have been reported to be tolerant of organic pollution.

1a The body of the organisms comprising a single microscopic independent cell, or many similar and independently functioning cells associated in a colony, with little or no difference between the cells (i.e., without forming tissues); or comprising masses of multinucleate protoplasm; mostly microscopic. Single celled animals..... Phylum Protozoa

1b The body of the organism comprising many cells of different kinds (i.e, forming tissue) may be microscopic or macroscopic..... 2

2a Body of colony usually forming irregular masses or layers sometimes cylindrical, goblet-shaped, vase-shaped, elongate, serrate, or tree-like. Range from barely visible to large..... 3

2b Body or colony shows some type of definite symmetry..... 15

3a Colony forming irregular masses, clumps, or aggregations of material growing around, over, or clinging to surface of rocks, sticks, pipes, etc..... 4

3b Irregular, or not obviously symmetrical, but essentially erect or plant-like individuals or colonies..... 9

4a Freshwater..... 8

4b Marine..... 5

5a Surface of colony usually slightly rough or bristly in appearance under the microscope or hand lens..... 7

5b Surface smooth..... 6

5c Colony or organism erect, plant-like, branched or unbranched..... 12

6a Mass very thin, usually pink or white (Coral algae)..... 13b

6b Mass gelatinous, either as relatively thin sheet or clumps of tongue-like club shaped projections. Transparent or colored. Star shaped groups of individual organisms often evident. Colonial tunicates or ascideans. Phylum Chordata, Class Tunicata (Cf:12b)

7a Surface webbed or bristly in appearance, stick-like crystals or "spicules" often protruding from the general surface. Pores numerous, one or more usually large and conspicuous. Sponges. ..... Phylum Porifera

7b Colonies very thin, surface slightly roughened. Composed of minute individual cells or compartments arranged in various lacy or coral-like patterns. Each compartment inhabited by a minute bilaterally symmetrical animal which protrudes a crown of delicate tentacles. Moss animals, bryozoans.... Phylum Bryozoa

8a Surface of colony rough or bristly in appearance under the microscope or hand lens. Grey, green, or brown. Sponges. (Figure 1)..... Phylum Porifera

8b Surface colony relatively smooth, general texture of mass gelatinous, transparent. Clumps of minute individual organisms variously distributed. Moss animals, bryozoans. (Figure 2).. Phylum Bryozoa

9a Freshwater..... 10

9b Marine..... 11

10a Surface of structures rough or bristly under the microscope..... 8a

10b Individuals or branches tubular. Usually covered with brownish cuticle to which much sediment may adhere. Moss animals, bryozoans.... Phylum Bryozoa (Cf:8b) (Figure 2)

11a Surface of structure appears web-like or bristly under the microscope..... 7a

11b Surface of structure an unbroken skin or cuticle, except for a limited number of functional apertures..... 13

12a Colonies minute, plant-like or coral-like. (Figure 2)..... 13

12b Solitary, sac-like organism, either stalked or growing directly on substrate. Transparent, covered with mud or sand, or variously colored. Bilateral symmetry revealed on close investigation. Becomes flaccid when exposed and relaxed at low tide. Up to several inches in length. Seasquirts or tunicates.. Phylum Chordata, Class Tunicata

13a Compartments or apertures for separate individual animals evident..... 14

13b No evidence of the presence of individual animals. Structure profusely jointed. Individual segments hard, smooth, usually pink or white. May also grow as hard smooth thin sheet over surface of rocks. Calcareous algae or coral algae. A common Genus: ..... Corallina

14a Minute organisms protruding from holes in colony are radially symmetrical... 17b

14b Organisms protruding are bilaterally symmetrical. (This may be assumed if they are not obviously radial) Moss animals, bryozoans. (Figure 2) (Cf:7b) ..... Phylum Bryozoa

15a Radially symmetrical (body arranged around an axis)..... 16

15b Fundamentally bilaterally symmetrical. 18

15c May be superficially spiral as Figure 13 35

16a Plan involves multiples of two, or else rather large numbers of tentacles around the margin of an umbrella; saucer-like, or flower-like structure.....17

16b Body plan based on multiples of five. Outer surface hard or rough to the touch. Starfishes, brittle stars, sea urchins, sand dollars, sea cucumbers. ..... Phylum Echinodermata

17a Jelly-like body, massive or ovoid, free swimming nearly transparent in nature. Eight rows comb-like paddle plates: Comb Jellies..... Phylum Ctenophora

17b Body tube-like or umbrella-like, tentacles around mouth, margin of disc, or both. Jellyfishes, corals, hydroids, sea anemones, etc... Phylum Coelenterata

18a Microscopic. Action of two ciliated lobes at anterior end in life often gives appearance of wheels. Body often segmented, accordion-like. Free swimming or attached. Rotifers or wheel animalcules (Figure 3). Phylum Trochelminthes (Rotifera)

18b Often larger, worm-like or else having strong skeleton or shell..... 19

19a Skeleton (or shell) present may be internal or external..... 30

19b Body soft and/or worm-like. Skin may range from soft to parchment-like... 20

20a Three or more pairs of well-formed (though possibly small) jointed legs present. (Phylum Arthropoda)..... 36

20b Legs or appendages, if present, limited to pairs of bumps or hooks. Lobes or tentacles if present soft and fleshy, not jointed..... 21

21a Body round, oval or flat in cross section 22

21b Inverted U-shaped cross section, with flat slimy undersurface or foot tapering off behind. Head with two pairs of sensitive retractile tentacles. Marine or terrestrial. Marine forms have fancy gill structures on back. Slugs. Class Gastropoda (Cf:35a) (Phylum Mollusca)..... .35

22a Strongly depressed or flattened..... 23

22b Oval or round in cross section, or, if flat, with single sucker disc at each end. 25

23a Parasitic in bodies of higher animals. Extremely long and flat, divided into sections (like a Roman girdle). Life history may involve intermediate host. Tape worms. (Figure 5) Class Cestoda

23b Parasitic on bodies of higher animals, very muscular, strong external sucker discs (Figure 9)..... 29b

23c Body a single unit. Mouth and digestive system present (but no anus)..... 24

24a External or internal parasite on higher animals. Sucking discs present for attachment. Life history may involve two or more intermediate hosts or stages. Flukes..... Class Trematoda

24b Free-living. Entire body surface covered with locomotor cilia. Eyed areas in head often appear "crossed." Pol. Free-living flatworms (Figure 6). Class Turbellaria

25a Long, slender, with snake-like motion in life. Covered with glistening cuticle. Parasitic or free-living. Microscopic to six feet in length. Round worms (Figure 7).... Phylum Nemathelminthes

25b Divided into sections or segments (Figures 8 and 9)..... 26

26a Head a more or less well-formed hard capsule with jaws, eyes, and antennae. Pol. (Figure 8) Class Insecta, (Cf:49a)..... Order Diptera

26b Head structure soft (except jaws, if present) (Figure 9)..... 27

27a Head conical or rounded, lateral appendages not conspicuous or numerous..... 28

27b Head somewhat broad and blunt. Retractile jaws usually present. Soft fleshy lobes or tentacles often present in head region. Tail usually narrower. Lateral lobes or fleshy appendages on each segment. (Figure 9A) ..... Phylum Annelida..... 29

28a Minute dark colored retractile jaws present, body tapering somewhat at both ends, pairs or rings of bumps or "legs" often present, even near tail. Class Insecta. (Cf:49a) ..... Order Diptera

28b No jaws, sides of body generally parallel except at ends, (similar to Figure 9).

Thickened area or ring usually present part way back on body. Clumps of minute bristles on most segments. ..... (Phylum Annelida) 29

29a Segments with bristles, and/or fleshy lobes, or other extensions. Tube builder, borer, or burrower. Often reddish or greenish in color. Pol. Earthworms, sludge worms, clam worms, etc. ..... Class Chaetopoda

29b A sucker disc at each end, the larger one posterior. External bloodsucking parasites on higher animals, often found unattached to any host. Pol. Leeches. (Figure 9B). ..... Class Hirudinea

30a Skeleton internal, of true bone ..... (Vertebrates) 58

30b Body covered with external skeleton or shell. (Figure 4)..... 31

31a External jointed skeleton or shell covers legs and other appendages, often leathery in nature. Jointed legged animals. (Figures 11, 12, and 14 through 29) .....(Phylum Arthropoda) 36

31b Shells limey or calcareous. (Figures 10 and 13)..... 32

32a Marine, attached, shell conical, or purse-shaped on fleshy stalk. Crowded individuals may be more or less cylindrical. Shell composed of several parts, two of which spread apart to permit extension of "hand" for feeding. Barnacles. Phylum Arthropoda, Class Cirripedia

32b Shells one or two, or several arranged as transverse plates..... 33

33a Half inch or less in length. Two clam-like shells. Soft parts inside include delicate flattened jointed appendages. Phyllopods or branchipods (Figures 11 and 12) Phylum Arthropoda, Class Crustacea, Subclass Branchipoda (Cf:39a)

33b Soft parts covered with thin skin, often slimy.....(Phylum Mollusca) 34

34a Marine, oval in shape, eight shells transversely jointed. Chitons. ..... Class Amphineura

34b Shells one or two in number. Snails, clams, etc. (Figures 10-13)..... 35

35a Shell, single may be a spiral, a flattened cone, degenerate, or missing. Pol. Snails and slugs..... Class Gastropoda

35b Shell, double, right and left halves, hinged at one point. Mussels, clams, oysters. ..... Class Pelecypoda

36a Head, thorax, and abdominal body regions usually distinct. Three pairs of regular walking legs, or their rudiments. Wings present in all adults and rudiments in some larvae. Leglike appendages or gills present on abdomens of some larvae. No marine forms. (Phylum Arthropoda, ...Class Insecta)..... 47

36b More than three pairs of legs apparently present. (Figures 14-19)..... 37

37a Body elongated, head broad and flat with strong jaws. Appendages following first three pairs of legs are rounded tapering filaments. Up to 3 inches long. Dobson fly and fish fly larvae. Class Insecta, ... Order Megaloptera

37b Four or more pairs of true legs..... 38

38a Four pairs of legs. Body rounded, bulbous, head minute. Often brown or red. Water mites. (Figure 15) Phylum Arthropoda, Class Arachnida, Order Acari

38b Five or more pairs of locomotor appendages (walking or swimming legs) gills, two pairs of atenna. (Figures 16-19, 11-12) Crustaceans. Phylum Arthropoda, Class Crustacea..... 39

39a Ten or more pairs of flattened, leaf-like swimming (and respiratory) appendages. Many species swim constantly in life, some normally upside down. (Figure 16) Fairy shrimps, phyllopods, or branchipods. ..... Subclass Branchipoda

39b Less than ten pairs of locomotor appendages..... 40

40a Body and legs enclosed in bivalved (2 halves) shell which may or may not completely hide them. (Figures 11-12) 41

40b Appendages more rounded than leaf-like in cross section, or if appendages are flattened for swimming, eyes are on stalks (pelagic shrimps). May be large or minute..... 43

41a One pair of branched antennae enlarged for locomotion, extend outside of shell (carapace). Single eye usually visible. Cyclops or cladocera. Subclass Cladocera

41b Locomotion accomplished by body legs (not antennae). (Figure 16).....42

42a Appendages leaf-like, flattened, more than ten pairs. (Branchipods)..... 39a

42b Animal less than three mm in length. Appendages more or less slender and jointed, often used for walking. (Figure 11) Shells opaque. Ostracodes. ..... Subclass Ostracoda

43a Body a series of six or more essentially similar segments, differing mainly in size..... 44

43b Front part of body enlarged into a somewhat separate body unit (cephalothorax) often covered with a single piece of shell (carapace). Back part (abdomen) may be relatively small, even folded underneath front part. (Figure 19)..... 45

44a Body compressed laterally, i.e., high and thin. Scuds, amphipods. (Figure 17) ..... Subclass Amphipoda

44b Body depressed, i.e., low and broad. Flat gills contained in chamber beneath tail. (Figure 18) Sowbugs. ..... Subclass Isopoda

45a Minute in size. Often somewhat elongated and cylindrical, with abdomen extending straight out behind ending in two small projections. Two relatively enormous masses of eggs are often attached to female. Locomotion by means of two enlarged, unbranched antennae, the only large appendages on the body. (Figure 19) Copepods..... Subclass Copepoda

45b Larger. Carapace (shell of cephalothorax, the enlarged front section) usually more conspicuous. 5 pairs of walking legs including claws, if present. Appendages present on abdomen..... 46

46a Eyes immovable, in broad shell shaped like a horseshoe, a mud dweller. Long sharp pointed triangular tail extends out behind. Flat "book" gills beneath abdomen. Horseshoe crab. Subclass Arachnoidea, ... Genus Limulus

46b Eyes on movable stalks. Abdomen tightly folded back under cephalothorax (crabs) or continued out behind thorax to end in an expanded "flipper" or swimming paddle. Crabs, shrimps, crayfishes, lobsters, etc..... Subclass Decapoda

47a Two pairs of functional wings (one pair may be more or less hardened as protection for the other pair). Adult insects which normally live on or in the water (Figures 22, 25, 28, 29) 57

47b No functional wings, though "pads" in which they are developing may be visible. Some resemble adults very closely, others differ extremely (Figures 8, 23, 24, 26, 27) 48

48a External pads or cases in which wings develop clearly visible..... 53

48b More or less worm-like, no external evidence of wing development..... 49

49a No jointed legs (sucker discs, hooks, prolegs, breathing tubes, and other such structures may be present. (Figures 8, 9D) Larvae of two winged flies, midges, etc. Pol..... Order Diptera

49b Three pairs of jointed thoracic legs, head capsule well-formed..... 50

50a Minute (2 - 4mm), living on surface film. Tail a strong organ that can be hooked into

a "catch" beneath thorax. When released, animal jumps into the air. Not usually classed as aquatic. These are adults, no wings are ever grown. (Figure 20) Springtails..... Order Collembola

50b Larger (rarely collected under 5 mm), worm-like, living beneath the surface. 51

51a Construct fixed or movable cases of bottom materials. (Figure 21) Abdominal segments often with minute gill filaments, no long lateral filaments. Generally cylindric in shape. Caddisfly larvae. ..... Order Trichoptera

51b Free living, i.e., not case-building. 52

52a Somewhat flattened and relatively massive in appearance. First segment of thorax about same size as head, following two segments smaller. Each abdominal segment with rather stout, tapering lateral filament about as long as body is wide. (Figure 14) Dobsonfly, alderfly, and fishfly larvae. Order Megaloptera

52b Generally rounded in cross section. Lateral filaments if present tend to be longer and thinner. (Figure 23) (A few forms extremely flattened, like a suction cup: Psephenidae). Beetle larvae. ..... Order Coleoptera

53a Two or three filaments or other structures extending out from end of abdomen. (Figures 24B, 24D, 26, 27)..... 55

53b Abdomen ending abruptly, unless terminal segment itself is extended as single structure. (Figure 24C)..... 54

54a Head broad, eyes widely spaced. Front of face covered by extensible folded mouth-parts often called a "mask." Larvae of dragonflies or darning needles. Pol. (Figure 24E)..... Order Odonata ..... (Suborder Anisoptera)

54b Mouthparts for piercing and sucking. Body form varies extremely. Water bugs, walking sticks, water boatmen, back-swimmers, electric light bugs, water striders, and others. (Figure 25) ..... Order Hemiptera

55a Tail extensions (caudal filaments) two. Stonefly larvae. (Figure 26) ..... Order Plecoptera

55b Tail extensions usually three. (Figures 24B, 24D, 27)..... 56

56a Tail extensions long and slender. Rows of hairs may give them a feather-like appearance. Larvae of mayflies. ..... Order Ephemeroptera

56b Tail extensions flat, elongated plates. Head broad with widely spaced eyes, abdomen relatively long and slender. Larvae of damselflies. Order Odonata ..... (Suborder Zygoptera)

57a External wings or wing covers form a hard protective dome over the inner wings folded beneath, and the abdomen. Beetles. (Figure 28)..... Order Coleoptera

57b External wings somewhat membranous, usually relatively flat as compared to wing covers of beetles. Mouthparts for piercing and sucking. Body form various, (Cf:53b). True bugs. (Figure 25) Order Hemiptera

58a Appendages present in pairs (fins, legs, wings)..... 60

58b Mouth a round suction disc. No paired appendages..... 59

59a Body long and slender. Several holes alongside of head. Lampreys and hagfishes. Sub-phylum Vertebrata. Class Cyclostomata

59b Body plump, oval, tail extending out abruptly. Larvae of frogs and toads. Legs appear one at a time during metamorphosis to adult form. Tadpoles... Class Amphibia

60a Paired appendages are legs..... 62

60b Paired appendages are fins..... 61

61a Gills covered with a flap or "operculum." True fishes..... Class Pisces

61b Gill chambers open directly to the outside. Skin often rough and scratchy or penetrated by clumps of short sharp spines. Essentially marine. Sharks, skates, and rays. ..... Class Elasmobranchii

62a Digits with claws, nails, or hoofs.... 63

62b Skin naked, no claws on digits. Frogs, toads, and salamanders. Class Amphibia

63a Warm blooded..... 64

63b Body covered with horny scales or plates. Reptiles.....Class Reptilia

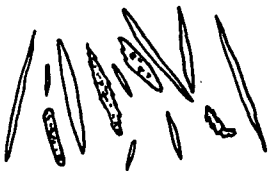
64a Body covered with feathers. Birds. .....Class Aves

64b Body covered with hair. Mammals. .....Class Mammalia

APPENDIX A

LIST OF INCLUDED GROUPS

Phyla or Subphyla	Classes or Subphyla	Classes or Orders	Other
Protozoa	-----		
Porifera	-----		
Coelenterata	-----		
Platyhelminthes	Turbellaria		
	Trematoda		
	Cestoda		
Rotifera	-----		
Nemathelminthes	-----		
Bryozoa	-----		
Annelida	Chaetopoda		
	Hirudinea		
Arthropoda	Crustacea	Branchiopoda	
		Cladocera	
		Ostracoda	
		Amphipoda	
		Isopoda	
		Copepoda	
		Decapoda	
		Cirripedia	
	Arachnoidea	Acari	
		Xiphosura	Limulus
	Insecta	Collembola	
		Plecoptera	
		Ephemeroptera	
		Odonata	
		Hemiptera	
		Megaloptera	
		Trichoptera	
		Coleoptera	
		Diptera	
Mollusca	Gastropoda		
	Pelecypoda		
Enchinodermata	-----		
Chordata	Tunicata		
	Cyclostomata		
	Pisces		
	Amphibia		
	Reptilia		
	Aves		
	Mammalia		
Algae	-----		Corallina

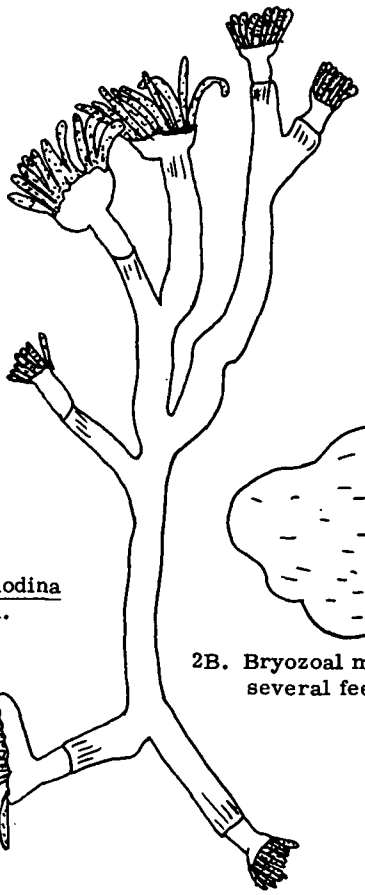


1. Spongilla spicules
Up to .2 mm. long.

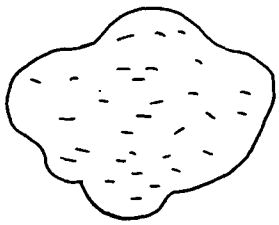
3A. Rotifer, Polyarthra
Up to .3 mm.

3B. Rotifer, Keratella
Up to .3 mm.

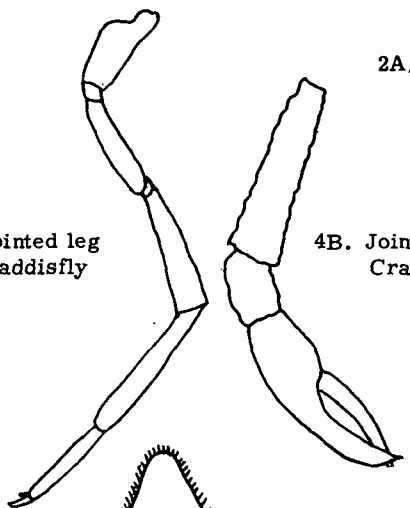
3C. Rotifer, Philodina
Up to .4 mm.



2A. Bryozoa, Plumatella. Individuals up to 2 mm. Intertwined masses may be very extensive.



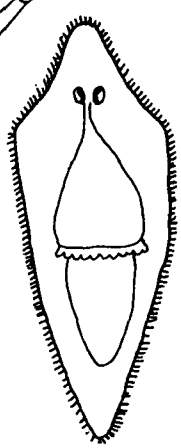
2B. Bryozoal mass. Up to several feet diam.



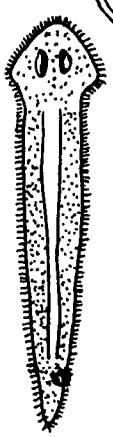
4A. Jointed leg
Caddisfly

4B. Jointed leg
Crayfish

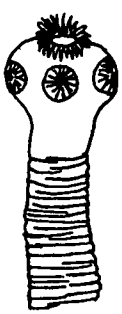
4C. Jointed leg
Ostracod



6A. Turbellaria, Mesostoma
Up to 1 cm.

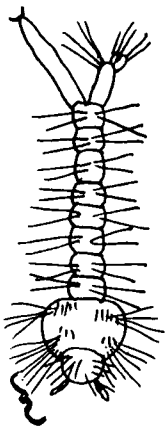


6B. Turbellaria, Dugesia
Up to 1.6 cm.



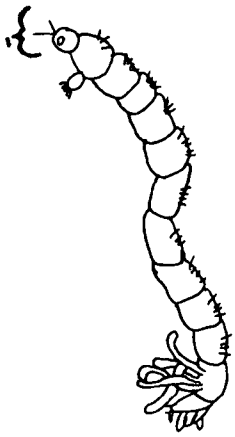
5. Tapeworm head,
Taenia. Up to
25 yds. long

7. Nematodes. Free living forms commonly up to 1 mm., occasionally more.



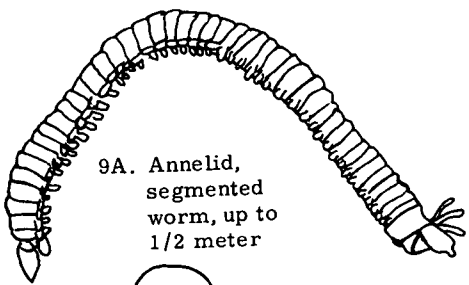
8A. Diptera, Mosquito larvae
Up to 15 mm. long.

8B. Diptera, Mosquito
pupa. Up to 5 mm.

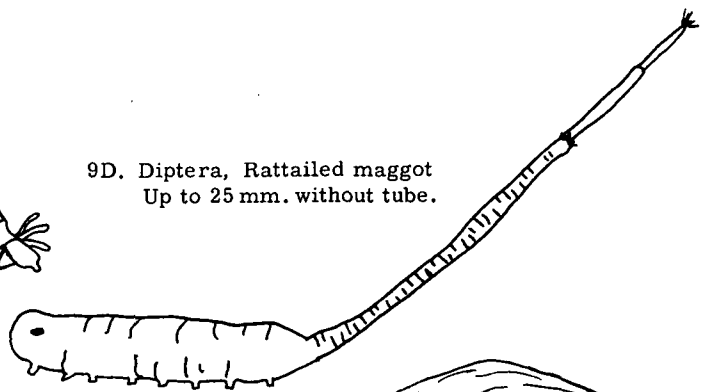


8C. Diptera, Tendiped
larvae. Up to 2 cm.

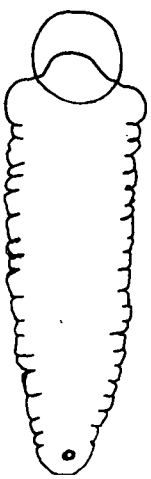
8E. Diptera, crane fly
pupa. Up to 2.5 cm.



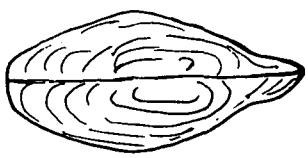
9A. Annelid,
segmented
worm, up to
1/2 meter



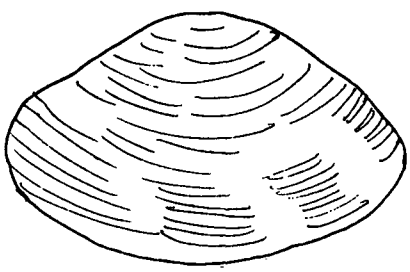
9D. Diptera, Rattailed maggot
Up to 25 mm. without tube.



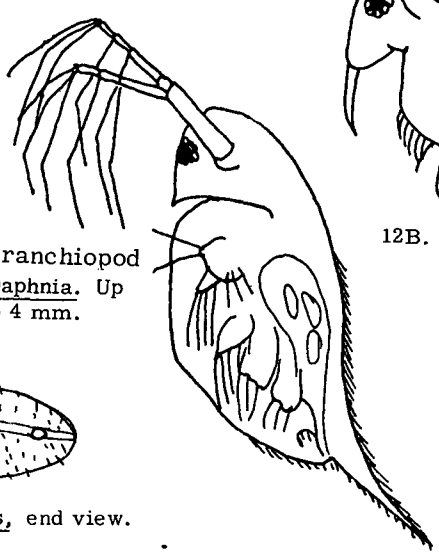
9B. Annelid, leech up to 20 cm.



10B. Alasmidonta, end view.

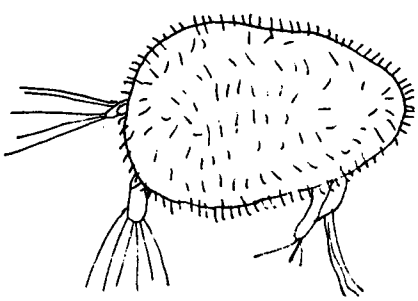


10A. Pelecyopod, Alasmidonta
Side view, up to 18 cm. long.

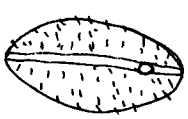


12A. Branchiopod
Daphnia. Up
to 4 mm.

12B. Branchiopod
Bosmina. Up
to 2 mm.

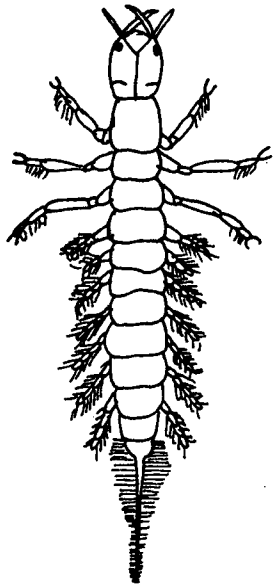


11A. Ostracod, Cypericus
Side view, up to 7 mm.

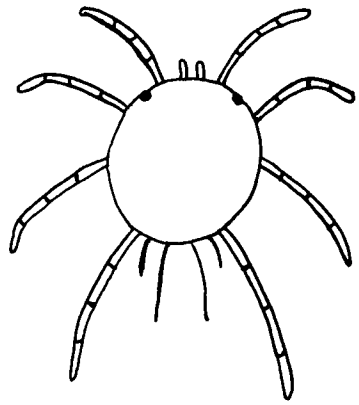


11B. Cypericus, end view.

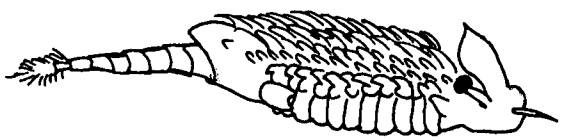
13. Gastropod, Viviparus
Up to 3 inches.



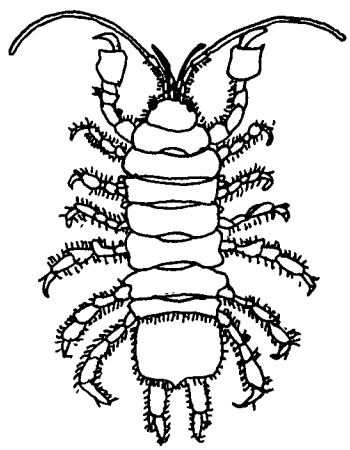
14. Megaloptera,
Alderfly larvae
Up to 25 mm.



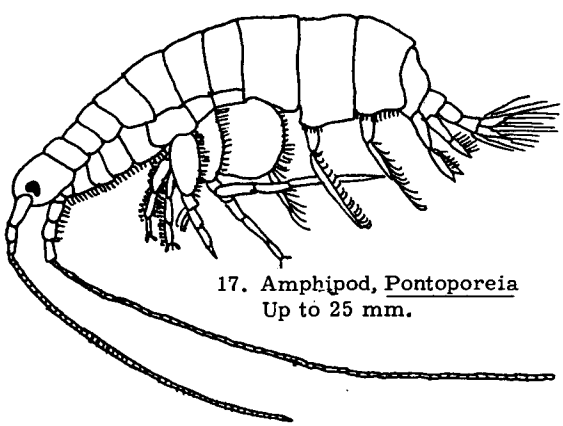
15. Water mite,
Up to 3 mm.



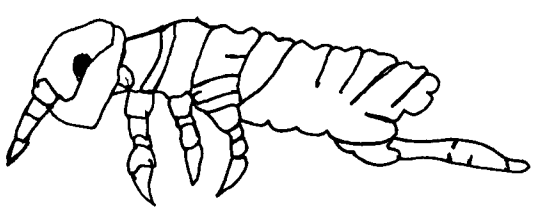
16. Fairy Shrimp, Eubranchipus
Up to 5 cm.



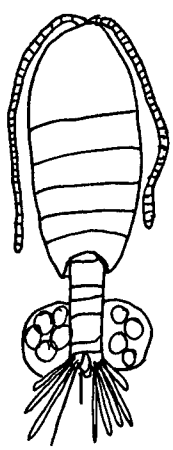
18. Isopod, Asellus
Up to 25 mm.



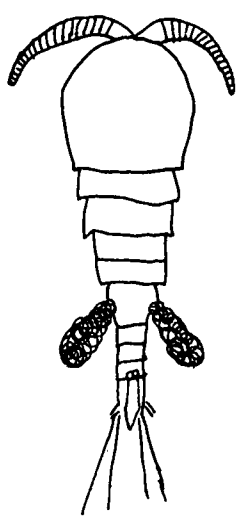
17. Amphipod, Pontoporeia
Up to 25 mm.



20. Collembola, Podura
Up to 2 mm. long



19A. Calanoid copepod,
Female
Up to 3 mm.



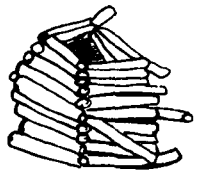
19B. Copepoid copepod,
Female
Up to 25 mm.

21A.

21B.

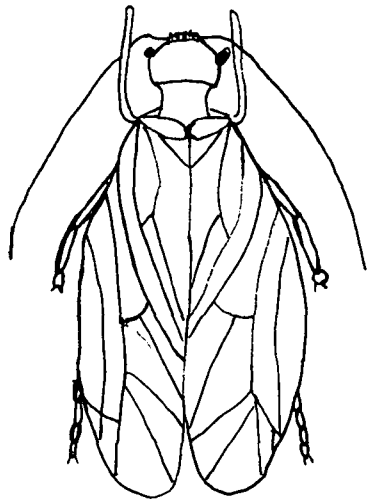
21C.

21D.

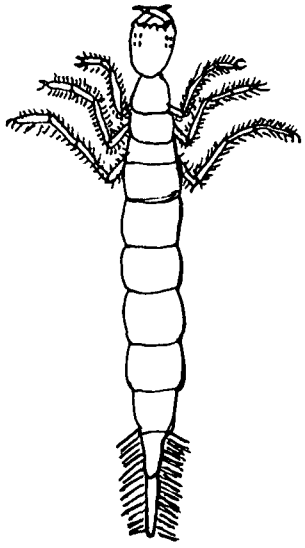


21E.

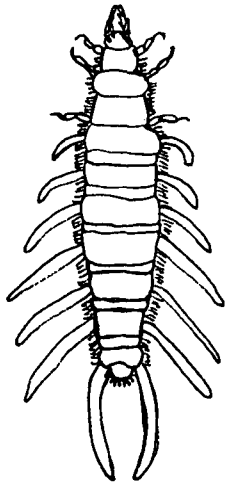
21. Tricoptera, Larvae cases, mostly 1-2 cm.



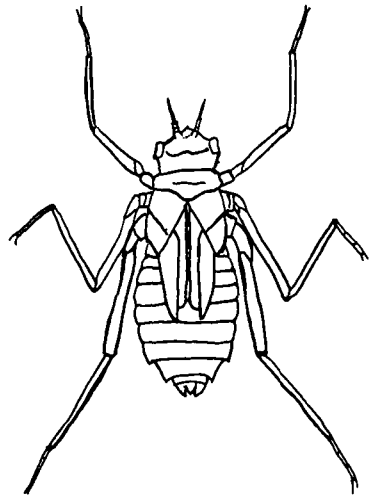
22. Megaloptera, alderfly
Up to 2 cm.



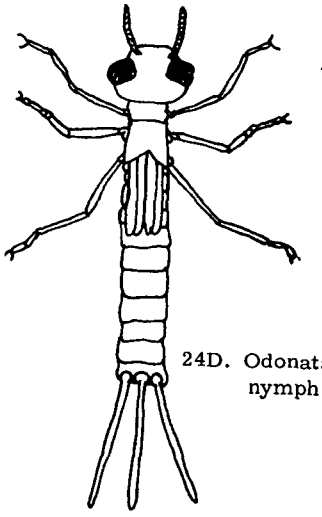
23A. Beetle larvae,
Dytisidae,
Usually about $\frac{1}{2}$ cm.



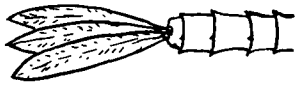
23B. Beetle larvae,
Hydrophilidae,
Usually about 1 cm.



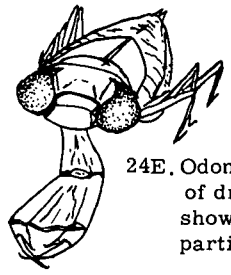
24A. Odonata, dragonfly
nymph up to 3 or 4
cm.



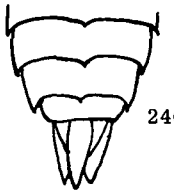
24D. Odonata, damselfly
nymph (top view)



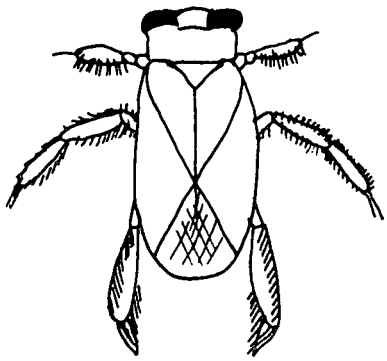
24B. Odonata, tail of
damselfly nymph
(side view)



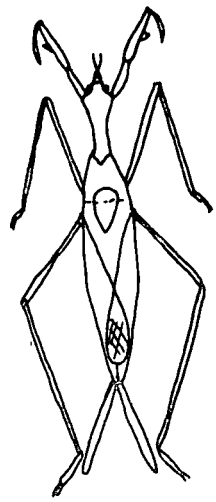
24E. Odonata, front view
of dragonfly nymph
showing "mask"
partially extended



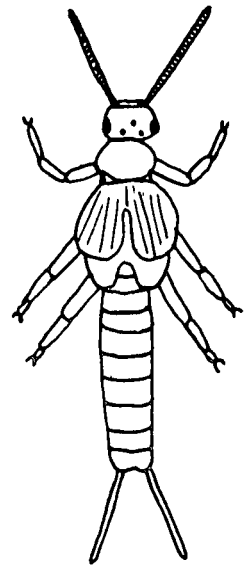
24C. Odonata, tail of
dragonfly nymph
(top view)



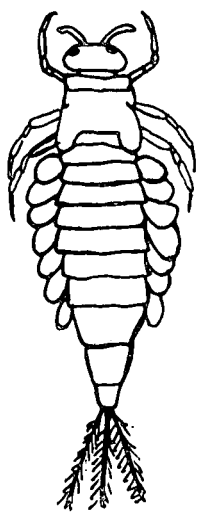
25A. Hemiptera,
Water Boatman
About 1 cm.



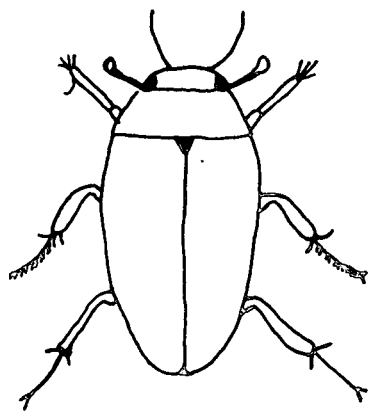
25B. Hemiptera,
Water Scorpion
About 4 cm.



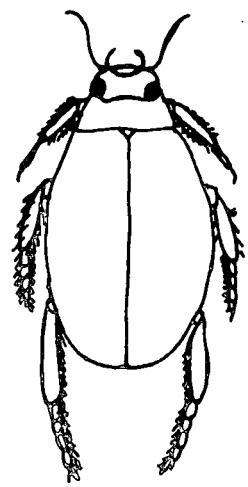
26. Plecoptera,
Stonefly nymph
Up to $\frac{1}{2}$ cm.



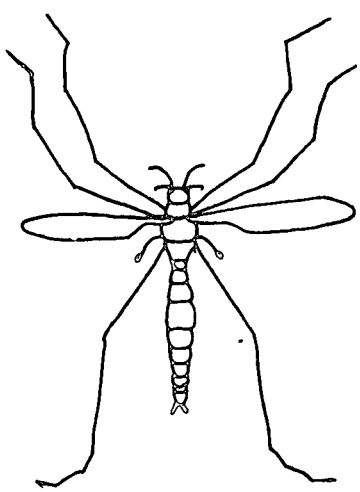
27. Ephemeroptera,
Mayfly nymph
Up to $\frac{1}{2}$ cm.



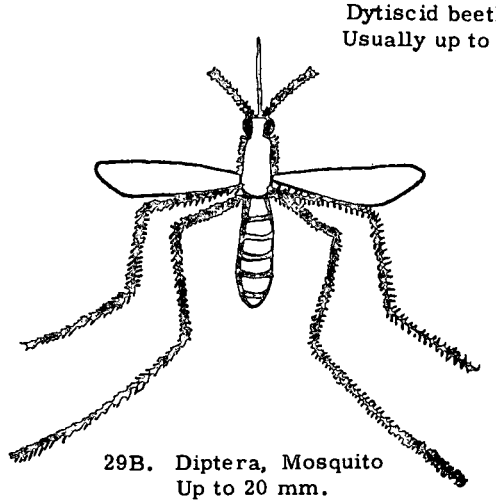
28A. Coleoptera,
Water scavenger
beetle. Up to 4 cm.



28B. Coleoptera,
Dytiscid beetle
Usually up to $2\frac{1}{2}$ cm.



29A. Diptera, Crane
fly. Up to $2\frac{1}{2}$ cm.



29B. Diptera, Mosquito
Up to 20 mm.

REFERENCES - General

Keeler, C.R. Books on Aquatic Biology. Freshwater and Marine.Facile Press, Tallahassee, Florida. 1965.

REFERENCES - Invertebrates

1 Eddy, S. and Hodson, A.C. Taxonomic Keys to the Common Animals of the North Central States. Burgess Publ. Co., Minneapolis. 1955.

2 Edmondson, W.T., Ed. Ward and Whipple's Freshwater Biology. John Wiley & Sons. New York. 1959.

3 Hedgepeth, J., and Hinton, X. Common Seashore Life of Southern California. Naturegraph Company, Heraldsburg, California. 1961.

4 Jahn, T.L., and Jahn, F.F. How to Know the Protozoa. Wm. C. Brown Co., Dubuque, Iowa. 1949.

5 Kudo, R. Protozology. Charles C. Thomas, Publisher, Springfield, Illinois. 1950.

6 Light, S. F., et al. Intertidal Invertebrates of the Central California Coast. University of California Press, Berkeley. 1961.

7 MacGinitie, G.E., and N. Natural History of Marine Animals. McGraw-Hill. 1949.

8 Miner, R.W. Fieldbook of Seashore Life. G.B. Putnam's Sons, New York. 1960.

9 Pennak, R.W. Freshwater Invertebrates of the United States. The Ronald Press Co., New York. 1953.

10 Pratt, H.W. A Manual of the Common Invertebrate Animals Exclusive of Insects. The Blakiston Company, Philadelphia. 1951.

11 Ricketts, E., and Calvin, J. Between Pacific Tides. Revised by J. Hedgepeth, Stanford Univ. Press, Palo Alto, California. 1952.

12 Smith, R.I. Keys to Marine Invertebrates of the Woods Hole Region, Contribution No. 11, MBL, Woods Hole, Mass. 1964.

REFERENCES - Fishes

1 American Fisheries Society. A List of Common and Scientific Names of Fishes from the United States and Canada. Special Publication No. 2. Am. Fish Soc. Dr. E.A. Seaman, Sec. - Treas. Box 483. McLean, Va. 1960. (Price $1.00 paper, $2.00 cloth)

2 Anon. Check List of the Florida Game and Commercial Marine Fishes with Common Names. Florida State Board of Cons. Educational Bull. No. 12 (Free).

3 Benton, A.H., and Stewart. Keys to the Vertebrates of the Northeastern States. Burgess Pub. Co. 1964.

4 Breder, C.M. Fieldbook of Marine Fishes of the Atlantic Coast. G.B. Putnam's Sons, New York.

5 Clemens, W.A., and Wilby, G.V. Fishes of the Pacific Coast of Canada. Fisheries Research Board of Canada. Bulletin No. 68, 2nd Ed. 1961.

6 Hubbs, C.L., and Lagler, K.F. Fishes of the Great Lakes Region. Bull. Cranbrook Inst. Science. Bloomfield Hills, Michigan. 1949.

7 Perlmutter, A. Guide to Marine Fishes. New York University, NY. 1961.

8 Schrenkeisen, R. Fieldbook of Freshwater Fishes of North America, north of Mexico. Putnam's Sons. 1963.

9 Zim, H. S., and Shoemaker, H. H. Fishes--A guide to Fresh and Salt Water Species. Golden. 1959.

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Descriptors:
Aquatic Life; Systematics

AQUATIC ORGANISMS OF SIGNIFICANCE IN POLLUTION SURVEYS

I INTRODUCTION

A Any organism encountered in a survey is of significance. Our problem is thus not to determine which are of significance but rather to decide "what is the significance of each?"

B The first step in interpretation is recognition. "The first exercise in ecology is systematics."

C Recognition implies identification and an understanding of general relationships (systematics). The following outline will thus review the general relationships of living (as contrasted to fossil) organisms and briefly describe the various types.

D The species problem

1 Necessity of identifying species

Studies of the ecology of any habitat require the identification of the organisms found in it. One cannot come up with definitive evaluations of stress on the biota of a system unless we can say what species constitute the biota. Species vary in their responses to the impact of the environment.

2 Solutions to the problem

a Evasion

Treat the ecosystem as a "black box"--a unit--while ignoring the constitution of the system. This may produce some broad generalizations and will certainly yield more questions than answers.

b Compromise

Work only with those taxonomic categories with which one has the competence to deal. Describe the biotic component as a taxocenosis limited to one or two numerically dominant taxonomic categories, bearing in mind that numerically taxa which are ignored may be very important to the ecology of the ecosystem.

c Comprehensive description

Attempt a comprehensive description of the biota. No one can claim competence to deal with more than one or two groups. The cooperation of experts must be obtained. The Smithsonian Institution has a clearinghouse for this sort of thing.[1] Lists of expert taxonomists can be obtained. There will be none for some groups. Also collaboration is time consuming.

3 Fungi: extracellular digestion (enzymes secreted externally.) Food material then taken in through cell membrane where it is metabolized and reduced to the mineral condition. Ecologically known as REDUCERS.

E Each of these groups includes simple, single-celled representatives, persisting at lower levels on the evolutionary stems of the higher organisms.

1 These groups span the gaps between the higher kingdoms with a multitude of transitional forms. They are collectively called the PROTISTA.

2 Within the protista, two principal sub-groups can be defined on the basis of relative complexity of structure.

a The bacteria and blue-green algae, lacking a nuclear membrane may be considered as the lower protista (or Monera).

b The single-celled algae and protozoa are best referred to as the higher protista.

F Distributed throughout these groups will be found most of the traditional "phyla" of classic biology.

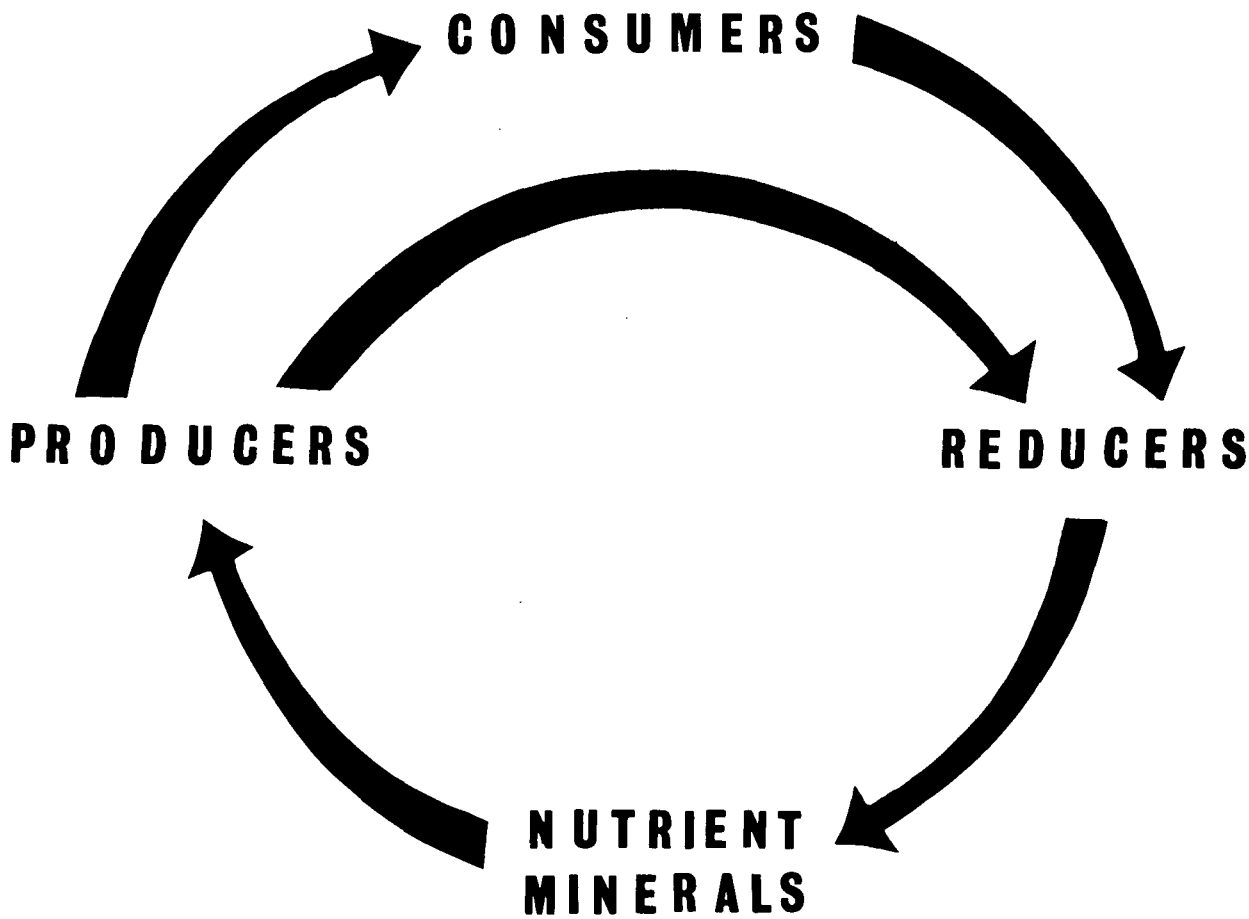


Figure 1. BASIC CYCLES OF LIFE

II PLANTS

A The vascular plants are usually larger and possess roots, stems, and leaves.

1 Some types emerge above the surface (emersed).

2 Submersed typed typically do not extend to the surface.

3 Floating types may be rooted or free-floating.

B Algae generally smaller, more delicate, less complex in structure, possess chlorophyll like other green plants. For convenience the following artificial grouping is used in sanitary science:

1 "Blue-green algae" are typically small and lack an organized nucleus, pigments are dissolved in cell sap. Structure very simple.

2 "Pigmented flagellates" possess nuclei, chloroplasts, flagellae and a red eye spot. This is an artificial group containing several remotely related organisms, may be green, red, brown, etc.

3 "Diatoms" have "pillbox" structure of SiO_2 - may move. Extremely common. Many minute in size, but colonial forms may produce hair-like filaments. Golden brown in color.

4 "Non-motile green algae" have no locomotor structure or ability in mature condition. Another artificial group.

a Unicellular representatives may be extremely small.

b Multicellular forms may produce great floating mats of material.

III FUNGI

Lack chlorophyll and consequently most are dependent on other organisms. They secrete extracellular enzymes and reduce complex organic material to simple compounds which they can absorb directly through the cell wall.

A Schizomycetes or bacteria are typically very small and do not have an organized nucleus.

1 Autotrophic bacteria utilize basic food materials from inorganic substrates. They may be photo-synthetic or chemosynthetic.

2 Heterotrophic bacteria are most common. The require organic material on which to feed.

B "True fungi" usually exhibit hyphae as the basis of structure.

IV ANIMALS

A Lack chlorophyll and consequently feed on or consume other organisms. Typically ingest and digest their food.

B The Animal Phyla

1 PROTOZOA are single celled organisms; many resembling algae but lacking chlorophyll (cf: illustration in "Oxygen" lecture).

2 PORIFERA are the sponges; both marine and freshwater representatives.

3 CNIDARIA (= COELENTERATA) include corals, marine and freshwater jelly fishes, marine and freshwater hydroids.

4 PLATYHELMINTHES are the flat worms such as tape worms, flukes and Planeria.

5 NEMATHELMINTHES are the round worms and include both free-living forms and many dangerous parasites.

6 ROTIFERS are multicellular microscopic predators.

7 BRYOZOA are small colonial sessile forms, marine or freshwater.

8 MOLLUSCA include snails and slugs, clams, mussels and oysters, squids, and octopi.

9 BRACHIOPODS are bivalved marine organisms usually observed as fossils.

10 ANNELIDS are the segmented worms such as earthworms, sludge worms and many marine species.

11 ECHINODERMS include starfish, sea urchins and brittle stars. They are exclusively marine.

12 CTENOPHORES, or comb jellies, are delicate jelly-like marine organisms.

13 ARTHROPODA, the largest of all animal phyla. They have jointed appendages and a chitinous exoskelton.

a CRUSTACEA are divided into a cephalothoraz and abdomen, and have many pairs of appendages, including paired antennae.

1) CLADOCERA include Daphina a common freshwater microcrustacean; swim by means of branched antennae.

2) ANOSTRACA (=PHYLLOPODS) are the fairy shrimps, given to eruptive appearances in temporary pools.

3) COPEPODES are marine and freshwater microcrustacea--swim by means of unbranced antennae.

4) OSTRACODS are like microscopic "clams with legs."

5) ISOPODS are dorsoventrally compressed; called sowbugs. Terrestrial and aquatic, marine and freshwater.

6) AMPHIPODA - known as scuds, laterally compressed. Marine and freshwater.

7) DECAPODA - crabs, shrimp, crayfish, lobsters, etc. Marine and freshwater.

b INSECTA - body divided into head, thorax and abdomen; 3 paris of legs; adults typically with 2 pairs of wings and one pair of antennae. No common marine species. Nine of the twenty-odd orders include species with freshwater-inhabiting stages in their life history as follows:

1) DIPTERA - two-winged flies

2) COLEOPTERA - beetles

3) EPHEMEROPTERA - may flies

4) TRICHOPTERA - caddis flies

5) PLECOPTERA - stone flies

6) ODONATA - dragon flies and damsel flies

7) NEUROPTERA - alder flies, Dobson flies and fish flies.

8) HEMIPTERA - true bugs, sucking insects such as water striders, electric light bugs and water boatman

9) LEPIDOPTERA - butterflies and moths, includes a few freshwater moths

c ARACHINIDA - body divided into cephalothorax and abdomen; 4 pairs of legs - spiders, scorpions, ticks and mites. Few aquatic representatives except for the freshwater mites and tardigrades.

C CHORDATA

1 PROTOCHORDATES - primitive marine forms such as acorn worms, sea squirts and lancelets

2 VERTEBRATES - all animals which have a backbone

a PICES or fishes: including such forms as sharks and rays, lampreys, and higher fishes; both marine and freshwater

b AMPHIBIA - frogs, toads, and salamanders - marine species rare.

c REPTILA - snakes, lizards and turtles.

d MAMMALS - whales and other warm-blooded vertebrates with hair.

e AVES - birds - warm-blooded vertebrates with feathers.

REFERENCE

Whittaker, R. H. New Concepts of Kingdoms of Organisms. Science 163:150-160. 1969.

This outline was prepared by H. W. Jackson, formerly Chief Biologist, National Training Center, Water Program Operations, EPA, Cincinnati, OH 45268, and revised by Ralph M. Sinclair, Aquatic Biologist, National Training Center.

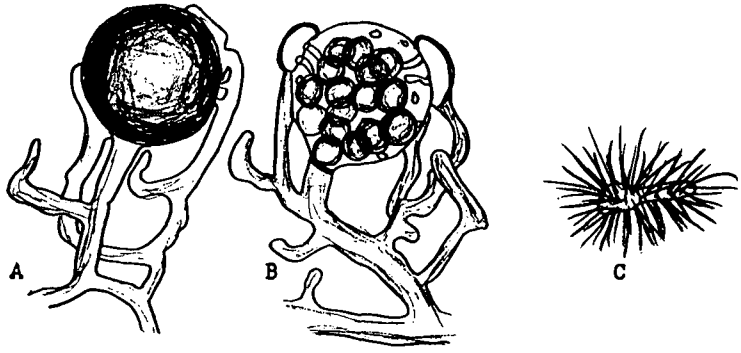
Descriptors:
Aquatic Life; Systematics, Streams, Surveys, Stream Pollution

FUNGI

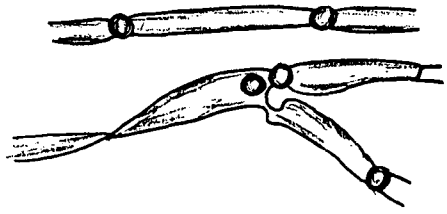
3/4

Schizomycetes - Bacteria, free living representatives

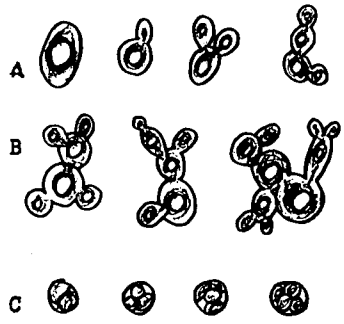
Aerobacter aerogenes | Rhizobium radicicola | Azotobacter



Phycomycetes - Saprolegnia; A, detail of immature reproductive stages; B, mature oogonium and antheridia, with eggs and fertilization tubes; C, dead tadpole with growth of S.



Phycomycete - Leptomitus, this genus includes pollution tollerant species.



Ascomycete - Saccharomyces, a yeast including poll. tollerant species. A, single cell; B, budding; C, ascospore formation.

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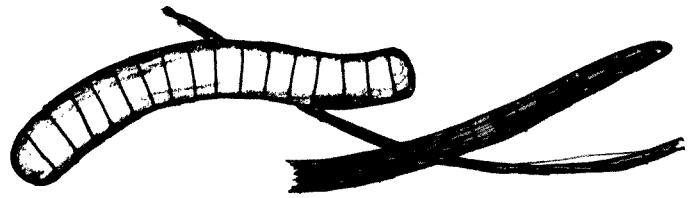
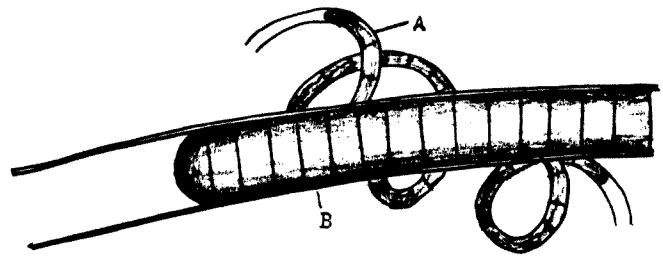
PLATE I

BLUE GREEN ALGAE

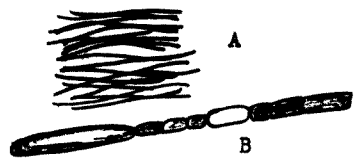
3/4



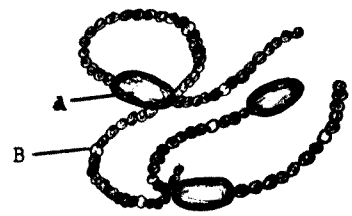
Oscillatoria spp., filaments (trichomes) range from .6 to over 60 μ in diameter. Ubiquitous, pollution tollerant.



Lyngbia spp., similar to Oscillatoria but has a sheath.
A, Lyngbia contorta, reported to be generally intollerant of pollution; B, L. birgei.



Aphanizomenon flos-aquae
A, colony; B, filament



Anabaena flos-aquae
A, akinete; B, heterocyst

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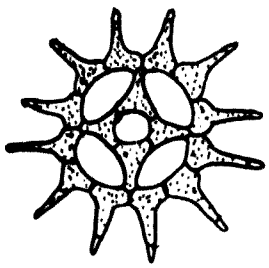
PLATE II

1/67

NON-MOTILE GREEN ALGAE: COCCOID
(CHLOROPHYCEAE)



Pediastrum

Species of the Genus Scenedesmus

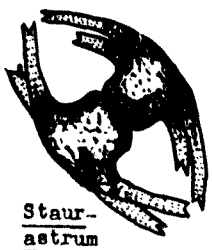
S. caudatus

S. abundans

S. dimorphus

Desmids

Closterium



Staur-
astrum

Cosmarium

PLATE III

11/3 NON-MOTILE GREEN ALGAE: FILAMENTOUS

(Chlorophyceae)

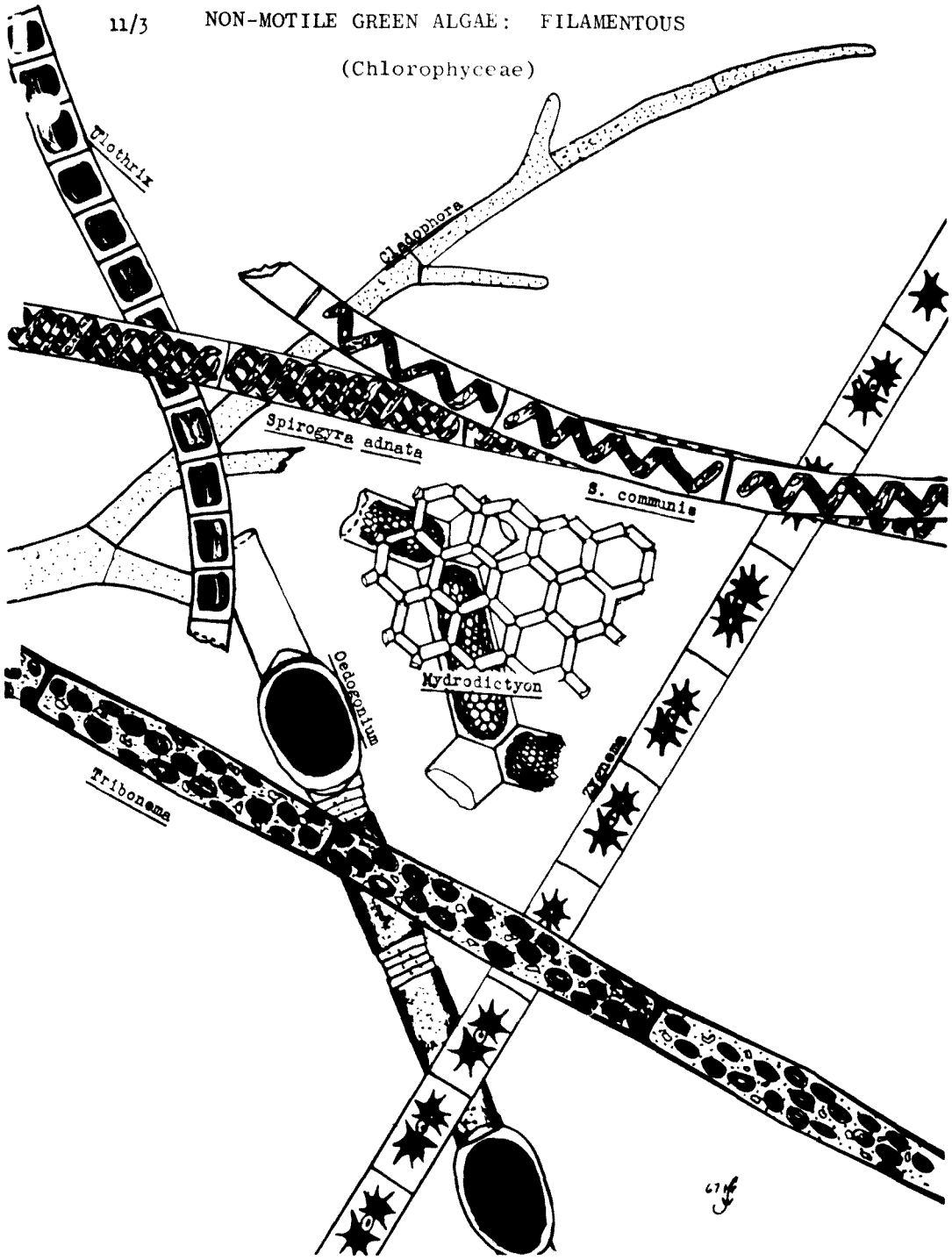
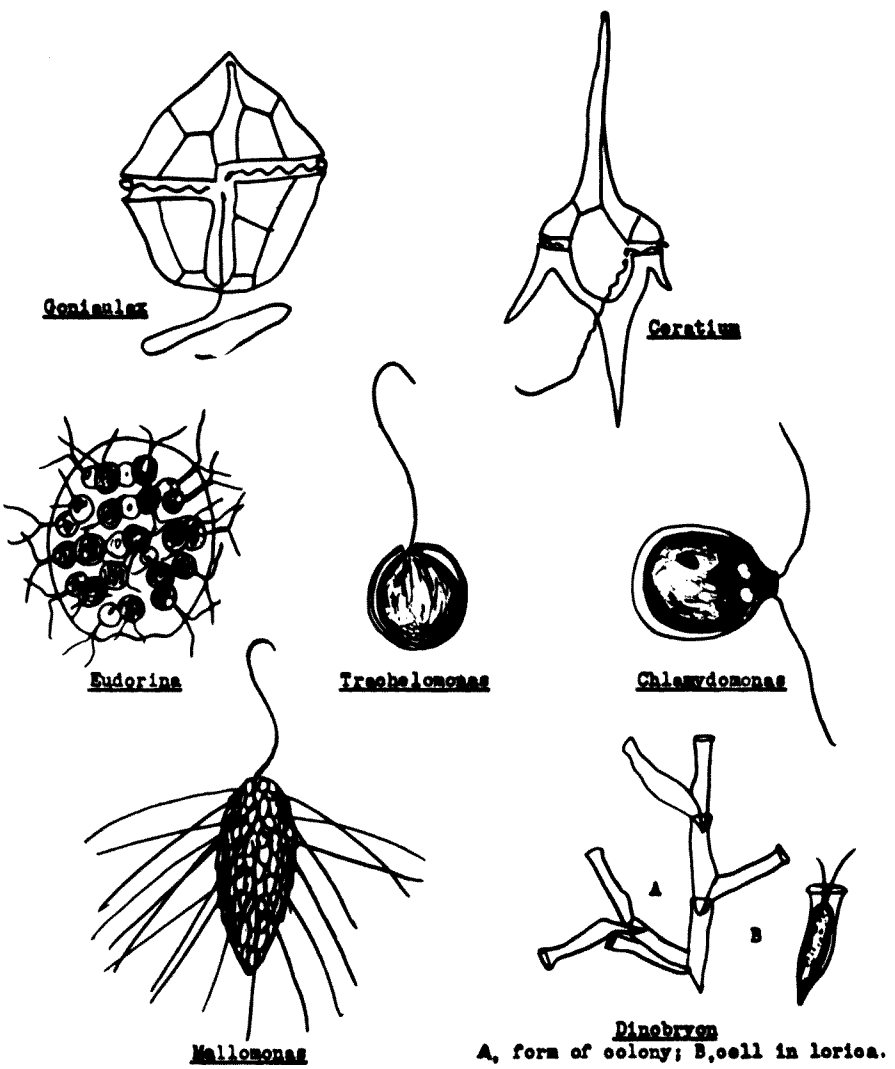


PLATE IV

PIGMENTED FLAGELLATES

3/4



A, form of colony; B, cell in lorica.

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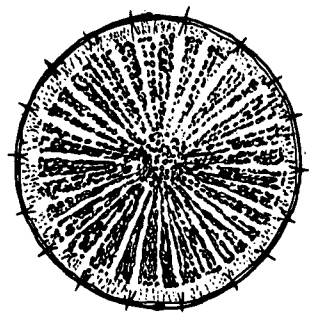
PLATE V

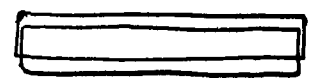
DIATOMS

3/4



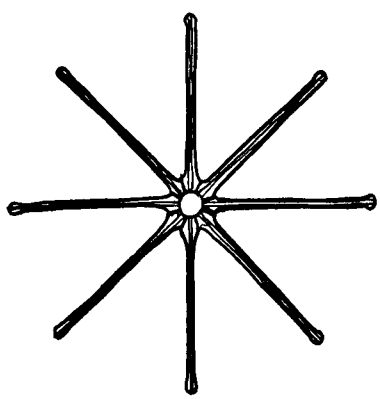
Valve views

Girdle views, stylized to show basic diatom structure.

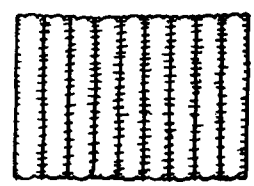


A discoid or central diatom such as Stephanodiscus

A pennate or naviculer diatom such as Fragillaria



A colony of Asterionella (girdle views)



A colony of Fragillaria (girdle views)

Gomphonema
A, valve view; B, girdle view.

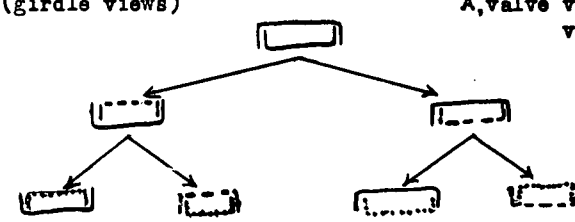


Diagram showing progressive diminution in the size of certain frustules through successive cell generations of a diatom.

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PLATE VI

3/4

FREE LIVING PROTOZOA

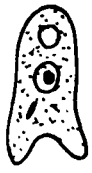
I. Flagellated Protozoa, Class Mastigophora

Anthophysis
Pollution tolerant
6 μ

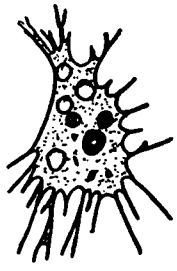
Bodo
Pollution tolerant
19 μ

Colony of Poteriodendron
Pollution tolerant , 35 μ

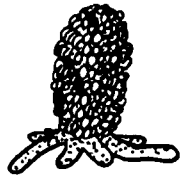
II. Ameboid Protozoa, Class Sarcodina



Dimastigamoeba
Pollution tolerant
10-50 μ



Nuclearia, reported to be intolerant of **pollution, 45 μ.**



Difflugia
Pollution tolerant
60-500 μ

III. Ciliated Protozoa, Class Ciliophora

Colpoda
Pollution tolerant
20-120 μ

Holophrya, reported to be intolerant **of pollution, 35 μ**

Epistylis, pollution tolerant **Colonies often macroscopic.**

PLATE VII

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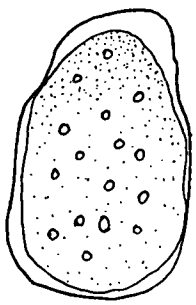
PLANKTONIC PROTOZOA

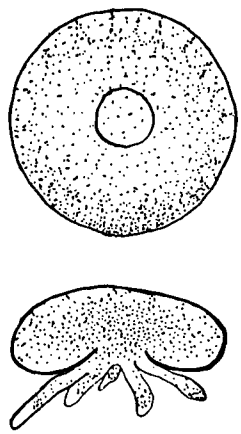
Peranema trichophorum



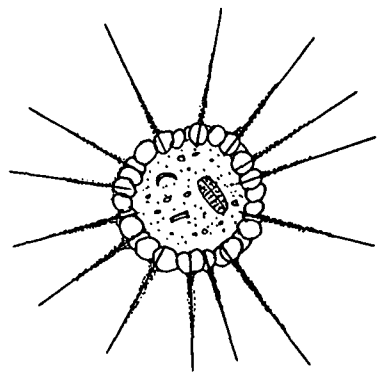
Chaos

Top

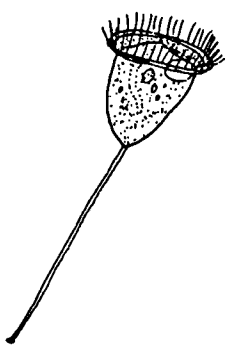
Side



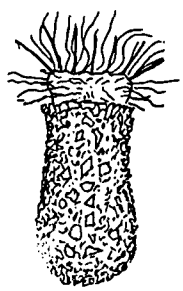
Arcella vulgaris



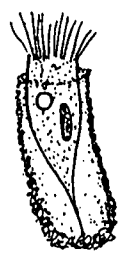
Actinosphaerium



Vorticella



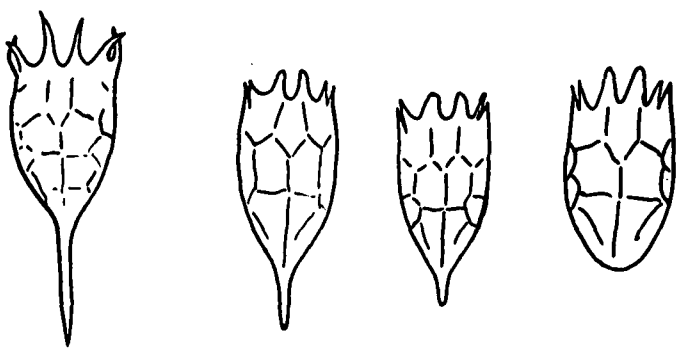
Codonella cratera



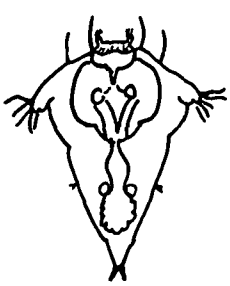
Tintinnidium fluviatle

PLATE VIII

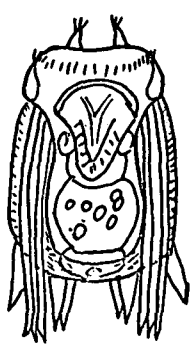
PLANKTONIC ROTIFERS



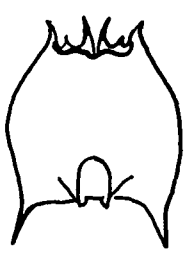
Various Forms of Keratella cochlearis



Synchaeta pectinata



Polygarthra vulgaris



Brachionus quadridentata

Rotaria sp

PLATE IX

FREE LIVING NEMATHELMINTHES, OR ROUND WORMS

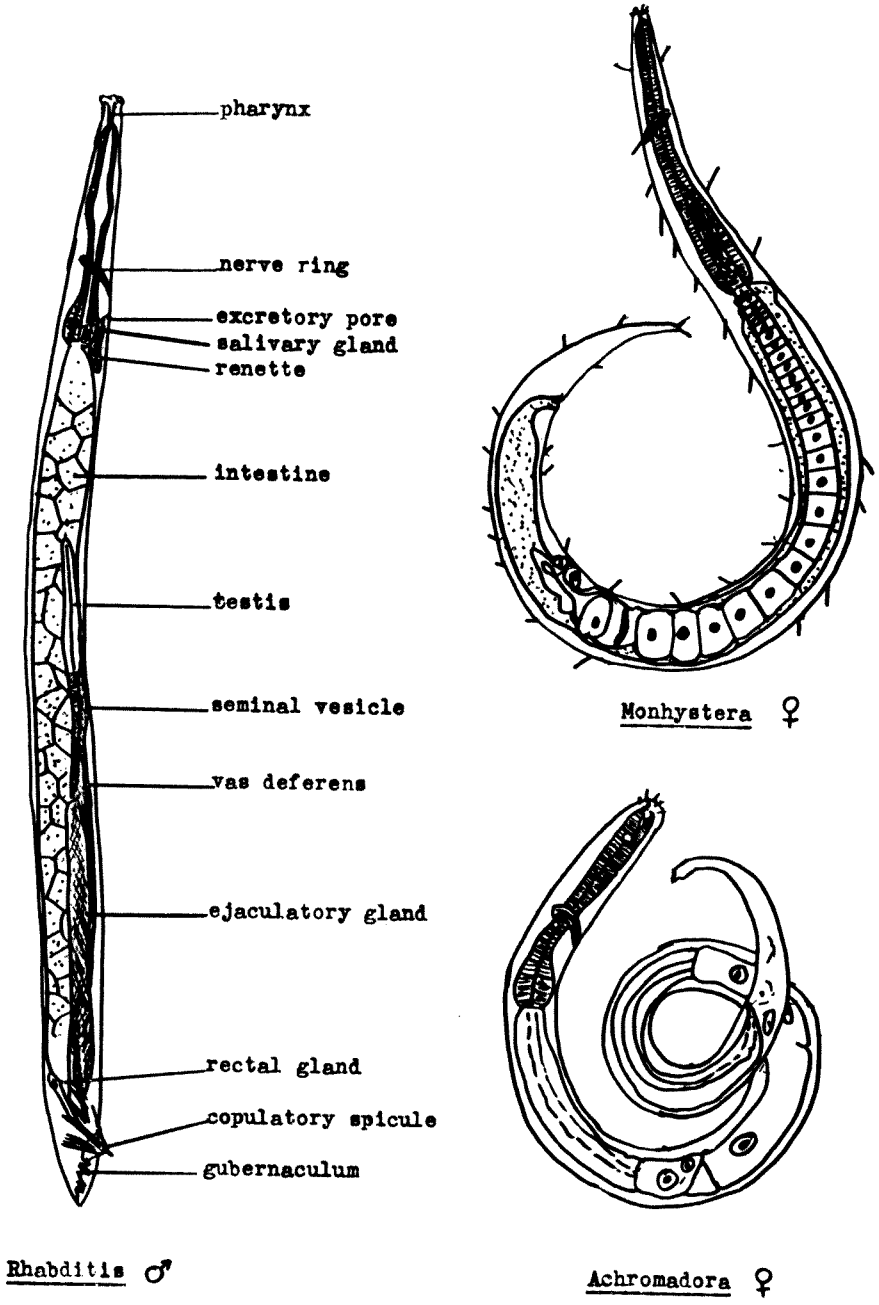
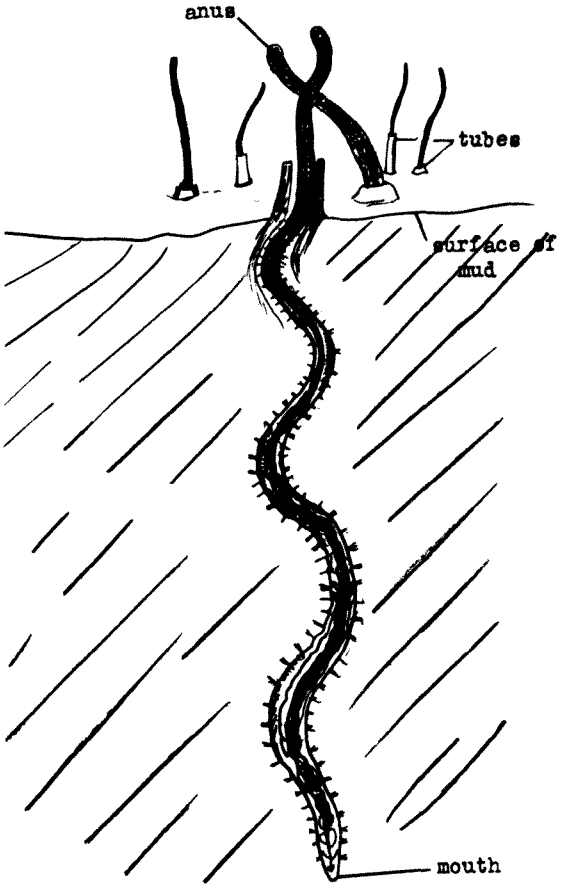


PLATE X

FRESH WATER ANNELID WORMS

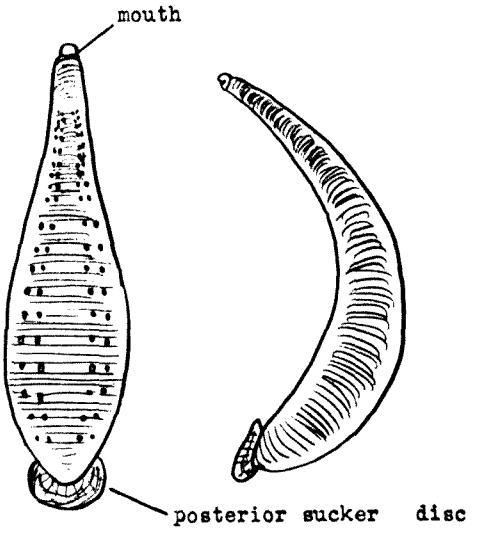
Phylum Annelida



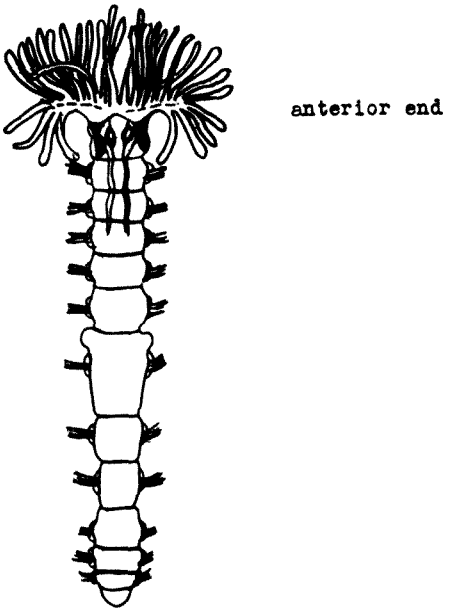
Class Oligochaeta, earthworms

Ex: Tubifex , the sludgeworm

(After Liebman)



Class Hirudinea, leeches
(After Hegner)



Class Polychaeta , polychaet worms

Ex: Manayunkia, a minute, rare, tube building worm.

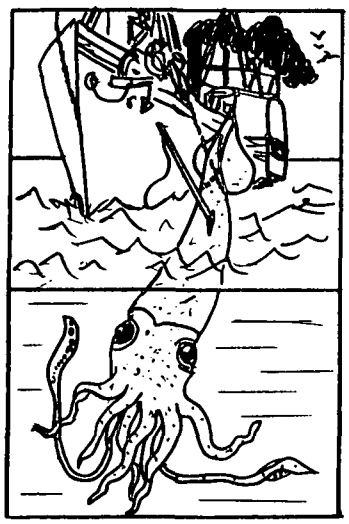
(After Leidy)

H.W.Jackson

PLATE XI

SOME MOLLUSCAN TYPES

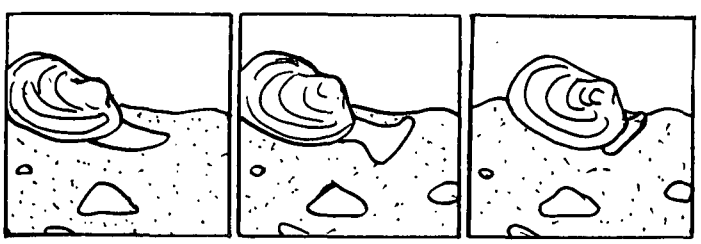
Class: Cephalopoda: Squids, octopus, cuttlefish.



Exclusively marine. The giant squid shown was captured in the Atlantic in the early ninteenth century. (After Hegner)

Limax, a slug — Lymnaea, an air breathing snail — Campeloma, a water breathing snail

Class: Gastropoda; snails and slugs. (After Buchsbaum)



Class: Pelecypoda; clams, mussels, oysters.

Locomotion of a freshwater clam, showing how foot is extended, the tip expanded, and the animal pulled along to its own anchor. (After Buchsbaum)

H.W.Jackson

PLATE XII

Class CRUSTACEA

3/4

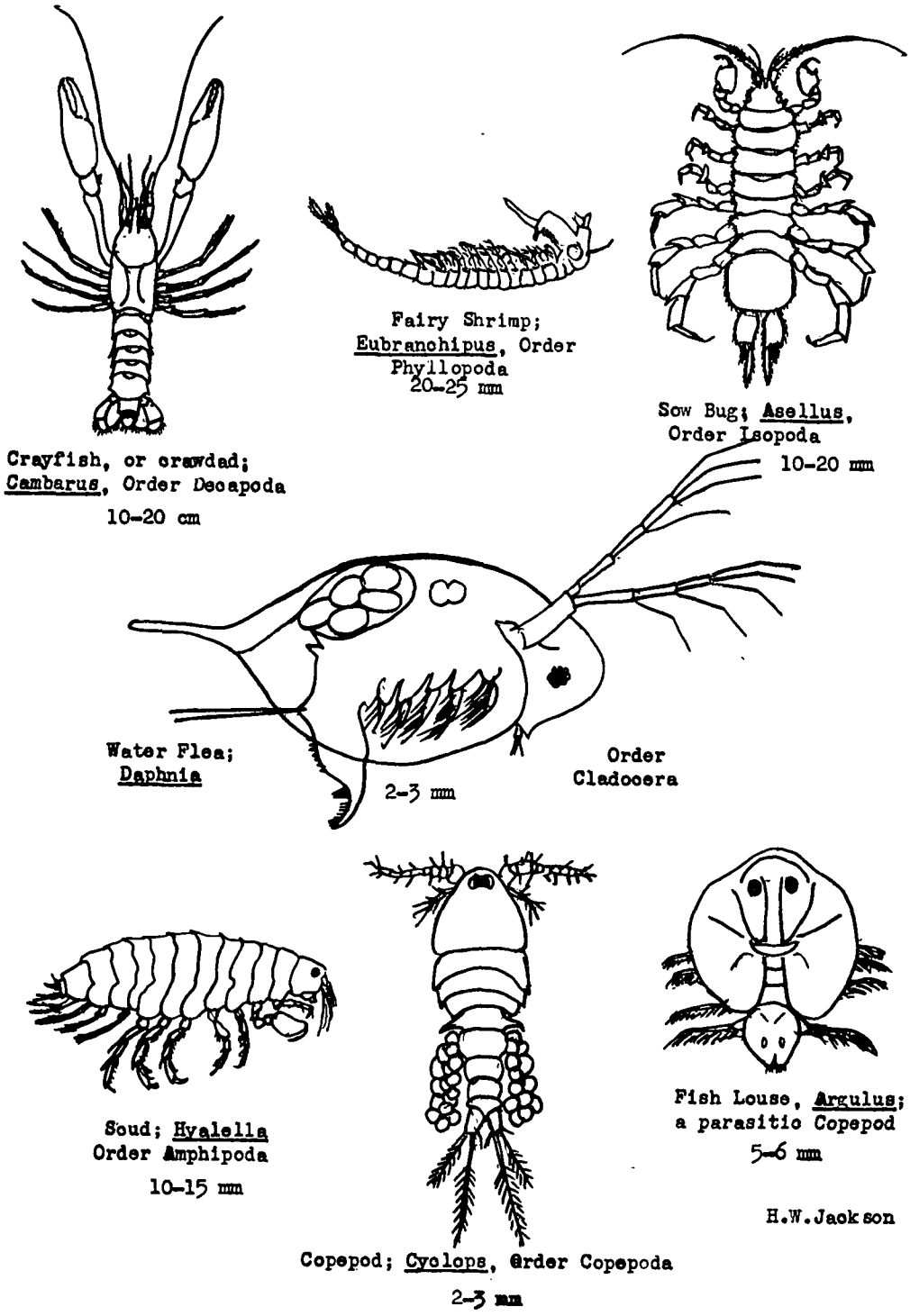
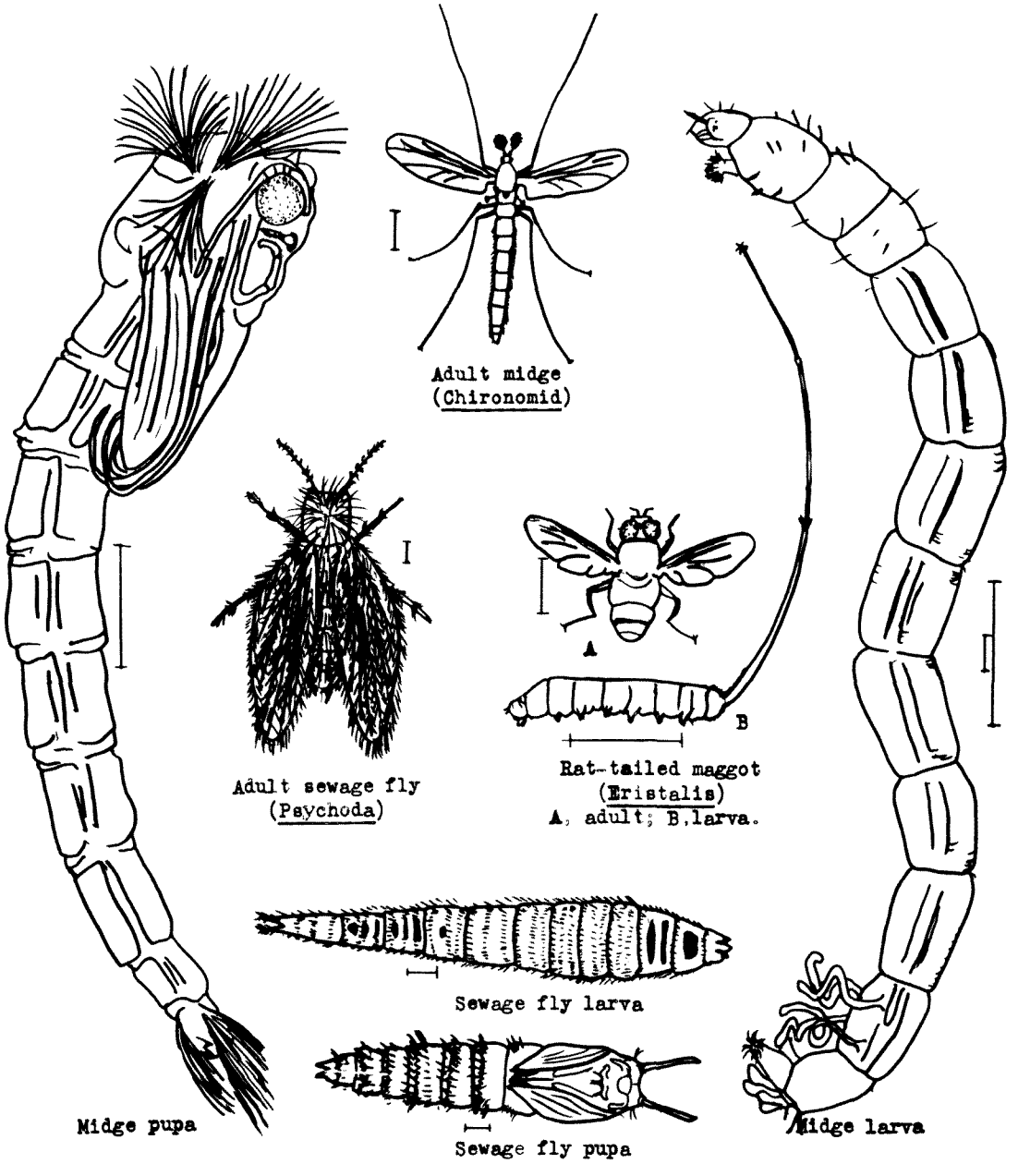


PLATE XIII

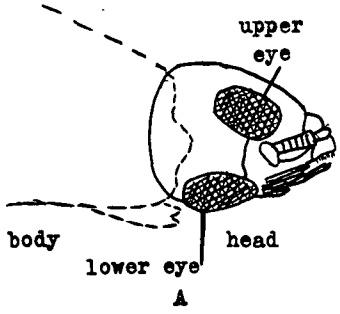
Two Winged Flies
Order DIPTERA

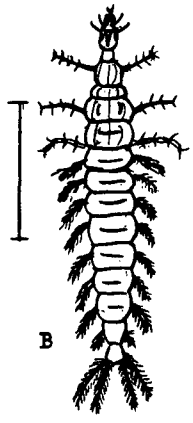


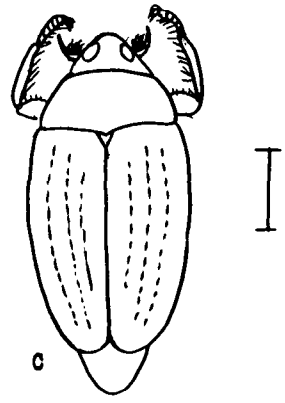
H.W.Jackson
After various authors

PLATE XIV

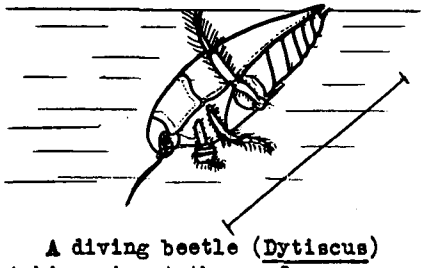
Beetles
Order COLEOPTERA



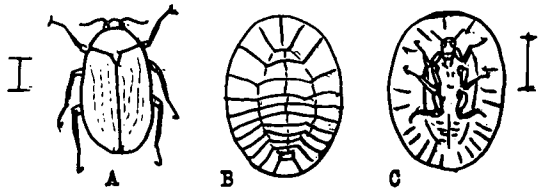




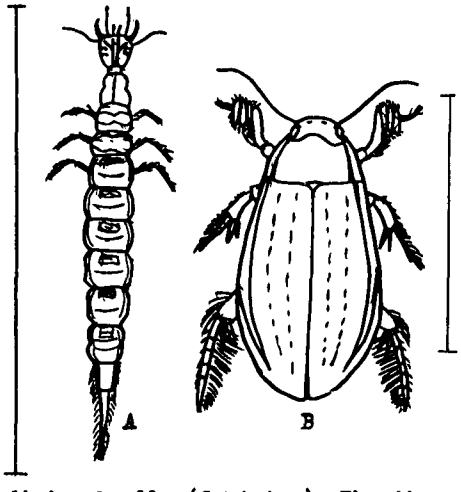
Whirligig beetle (Gyrinus) A, Side view of head of adult showing divided eye; B, Larva; C, Adult. Carnivorous.



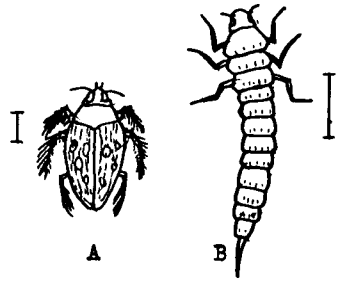
A diving beetle (Dytiscus) taking air at the surface.



The riffle beetle (Psephenus); A, adult; B, dorsal side of larva; C, ventral side of larva. Predominantly herbivorous.



A diving beetle (Cybister). The diving beetles include some of the largest and most voracious of aquatic insects. A, larva; B, adult.



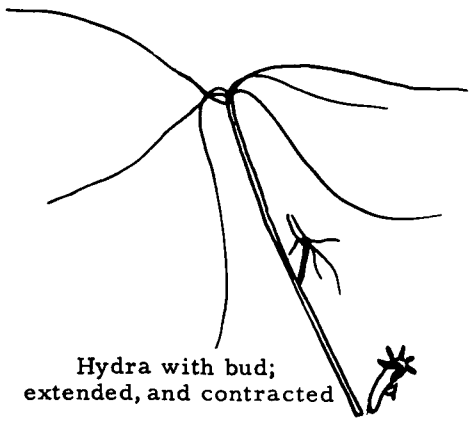
Crawling water beetle; A; adult; B, larva. Predominantly herbivorous.

H.W.Jackson. After Needham, Pennak, Morgan, and others.

PLATE XV

MINOR PHYLA

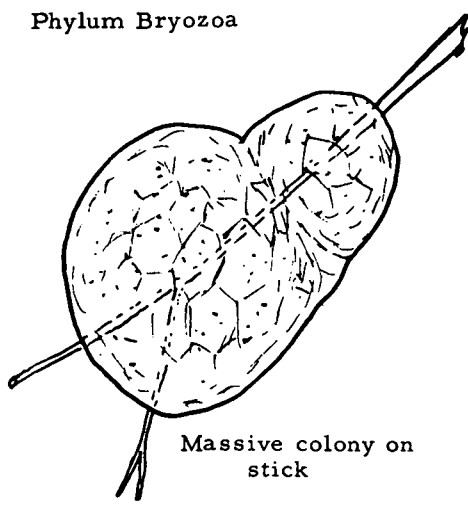
Phylum Coelenterata



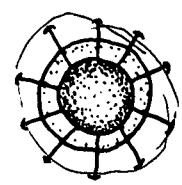
Hydra with bud;
extended, and contracted

Medusa of
Craspedacusta

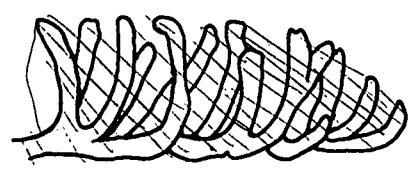
Phylum Bryozoa



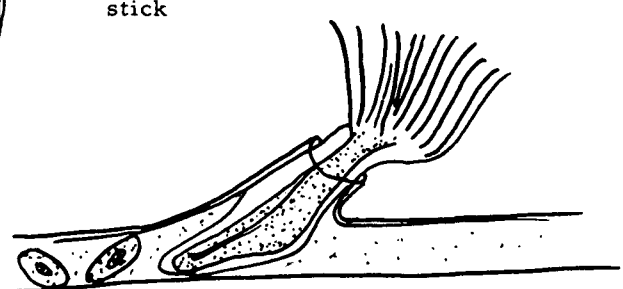
Massive colony on
stick



Statoblast



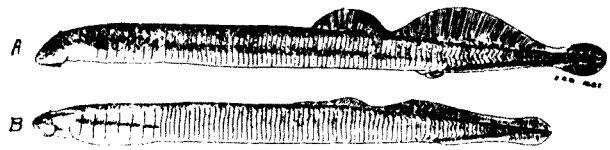
Creeping colony
on rock



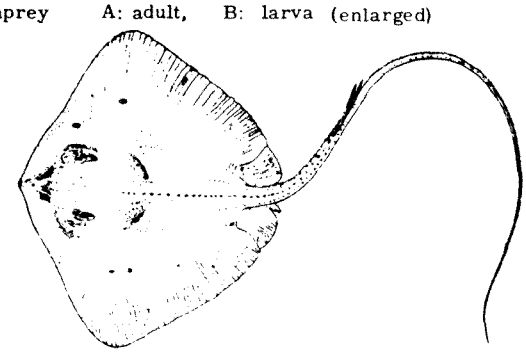
Single zooid, young statoblasts in tube

PLATE XVI

SOME PRIMITIVE FISHES

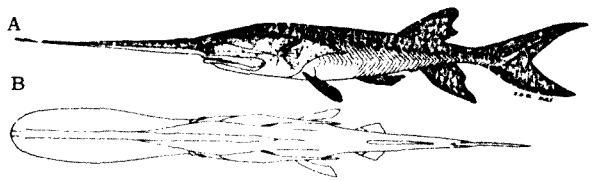


Class Agnatha, jawless fishes (lampreys and hagfishes) - Family PETROMYZONTIDAE, the lampreys. Lampetra aepyptera, the Brook Lamprey A: adult, B: larva (enlarged)

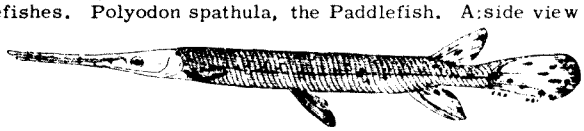


Class Chondrichthyes - cartilagenous fishes (sharks, skates, rays) Family DASYATIDAE - stingrays. Dasyatis centroura, the Roughtail Stingray

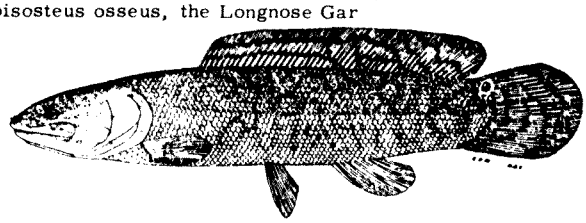
Class Osteichthyes - bony fishes - Family ACIPENSERIDAE, sturgeon. Acipenser fulvescens, the Lake Sturgeon



Class Osteichthyes - bony fishes - Family POLYODONTIDAE, the paddlefishes. Polyodon spathula, the Paddlefish. A:side view B:top view



Class Osteichthyes - bony fishes - Family LEPISOSTEIDAE - gars Lepisosteus osseus, the Longnose Gar



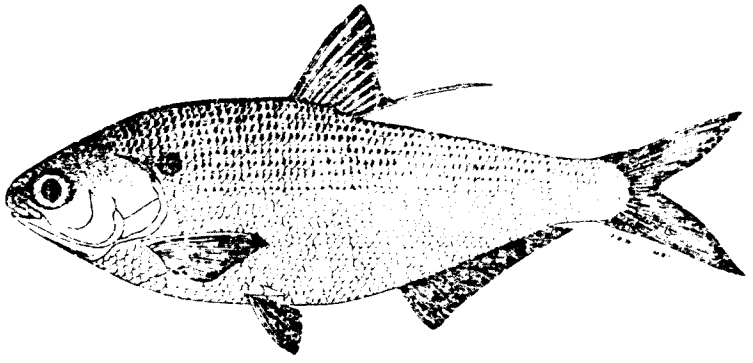
Class Osteichthyes - bony fishes - Family AMIIDAE, bowfins Aniia calva, the Bowfin

Reproduced with permission; Trautman, 1957.

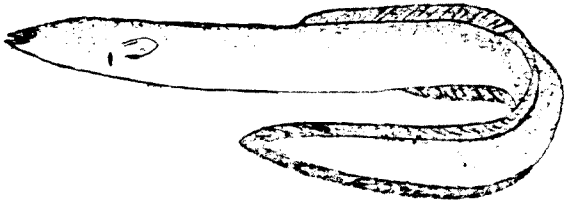
Bl. AQ. pl. 91. 6. 60

PLATE XVII

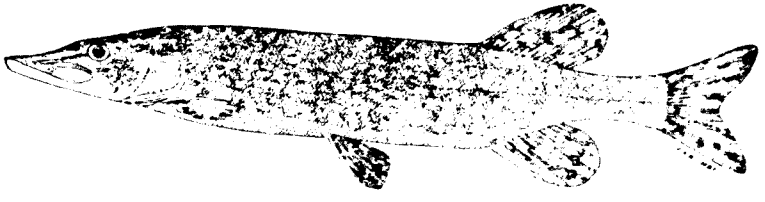
TYPES OF BONY FISHES



Family CLUPEIDAE - herrings
Dorosoma cepedianum - the eastern gizzard shad



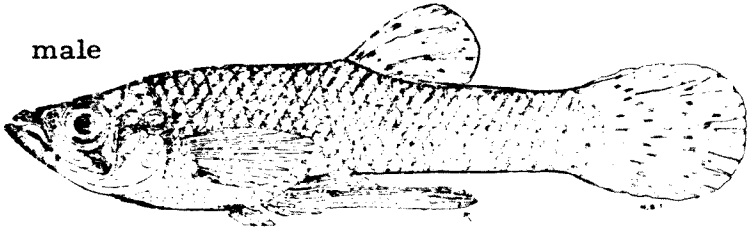
Family ANGUILLEDAE - freshwater eels
Anguilla rostrata - the American eel



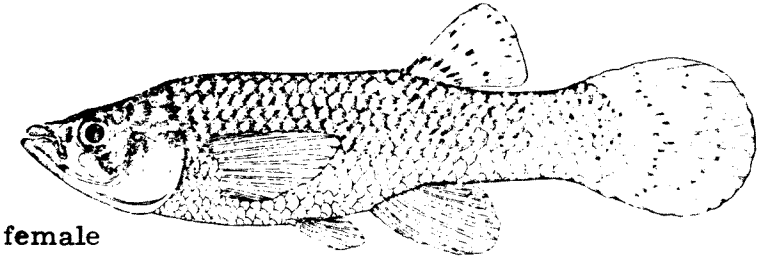
Family ESOCIDAE - pikes
Esox lucius - the northern pike

Reproduced with permission; Trautman, 1957.

BI. AQ. pl. 9m. 6. 60

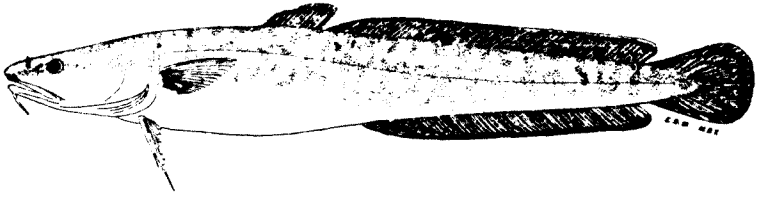


male

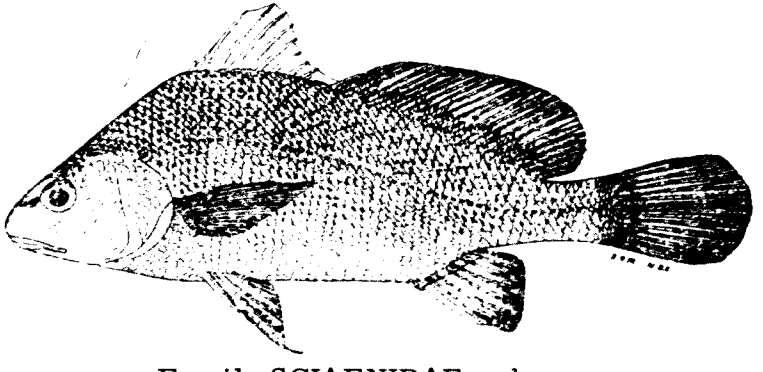


female

Family POECILIIDAE - livebearers
Gambusia affinis - the mosquitofish



Family GADDIDAE - codfishes, hakes, haddock, burbot
Lota lota - the eastern burbot



Family SCIAENIDAE - drums
Aplodinotus grunniens - the freshwater drum

KEY TO SELECTED GROUPS OF FRESHWATER ANIMALS

The following key is intended to provide an introduction to some of the more common freshwater animals. Technical language is kept to a minimum.

In using this key, start with the first couplet (1a, 1b), and select the alternative that seems most reasonable. If you selected "1a" you have identified the animal as a member of the group, Phylum PROTOZOA. If you selected "1b", proceed to the couplet indicated. Continue this process until the selected statement is terminated with the name of a group.

If you wish more information about the group, consult references. (See reference list.)

1a The body of the organism comprising a single microscopic independent cell, or many similar and independently functioning cells associated in a colony with little or no difference between the cells, i.e., without forming tissues; or body comprised of masses of multinucleate protoplasm. Mostly microscopic, single celled animals.

Phylum PROTOZOA

1b The body of the organism comprised of many cells of different kinds, i.e., forming tissues. May be microscopic or macroscopic. 2

2a Body or colony usually forming irregular masses or layers sometimes cylindrical, goblet shaped, vase shaped, or tree like. Size range from barely visible to large. 3

2b Body or colony shows some type of definite symmetry. 4

3a Colony surface rough or bristly in appearance under microscope or hand lens. Grey, green, or brown. Sponges.
Phylum PORIFERA (Fig. 1)

3b Colony surface relatively smooth. General texture of mass gelatinous, transparent. Clumps of minute individual organisms variously distributed. Moss animals, bryozoans.
Phylum BRYOZOA (Fig. 2)

4a Microscopic. Action of two ciliated (fringed) lobes at anterior (front) end in life often gives appearance of wheels. Body often segmented, accordian-like. Free swimming or attached. Rotifers or wheel animalcules.
Phylum TROCHELMINTHES (Rotifera) (Fig. 3)

4b Larger, wormlike, or having strong skeleton or shell. 5

5a Skeleton or shell present. Skeleton may be external or internal. 15

5b Body soft and/or wormlike. Skin may range from soft to parchment-like. 6

6a Three or more pairs of well formed jointed legs present. 19
Phylum ARTHROPODA (Fig. 4)

6b Legs or appendages, if present, limited to pairs of bumps or hooks. Lobes or tenacles, if present, soft and fleshy, not jointed. 7

7a Body strongly depressed or flattened in cross section. 8

7b Body oval, round, or shaped like an inverted "U" in cross section. 10

8a Parasitic inside bodies of higher animals. Extremely long and flat, divided into sections like a Roman girdle. Life history may involve an intermediate host. Tape worms.
Class CESTODA (Fig. 5)

8b Body a single unit. Mouth and digestive system present, but no anus. 9

9a External or internal parasite of higher animals. Sucking discs present for attachment. Life history may involve two or more intermediate hosts or stages. Flukes.
Class TREMATODA

9b Free living. Entire body covered with locomotive cilia. Eye areas in head often appear "crossed". Free living flatworms.
Class TURBELLARIA (Fig. 6)

10a Long, slender, with snake-like motion in life. Covered with glistening cuticle. Parasitic or free-living. Microscopic to six feet in length. Round worms.
Phylum NEMATHELMINTHES (Fig. 7)

10b Divided into sections or segments 11

10c Unsegmented, head blunt, one or two retractile tentacles. Flat pointed, tail. 18

11a Head a more or less well-formed, hard, capsule with jaws, eyes, and antennae.
Class INSECTA order DIPTERA (Figs. 8A, 8C)

11b Head structure soft, except jaws (if present). Fig. 8E.) 12

12a Head conical or rounded, lateral appendages not conspicuous or numerous. 13

12b Head somewhat broad and blunt. Retractile jaws usually present. Soft fleshy lobes or tentacles, often somewhat flattened, may be present in the head region. Tail usually narrow. Lateral lobes or fleshy appendages on each segment unless there is a large sucker disc at rear end. 14
Phylum ANNELIDA (Fig. 9)

13a Minute dark colored retractile jaws present, body tapering somewhat at both ends, pairs or rings of bumps or "legs" often present, even near tail.
Class INSECTA Order DIPTERA (Fig. 8)

13b No jaws, sides of body generally parallel except at ends. Thickened area or ring usually present if not all the way back on body. Clumps of minute bristles on most segments. Earthworms, sludge-worms. 14
Order OLIGOCHAETA

14a Segments with bristles and/or fleshy lobes or other extensions. Tube builders, borers, or burrowers. Often reddish or greenish in color. Brackish or fresh water. Nereid worms.
Order POLYCHAETA (Fig. 9A)

14b Sucker disc at each end, the large one posterior. External blood-sucking parasites on higher animals, often found unattached to host. Leaches.
Class HIRUDINEA (Fig. 9B)

15a Skeleton internal, of true bone. (Vertebrates) 40

15b Body covered with an external skeleton or shell. 16
(Figs. 10, 13, 17, 18, 24, 25, 28)

16a External skeleton jointed, shell covers legs and other appendages, often leathery in nature. 19
Phylum ARTHROPODA

16b External shell entire, not jointed, unless composed of two clam-like halves. 17
(Figs. 10, 11, 12)

17a Half inch or less in length. Two leathery, clam-like shells. Soft parts inside include delicate, jointed appendages. Phyllopods or branchiopods.
Class CRUSTACEA, Subclasses BRANCHIOPODA (Fig. 12) and OSTRACODA (Fig. 11)

17b Soft parts covered with thin skin, mucous produced, no jointed legs. 18
Phylum MOLLUSCA

18a Shell single, may be a spiral cone. Snails.
Class GASTROPODA (Fig. 13)

18b Shell double, two halves, hinged at one point. Mussels, clams.
Class BIVALVIA (Fig. 10)

19a Three pairs of regular walking legs, or their rudiments. Wings present in all adults and rudiments in some larvae. 29
Class INSECTA (Figs. 22, 24D, 25, 26, 28, 29)

19b More than three pairs of legs apparently present. 20

20a Body elongated, head broad and flat

with strong jaws. Appendages following first three pairs of legs are rounded tapering filaments. Up to 3 inches long. Dobson fly and fish fly larvae.
Class INSECTA Order MEGALOPTERA (Fig. 14)

20b Four or more pairs of legs. 21

21a Four pairs of legs. Body rounded, bulbous, head minute. Often brown or red. Water mites.

Phylum ARTHROPODA, Class ARACHNIDA, Order ACARI (Fig. 15)

21b Five or more pairs of walking or swimming legs; gills, two pairs of antennae. Crustaceans. 22
Phylum ARTHROPODA, Class CRUSTACEA

22a Ten or more pairs of flattened, leaflike swimming and respiratory appendages. Many species swim constantly in life; some swim upside down. Fairy shrimps, phyllopods, or branchipods.
Subclass BRANCHIOPODA (Fig. 16)

22b Less than ten pairs of swimming or respiratory appendages. 23

23a Body and legs inclosed in bivalved (2 halves) shell which may or may not completely hide them. 24

23b Body and legs not enclosed in bivalve shell. May be large or minute. 26
(Figs. 17, 18, 19)

24a One pair of branched antennae enlarged for locomotion, extend outside of shell (carapace). Single eye usually visible. "Water fleas"
Subclass CLADOCERA (Fig. 12)

24b Locomotion accomplished by body legs, not by antennae. 25

25a Appendages leaflike, flattened, more than ten pairs.
Subclass BRANCHIOPODA (See 22 a)

25b Animal less than 3 mm, in length. Appendages more or less slender and jointed, often used for walking. Shells opaque. Ostracods.
(Fig. 11) Subclass OSTRACODA

26a Body a series of six or more similar segments, differing mainly in size. 27

26b Front part of body enlarged into a somewhat separate body unit (cephalothorax) often covered with a single piece of shell (carapace). Back part (abdomen) may be relatively small, even folded underneath front part. (Fig. 19b) 28

27a Body compressed laterally, i.e., organism is tall and thin. Scuds. amphipods.
Subclass AMPHIPODA (Fig. 17)

27b Body compressed dorsoventrally, i.e., organism low and broad. Flat gills contained in chamber beneath tail. Sowbugs.
Subclass ISOPODA (Fig. 18)

28a Abdomen extending straight out behind, ending in two small projections. One or two large masses of eggs are often attached to female. Locomotion by means of two enlarged, unbranched antennae, the only large appendages on the body. Copepods.
Subclass COPEPODA (Fig. 19)

28b Abdomen extending out behind ending in an expanded "flipper" or swimming paddle. Crayfish or craw fish. Eyes on movable stalks. Size range usually from one to six inches.
Subclass DECAPODA

29a Two pairs of functional wings, one pair may be more or less hardened as protection for the other pair. Adult insects which normally live on or in the water. (Figs. 25, 28) 39

29b No functional wings, though pads in which wings are developing may be visible. Some may resemble adult insects very closely, others may differ extremely from adults. 30

30a External pads or cases in which wings develop clearly visible.(Figs. 24, 26, 27) 35

30b More or less wormlike, or at least no external evidence of wing development. 31

31a No jointed legs present. Other structures such as hooks, sucker discs, breathing tubes may be present. Larvae of flies, midges, etc.
Order DIPTERA (Fig. 8)

31b Three pairs of jointed thoracic legs, head capsule well formed. 32

32a Minute (2-4mm) living on the water surface film. Tail a strong organ that can be hooked into a "catch" beneath the thorax. When released animal jumps into the air. No wings are ever grown. Adult springtails.
Order COLLEMBOLA (Fig. 20)

32b Larger (usually over 5 mm) wormlike, living beneath the surface. 33

33a Live in cases or webs in water. Cases or webs have a silk foundation to which tiny sticks, stones, and/or bits of debris are attached. Abdominal segments often with minute gill filaments. Generally cylindric in shape. Caddisfly larvae.
Order TRICHOPTERA (Fig. 21)

33b Free living, build no cases. 34

34a Somewhat flattened in cross section and massive in appearance. Each abdominal segment with rather stout, tapering, lateral filaments about as long as body is wide. Alderflies, fishflies, and dobsonflies.
Order MEGALOPTERA (Fig. 22, 14)

34b Generally rounded in cross section. Lateral filaments if present tend to be long and thin. A few forms extremely flattened, like a suction cup. Beetle larvae.
Order COLEOPTERA (Fig. 23)

35a Two or three filaments or other structures extending out from end of abdomen. 37

35b Abdomen ending abruptly, unless terminal segment itself is extended as single structure.(Figs. 24A, 24C) 36

36a Mouth parts adopted for chewing. Front of face covered by extensible folded mouthparts often called a "mask". Head broad, eyes widely spaced. Nymphs of dragonflies or darning needles.
Order ODONATA (Figs.24A, 24C, 24E)

36b Mouthparts for piercing and sucking. Legs often adapted for water locomotion. Body forms various. Water bugs, water scorpions, water boatmen, backswimmers, electric light bugs, water striders, water measurers, etc.
Order HEMIPTERA (Fig. 25)

37a Tail extensions (caudal filaments) two. Stonefly larvae.
Order PLECOPTERA (Fig. 26)

37b Tail extensions three, at times greatly reduced in size. 38

38a Tail extensions long and slender. Rows of hairs may give extensions a feather-like appearance. Mayfly larvae.
Order EPHEMEROPTERA (Fig. 27)

38b Tail extensions flat, elongated plates. Head broad with widely spaced eyes, abdomen relatively long and slender. Damselfly nymths.
Order ODONATA (Fig. 24D)

39a External wings or wing covers form a hard protective dome over the inner wings folded beneath, and over the abdomen. Beetles.
Order COLEOPTERA (Fig. 28)

39b External wings leathery at base, Membranaceous at tip. Wings sometimes very short. Mouth-parts for piercing and sucking. Body form various. True bugs.
Order HEMIPTERA (Fig. 25)

40a Appendage present in pairs. (fins, legs, wings). 42

40b No paired appendages. Mouth a round suction disc. 41

41a Body long and slender. Several holes along side of head. Lampreys.
Sub Phylum VERTEBRATA, Class CYCLOSTOMATA

41b Body plump, oval. Tail extending out abruptly. Larvae of frogs and toads. Legs appear one at a time during metamorphosis to adult form. Tadpoles.
Class AMPHIBIA

42a Paired appendages are legs 43

42b Paired appendages are fins, gills covered by a flap (operculum). True fishes.
Class PISCES

43a Digits with claws, nails, or hoofs 44

43b Skin naked. No claws or digits. Frogs, toads, and salamanders.
Class AMPHIBIA

44a Warm blooded 45

44b Cold blooded. Body covered with horny scales or plates.
Class REPTILIA

45a Body covered with feathers. Birds.
Class AVES

45b Body covered with hair. Mammals.
Class MAMMALIA

REFERENCES - Invertebrates

1 Eddy, Samuel and Hodson, A.C. Taxonomic Keys to the Common Animals of the North Central States. Burgess Pub. Company, Minneapolis. 162 p. 1961.

2 Edmondson, W.T. (ed.) and Ward and Whipple's Freshwater Biology. John Wiley & Sons, New York. pp. 1-1248. 1959.

3 Jahn, T.L. and Jahn, F.F. How to Know the Protozoa. Wm. C. Brown Company, Dubuque, Iowa. pp. 1-234. 1949.

4 Klots, Elsie B. The New Field Book of Freshwater Life. G.P. Putnam's Sons. 398 pp. 1966.

5 Kudo, R. Protozoology. Charles C. Thomas, Publisher, Springfield, Illinois. pp. 1-778. 1950.

6 Palmer, E. Lawrence. Fieldbook of Natural History. Whittlesey House, McGraw-Hill Book Company, Inc. New York. 1949.

7 Pennak, R.W. Freshwater Invertebrates of the United States. The Ronald Press Company, New York. pp. 1-769. 1953.

8 Pimentel, Richard A. Invertebrate Identification Manual. Reinhold Publishing Corp. 151 pp. 1967.

9 Pratt, H.W. A Manual of the Common Invertebrate Animals Exclusive of Insects. The Blaikston Company, Philadelphia, Pa. pp. 1-854. 1951.

REFERENCES - Fishes

1 American Fisheries Society. A List of Common and Scientific Names of Fishes from the United States and Canada. Special Publication No. 2, Am. Fish Soc. Executive Secretary AFS. Washington Bld. Suite 1040, 15th & New York Avenue, N.W. Washington, DC 20005. (Price $4.00 paper, $7.00 cloth). 1970.

2 Bailey, Reeve M. A Revised List of the Fishes of Iowa with Keys for Identification, IN: Iowa Fish and Fishing. State of Iowa, Super. of Printing. 1956. (Excellent color pictures).

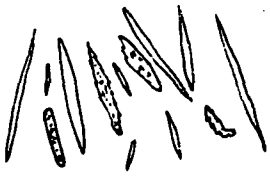
3 Eddy, Samuel. How to Know the Freshwater Fishes. Wm. C. Brown Company, Dubuque, Iowa. 1957.

4 Hubbs, C.L. and Lagler, K.F. Fishes of the Great Lakes Region. Bull. Cranbrook Inst. Science, Bloomfield Hills, Michigan. 1949.

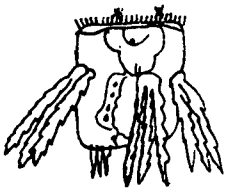
5 Lagler, K.F. Freshwater Fishery Biology. Wm. C. Brown Company, Dubuque, Iowa. 1952.

6 Trautman, M.B. The Fishes of Ohio. Ohio State University Press, Columbus. 1957. (An outstanding example of a State study).

Descriptors: Aquatic Life, Systematics.



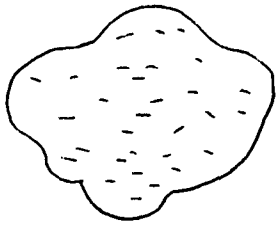
1. Spongilla spicules
Up to .2 mm. long.



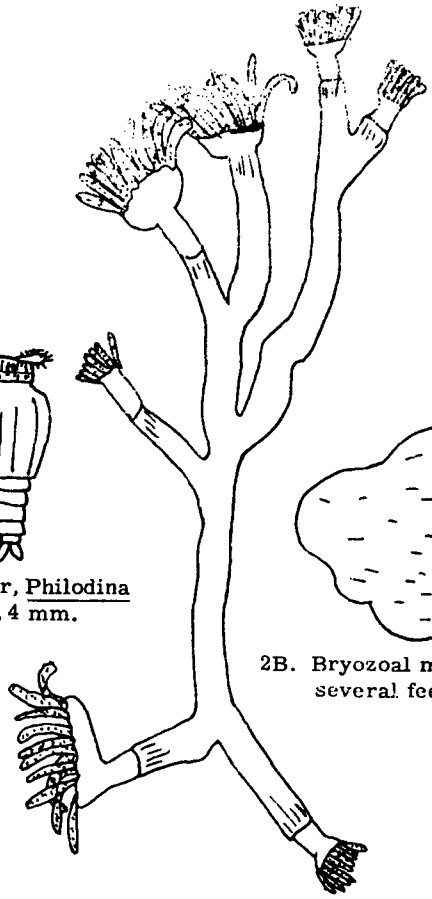
3A. Rotifer, Polyarthra
Up to .3 mm.

3B. Rotifer, Keratella
Up to .3 mm.

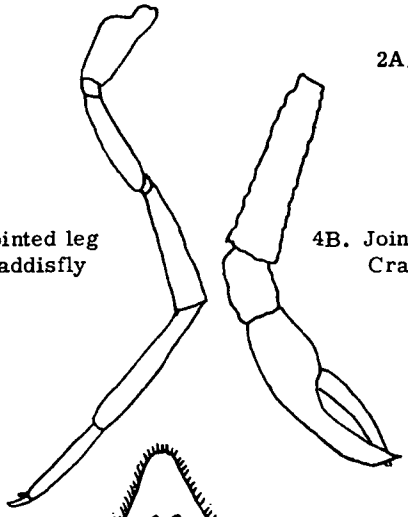
3C. Rotifer, Philodina
Up to .4 mm.



2B. Bryozoal mass. Up to several feet diam.

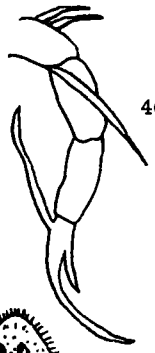


2A. Bryozoa, Plumatella. Individuals up to 2 mm. Intertwined masses may be very extensive.

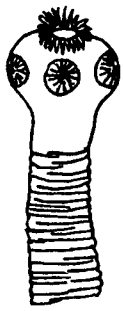


4A. Jointed leg
Caddisfly

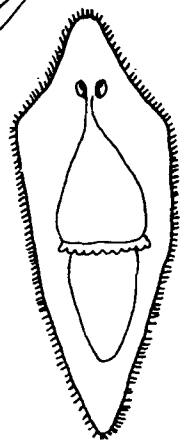
4B. Jointed leg
Crayfish



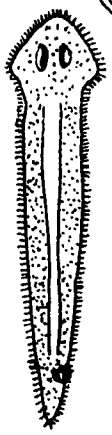
4C. Jointed leg
Ostracod



5. Tapeworm head,
Taenia. Up to
25 yds. long

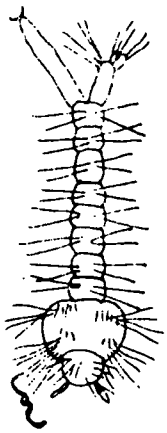


6A. Turbellaria, Mesostoma
Up to 1 cm.



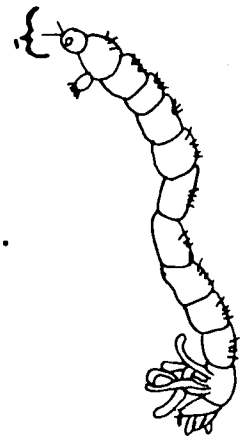
6B. Turbellaria, Dugesia
Up to 1.6 cm.

7. Nematodes. Free living forms commonly up to 1 mm., occasionally more.



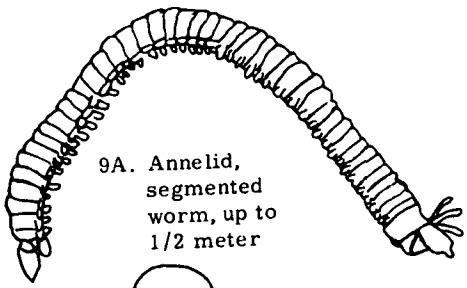
8A. Diptera, Mosquito larvae Up to 15 mm. long.

8B. Diptera, Mosquito pupa. Up to 5 mm.

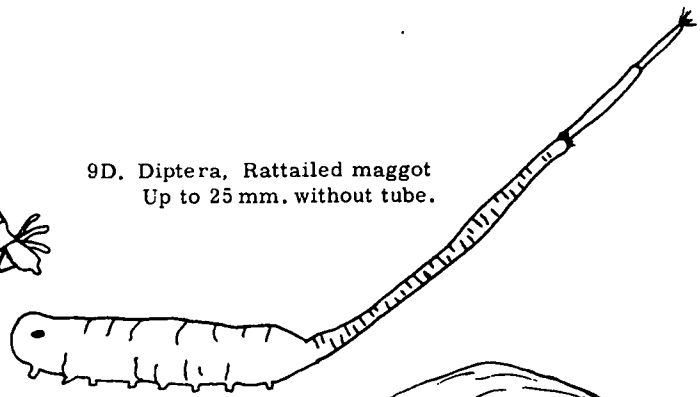


8C. Diptera, chironomid larvae. Up to 2 cm.

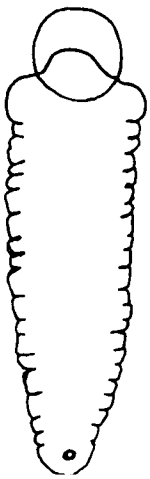
8E. Diptera, crane fly pupa. Up to 2.5 cm.



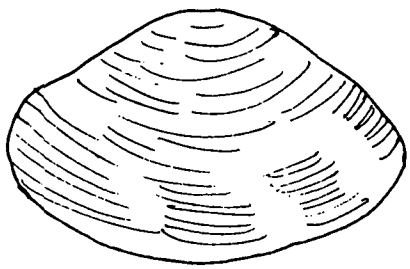
9A. Annelid, segmented worm, up to 1/2 meter



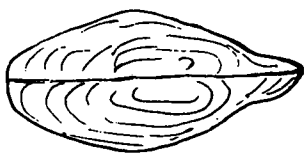
9D. Diptera, Rattailed maggot Up to 25 mm. without tube.



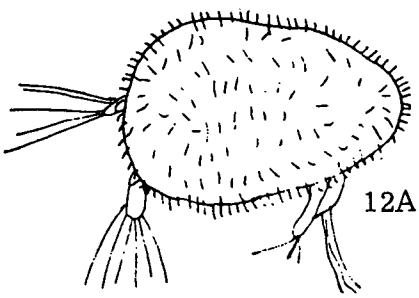
9B. Annelid, leech up to 20 cm.



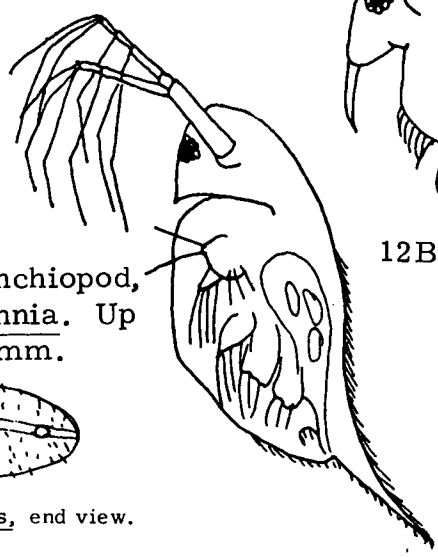
10A. Pelecyopod, Alasmidonta Side view, up to 18 cm. long.



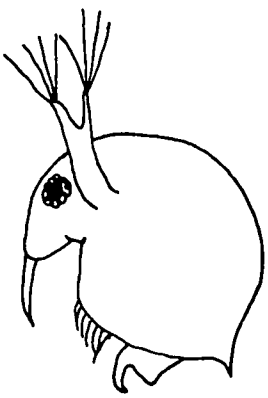
10B. Alasmidonta, end view.



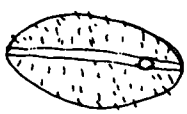
11A. Ostracod, Cypericus Side view, up to 7 mm.



12A. Branchiopod, Daphnia. Up to 4mm.

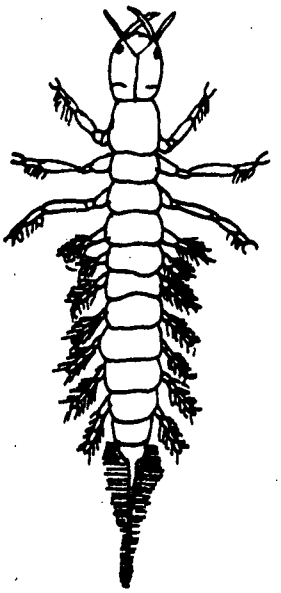


12B. Branchiopod, Bosmina. Up to 2mm.

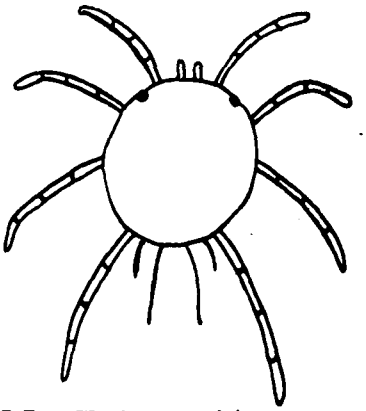


11B. Cypericus, end view.

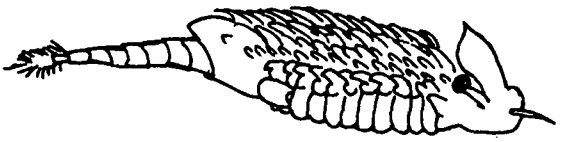
13. Gastropod, Campeloma
Up to 3 inches.



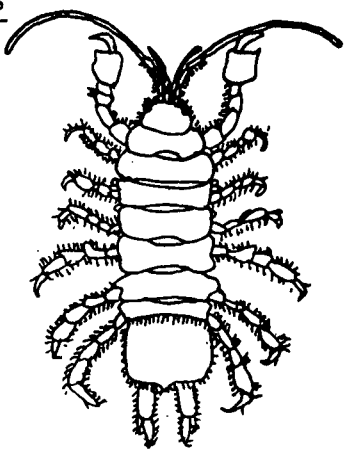
14. Megaloptera, Sialis
Alderfly larvae
Up to 25 mm.



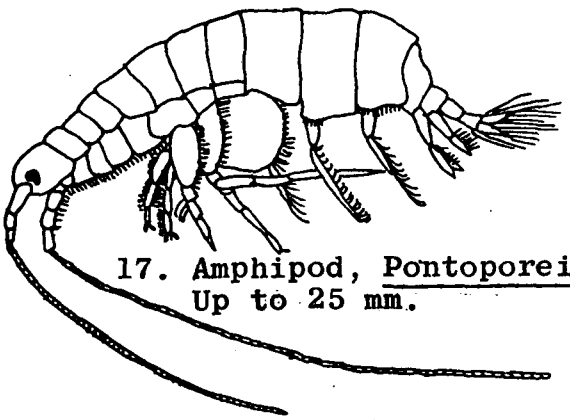
15. Water mite,
up to 3 mm.



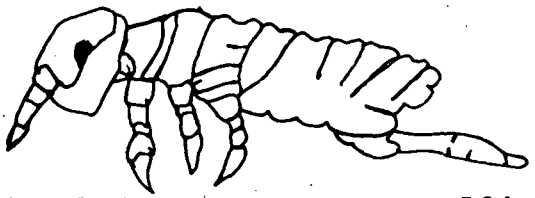
16. Fairy Shrimp, Eubranchipus
Up to 5 cm.



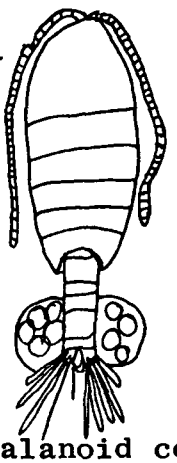
18. Isopod, Asellus
Up to 25 mm.



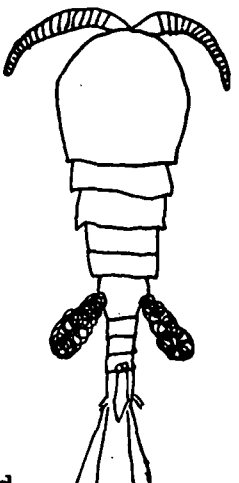
17. Amphipod, Pontoporeia
Up to 25 mm.



20. Collembola, Podura
Up to 2 mm. long



19A. Calanoid copepod,
Female
Up to 3 mm.



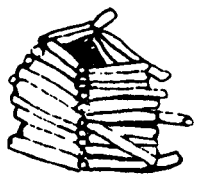
19B. Cyclopoid copepod
Female
Up to 25 mm.

21A.

21B.

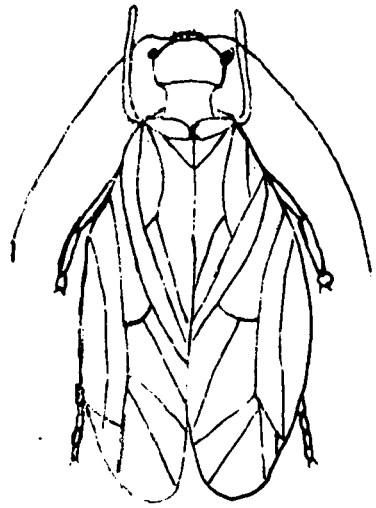
21C.

21D.

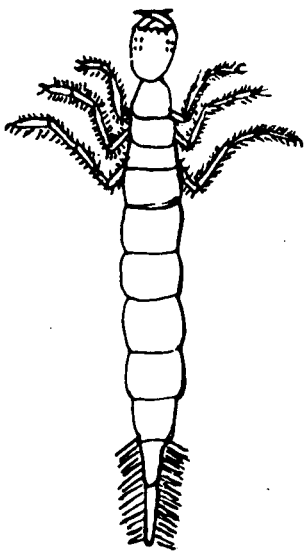


21E.

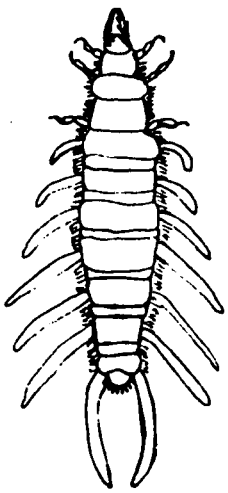
21. Trichoptera, larval cases, mostly 1-2 cm.



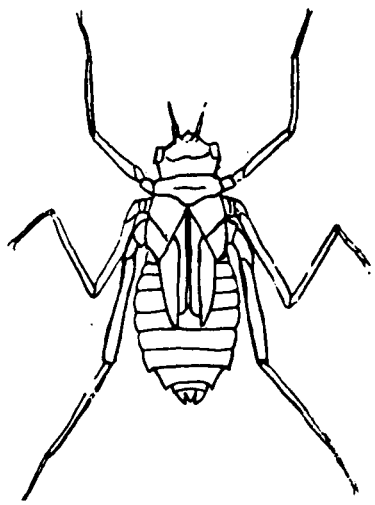
22. Megaloptera, alderfly Up to 2 cm.



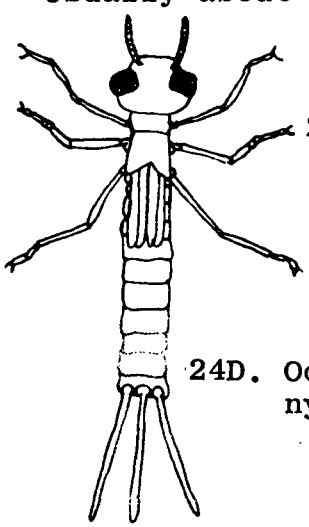
23A. Beetle larvae, Dytisidae, Usually about 2 cm.



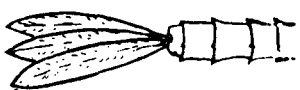
23B. Beetle larvae, Hydrophilidae Usually about 1 cm.



24A. Odonata, dragonfly nymph up to 3 or 4 cm



24D. Odonata, damselfly nymph (top view)

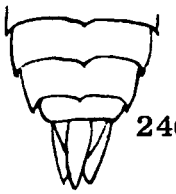


24B. Odonata, tail of damselfly nymph (side view)

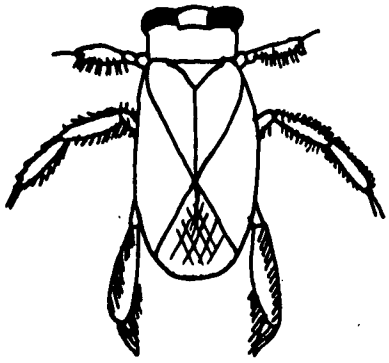
Suborder Zygoptera (24B, D)

24E. Odonata, front view of dragonfly nymph showing "mask" partially extended

Suborder Anisoptera (24A, E, C)

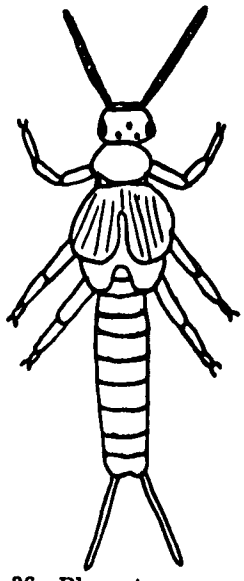


24C. Odonata, tail of dragonfly nymph (top view)



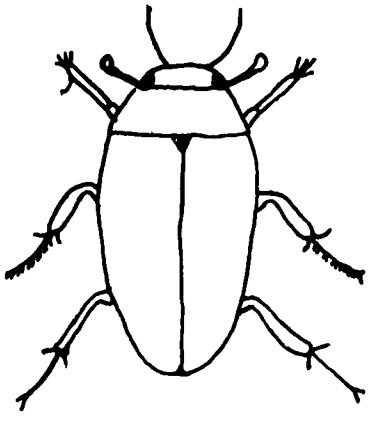
25A. Hemiptera,
Water Boatman
About 1 cm.

25B. Hemiptera,
Water Scorpion
About 4 cm.

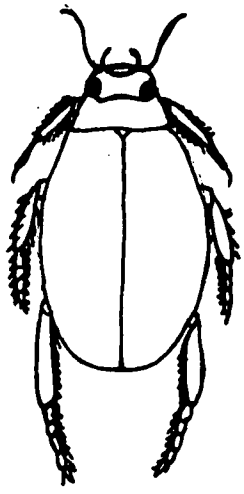


26. Plecoptera,
Stonefly nymph
Up to 5cm.

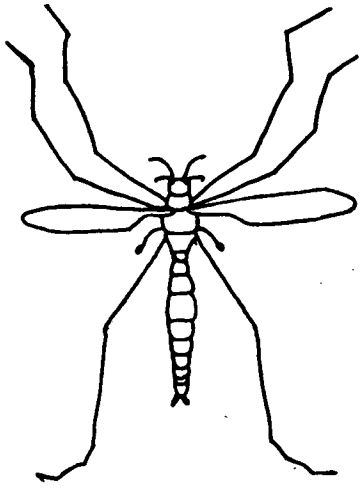
27. Ephemeroptera,
Mayfly nymph
Up to 3cm.



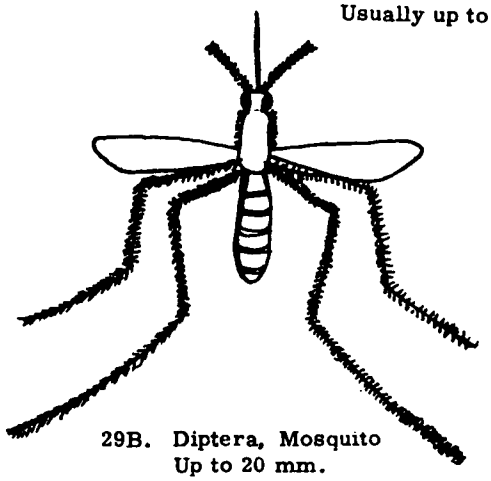
28A. Coleoptera,
Water scavenger
beetle. Up to 4 cm.



28B. Coleoptera,
Dytiscid beetle
Usually up to 4 cm.



29A. Diptera, Crane
fly. Up to $2\frac{1}{2}$ cm.



29B. Diptera, Mosquito
Up to 20 mm.

USING BENTHIC BIOTA IN WATER QUALITY EVALUATIONS

I BENTHOS ARE ORGANISMS GROWING ON OR ASSOCIATED PRINCIPALLY WITH THE BOTTOM OF WATERWAYS

Benthos is the noun.

Benthonic, benthal and benthic are adjectives.

II THE BENTHIC COMMUNITY

A Composed of a wide variety of life forms that are related because they occupy "common ground"--the waterways bottom substrates. Usually they are attached or have relatively weak powers of locomotion. These life forms are:

1 Bacteria

A wide variety of decomposers work on organic materials, breaking them down to elemental or simple compounds (heterotrophic). Other forms grow on basic nutrient compounds or form more complex chemical compounds (autotrophic).

2 Algae

Photosynthetic plants having no true roots, stems, and leaves. The basic producers of food that nurtures the animal components of the community.

3 Flowering Aquatic Plants (Pondweeds)

The largest flora, composed of complex and differentiated tissues. Many are rooted.

4 Microfauna

Animals that pass through a U.S. Standard Series No. 30 sieve, but are retained on a No. 100 sieve. Examples are rotifers and microcrustaceans. Some forms have organs for attachment to substrates, while others burrow into soft materials or occupy the interstices between rocks, floral or faunal materials.

5 Meiofauna

Animals, mostly metazoans, that can pass a 1.0 mm to 0.5 mm screen. Examples are naiad worms and flatworms.

6 Macrofauna

Animals that are retained on a No. 30 sieve. This group includes the insects, worms, molluscs, and occasionally fish. Fish are not normally considered as benthos, though there are bottom dwellers such as sculpins and darters.

B It is a self-contained community, though there is interchange with other communities. For example: Plankton settles to it, fish prey on it and lay their eggs there, terrestrial detritus leaves are added to it, and many aquatic insects migrate from it to the terrestrial environment for their mating cycles.

C It is a stationary water quality monitor. The low motility of the biotic components requires that they "live with" the quality changes of the over-passing waters. Changes imposed in the long-lived components remain visible for extended periods, even after the cause has been eliminated. Only time will allow a cure for the community by drift and reproduction.

III HISTORY OF BENTHIC OBSERVATIONS

A Ancient literature records the vermin associated with fouled waters.

B 500-year-old fishing literature refers to animal forms that are fish food and used as bait.

C The scientific literature associating biota to water pollution problems is over 100 years old (Mackenthun and Ingram, 1964).

D Early this century, applied biological investigations were initiated.

1 The entrance of State boards of Health into water pollution control activities.

2 Creation of state conservation agencies.

3 Industrialization and urbanization.

4 Growth of limnological programs at universities.

E A decided increase in benthic studies occurred in the 1950 decade, and much of today's activities are strongly influenced by developmental work conducted during this period. Some of the reasons for this are:

1 Movement of the universities from "academic biology" to applied pollution programs.

2 Entrance of the federal government into enforcement aspects of water pollution control.

3 A rising economy and the development of federal grant systems.

4 Environmental Protection Programs are a current stimulus.

IV WHY THE BENTHOS?

A It is a natural monitor

B The community contains all of the components of an ecosystem.

1 Reducers

2 Producers

3 Consumers

a Detritivores and bacterial feeders

b Herbivores

c Predators

C Economy of Survey

1 Manpower

2 Time

3 Equipment

D Extensive Supporting Literature

E Advantages of the Macrobenthos

1 Relatively sessile

2 Life history length

3 Fish food organisms

4 Reliability of Sampling

5 Dollars/information

6 Predictability

7 Universality

V REACTIONS OF THE COMMUNITY TO POLLUTANTS

A Destruction of Organism Types

1 Beginning with the most sensitive forms, pollutants kill in order of sensitivity until the most tolerant form is the last survivor. This results in a reduction of variety or diversity of organisms.

2 The usual order of macroinvertebrate disappearance on a sensitivity scale below pollution sources is shown in Figure 2.

Water Quality Deteriorating ↓	Stoneflies Mayflies Caddisflies Amphipods Isopods Midges Oligochaetes	↑ Water Quality Improving

As water quality improves, these reappear in the same order.

B The Number of Survivors Increase

1 Competition and predation are reduced between forms.

2 When the pollutant is a food (plants, fertilizers, animals, organic materials)

C The Number of Survivors Decrease

1 The material added is toxic or has no food value.

2 The material added produces toxic conditions as a byproduct of decomposition (e.g., large organic loadings produce an anaerobic environment resulting in the production of toxic sulfides, methanes, etc.)

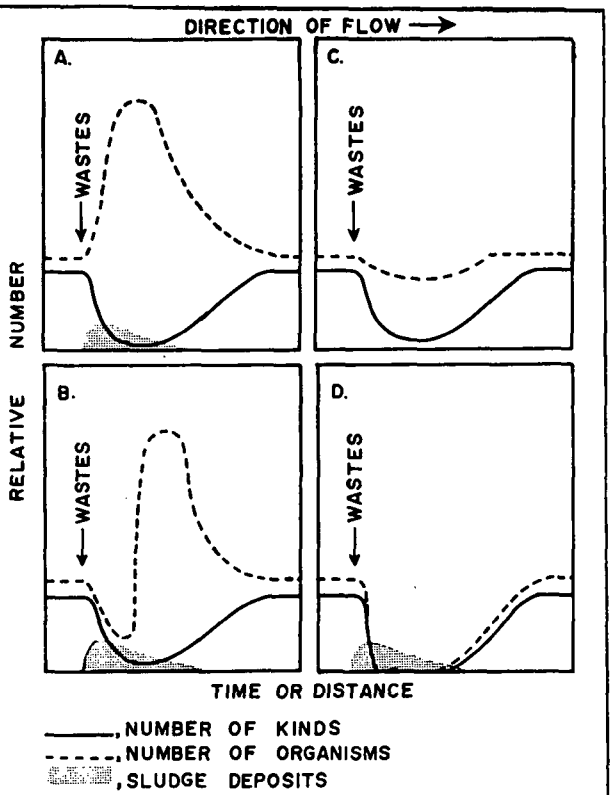
D The Effects May be Manifest in Combinations

1 Of pollutants and their effects.

2 Vary with longitudinal distribution in a stream. (Figure 1)

E Tolerance to Enrichment Grouping (Figure 2)

Flexibility must be maintained in the establishment of tolerance lists based on the response of organisms to the environment because of complex relationships among varying environmental conditions. Some general tolerance patterns can be established. Stonefly nymphs, mayfly naiads, hellgrammites, and caddisfly larvae represent a grouping (sensitive or intolerant) that is generalized quite sensitive to environmental changes. Blackfly larvae, scuds, sowbugs, snails, fingernail clams, dragonfly nymphs, damselfly nymphs, and most kinds of midge larve are intermediate (facultative or intermediate) in tolerance. Sludge-worms, some kinds of midge larvae (bloodworms), and some leeches are tolerant to comparatively heavy loads of organic pollutants. Sewage mosquitoes and rat-tailed maggots are tolerant of anaerobic environments.



Four basic responses of bottom animals to pollution. A. Organic wastes eliminate the sensitive bottom animals and provide food in the form of sludges for the surviving tolerant forms. B. Large quantities of decomposing organic wastes eliminate sensitive bottom animals and the excessive quantities of byproducts of organic decomposition inhibit the tolerant forms; in time, with natural stream purification, water quality improves so that the tolerant forms can flourish, utilizing the sludges as food. C. Toxic materials eliminate the sensitive bottom animals; sludge is absent and food is restricted to that naturally occurring in the stream, which limits the number of tolerant surviving forms. Very toxic materials may eliminate all organisms below a waste source. D. Organic sludges with toxic materials reduce the number of kinds by eliminating sensitive forms. Tolerant survivors do not utilize the organic sludges because the toxicity restricts their growth.

Figure 1

F Structural Limitations

1 The morphological structure of a species limits the type of environment it may occupy.

a Species with complex appendages and exposed complicated respiratory structures, such as stonefly nymphs, mayfly nymphs, and caddisfly larvae, that are subjected to a constant deluge of setteable particulate matter soon abandon the polluted area because of the constant preening required to maintain mobility or respirotory functions; otherwise, they are soon smothered.

b Benthic animals in depositing zones may also be burdened by "sewage fungus" growths including stalked protozoans. Many of these stalked protozoans are host specific.

2 Species without complicated external structures, such as bloodworms and sludgeworms, are not so limited in adaptability.

a A sludgeworm, for example, can burrow in a deludge of particulate organic matter and flourish on the abundance of "manna."

b Morphology also determines the species that are found in riffles, on vegetation, on the bottom of pools, or in bottom deposits.

VI SAMPLING PROCEDURES

A Fauna

1 Qualitative sampling determines the variety of species occupying an area. Samples may be taken by any method that will capture representatives of the species present. Collections from such samplings indicate changes in the environment, but generally do not accurately reflect the degree of change. Mayflies, for example, may be reduced from 100 to 1 per square foot. Qualitative data would indicate the presence of both species, but might not necessarily delineate the change in predominance from mayflies to sludgeworms. The stop net or kick sampling technique is often used.

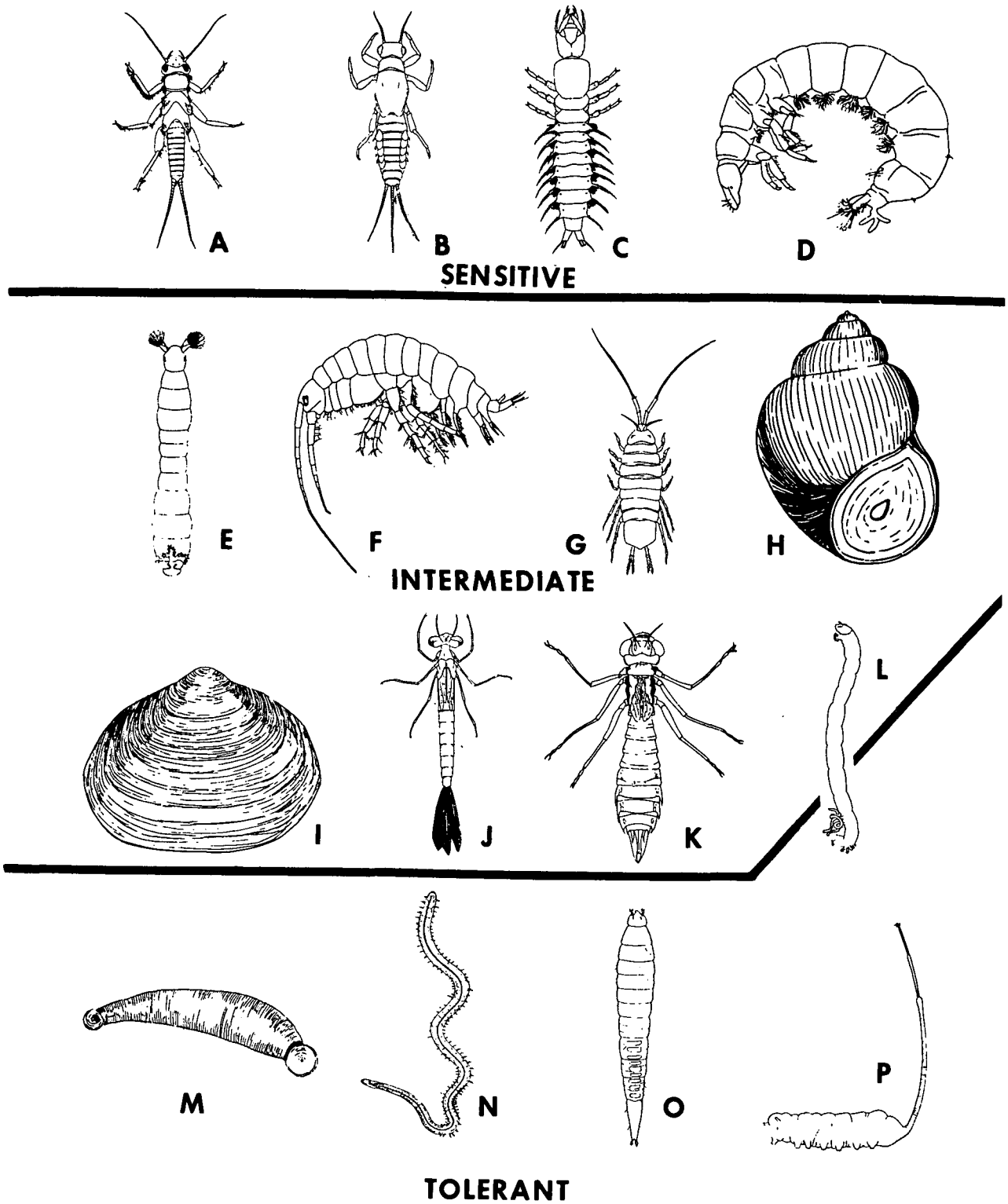
2 Quantitative sampling is performed to observe changes in predominance. The most common quantitative sampling tools are the Petersen and Elkman

REPRESENTATIVE BOTTOM-DWELLING MACROANIMALS

Drawings from Geckler, J., K. M. Mackenthun and W. M. Ingram, 1963. Glossary of Commonly Used Biological and Related Terms in Water and Waste Water Control, DHEW, PHS, Cincinnati, Ohio, Pub. No. 999-WP-2.

A	Stonefly nymph	(Plecoptera)	I	Fingernail clam	(Sphaeriidae)
B	Mayfly nymph	(Ephemeroptera)	J	Damselfly nymph	(Zygoptera)
C	Hellgrammite or Dobsonfly larvae	(Megaloptera)	K	Dragonfly nymph	(Anisoptera)
D	Caddisfly larvae	(Trichoptera)	L	Bloodworm or midge fly larvae	(Chironomidae)
E	Black fly larvae	(Simuliidae)	M	Leech	(Hirudinea)
F	Scud	(Amphipoda)	N	Sludgeworm	(Tubificidae)
G	Aquatic sowbug	(Isopoda)	O	Sewage fly larvae	(Psychodidae)
H	Snail	(Gastropoda)	P	Rat-tailed maggot	(Tubifera-Eristalis)

KEY TO FIGURE 2


A
B
C
D
SENSITIVE
E
F
G
H
INTERMEDIATE
I
J
K
L
M
N
O
P
TOLERANT

grabs and the Surber stream bottom or square-foot sampler. Of these, the Petersen grab samples the widest variety of substrates. The Ekman grab is limited to fine-textured and soft substrates, such as silt and sludge.

The Surber sampler is designed for sampling riffle areas; it requires moving water to transport dislodged organisms into its net and is limited to depths of two feet or less.

3 The collected sample is screened with a standard sieve to concentrate the organisms; these are sorted from the retained material, and the number of each kind determined. Data are then adjusted to number per unit area, usually to number of bottom per square meter.

4 Independently, neither qualitative not quantitative data suffice for thorough analyses of environmental conditions. A cursory examination to detect damage may be made with either method, but a combination of the two gives a more precise determination. If a choice must be made, quantitative sampling would be best, because it incorporates a partial qualitative sample.

B Flora

1 Direct quantitative sampling of naturally growing bottom algae is difficult. It is basically one of collecting algae from a standardor uniform area of the bottom substrates without disturbing the delicate growths and thereby distort the sample. Indirect quantitative sampling is the best available method.

Artificial substrates, such as wood blocks, glass or plexiglass slides, bricks, etc., are placed in a stream. Bottom-attached algae will grow on these artificial substrates. After two or more weeks, the artificial substrates are removed for analysis. Algal growths are scraped from the substrates and the qunatity measured. Since the exposed substrate area and exposure periods are equal at all of the sampling sites, differences in the quantity of algae can be related to changes in the quality of water flowing over the substrates.

2 The quantity of algae on artificial substrates can be measured in several ways. Microscopic counts of algal cells and dry weight of algal material are long established methods.

Microscopic counts involve thorough scraping, mixing, and suspension of the algal cells. From this mixture an aliquot of cells is withdrawn for enumeration under a microscope. Dry weight is determined by drying and weighing the algal sample, then igniting the sample to burn off the algal materials, leaving inert inorganic materials that are again weighed. The difference between initial weight and weight after ignition is attributed to algae.

Any organic sediments, however, that settle on the artificial substrate along with the algae are processed also. Thus, if organic wastes are present appreciable errors may enter into this method.

3 During the past decade, chlorophyll analysis has become a popular method for estimating algal growth. Chlorophyll is extracted from the algae and is used as an index of the quantity of algae present. The advantages of chlorophyll analysis are rapidity, simplicity, and vivid pictorial results.

The algae are scrubbed from the artificial substrate samples, ground, then each sample is steeped in equal volumes, 90% aqueous acetone, which extracts the chlorophyll from the algal cells. The chlorophyll extracts may be compared visually.

Because the chlorophyll extracts fade with time, colorimentry should be used for permanent records. For routine records, simple colorimeters will suffice. At very high chlorophyll densities, interference with colorimetry occurs, which must be corrected through serial dilution of the sample or with a nomograph.

4 Autotrophic Index

The chlorophyll content of the periphyton is used to estimate the algal biomass and as an indicator of the nutrient content (or trophic status) or toxicity of the water and the taxonomic composition of the community. Periphyton growing in surface water relatively free of organic pollution consists largely of algae, which contain approximately 1 to 2 percent chlorophyll a by dry weight. If dissolved or particulate organic matter is present in high concentrations, large populations of filamentous bacteria, stalked protozoa, and other nonchlorophyll bearing microorganisms develop and the percentage of chlorophyll a is then reduced. If the biomass-chlorophyll a relationship is expressed as a ratio (the autotrophic index), values greater than 100 may result from organic pollution (Weber and McFarland, 1969; Weber, 1973).

$$\text{Autotrophic Index} = \frac{\text{Ash-free Wgt (mg/m}^2)}{\text{Chlorophyll a (mg/m}^2)}$$

VII FACTORS INVOLVED IN DATA INTERPRETATION

Two very important factors in data evaluation are a thorough knowledge of conditions under which the data were collected and a critical assessment of the reliability of the data's representation of the situation.

A Maximum-Minimum Values

The evaluation of physical and chemical data to determine their effects on aquatic organisms is primarily dependent on maximum and minimum observed values. The mean is useful only when the data are relatively uniform. The minimum or maximum values usually create acute conditions in the environment.

B Identification

Precise identification of organisms to species requires a specialist in limited taxonomic groups. Many immature aquatic forms have not been associated with the adult species. Therefore, one who is certain of the genus but not the species should utilize the generic name, not a potentially incorrect species name. The method of interpreting biological data on the basis of numbers of kinds and numbers of organisms will typically suffice.

C Lake and Stream Influence

Physical characteristics of a body of water also affect animal populations. Lakes or impounded bodies of water support different faunal associations from rivers. The number of kinds present in a lake may be less than that found in a stream because of a more unifrom habitat. A lake is all pool, but a river is composed of both pools and riffles. The nonflowing water of lake exhibits a more complete settling of particulate organic matter that naturally supports a higher population of detritus consumers. For these reasons, the bottom fauna of a lake or impoundment cannot be directly compared with that of a flowing stream.

D Extrapolation

How can bottom-dwelling macrofauna data be extrapolated to other environmental components? It must be borne in mind that a component of the total environment is being sampled. If the sampled component exhibits changes, then so must the other interdependent components of the environment. For example, a clean stream with a wide variety of desirable bottom organisms

would be expected to have a wide variety of desirable bottom fishes; when pollution reduces the number of bottom organisms, a comparable reduction would be expected in the number of fishes. Moreover, it would be logical to conclude that any factor that eliminates all bottom organisms would eliminate most other aquatic forms of life.

VII IMPORTANT ASSOCIATED ANALYSES

A The Chemical Environment

1 Dissolved oxygen

2 Nutrients

3 Toxic materials

4 Acidity and alkalinity

5 Etc.

B The Physical Environment

1 Suspended solids

2 Temperature

3 Light penetration

4 Sediment composition

5 Etc.

IX AREAS IN WHICH BENTHIC STUDIES CAN BEST BE APPLIED

A Damage Assessment

If a stream is suffering from pollutants, the biota will so indicate. A biologist can determine damages by looking at the "critter" assemblage in a matter of hours. Usually, if damages are not found, it will not be necessary to alert the remainder of the agency's staff, pack all the equipment, pay travel and per diem, and then wait five days before enough data can be assembled to begin evaluation.

B By determining what damages have been done, the potential cause "list" can be reduced to a few items for emphasis and the entire "wonderful worlds" of science and engineering need not be practiced with the result that much data are discarded later because they were not applicable to the problem being investigated.

C Good benthic data associated with chemical, physical, and engineering can be data used to predict the direction of future changes and to estimate the amount of pollutants that need to be removed from the waterways.

REFERENCES

1 Hynes, H.B.N. The Ecology of Running Waters. Univ. Toronto Press. 1970.

2 Keup, L.E., Ingram, W.M. and Mackenthun, K.M. The Role of Bottom Dwelling Macrofauna in Water Pollution Investigations. USPHS Environmental Health Series Publ. No. 999-WP-38, 23 pp. 1966.

3 Keup, L.E., Ingram, W.M. and Mackenthun, K.M. Biology of Water Population: A Collection of Selected Papers on Stream Pollution, Waste Water, and Water Treatment. Federal Water Pollution Control Administration Pub. No. CWA-3, 290 pp. 1967.

4 Mackenthun, K.M. The Practice of Water Pollution Biology. FWQA. 281 pp. 1969.

5 Stewart, R.K., Ingram, W.M. and Mackenthun, K.M. Water Pollution Control, Waste Treatment and Water Treatment: Selected Biological References on Fresh and Marine Waters. FWPCA Pub. No. WP-23, 126 pp. 1966.

6 Weber, Cornelins I., Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. U.S. Environmental Protection Agency, NERC, Cincinnati, OH. Environmental Monitoring Series 670/4.73.001 July 1973

This outline was prepared by Lowell E. Keup, Chief, Technical Studies Branch, Div. of Technical Support, EPA, Washington, D. C. 20242, and revised by Ralph M. Sinclair, Aquatic Biologist, National Training Center, EPA, WPO, Cincinnati, OH 45268.

Descriptors:
Aquatic Life, Benthos, Water Quality, Degradation, Environmental Effects, Trophic Level, Biological Communities, Ecological Distributions

AUTOMATIC INSTRUMENTS FOR WATER QUALITY MEASUREMENTS

I NEED FOR CONTINUOUS MONITORING OF WATER QUALITY

Satisfactory evaluation of the quality of water depends upon the availability of adequate data. Such data must be not only as accurate as economically practical but must also be of sufficient quantity to support reliable conclusions. In general, the more information available, the more reliable will be the interpretations.

In the past, information on the chemical, physical, and bacteriological quality of most surface waters has been obtained by periodic stream surveys or infrequent "spot" analyses. Although raw water supplies are sampled daily, the data obtained are restricted to those tests of importance in water treatment and do not include other pollutional parameters or water resources not at present being used for water supply.

It is apparent that in many situations significant changes in water quality may occur often and abruptly. Seasonal changes in flow, the occurrence of unpredictable industrial discharges or spills, and the changes in flow from impoundments may alter the concentration of many of the substances of interest to the water user. In some cases these changes may occur within a few hours: for example, the diurnal fluctuations in dissolved oxygen and the salinity changes in tidal estuaries. Thus considerable advantage is gained if continuous monitoring of water quality can be accomplished. The use of manual sampling and standard laboratory analyses would be far too expensive; therefore a wide variety of automatic instruments have been developed.

II ELECTRICAL INSTRUMENTS

The diagram below illustrates a typical system for monitoring instruments which utilize electrical sensors.

The sensor produces an electrical signal representative of concentration, the signal is converted, and amplified, then passed to the recorder to develop a permanent record of concentration levels. For a clear understanding of the operational characteristics, the parts of the system are discussed separately.

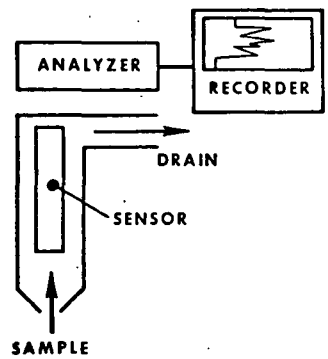


Figure 1

A Sensors

The sensor is the part of the system in contact with the sample. Sensing elements may be immersed directly in the stream, or placed in flow cells through which the sample is pumped. Both methods have advantages and limitations.

When the sensor is placed in the stream the determination is made "in situ." Therefore the sample is not affected by pumping, temperature changes, or time of travel through the instrument. Such an installation, however, presents certain problems. The sensing elements must be protected from floating debris and must be mounted so as to remain in a fixed position in spite of changes in velocity or direction of current. In bodies of water which fluctuate in surface level, such as impoundments, or estuaries, the relative depth of the sensor may change. In addition, frequent inspection of the sensing elements for attached growths or physical damage is difficult and apt to be neglected.

The use of the shore-based system, where the stream sample is pumped through flow cells within the instrument housing, is free from some of the difficulties mentioned previously. Inspection of the sensors is easy, cleaning is simplified, and replacement of sensing elements is readily accomplished. It should be recognized, however, that the precautions required for satisfactory mounting and protection of sensor units in the stream apply equally well to the pump intake. Further, it is essential that the sample being tested in the flow cell is truly representative of the stream water. If dissolved oxygen is included in the parameters, a submersible pump is required, to avoid cavitation and prevent suction removal of dissolved gases. The intake screen must be carefully designed, since a fine screen will quickly clog, while a coarse screen may permit floating material to enter the system.

Electrical sensors may be conductimetric, potentiometric, polarographic, or coulimetric. The sensor may directly measure a constituent or property of the sample or it may be used as an indicating mechanism in automatic titration.

B Analyzer-Amplifier

The function of the analyzer is to convert the signal from the sensor to a standard EMF, usually ranging from 0-50 millivolts. Often bridge circuits are employed. The analyzer must be rugged to avoid shock damage and the electronic circuitry must be stable over a wide range of environmental conditions. Considerable advantage is gained by the use of standard, readily available components. Provision should be made for a standard signal to permit internal checking of the circuits.

In most cases the signal must be amplified to provide sufficient voltage to drive a recorder. Amplification is generally built into the analyzer circuitry.

C Recorder

Although read-out meters can be used, a permanent record is desirable. Strip-chart recorders are the most popular even though they require line voltage. A slow chart speed is necessary, since observation periods of several days are common. Difficulties with pens are often encountered due to varying conditions of humidity and temperature. The use of pressure-sensitive paper may offer distinct advantage.

Circular charts have long been used but they have certain disadvantages. Unless the chart is inconveniently large the chart divisions are very close together making hourly fluctuations difficult to interpret. In addition, the circular chart does not lend itself to mechanical handling of data for computer processing. Chart drive by clock mechanism is possible, however, eliminating the need for power connection.

A preferable system for the recording of data is the use of digital output. Water quality instruments can be equipped with analog-to-digital converters which change the amplitude of the signal to a digital value. By the use of a punch-tape readout the data is recorded on paper tape rather than on a chart. The punched tape may then be transferred to a computer for data processing. Therefore, a monitor equipped with digital tape readout permits the recording of original signal and the performance of statistical computations without hand transcription.

When the Surveillance Program includes several automatic monitors on a river system , consideration should be given to a central data logging facility. By transmitting the data from the monitors directly to a small computer (by leased phone lines or radio), the information on water quality of the entire river basin is immediately available. The central facility also provides for the detection of unusual discharges which may affect downstream water users.

D Parameters Measured Electrically

The following water quality parameters can be measured by the use of electrodes:

Temperature	Chloride
Conductivity	Residual chlorine
pH	Oxidation-reduction potential
Oxygen	

A prominent instrument manufacturer is now conducting research into the development of a number of glass electrodes specifically designed for the measurement of other ions. It appears that the list above may be considerably expanded in the future.

In most cases precision and accuracy is not as good as with standard laboratory tests, but the advantages of continuous recording outweigh the limitations in performance.

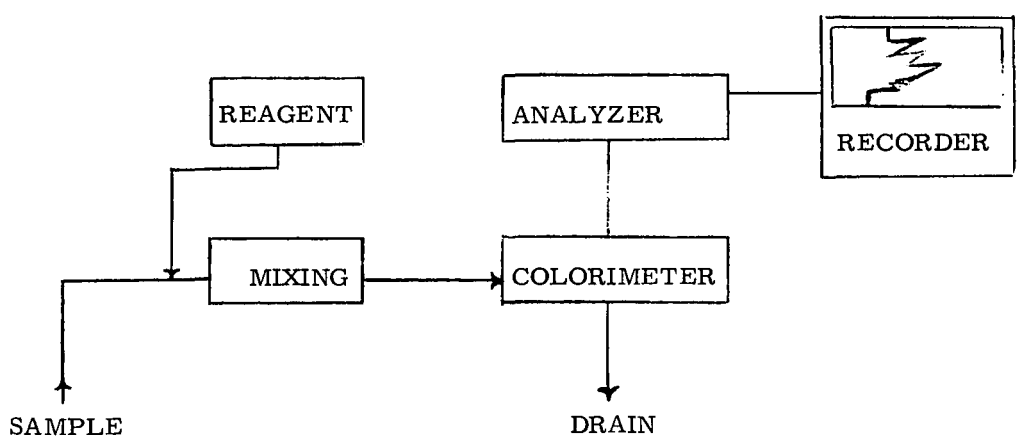
III PHOTOMETRIC INSTRUMENTS

Because of the importance of colorimetric analysis in the water laboratory, considerable attention has been given to the development of continuous monitoring instruments employing this principle. A typical system is shown in Figure 2.

It should be noted that the sample must be pumped through the instrument with the attendant problems described above.

A Measurement of Turbidity and Color

In the simplest photometric instrument, a property of the sample, such as turbidity or color, is measured directly. These relatively simple parameters, however, are rather difficult to determine. Turbidity measurements are affected by particle size and by the true color of the sample. Conversely, color determinations are subject to errors caused by turbidity and by the fact that the wave length of the color in the sample may vary widely. Instruments of this type currently available do not satisfactorily compensate for these interferences and the data obtained from them does not correlate well with standard methods values.



B Colorimetric Analyzers

A second type of photometric instrument is designed to reproduce laboratory colorimetric procedures. That is, reagents are added to the sample to produce a color change proportional to the concentration of the material being determined. Since the sample is flowing continuously the reagents must be metered accurately and mixed thoroughly before photometric measurement. In a properly designed system, almost any colorimetric procedure can be duplicated and therefore the potential range of determinations is much wider than in the electrometric instruments.

A modification of the colorimetric instrument is the continuous titrator. In this system an indicator is added to the flowing sample and the reagent is added at a variable rate to maintain a constant color. The amount of reagent required is proportional to the concentration of the reactant in the sample, and the current used by the metering pump acts as a signal for the analyzer.

In spite of the apparent advantages of the photometric systems, certain special problems are inherent. The color and turbidity of the sample may interfere, the accumulation of slime in the cells may seriously reduce the sensitivity, and the limitations of the filter photometer (wide band pass) must be considered. Further, in most colorimetric procedures the amount of reagent required is proportional to the concentration range of the sample, a factor which would limit the applicability of the instrument.

C Parameters Measured Photometrically

Continuous analytical procedures have been developed for:

Turbidity	Fluoride
Color	Silica
Hardness	Phosphate
Residual Chlorine	Phenols
Alkalinity	Chemical Oxygen Demand

IV PERFORMANCE

To illustrate the quality of the data which may be obtained from an integrated water quality monitor, the following table shows the results of a performance test conducted on a prototype instrument supplied by one manufacturer. The "Acceptable" tolerances were selected as representative of the usual requirements for continuous data acquisition and may be too high or too low, depending upon the accuracy deemed necessary.

V CALIBRATION AND MAINTENANCE

A Calibration

In the parlance of the instrument manufacturer, calibration involves two steps: (1) the setting of the readout to zero value in the absence of sensor signal, and (2) setting of the readout to some standard value, such as 5mv. when the sensor signal is replaced by a standard signal. It is readily apparent that a calibration of this type adjusts the meter and amplifier to reproduce correctly the signal received from the sensing elements. It does not, however, "calibrate" the total instrument in terms of the concentration of measured substance in the sample.

For proper calibration, it is essential that the final readout of the instrument be adjusted to correspond to a true value for the measured material. Thus the only adequate calibration must involve the measurement of a standard solution, such as a buffer of known pH or a salt solution of known conductance. Since instrument systems may lack linear response to a wide signal range, the instrument should be calibrated at several points over the range of values anticipated. In some cases current instrument design has not included adequate means of replacing the sample with standard solutions for calibration purposes.

The frequency of recalibration depends upon both the stability of the analyzer and the sensor. In general, analyzer-amplifiers are more stable than the sensing systems now in use. The Basic Data Program,

PERFORMANCE DATA

Test	Acceptable deviation	Mean deviation found	% Acceptable
pH	± 0.1 unit	0.1 unit	80%
Temperature	± 1°	0.2°	85%
Conductance	± 5%	17 μmhos	99%
Dissolved Oxygen	± 0.5 mg/l	0.3 mg/l	73%

FWPCA, in performance specifications for monitoring instruments,has established a two-week period for unattended performance. While such instrument stability is desirable, circumstances of site location and sample characteristics, often necessitate more frequent checking and recalibration.

B Maintenance

It is unwise to assume that any stream monitoring instrument, no matter how well designed and built, can function adequately for extended periods of time without maintenance. Sensing elements are subject to physical, chemical, and biological actions, analyzer components may fail or perform erratically, and recorders may stop or fail to print. The frequency of maintenance depends upon a large number of factors both controllable and accidental and can be determined only by long-term testing and actual field experience. Ease of maintenance can be designed into the instrument by the use of replaceable electronic components and accessibility for cleaning of flow cells and sample lines.

The availability of trained personnel for checking and maintaining the instruments in the field is a key factor in the successful stream monitoring program. Such personnel need not be either electronic specialists or experienced analysts but they should have a working knowledge of the instrument system and a familiarity with the chemical principles involved in the use of standard solutions for calibration.

VI APPLICATIONS

Stream monitoring systems are now in use in a variety of locations throughout the United States. In most cases the instruments measure a single parameter or condition of the stream rather than a number of parameters. Some of the instruments employ well established measurement systems, for example pH and conductance, while others, are still largely experimental.

Because of the need for continuous stream data, the intensive interest on the part of regulatory agencies, and the development programs by instrument makers, it is safe to assume that the use of water quality monitoring instruments will increase significantly in the near future.

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Descriptors: Analytical Techniques, Instrumentation, Testing Monitoring, Control Systems, Data Collections, Data Transmission, Remote Sensing

DISSOLVED OXYGEN

Determination By The Winkler Iodometric Titration and Azide Modification

The basic Winkler procedure (1888) has been modified many times to improve its workability in polluted waters. None of these modifications have been completely successful. The most useful modification was proposed by Alsterberg and consists of the addition of sodium azide to control nitrite interference during the iodometric titration. The Azide modification of the iodometric titration is recommended as official by the EPA-WPO Quality Control Committee for relatively clean waters.

A Reactions

1 The determination of DO involves a complex series of interactions that must be quantitative to provide a valid DO result. The number of sequential reactions also complicates interference control. The reactions will be presented first followed by discussion of the functional aspects.

$$MnSO_4 + 2\ KOH \rightarrow Mn(OH)_2 + K_2SO_4 \quad (a)$$

$$2\ Mn(OH)_2 + O_2 \rightarrow 2\ MnO(OH)_2 \quad (b)$$

$$MnO(OH)_2 + 2\ H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O \quad (c)$$

$$Mn(SO_4)_2 + 2\ KI \rightarrow MnSO_4 + K_2SO_4 + I_2 \quad (d)$$

$$I_2 + 2\ Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2\ NaI \quad (e)$$

2 Reaction sequence

The series of reactions involves five different operational steps in converting dissolved oxygen in the water into a form in which it can be measured.

a $O_2 \rightarrow MnO(OH)_2 \rightarrow Mn(SO_4)_2 \rightarrow I_2 \rightarrow$ Thiosulfate (thio) or phenylarsine oxide (PAO) titration.

b All added reagents are in excess to improve contact possibilities and to force the reaction toward completion.

3 The first conversion, $O_2 \rightarrow MnO(OH)_2$ (reactions a, b) is an oxygen transfer operation where the dissolved oxygen in the water combines with manganous hydroxide to form an oxygenated manganic hydroxide.

a The manganous salt can react with oxygen only in a highly alkaline media.

b The manganous salt and alkali must be added separately with addition below the surface of the sample to minimize reaction with atmospheric oxygen via air bubbles or surface contact. Reaction with sample dissolved oxygen is intended to occur upon mixing of the reagents and sample after stoppering the full bottle (care should be used to allow entrained air bubbles to rise to the surface before adding reagents to prevent high results due to including entrained oxygen).

c Transfer of oxygen from the dissolved state to the precipitate form involves a two phase system of solution and precipitate requiring effective mixing for quantitative transfer. Normally a gross excess of reagents is used to limit mixing requirements. Mixing by rapid inversion 25 to 35 times will accomplish the purpose. Less energy is required by inversion 5 or 6

times, allowing the solids to settle half way and repeat the process. Reaction is rapid; contact is the principal problem in the two phase system.

d If the alkaline floc is white, no oxygen is present.

4 Acidification (reactions c and d) changes the oxygenated manganic hydroxide to manganic sulfate which in turn reacts with potassium iodide to form elemental iodine. Under acid conditions, oxygen cannot react directly with the excess manganous sulfate remaining in solution.

5 Iodine (reaction e) may be titrated with sodium thiosulfate standard solution to indicate the amount of dissolved oxygen originally present in the sample.

a The blue color of the starch-iodine complex commonly is used as an indicator. This blue color disappears when elemental iodine has been reacted with an equivalent amount of thiosulfate.

b Phenylarsine oxide solutions are more expensive to obtain but have better keeping qualities than thiosulfate solutions. Occasional use, field operations and situations where it is not feasible to calibrate thio solutions regularly, usually encourage use of purchased PAO reagents.

6 For practical purposes the DO determination scheme involves the following operations.

a Fill a 300 ml bottle* under conditions minimizing DO changes. This means that the sample bottle must be flushed with test solution to displace the air in the bottle with water characteristic of the tested sample*.

*DO test bottle volumes should be checked - discard those outside of the limits of 300 ml + or - 3 ml.

b To the filled bottle:

1) Add $MnSO_4$ reagent (2 ml)

2) Add KOH, KI, NaN_3 reagent (2 ml)
Stopper, mix by inversion, allow to settle half way and repeat the operation. Highly saline test waters commonly settle very slowly at this stage and may not settle to the half way point in the time allotted.

c To the alkaline mix (settled about half way) add 2 ml of sulfuric acid, stopper and mix until the precipitate dissolves.

d Transfer the contents of the bottle to a 500 ml Erlenmeyer flask and titrate with 0.0375 Normal Thiosulfate*. Each ml of reagent used represents 1 mg of DO/liter of sample.

The same thing applies for other sample volumes when using an appropriate titrant normality such as:

1) For a 200 ml sample, use 0.025 N Thio

2) For a 100 ml sample, use 0.0125 N Thio

*EPA-WPO Method

7 The addition of the first two DO reagents, ($MnSO_4$ and the KOH, KI and NaN_3 solutions) displaces an equal quantity of the sample. This is not the case when acid is added because the clear liquid above the floc does not contain dissolved oxygen as all of it should be converted to the particulate $MnO(OH)_2$. Some error is introduced by this displacement of sample during dosage of the first two reagents. The error upon addition of 2 ml of each reagent to a 300 ml sample is $\frac{4}{300} \times 100$ or 1.33% loss in DO. This may be corrected by an appropriate factor or by adjustment of reagent normality. It is generally considered small in relation to other errors in sampling, manipulation and interference, hence this error may be recognized but not corrected.

8 Reagent preparation and procedural details can be found in reference 1.

IV The sequential reactions for the Chemical DO determination provides several situations where significant interference may occur in application on polluted water, such as:

A Sampling errors may not be strictly designated as interference but have the same effect of changing sample DO. Inadequate flushing of the bottle contents or exposure to air may raise the DO of low oxygen samples or lower the DO of supersaturated samples.

B Entrained air may be trapped in a DO bottle by:

1 Rapid filling of vigorously mixed samples without allowing the entrained air to escape before closing the bottle and adding DO reagents.

2 Filling a bottle with low temperature water holding more DO than that in equilibrium after the samples warm to working temperature.

3 Aeration is likely to cool the sample permitting more DO to be introduced than can be held at the room or incubator temperatures.

4 Samples warmer than working incubator temperatures will be only partially full at equilibrium temperatures.

Addition of DO reagents results in reaction with dissolved or entrained oxygen. Results for DO are invalid if there is any evidence of gas bubbles in the sample bottle.

C The DO reagents respond to any oxidant or reductant in the sample capable of reacting within the time allotted. HOCl or H_2O_2 may raise the DO titration while H_2S, & SH^- may react with sample oxygen to lower the sample titration. The items mentioned react rapidly and raise or lower the DO result promptly. Other items such as Fe^{++} or $SO_3^=$ may or may not react completely within the time allotted for reaction. Many organic materials or complexes from benthic deposits may have an effect upon DO results that are difficult to predict. They may have one effect during the alkaline stage to release iodine from Kl while favoring irreversible absorption of iodine during the acid stage. Degree of effect may increase with reaction time. It is generally inadvisable to use the iodometric titration on samples containing large amounts of organic contaminants or

benthic residues. It would be expected that benthic residues would tend toward low results because of the reduced iron and sulfur content - they commonly favor high results due to other factors that react more rapidly, often giving the same effect as in uncontrolled nitrite interference during titration.

D Nitrite is present to some extent in natural waters or partially oxidized treatment plant samples. Nitrite is associated with a cyclic reaction during the acid stage of the DO determination that may lead to erroneous high results.

1 These reactions may be represented as follows:

$$2HNO_2 + 2\,HI \rightarrow I_2 + 4H_2O + N_2O_2 \quad (a)$$

$$2HNO_2 \leftarrow + 1/2O_2 + H_2O + N_2O_2 \quad (b)$$

These reactions are time, mixing and concentration dependent and can be minimized by rapid processing.

2 Sodium azide (NaN_3) reacts with nitrite under acid conditions to form a combination of $N_2 + N_2O$ which effectively blocks the cyclic reaction by converting the HNO_2 to noninterfering compounds of nitrogen.

3 Sodium azide added to fresh alkaline KI reagent is adequate to control interference up to about 20 mg of NO_2^- N/liter of sample. The azide is unstable and gradually decomposes. If resuspended benthic sediments are not detectable in a sample showing a returning blue color, it is likely that the azide has decomposed in the alkaline KI azide reagent.

E Surfactants, color and Fe^{+++} may confuse endpoint detection if present in significant quantities.

F Polluted water commonly contains significant interferences such as C. It is advisable to use a membrane protected sensor of the electronic type for DO determinations in the presence of these types of interference.

G The order of reagent addition and prompt completion of the DO determination is critical. Stable waters may give valid DO results after extended delay of titration during the acidified stage. For unstable water, undue delay at any stage of processing accentuates interference problems.

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Descriptors: Chemical Analysis, Dissolved Oxygen, Oxygen, Water Analysis

DISSOLVED OXYGEN
DETERMINATION BY ELECTRONIC MEASUREMENT

I INTRODUCTION

A Electronic measurement of DO is attractive for several reasons:

1 Electronic methods are more readily adaptable for automated analysis, continuous recording, remote sensing or portability.

2 Application of electronic methods with membrane protection of sensors affords a high degree of interference control.

3 Versatility of the electronic system permits design for a particular measurement, situation or use.

4 Many more determinations per man-hour are possible with a minor expenditure of time for calibration.

B Electronic methods of analysis impose certain restrictions upon the analyst to insure that the response does, in fact, indicate the item sought.

1 The ease of reading the indicator tends to produce a false sense of security. Frequent and careful calibrations are essential to establish workability of the apparatus and validity of its response.

2 The use of electronic devices requires a greater degree of competence on the part of the analyst. Understanding of the behavior of oxygen must be supplemented by an understanding of the particular instrument and its behavior during use.

C Definitions

1 Electrochemistry - a branch of chemistry dealing with relationships between electrical and chemical changes.

2 Electronic measurements or electrometric procedures - procedures using the measurement of potential differences as an indicator of reactions taking place at an electrode or plate.

3 Reduction - any process in which one or more electrons are added to an atom or an ion, such as $O_2 + 2e \rightarrow 2O^-$ The oxygen has been reduced.

4 Oxidation - any process in which one or more electrons are removed from an atom or an ion, such as $Zn^o - 2e \rightarrow Zn^{+2}$. The zinc has been oxidized.

5 Oxidation - reduction reactions - in a strictly chemical reaction, reduction cannot occur unless an equivalent amount of some oxidizable substance has been oxidized. For example:

$$2H_2 + O_2 \rightleftarrows 2H_2O$$
$$2H_2 - 4e \rightleftarrows 4H^{+1} \quad \text{hydrogen oxidized}$$
$$O_2 + 4e \rightleftarrows 2O^{-2} \quad \text{oxygen reduced}$$

Chemical reduction of oxygen may also be accomplished by electrons supplied to a noble metal electrode by a battery or other energizer.

6 Anode - an electrode at which oxidation of some reactable substance occurs.

7 Cathode - an electrode at which reduction of some reactable substance occurs. For example in I. C. 3, the reduction of oxygen occurs at the cathode.

8 Electrochemical reaction - a reaction involving simultaneous conversion of chemical energy into electrical energy or the reverse. These conversions are

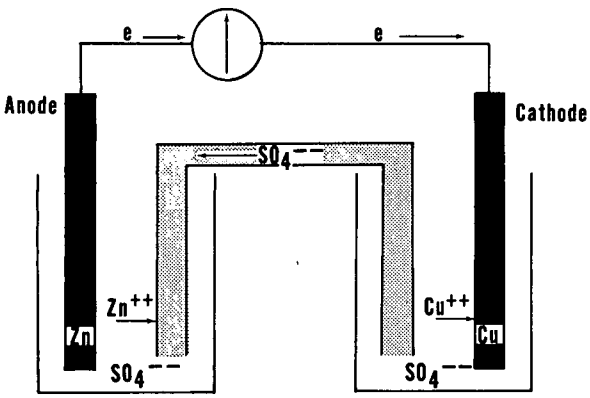
Note: Mention of Commercial Products and Manufacturers Does Not Imply Endorsement by the Environmental Protection Agency.

equivalent in terms of chemical and electrical energy and generally are reversible.

9 Electrolyte a solution, gel, or mixture capable of conducting electrical energy and serving as a reacting media for chemical changes. The electrolyte commonly contains an appropriate concentration of selected mobile ions to promote the desired reactions.

10 Electrochemical cell - a device consisting of an electrolyte in which 2 electrodes are immersed and connected via an external metallic conductor. The electrodes may be in separate compartments connected by tube containing electrolyte to complete the internal circuit.

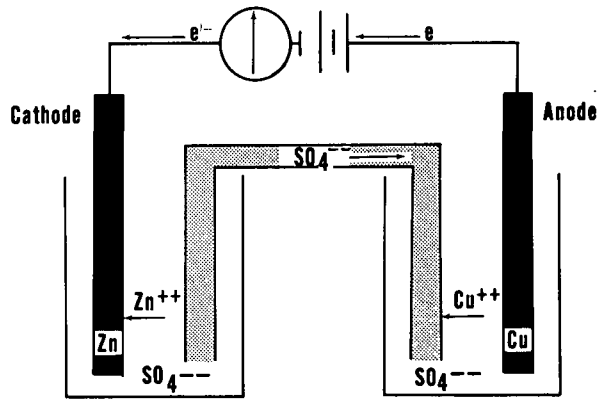
a Galvanic (or voltaic) cell - an electrochemical cell operated in such a way as to produce electrical energy from a chemical change, such as a battery (See Figure 1).



GALVANIC CELL

Figure 1

b Polarographic (electrolytic) cell - an electrochemical cell operated in such a way as to produce a chemical change from electrical energy (See Figure 2).



POLAROGRAPHIC CELL

Figure 2

D As indicated in I. C. 10 the sign of an electrode may change as a result of the operating mode. The conversion by the reactant of primary interest at a given electrode therefore designates terminology for that electrode and operating mode. In electronic oxygen analyzers, the electrode at which oxygen reduction occurs is designated the cathode.

E Each cell type has characteristic advantages and limitations. Both may be used effectively.

1 The galvanic cell depends upon measurement of electrical energy produced as a result of oxygen

reduction. If the oxygen content of the sample is negligible, the measured current is very low and indicator driving force is negligible, therefore response time is longer.

2 The polarographic cell uses a standing current to provide energy for oxygen reduction. The indicator response depends upon a change in the standing current as a result of electrons released during oxygen reduction. Indicator response time therefore is not dependent upon oxygen concentration.

3 Choice may depend upon availability, habit, accessories, or the situation. In each case it is necessary to use care and judgment both in selection and use for the objectives desired.

II ELECTRONIC MEASUREMENT OF DO

A Reduction of oxygen takes place in two steps as shown in the following equations:

1 $O_2 + 2H_2O + 2e \rightarrow H_2O_2 + 2OH^-$

2 $H_2O_2 + 2e^- \rightarrow 2OH^-$

Both equations require electron input to activate reduction of oxygen. The first reaction is more important for electronic DO measurement because it occurs at a potential (voltage) which is below that required to activate reduction of most interfering components (0.3 to 0.8 volts relative to the saturated calomel electrode - SCE). Interferences that may be reduced at or below that required for oxygen usually are present at lower concentrations in water or may be minimized by the use of a selective membrane or other means. When reduction occurs, a definite quantity of electrical energy is produced that is proportional to the quantity of reductant entering the reaction. Resulting current measurements thus are more specific for oxygen reduction.

B Most electronic measurements of oxygen are based upon one of two techniques for evaluating oxygen reduction in line with equation II.A.1. Both require activating energy, both produce a current proportional to the quantity of reacting reductant. The techniques differ in the means of supplying the activating potential; one employs a source of outside energy, the other uses spontaneous energy produced by the electrode pair.

1 The polarographic oxygen sensor relies upon an outside source of potential to activate oxygen reduction. Electron gain by oxygen changes the reference voltage.

a Traditionally, the dropping mercury electrode (DME) has been used for polarographic measurements. Good results have been obtained for DO using the DME but the difficulty of maintaining a constant mercury drop rate, temperature control, and freedom from turbulence makes it impractical for field use.

b Solid electrodes are attractive because greater surface area improves sensitivity. Poisoning of the solid surface electrodes is a recurrent problem. The use of selective membranes over noble metal electrodes has minimized but not eliminated electrode contamination. Feasibility has been improved sufficiently to make this type popular for regular use.

2 Galvanic oxygen electrodes consist of a decomposable anode and a noble metal cathode in a suitable electrolyte to produce activating energy for oxygen reduction (an air cell or battery). Lead is commonly used as the anode because its decomposition potential favors spontaneous reduction of oxygen. The process is continuous as long as lead and oxygen are in contact in the electrolyte and the electrical energy released at the cathode may be dissipated by an outside circuit. The anode may be conserved by limiting oxygen availability. Interrupting the outside circuit may produce erratic behavior for a time after reconnection. The resulting

current produced by oxygen reduction may be converted to oxygen concentration by use of a sensitivity coefficient obtained during calibration. Provision of a pulsed or interrupted signal makes it possible to amplify or control the signal and adjust it for direct reading in terms of oxygen concentration or to compensate for temperature effects.

III ELECTRONIC DO ANALYZER APPLICATION FACTORS

A Polarographic or galvanic DO instruments operate as a result of oxygen partial pressure at the sensor surface to produce a signal characteristic of oxygen reduced at the cathode of some electrode pair. This signal is conveyed to an indicating device with or without modification for sensitivity and temperature or other influences depending upon the instrument capabilities and intended use.

1 Many approaches and refinements have been used to improve workability, applicability, validity, stability and control of variables. Developments are continuing. It is possible to produce a device capable of meeting any reasonable situation, but situations differ.

2 Most commercial DO instruments are designed for use under specified conditions. Some are more versatile than others. Benefits are commonly reflected in the price. It is essential to determine the requirements of the measurement situation and objectives for use. Evaluation of a given instrument in terms of sensitivity, response time, portability, stability, service characteristics, degree of automation, and consistency are used for judgment on a cost/benefit basis to select the most acceptable unit.

B Variables Affecting Electronic DO Measurement

1 Temperature affects the solubility of oxygen, the magnitude of the resulting signal and the permeability of the protective membrane. A curve of oxygen solubility in water versus increasing temperature may be concave downward while a similar curve of sensor response versus temperature is concave upward. Increasing temperature decreases oxygen solubility and increases probe sensitivity and membrane permeability. Thermistor actuated compensation of probe response based upon a linear relationship or average of oxygen solubility and electrode sensitivity is not precisely correct as the maximum spread in curvature occurs at about 17°C with lower deviations from linearity above or below that temperature. If the instrument is calibrated at a temperature within + or - 5°C of working temperature, the compensated readout is likely to be within 2% of the real value. Depending upon probe geometry, the laboratory sensor may require 4 to 6% correction of signal per °C change in liquid temperature.

2 Increasing pressure tends to increase electrode response by compression and contact effects upon the electrolyte, dissolved gases and electrode surfaces. As long as entrained gases are not contained in the electrolyte or under the membrane, these effects are negligible.

Inclusion of entrained gases results in erratic response that increases with depth of immersion.

3 Electrode sensitivity changes occur as a result of the nature and concentration of contaminants at the electrode surfaces and possible physical chemical or electronic side reactions produced. These may take the form of a physical barrier, internal short, high residual current, or chemical changes in the metal surface. The membrane is intended to allow dissolved gas penetration but to exclude passage of ions or particulates. Apparently some ions or materials producing extraneous ions within the electrode vicinity are able to pass in limited amounts which

become significant in time. Dissolved gases include 1) oxygen, 2) nitrogen, 3) carbon dioxide, 4) hydrogen sulfide, and certain others. Item 4 is likely to be a major problem. Item 3 may produce deposits in alkaline media; most electrolytes are alkaline or tend to become so in line with reaction II.A.1. The usable life of the sensor varies with the type of electrode system, surface area, amount of electrolyte and type, membrane characteristics, nature of the samples to which the system is exposed and the length of exposure. For example, galvanic electrodes used in activated sludge units showed that the time between cleanup was 4 to 6 months for electrodes used for intermittent daily checks of effluent DO; continuous use in the mixed liquor required electrode cleanup in 2 to 4 weeks. Each electrometric cell configuration and operating mode has its own response characteristics. Some are more stable than others. It is necessary to check calibration frequency required under conditions of use as none of them will maintain uniform response indefinitely. Calibration before and after daily use is advisable.

4 Electrolytes may consist of solutions or gels of ionizable materials such as acids, alkalies or salts. Bicarbonates, KCl and KI are frequently used. The electrolyte is the transfer and reaction media, hence, it necessarily becomes contaminated before damage to the electrode surface may occur. Electrolyte concentration, nature, amount and quality affect response time, sensitivity, stability, and specificity of the sensor system. Generally a small quantity of electrolyte gives a shorter response time and higher sensitivity but also may be affected to a greater extent by a given quantity of contaminating substances.

5 Membranes may consist of teflon, polyethylene, rubber, and certain other polymeric films. Thickness may vary from 0.5 to 3 mils (inches × 1/1000). A thinner membrane will decrease response time and increase sensitivity but is less selective and may be ruptured more easily. The choice of material and its uniformity affects response time, selectivity and durability. The area of the membrane and its permeability are directly related to the quantity of transported materials that may produce a signal. The permeability of the membrane material is related to temperature and to residues accummulated on the membrane surface or interior. A cloudy membrane usually indicates deposition and more or less loss of signal.

6 Test media characteristics control the interval of usable life between cleaning and rejuvenation for any type of electrode. More frequent cleanup is essential in low quality waters than for high quality waters. Reduced sulfur compounds are among the more troublesome contaminants. Salinity affects the partial pressure of oxygen at any given temperature. This effect is small compared to most other variables but is significant if salinity changes by more than 500 mg/l.

7 Agitation of the sample in the vicinity of the electrode is important because DO is reduced at the cathode. Under quiescent conditions a gradient in dissolved oxygen content would be established on the sample side of the membrane as well as on the electrode side, resulting in atypical response. The sample should be agitated sufficiently to deliver a representative portion of the main body of the liquid to the outer face of the membrane. It is commonly observed that no agitation will result in a very low or negigible response after a short period of time. Increasing agitation will cause the response to rise gradually until some minimum liquid velocity is reached that will not cause a further increase in response with increased mixing energy. It is important to check mixing velocity to reach a stable high signal that is independent of a reasonable change in sample mixing. Excessive

mixing may create a vortex and expose the sensing surface to air rather than. sample liquid. This should be avoided. A linear liquid velocity of about 1 ft/sec at the sensing surface is usually adequate.

8 DO sensor response represents a potential or current signal in the milli-volt or milli-amp range in a high resistance system. A high quality electronic instrument is essential to maintain a usable signal-to-noise ratio. Some of the more common difficulties include:

a Variable line voltage or low batteries in amplifier power circuits.

b Substandard or unsteady amplifier or resistor components.

c Undependable contacts or junctions in the sensor, connecting cables, or instrument control circuits.

d Inadequately shielded electronic components.

e Excessive exposure to moisture, fumes or chemicals in the wrong places lead to stray currents, internal shorts or other malfunction.

C Desirable Features in a Portable DO Analyzer

1 The unit should include steady state performance electronic and indicating components in a convenient but sturdy package that is small enough to carry.

2 There should be provisions for addition of special accessories such as bottle or field sensors, agitators, recorders, line extensions, if needed for specific requirements. Such additions should be readily attachable and detachable and maintain good working characteristics.

3 The instrument should include a sensitivity adjustment which upon calibration will provide for direct reading in terms of mg of DO/liter.

4 Temperature compensation and temperature readout should be incorporated.

5 Plug in contacts should be positive, sturdy, readily cleanable and situated to minimize contamination. Water seals should be provided where necessary.

6 The sensor should be suitably designed for the purpose intended in terms of sensitivity, response, stability, and protection during use. It should be easy to clean, and reassemble for use with a minimum loss of service time.

7 Switches, connecting plugs, and contacts preferably should be located on or in the instrument box rather than at the "wet" end of the line near the sensor. Connecting cables should be multiple strand to minimize separate lines. Calibration controls should be convenient but designed so that it is not likely that they will be inadvertently shifted during use.

8 Agitator accessories for bottle use impose special problems because they should be small, self contained, and readily detachable but sturdy enough to give positive agitation and electrical continuity in a wet zone.

9 Major load batteries should be rechargeable or readily replaceable. Line operation should be feasible wherever possible.

10 Service and replacement parts availability are a primary consideration. Drawings, parts identification and trouble shooting memos should be incorporated with applicable operating instructions in the instrument manual in an informative organized form.

D Sensor and Instrument Calibration

The instrument box is likely to have some form of check to verify electronics, battery or other power supply conditions for use. The sensor commonly is not included in this check. A known reference

sample used with the instrument in an operating mode is the best available method to compensate for sensor variables under use conditions. It is advisable to calibrate before and after daily use under test conditions. Severe conditions, changes in conditions, or possible damage call for calibrations during the use period. The readout scale is likely to be labeled - calibration is the basis for this label.

The following procedure is recommended:

1 Turn the instrument on and allow it to reach a stable condition. Perform the recommended instrument check as outlined in the operating manual.

2 The instrument check usually includes an electronic zero correction. Check each instrument against the readout scale with the sensor immersed in an agitated solution of sodium sulfite containing sufficient cobalt chloride to catalyze the reaction of sulfite and oxygen. The indicator should stabilize on the zero reading. If it does not, it may be the result of residual or stray currents, internal shorting in the electrode, or membrane rupture. Minor adjustments may be made using the indicator rather than the electronic controls. Serious imbalance requires electrode reconditioning if the electronic check is O.K. Sulfite must be carefully rinsed from the sensor until the readout stabilizes to prevent carry over to the next sample.

3 Fill two DO bottles with replicate samples of clarified water similar to that to be tested. This water should not contain significant test interferences.

4 Determine the DO in one by the azide modification of the iodometric titration.

5 Insert a magnetic stirrer in the other bottle or use a probe agitator. Start agitation after insertion of the sensor assembly and note the point of stabilization.

a Adjust the instrument calibration control if necessary to compare with the titrated DO.

b If sensitivity adjustment is not possible, note the instrument stabilization point and designate it as ua. A sensitivity coefficient, ϕ is equal to $\frac{ua}{DO}$ where DO is the titrated value for the sample on which ua was obtained. An unknown DO then becomes DO = $\frac{ua}{\phi}$. This factor is applicable as long as the sensitivity does not change.

6 Objectives of the test program and the type of instrument influence calibration requirements. Precise work may require calibration at 3 points in the DO range of interest instead of at zero and high range DO. One calibration point frequently may be adequate.

Calibration of a DO sensor in air is a quick test for possible changes in sensor response. The difference in oxygen content of air and of water is too large for air calibration to be satisfactory for precise calibration for use in water.

IV This section reviews characteristics of several sample laboratory instruments. Mention of a specific instrument does not imply EPA endorsement or recommendation. No attempt has been made to include all the available instruments; those described are used to indicate the approach used at one stage of development which may or may not represent the current available model.

A The electrode described by Carrit and Kanwisher (1) is illustrated in Figure 3. This electrode was an early example of those using a membrane. The anode was a silver - silver oxide reference cell with a platinum disc cathode (1-3 cm diameter). The salt bridge consisted of N/2 KCl and

KOH. The polyethylene membrane was held in place by a retaining ring. An applied current was used in a polarographic mode. Temperature effects were relatively large. Thermistor correction was studied but not integrated with early models.

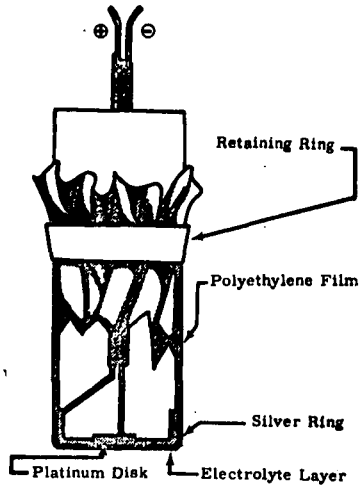


Figure 3

B The Beckman oxygen electrode is another illustration of a polarographic DO sensor (Figure 4). It consists of a gold cathode, a silver anode, an electrolytic gel containing KCl, covered by a teflon membrane. The instrument has a temperature readout and compensating thermistor, a source polarizing current, amplifier with signal adjustment and a readout DO scale with recorder contacts.

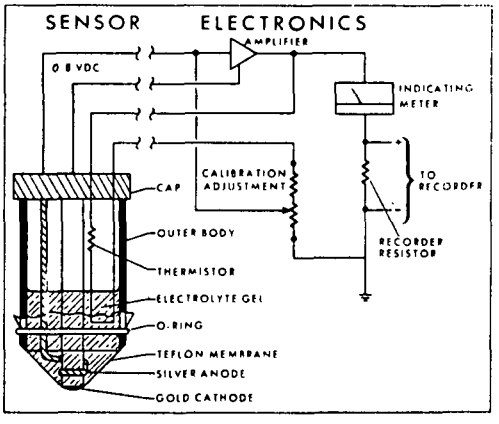


Figure 4. THE BECKMAN OXYGEN SENSOR

C The YSI Model 51 (3) is illustrated in Figure 5. This is another form of polarographic DO analyzer. The cell consists of a silver anode coil, a gold ring cathode and a KCl electrolyte with a teflon membrane. The instrument has a sensitivity adjustment, temperature and DO readout. The model 51 A has temperature compensation via manual preset dial. A field probe and bottle probe are available.

YSI Model 51 DO Sensor

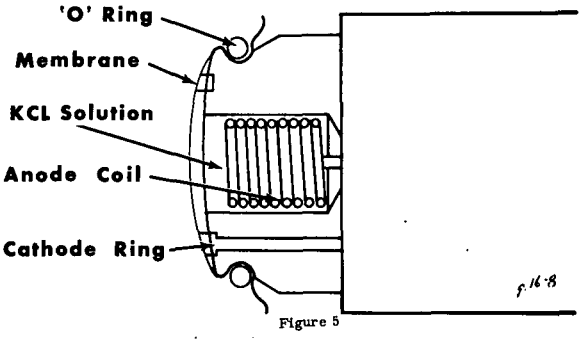


Figure 5

D The Model 54 YSI DO analyzer (4) is based upon the same electrode configuration but modified to include automatic temperature compensation, DO readout, and recorder jacks. A motorized agitator bottle probe is available for the Model 54 (Figure 6).

YSI Model 54 Agitator Probe

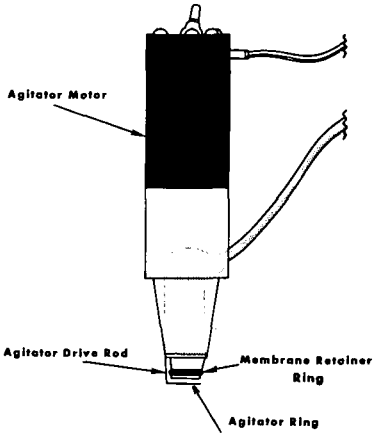


Figure 6

E The Galvanic Cell Oxygen Analyzer (7, 8) employs an indicator for proportional DO signal but does not include thermistor compensation or signal adjustment. Temperature readout is provided. The sensor includes a lead anode ring, and a silver cathode with KOH electrolyte (4 molar) covered by a membrane film (Figure 7).

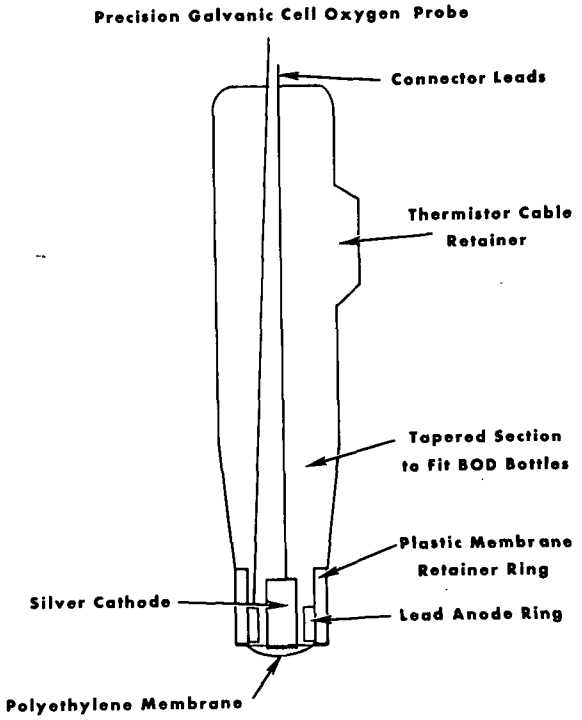


Figure 7

F The Weston and Stack Model 300 DO Analyzer (8) has a galvanic type sensor with a pulsed current amplifier adjustment to provide for signal and temperature compensation. DO and temperature readout is provided. The main power supply is a rechargeable battery. The sensor (Figure 8) consists of a lead anode coil recessed in the electrolyte cavity (50% KI) with a platinum cathode in the tip. The sensor is covered with a teflon membrane. Membrane retention by rubber band or by a plastic retention ring may be used for the bottle agitator or depth sampler respectively. The thermistor and agitator are mounted in a sleeve that also provides protection for the membrane.

G The EIL Model 15 A sensor is illustrated in Figure 9. This is a galvanic cell with thermistor activated temperature compensation and readout. Signal adjustment is provided. The illustration shows an expanded scheme of the electrode which when assembled compresses into a sensor approximating 5/8 inch diameter and 4 inch length exclusive of the enlargement at the upper end. The anode consists of compressed lead shot in a replaceable capsule (later models used fine lead wire coils), a perforated silver cathode sleeve around the lead is covered by a membrane film. The electrolyte is saturated potassium bicarbonate. The large area of lead surface, silver and membrane provides a current response of 200 to 300 microamperes in oxygen saturated water at 20° C for periods of up to 100 days use (8). The larger electrode displacement favors a scheme described by Eden (9) for successive DO readings for BOD purposes.

V Table 1 summarizes major characteristics of the sample DO analyzers described in Section IV. It must be noted that an ingenious analyst may adapt any one of these for special purposes on a do-it-yourself program. The sample instruments are mainly designed for laboratory or portable field use. Those designed for field monitoring purposes may include similar designs or alternate designs generally employing larger anode, cathode, and electrolyte capacity to approach better response stability with some sacrifice in response time and sensitivity. The electronic controls, recording, telemetering, and accessory apparatus generally are semi-permanent installations of a complex nature.

ACKNOWLEDGMENTS:

This outline contains certain materials from previous outlines by D. G. Ballinger, N.C. Malof, and J.W. Mandia. Additional information was provided by C.R. Hirth, C.N. Shadix, D.F. Krawczyk, J. Woods, and others.

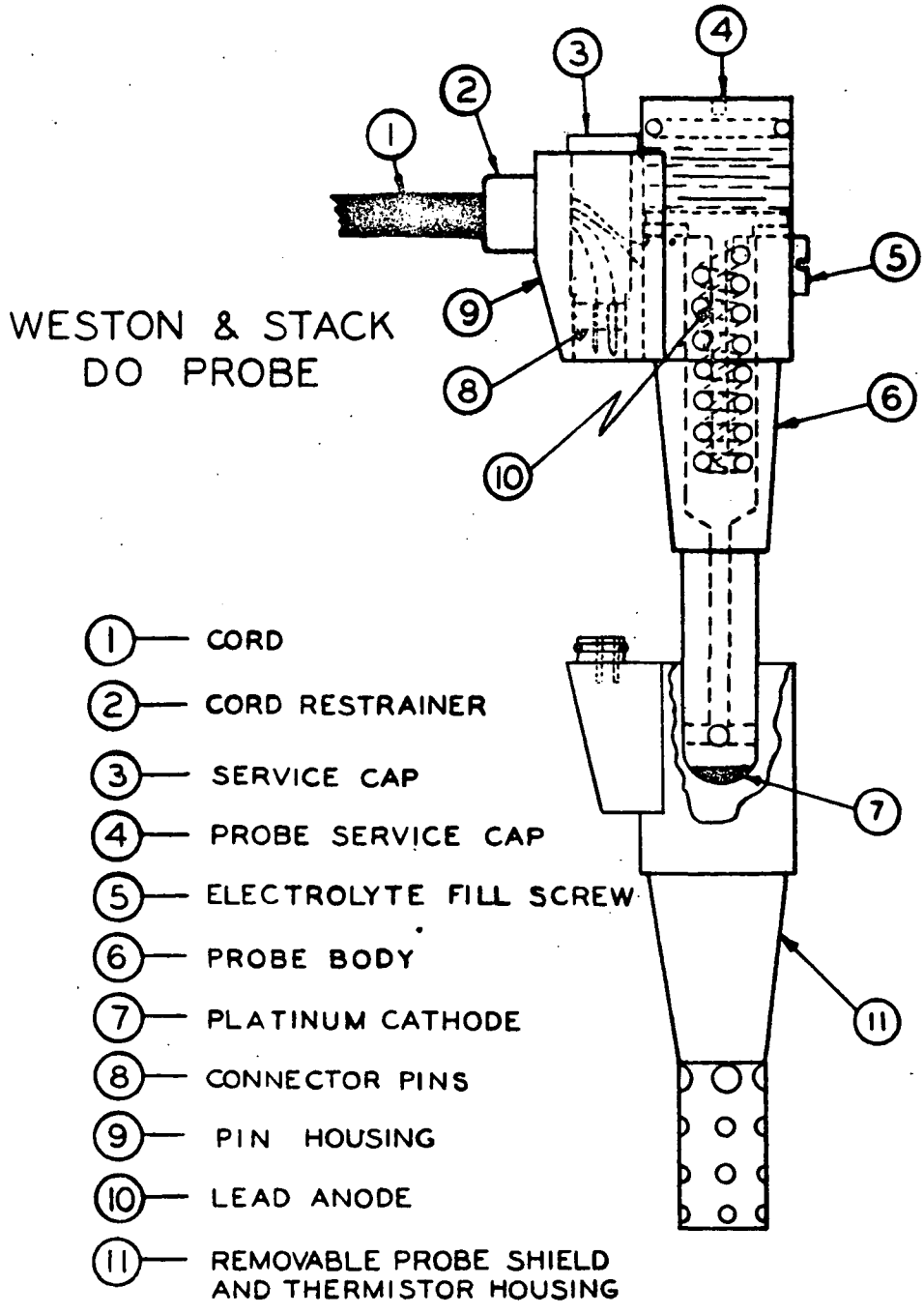
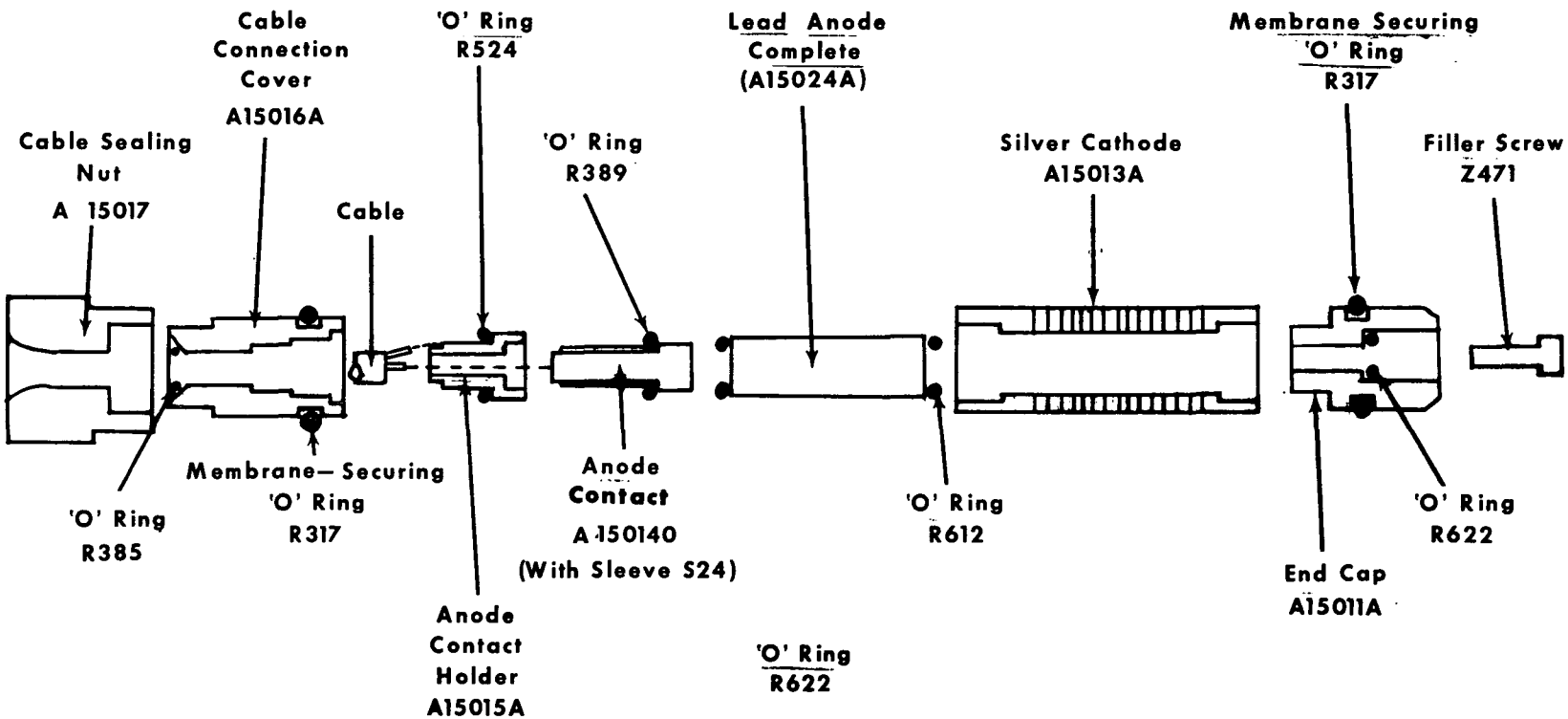

WESTON & STACK
DO PROBE
1 — CORD
2 — CORD RESTRAINER
3 — SERVICE CAP
4 — PROBE SERVICE CAP
5 — ELECTROLYTE FILL SCREW
6 — PROBE BODY
7 — PLATINUM CATHODE
8 — CONNECTOR PINS
9 — PIN HOUSING
10 — LEAD ANODE
11 — REMOVABLE PROBE SHIELD AND THERMISTOR HOUSING

Figure 8

Model A15A ELECTRODE COMPONENT PARTS



Note: Red wire of cable connects to Anode Contact Holder

Black wire of cable connects to Anode Contact

Membrane not shown E. I. L. part number T22.

Figure 9

TABLE 1

CHARACTERISTICS OF VARIOUS LABORATORY DO INSTRUMENTS

	Anode	Cathode	Elec	Type	Membr	DO Sig. Adj.	Temp. Comp. Temp.	Rdg.	Accessories for which designed
Carrit & Kanwisher	silver-silver ox. ring	Pt disc	KCl KOH N/2	pol*	polyeth	no	no		Recording temp. & signal adj. self assembled
Beckman	Aq ring	Au disc	KCl gel	pol	teflon	yes	yes	yes	recording
Yellow Springs 51	Ag coil	Au ring	KCl soln sat.	pol	teflon	yes	no*	yes	field and bottle probe
Yellow Springs 54	"	"	"	"	"	yes	yes	yes	recording field bottle & agitator probes
Precision Sci	Pb ring	silver disc	KOH 4N	galv**	polyeth	no	no	yes	
Weston & Stack 300	Pb coil	Pt disc	KI 40%	galv	teflon	yes	yes	yes	agit. probe depth sampler
EIL	Pb	Ag	$KHCO_3$	galv	teflon	yes	yes	yes	recording
Delta 75	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes	no	field bottle & agitator probe
Delta 85	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes	yes	field bottle & agitator probe

*Pol - Polarographic (or amperometric)
**Galv - Galvanic (or voltametric)

REFERENCES

1 Carrit, D.E. and Kanwisher, J.W. Anal. Chem. 31:5. 1959.

2 Beckman Instrument Company. Bulletin 7015, A Dissolved Oxygen Primer, Fullerton, CA. 1962.

3 Instructions for the YSI Model 51 Oxygen Meter, Yellow Springs Instrument Company, Yellow Springs, OH 45387.

4 Instructions for the YSI Model 54 Oxygen Meter, Yellow Springs Instrument Company, Yellow Springs, OH 45387.

5 Technical Bulletin TS-68850 Precision Scientific Company, Chicago, IL 60647.

6 Mancy, K.H., Okun, D.A. and Reilley, C.N. J. Electroanal. Chem. 4:65. 1962.

7 Instruction Bulletin, Weston and Stack Model 300 Oxygen Analyzer. Roy F. Weston, West Chester, PA 19380.

8 Briggs, R. and Viney, M. Design and Performance of Temperature Compensated Electrodes for Oxygen Measurements. Jour. of Sci. Instruments 41:78-83. 1964.

9 Eden, R. E. BOD Determination Using a Dissolved Oxygen Meter. Water Pollution Control. pp. 537-539. 1967.

10. Skoog, D. A. and West, D. M. Fundamentals of Analytical Chemistry. Holt, Rinehart & Winston, Inc. 1966.

11 Methods for Chemical Analysis of Water and Wastes. EPA -AQCL, Cincinnati, OH, July, 1971.

This outline was prepared by F. J. Ludzack Chemist, EPA, WPO, National Training Center, Cincinnati, OH 45268 and Nate Malof, Chemist, EPA, WPO, National Field Investigations Center, Cincinnati, OH

Descriptors: Chemical Analysis, Dissolved Oxygen, Dissolved Oxygen Analyzers, Instrumentation, On-Site Tests, Water Analysis, Analysis, Wastewater

DISSOLVED OXYGEN

Factors Affecting DO Concentration in Water

I The Dissolved Oxygen determination is a very important water quality criteria for many reasons:

A Oxygen is an essential nutrient for all living organisms. Dissolved oxygen is essential for survival of aerobic organisms and permits facultative organisms to metabolize more effectively. Many desirable varieties of macro or micro organisms cannot survive at dissolved oxygen concentrations below certain minimum values. These values vary with the type of organisms, stage in their life history, activity, and other factors.

B Dissolved oxygen levels may be used as an indicator of pollution by oxygen demanding wastes. Low DO concentrations are likely to be associated with low quality waters.

C The presence of dissolved oxygen prevents or minimizes the onset of putrefactive decomposition and the production of objectionable amounts of malodorous sulfides, mercaptans, amines, etc.

D Dissolved oxygen is essential for terminal stabilization wastewaters. High DO concentrations are normally associated with good quality water.

E Dissolved oxygen changes with respect to time, depth or section of a water mass are useful to indicate the degree of stability or mixing characteristics of that situation.

F The BOD or other respirometric test methods for water quality are commonly based upon the difference between an initial and final DO determination for a given sample time interval and condition. These measurements are useful to indicate:

1 The rate of biochemical activity in terms of oxygen demand for a given sample and conditions.

2 The degree of acceptability (a bioassay technique) for biochemical stabilization of a given microbiota in response to food, inhibitory agents or test conditions.

3 The degree of instability of a water mass on the basis of test sample DO changes over an extended interval of time.

4 Permissible load variations in surface water or treatment units in terms of DO depletion versus time, concentration, or ratio of food to organism mass, solids, or volume ratios.

5 Oxygenation requirements necessary to meet the oxygen demand in treatment units or surface water situations.

G The DO test is the only chemical test included in all Water Quality Criteria, Federal, State, Regional or local.

II FACTORS AFFECTING THE DO CONCENTRATION IN WATER

A Physical Factors:

1 DO solubility in water for an air/water system is limited to about 9 mg DO/liter of water at 20°C. This amounts to about 0.0009% as compared to 21% by weight of oxygen in air.

2 Transfer of oxygen from air to water is limited by the interface area, the oxygen deficit, partial pressure, the conditions at the

interface area, mixing phenomena and other items.

Certain factors tend to confuse reoxygenation mechanisms of water aeration:

a The transfer of oxygen in air to dissolved molecular oxygen in water has two principal variables:

1) Area of the air-water interface.

2) Dispersion of the oxygen-saturated water at the interface into the body liquid.

The first depends upon the surface area of the air bubbles in the water or water drops in the air; the second depends upon the mixing energy in the liquid. If diffusors are placed in a line along the wall, dead spots may develop in the core. Different diffusor placement or mixing energy may improve oxygen transfer to the liquid two or threefold.

b Other variables in oxygen transfer include:

3) Oxygen deficit in the liquid.

4) Oxygen content of the gas phase.

5) Time.

If the first four variables are favorable, the process of water oxygenation is rapid until the liquid approaches saturation. Much more energy and time are required to increase oxygen saturation from about 95 to 100% than to increase oxygen saturation from 0 to about 95%. For example: An oxygen-depleted sample often will pick up significant DO during DO testing; changes are unlikely with a sample containing equilibrium amounts of DO.

c The limited solubility of oxygen in water compared to the oxygen content of air does not require the interchange of a large mass of oxygen per unit volume of water to change DO saturation. DO increases from zero to 50% saturation are common in passage over a weir.

d Aeration of dirty water is practiced for cleanup. Aeration of clean water results in washing the air and transferring fine particulates and gaseous contaminants to the liquid.

e One liter of air at room temperature contains about 230 mg of oxygen. A 5 gal carboy of water with 2 liters of gas space above the liquid has ample oxygen supply for equilibration of DO after storage for 2 or 3 days or shaking for 30 sec.

f Aeration tends toward evaporative cooling. Oxygen content becomes higher than saturation values at the test temperature, thus contributing to high blanks.

3 Oxygen solubility varies with the temperature of the water. Solubility at 10° C is about two times that at 30° C. Temperature often contributes to DO variations much greater than anticipated by

solubility. A cold water often has much more DO than the solubility limits at laboratory temperature. Standing during warmup commonly results in a loss of DO due to oxygen diffusion from the supersaturated sample. Samples warmer than laboratory temperature may decrease in volume due to the contraction of liquid as temperature is lowered. The full bottle at higher temperature will be partially full after shrinkage with air entrance around the stopper to replace the void. Oxygen in the air may be transferred to raise the sample DO. For example, a volumetric flask filled to the 1000 ml mark at 30° C will show a water level about 1/2 inch below the mark when the water temperature is reduced to 20° C. BOD dilutions should be adjusted to 20° C + or - 1 1/2° before filling and testing.

4 Water density varies with temperature with maximum water density at 4° C. Colder or warmer waters tend to promote stratification of water that interferes with distribution of DO because the higher density waters tend to seek the lower levels.

5 Oxygen diffusion in a water mass is relatively slow, hence vertical and lateral mixing are essential to maintain relatively uniform oxygen concentrations in a water mass.

6 Increasing salt concentration decreases oxygen solubility slightly but has a larger effect upon density stratification in a water mass.

7 The partial pressure of the oxygen in the gas above the water interface controls the oxygen solubility limits in the water. For example, the equilibrium concentration of oxygen in water is about 9 mg DO/l under one atmospheric pressure of air, about 42 mg DO/liter in contact with pure oxygen and 0 mg DO/liter in contact with pure nitrogen (@ 20° C).

B Biological or Bio-Chemical Factors

1 Aquatic life requires oxygen for respiration to meet energy requirements for growth, reproduction, and motion. The net effect is to deplete oxygen resources in the water at a rate controlled by the type, activity, and mass of living materials present, the availability of food and favorability of conditions.

2 Algae, autotrophic bacteria, plants or other organisms capable of photosynthesis may use light energy to synthesize cell materials from mineralized nutrients with oxygen released in process.

a Photosynthesis occurs only under the influence of adequate light intensity.

b Respiration of alga is continuous.

c The dominant effect in terms of oxygen assets or liabilities of alga depends upon algal activity, numbers and light intensity. Gross algal productivity contributes to significant diurnal DO variations.

3 High rate deoxygenation commonly accompanies assimilation of readily available nutrients and conversion into cell mass or storage products. Deoxygenation due to cell mass respiration commonly occurs at some lower rate dependent upon the nature of the organisms present, the stage of decomposition and the degree of predation, lysis, mixing and regrowth. Relatively high

deoxygenation rates commonly are associated with significant growth or regrowth of organisms.

4 Micro-organisms tend to flocculate or agglomerate to form settleable masses particularly at limiting nutrient levels (after available nutrients have been assimilated or the number of organisms are large in proportion to available food).

a Resulting benthic deposits continue to respire as bed loads.

b Oxygen availability is limited because the deposit is physically removed from the source of surface oxygenation and algal activity usually is more favorable near the surface. Stratification is likely to limit oxygen transfer tothe bed load vicinity.

c The bed load commonly is oxygen deficient and decomposes by anaerobic action.

d Anaerobic action commonly is characterized by a dominant hydrolytic or solubilizing action with relatively low rate growth of organisms.

e The net effect is to produce low molecular weight products from cell mass with a correspondingly large fraction of feedback of nutrients to the overlaying waters. These lysis products have the effect of a high rate or immediate oxygen demand upon mixture with oxygen containing waters.

f Turbulence favoring mixing of surface waters and benthic sediments commonly are associated with extremely rapid depletion of DO. Recurrent resuspension of thin benthic deposits may contribute to highly erratic DO patterns.

g Long term deposition areas commonly act like point sources of new pollution as a result of the feedback of nutrients from the deposit. Rate of reaction may be low for old materials but a low percentage of a large mass of unstable material may produce excessive oxygen demands.

C Tremendous DO variations are likely in a polluted water in reference to depth, cross section or time of day. More stabilized waters tend to show decreased DO variations although it is likely that natural deposits such as leaf mold will produce differences related to depth in stratified deep waters.

ACKNOWLEDGMENTS

This outline contains significant materials from previous outlines by J. W. Mandia. Review and comments by C. R. Hirth and R. L. Booth are greatly appreciated.

REFERENCE

1 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, OH, July 1971.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268 and revised by Charles R. Feldmann, Chemist, National Training Center.

Descriptors: Aeration, Aerobic Conditions, Air-Water Interfaces, Anaerobic Conditions, Benthos, Biological Oxygen Demand, Dissolved Oxygen, Water Pollution, Water Quality

CHEMICAL OXYGEN DEMAND AND COD/BOD RELATIONSHIPS

I DEFINITION

A The Chemical Oxygen Demand (COD) test is a measure of the oxygen equivalent of that portion of the organic matter in a sample that is susceptible to oxidation under specific conditions of oxidizing agent, temperature and time.

B A variety of terms have been and are used for the test described here as COD:

1 Oxygen absorbed (OA) primarily in British practice.

2 Oxygen consumed (OC) preferred by some, but unpopular.

3 Chemical oxygen demand (COD) current preference.

4 Complete oxygen demand (COD) misnomer.

5 Dichromate oxygen demand (DOC) earlier distinction of the current preference for COD by dichromate or a specified analysis such as Standard Methods.

6 Others have been and are being used. Since 1960, terms have been generally agreed upon within most professional groups as indicated in I-A and B-3 and the explanation in B-5.

C The concept of the COD is almost as old as the BOD. Many oxidants and variations in procedure have been proposed, but none have been completely satisfactory.

1 Ceric sulfate has been investigated, but in general it is not a strong oxidant.

2 Potassium permanganate was one of the earliest oxidants proposed and until recently appeared in Standard Methods (9th ed.) as a standard procedure. It is currently used in British practice as a 4-hr. test at room temperature.

a The results obtained with permanganate were dependent upon concentration of reagent, time of oxidation, temperature, etc., so that results were not reproducible.

3 Potassium iodate or iodic acid is an excellent oxidant but methods employing this reaction are time-consuming and require a very close control.

4 A number of investigators have used potassium dichromate under a variety of conditions. The method proposed by Moore at SEC is the basis of the standard procedure.[1,2] Statistical comparisons with other methods are described.[3]

5 Effective determination of elemental carbon in wastewater was sought by Buswell as a water quality criteria.

a Van Slyke[4] described a carbon determination based on anhydrous samples and mixed oxidizing agents including sulfuric, chromic, iodic and phosphoric acids to obtain a yield comparable to the theoretical on a wide spectrum of components.

b Van Hall, et al.,[5] used a heated combustion tube with infrared detection to determine carbon quickly and effectively by wet sample injection.

6 Current development shows a trend to instrumental methods automating

conventional procedures or to seek elemental or more specific group determination.

II RELATIONSHIP OF THE COD TEST WITH OTHER OXIDATION CRITERIA IS INDICATED IN TABLE 1.

A Table 1

Test	Test Temp. °C	Reaction time	Oxidation system	Variables
BOD	20	days	Biol. prod. Enz. Oxidn.	Compound, environment, biota, time, numbers. Metabolic acceptability, etc.
COD	145	2 hrs.	50% H_2SO_4 $K_2Cr_2O_7$ May be catalyzed	Susceptibility of the test sample to the specified oxidation
IDOD	20	15'	Diss. oxyg.	Includes materials rapidly oxidized by direct action, Fe^{+2}, SH.
Van Slyke Carbon detn.	400+	1 hr.	H_3PO_4 HIO_3 H_2SO_4 $K_2Cr_2O_7$ Anhydrous	Excellent approach to theoretical oxidation for most compounds (N-nil)
Carbon by combustion +IR	950	minutes	Oxygen atm. catalyzed	Comparable to theoretical for carbon only.
Chlorine Demand	20	20 min.	HOCl soln.	Good NH_3 oxidn. Variable for other compounds.

B From Table 1 it is apparent that oxidation is the only common item of this series of separate tests.

1 Any relationships among COD & BOD or any other tests are fortuitous because the tests measure the oxidizability of a given sample under specified conditions, which are different for each test.

2 If the sample is primarily composed of compounds that are oxidized by both procedures (BOD and COD) a relationship may be established.

a The COD procedure may be substituted (with proper qualifications) for BOD or the COD may be used as an indication of the dilution required for setting up BOD analysis.

b If the sample is characterized by a predominance of material that can be chemically, but not biochemically oxidized, the COD will be greater than the BOD. Textile wastes, paper mill wastes, and other wastes containing high concentrations of cellulose have a high COD, low BOD.

c If the situation in item b is reversed the BOD will be higher than the COD. Distillery wastes or refinery wastes may have a high BOD, low COD, unless catalyzed by silver sulfate.

d Any relationship established as in 2a will change in response to sample history and environment. The BOD tends to decrease more rapidly than the COD. Biological cell mass or detritus produced by biological action has a low BOD but a relatively high COD. The COD/BOD ratio tends to increase with time, treatment, or conditions favoring stabilization.

III ADVANTAGES AND LIMITATIONS OF THE COD TEST[2] AS RELATED TO BOD

A Advantages

1 Time, manipulation, and equipment costs are lower for the COD test.

2 COD oxidation conditions are effective for a wider spectrum of chemical compounds.

3 COD test conditions can be standardized more readily to give more precise results.

4 COD results are available while the waste is in the plant, not several days later, hence, plant control is facilitated.

5 COD results are useful to indicate downstream damage potential in the form of sludge deposition.

6 The COD result plus the oxygen equivalent for ammonia and organic nitrogen is a good estimate of the ultimate BOD for many municipal wastewaters.

B Limitations

1 Results are not applicable for estimating BOD except as a result of experimental evidence by both methods on a given sample type.

2 Certain compounds are not susceptible to oxidation under COD conditions or are too volatile to remain in the oxidation flask long enough to be oxidized.

Ammonia, aromatic hydrocarbons, saturated hydrocarbons, pyridine, and toluene are examples of materials with a low analytical response in the COD test.

3 Dichromate in hot 50% sulfuric acid requires close control to maintain safety during manipulation.

4 Oxidation of chloride to chlorine is not closely related to BOD but may affect COD results.

5 It is not advisable to expect precise COD results on saline water.

IV BACKGROUND OF THE STANDARD METHODS COD PROCEDURE

A The COD procedure[1] considered dichromate oxidation in 33 and 50 percent sulfuric acid. Results indicated preference of the 50 percent acid concentration for oxidation of sample components. This is the basis for the present standard procedure.

B Muers[6] suggested addition of silver sulfate to catalyze oxidation of certain low molecular weight aliphatic acids and alcohols. The catalyst also improves oxidation of most other organic components to some extent but does not make the COD test universally applicable for all chemical pollutants.

C The unmodified COD test result (A) includes oxidation of chloride to chlorine. Each mg of chloride will have a COD equivalent of 0.23 mg. Chlorides must be determined in the sample and the COD result corrected accordingly.

1 For example, if a sample shows 300 mg of COD per liter and 200 mg Cl^- per liter the corrected COD result will be 300 -(200 x 0.23)or 300 - 46 = 254 mg COD/1 on a chloride corrected basis.

2 Silver sulfate addition as a catalyst tends to cause partial precipitation of silver chloride even in the hot acid solution. Chloride corrections are questionable unless the chloride is oxidized before addition of silver sulfate, i.e., reflux for 15 minutes for chloride oxidation, add Ag_2SO_4, and continue the reflux or use of $HgSO_4$(D).

D Dobbs and Williams [7] proposed prior complexation of chlorides with $HgSO_4$ to prevent chloride oxidation during the test. A ratio of about 10 of Hg^{++} to 1 of Cl^- (wt. basis) appears essential. The Cl^- must be complexed in acid solution before addition of dichromate and silver sulfate.

1 For unexplained reasons the $HgSO_4$ complexation does not completely prevent chloride oxidation in the presence of high chloride concentrations.

2 Factors have been developed to provide some estimate of error in the result due to incomplete control of chloride behavior. These tend to vary with the sample and technique employed.

E It is not likely that COD results will be precise for samples containing high chlorides. Sea water contains 18000 to 21000 mgCl^-/1 normally. Equivalent chloride correction for COD exceeds 4000 mg/1. The error in chloride determination may give negative COD results upon application of the correction.

Incomplete control of chloride oxidation with $HgSO_4$ may give equally confusing results.

$HgSO_4$ appears to give precise results for COD when chlorides do not exceed about 2000 mg/1. Interference increases with increasing chlorides at higher levels.

F The 12th edition of Standard Methods reduced the amount of sample and reagents to 40% of amounts utilized in previous editions. There has been no change in the relative proportions in the test. This step was taken to reduce the cost of providing expensive mercury and silver sulfates required. Results are comparable as long as the proportions are identical. Smaller aliquots of sample and reagents require more care during manipulation to promote precision.

G The EPA Methods for COD

1 For routine level COD (samples having an organic carbon concentration greater than 15 mg/liter and a chloride concentration less than 2000 mg/liter), the EPA specifies the procedures found in Standard Methods [2] and in ASTM[8].

2 For low level COD (samples with less than 15 mg/liter organic carbon and chloride concentration less than 2000 mg/liter), EPA provides an analytical procedure [9]. The difference from the routine procedure primarily involves a greater sample volume and more dilute solutions of dichromate and ferrous ammonium sulfate.

3 For saline samples (chloride level exceeds 2000 mg/liter), EPA provides an analytical procedure[9] involving preparation of a standard curve of COD versus mg/liter chloride to correct the calculations. Volumes and concentrations for the sample and reagents are adjusted for this type of determination.

V PRECISION AND ACCURACY[9]

Eighty six analysts in 58 laboratories analyzed a distilled water solution containing oxidizable organic material equivalent to 270 mg/l COD. The standard deviation was ± 17.76 mg/l COD with an accuracy as percent relative error (bias) of –4.7%. For a solution equivalent to 12.3 mg/l COD (low level), the standard deviation was ± 4.15 mg/l with an accuracy as percent relative error (bias) of 0.3%. (EPA Method Research Study 3)

VI REMARKS PERTINENT TO EFFECTIVE COD DETERMINATIONS INCLUDE:

A Sample size and COD limits for 0.250 N reagents are approximately as given. For 0.025 N reagents multiply COD by 0.1. Use the weak reagent for COD's in the range of 5-50 mg/1, (low level).

Sample Size	mg COD/1
20 ml	2000
10 ml	4000
5 ml	8000

B Most organic materials oxidize relatively rapidly under COD test conditions. A significant fraction of oxidation occurs during the heating upon addition of acid but the orange color of dichromate should remain. If the sample color changes from orange to green after acid addition the sample was too large. Discard without reflux and repeat with a smaller aliquot until the color after mixing does not go beyond a brownish hue. The dichromate color change is less rapid with sample components that are slowly oxidized under COD reaction conditions.

C Chloride concentrations should be known for all test samples so appropriate analytical techniques can be used.

D Special precautions advisable for the regular COD procdure and essential when using 0.025 N reagents include:

1 Keep the apparatus assembled when not in use.

2 Plug the condenser breather tube with glass wool to minimze dust entrance.

3 Wipe the upper part of the flask and lower part of the condenser with a wet towel before disassembly to minimize sample contamination.

4 Steam out the condenser after use for high concentration samples and periodically for regular samples. Use the regular blank reagent mix and heat, without use of condenser water, to clean the apparatus of residual oxidizable components.

5 Distilled water and sulfuric acid must be of very high quality to maintain low blanks on the refluxed samples for the 0.025 N oxidant.

VII NPDES METHODOLOGY

Under the National Pollutant Discharge Elimination System, the accepted method (Federal Register, Tuesday, October 16, 1973, Volume 38, number 199, Part II) for doing the Chemical Oxygen Demand Test is given in Standard Methods,[2] p. 495; ASTM,[8] p. 219 and the EPA Manual,[9] p. 20.

ACKNOWLEDGEMENT:

Certain portions of this outline contain training material from prior outlines by R. C. Kroner, R. J. Lishka, and J. W. Mandia.

REFERENCES:

1 Moore, W. A., Kroner, R. C. and Ruchhoft, C. C. Anal. Chem. 21:953, 1949.

2 Standard Methods, 13th Edition, APHA-AWWA-WPCF, 1971.

3 Moore, W. A., Ludzack, F. J. and Ruchhoft, C. C. Anal. Chem. 23:1297, 1951.

4 Van Slyke, D. D. and Folch, J. J. Biol. Chem. 136:509, 1940.

5 Van Hall, C. E., Safranko, J. and Stenger, V. A., Anal. Chem. 35:315, 1963.

6 Muers, M. M. J. Soc. Chem. Ind. (London) 55:711, 1936.

7 Dobbs, R. A. and Williams, R. T., Anal. Chem. 35:1064, 1963.

8 ASTM Standards, Part 23, Water: Atmospheric Analysis, 1972.

9 Methods for Chemical Analysis of Water and Wastes, EPA-MDQARL, Cincinnati, Ohio 45268, 1974.

This outline was prepared by F. J. Ludzack, Chemist, EPA, WPO, National Training Center, Cincinnati, OH 45268.

Descriptors: Analysis, Biochemical Oxygen Demand, Chemical Analysis, Chemical Oxygen Demand, Chlorides, Oxygen Demand, Wastewater, Water Analysis

LABORATORY PROCEDURE FOR ROUTINE LEVEL CHEMICAL OXYGEN DEMAND

I REAGENTS

A Standard Potassium Dichromate (0.250 N):

Dissolve 12.259 g of primary standard grade $K_2Cr_2O_7$, which had been dried at 103° C for two hours, in distilled water and dilute to one liter.

B Ferrous Ammonium Sulfate (0.1N):

Dissolve 39 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water.
Carefully add 20 ml of concentrated H_2SO_4.
Cool and dilute to one liter.

C Ferroin Indicator:

Dissolve 1.485 g 1, 10-phenanthroline monohydrate and 0.695 g $FeSO_4 \cdot 7H_2O$ in water and dilute to 100 ml. This indicator may be purchased already prepared.

D Concentrated Sulfuric Acid (36 N):

E Mercuric Sulfate: Analytical Grade

F Silver Sulfate: Analytical Grade

G Concentrated Sulfuric Acid - Silver Sulfate:

Dissolve 22 g of silver sulfate in a 9 lb bottle of concentrated sulfuric acid. (1-2 days required for dissolution)

II EQUIPMENT PREPARATION

Before use, the Erlenmeyer flask (500 ml, 24/40 standard taper joint) and reflux condenser (Friedrichs, 24/40 standard taper joint) should be steamed out to remove trace organic contaminants. Add 10 ml of 0.250 N $K_2Cr_2O_7$, 50 ml of distilled water, and several bumping stones to the flask. Carefully add 20 ml of concentrated H_2SO_4 and mix thoroughly. Connect the flask and condenser, but do not turn on the water to the condenser. Boil the mixture so that steam emerges from the top of the condenser for several minutes. Cool the mixture, carefully discard the acid, and rinse the condenser and flask with distilled water. In order to prevent contamination from air-borne particles, the top of the condenser should be lightly plugged with glass wool during storage and use.

III STANDARDIZATION OF FERROUS AMMONIUM SULFATE

A Dilute 10.0 ml of the standard potassium dichromate to about 100 ml with distilled water.

B Add 30 ml of concentrated H_2SO_4 and allow to cool.

C Add 2-3 drops of ferroin indicator and titrate to a reddish-brown end point with the ferrous ammonium sulfate. Calculate the normality, N, of the ferrous ammonium sulfate.

D Calculation

N of $Fe(NH_4)_2(SO_4)_2$ =

$$\frac{\text{ml } K_2Cr_2O_7 \times N \text{ of } K_2Cr_2O_7}{\text{ml } Fe(NH_4)_2(SO_4)_2}$$

IV PROCEDURE

A Place 0.4 g $HgSO_4$ in a 500 ml 24/40 standard taper Erlenmeyer flask. Add 20 ml of sample, or an aliquot diluted to 20 ml with distilled water.

B Add 3 ml of concentrated H_2SO_4 and swirl to dissolve the $HgSO_4$.

C Add 10.0 ml of the 0.250 N $K_2Cr_2O_7$ and mix.

D Carefully add 27 ml of the sulfuric acid - silver sulfate reagent.

E Add several pumice granules or glass beads to prevent bumping, and then swirl the mixture to insure complete mixing.

F Reflux the mixture for two hours.

G Allow the solution to cool, wash down the condenser with distilled water, and add about 75 ml water to bring the volume to about 150 ml.

H Add 2-3 drops of the ferroin indicator and titrate the solution to a reddish-brown end point with the ferrous ammonium sulfate.

I A blank consisting of 20 ml of distilled water and containing all reagents is refluxed and titrated in the same manner as the sample.

J Calculation

$$\text{mg COD/l} = \frac{(A-B)\ N \times 8 \times 1000}{\text{ml of sample}}$$

COD = chemical oxygen demand

A = ml $Fe(NH_4)_2(SO_4)_2$ used for blank

B = ml $Fe(NH_4)_2(SO_4)_2$ used for sample

N = N of $Fe(NH_4)_2(SO_4)_2$

8 = equivalent weight of oxygen

ACKNOWLEDGEMENT:

Portions of this outline were taken from an outline prepared by R. J. Liska.

REFERENCES

1 Methods for Chemical Analysis of Water and Wastes, EPA-MDQARL, Cincinnati, Ohio, 1974.

2 Standard Methods, APHA-AWWA-WPCF, 13th Edition, 1971.

This outline was prepared by Charles R. Feldmann, Chemist, EPA, WPO, National Training Center, Cincinnati, OH 45268.

Descriptors: Chemical Analysis, Chemical Oxygen Demand, Organic Compounds, Oxidation, Oxygen, Oxygen Demand, Oxygen Requirements, Water Analysis

ACIDITY, ALKALINITY, pH AND BUFFERS

I DEFINITIONS OF ACIDS AND BASES

A Arrhenius Theory of Acids and Bases (Developed about 1887)

1 Acid: A substance which produces, in aqueous solution, a hydrogen ion (proton), H^+.

2 Base: A substance which produces, in aqueous solution, a hydroxide ion, OH^-.

3 The Arrhenius theory was confined to the use of water as a solvent.

B Bronsted and Lowry Theory of Acids and Bases (Developed about 1923).

1 Acid: A substance which donates, in chemical reaction, a hydrogen ion (proton).

2 Base: A substance which accepts, in chemical reaction, a hydrogen ion (proton).

3 Bronsted and Lowry had expanded the acid-base concept into non-aqueous media; i.e., the solvent could, but did not have to be, water.

C There are other acid-base theories. The two above are probably the most commonly used ones when discussing wastewater topics however.

II DEFINITIONS OF ACIDITY, ALKALINITY AND NEUTRALITY

A Acidity

A condition in which there is a preponderance of acid materials present in the water.

B Alkalinity

A condition in which there is a preponderance of alkaline (or basic) materials present in the water.

C Neutrality

1 It is possible to have present in the water chemically equivalent amounts of acids and bases. The water would then be described as being neutral; i.e., there is a preponderance of neither acid nor basic materials. The occurrence of such a condition would be rare.

2 The term "neutralization" refers to the combining of chemically equivalent amounts of acids and bases. The two products of neutralization are a salt and water.

$$HCl + NaOH = NaCl + H_2O$$

Hydrochloric acid	Sodium Hydroxide	Sodium Chloride (a salt)

D The key word in the above definitions is "preponderance." It is possible to have a bas ion of acidity while there are basic materials present in the water, as well as conversely.

III HOW ARE DEGREES OF ACIDITY AND ALKALINITY EXPRESSED?

The pH scale is used to express various degrees of acidity and alkalinity. Values can range from 0 to 14. These two extremes are of theoretical interest and would never be encountered in a natural water or in a waste water. pH readings from 0 to just under 7 indicate an acidic condition; from just over 7 to 14, an alkaline condition. Neutrality exists if the pH value is exactly 7. pH paper, or a pH meter, provides the most convenient method of obtaining pH readings. It should be noted, that under NPDES Methodology, pH measurements are to be made using a pH meter. Some common liquids and pH values are listed in Table I.

TABLE 1. pH Values of Common Liquids	
Household lye	13.7
Bleach	12.7
Ammonia	11.3
Milk of magnesia	10.2
Borax	9.2
Baking soda	8.3
Sea water	8.0
Blood	7.3
Distilled water	7.0
Milk	6.8
Corn	6.2
Boric acid	5.0
Orange juice	4.2
Vinegar	2.8
Lemon juice	2.2
Battery acid	0.2

5.9 - 8.4 is the common pH range for most natural waters.

IV HARD AND SOFT WATERS

In addition to being acidic or basic, water can also be described as being hard or soft.

A Hard water contains large amounts of calcium, magnesium, strontium, manganese and iron ions, relative to the amount of sodium and potassium ions present. Hard water is objectionable because it forms insoluble compounds with ordinary soap.

B Soft water contains small amounts of calcium, magnesium, strontium, manganese and iron ions, relative to the amount of sodium and potassium ions present. Soft water does not form insoluble compounds with ordinary soap.

V TITRATIONS

A The conversion of pH readings into such quantities as milligrams (mg) of acidity, alkalinity, or hardness, is not easily carried out. These values are more easily obtained by means of a titration.

B In a titration, an accurately measured volume of sample (of unknown strength) is combined with an accurately measured volume of standard solution (of known strength) in the presence of a suitable indicator.

C The strength (called normality) of the sample is then found using the following expression:

milliliters (ml) of sample × normality (N) of sample = ml of standard solution × N of standard solution.

Three of the four quantities are known, and

N of sample = ml of standard solution × N of standard solution/ml of sample.

D In modified form, and a more specific application of the above equation, alkalinity is calculated in the following manner (13th ed. Standard Methods).

mg of alkalinity as mg $CaCO_3$/liter (l) = ml of standard H_2SO_4 × N of standard H_2SO_4 × 50 × 1000/ml sample.

VI INDICATORS

The term "suitable indicator" was used above. At the end of a titration, the pH of the solution will not necessarily be 7. It may be above or below 7. A suitable indicator, therefore, is one which undergoes its characteristic color change at the appropriate pH. Below are a few examples of indicators and the pH range in which they undergo their characteristic color changes. In some cases, mixed indicators may be used in order to obtain a sharper and more definite color change. Again it should be noted that under NPDES, pH meters are to be used for the measurement of pH.

Indicator	Operational pH Range
Methyl Yellow	2.8 - 4.0
Methyl Orange	3.1 - 4.4
Methyl Red	4.4 - 6.2
Cresol Purple	7.4 - 9.0
Phenolphthalein	8.0 - 9.6
Alizarine Yellow	10.0 - 12.0

TABLE 2. pH Range of Indicators

VII BUFFERS

A A buffer is a combination of substances which, when dissolved in water, resists a pH change in the water, as might be caused by the addition of acid or alkali. Listed below are a few chemicals which, when combined in the proper proportions, will tend to maintain the pH in the indicated range.

Chemicals	pH Range
Acetic Acid + Sodium Acetate	3.7 - 5.6
Sodium Dihydrogen Phosphate + Disodium Hydrogen Phosphate	5.8 - 8.0
Boric Acid + Borax	6.8 - 9.2
Borax + Sodium Hydroxide	9.2 - 11.0

TABLE 3. pH Range of Buffers

B A buffer functions by supplying ions which will react with hydrogen ions (acid "spill"), or with hydroxide ions (alkali "spill").

C In many instances, the buffer is composed of a weak acid and a salt of the weak acid; e.g., acetic acid and sodium acetate.

1 In water, acetic acid ionizes or "breaks down" into hydrogen ions and acetate ions.

$$HC_2H_3O_2 = H^+ + C_2H_3O_2^-$$

(acetic acid) (hydrogen ion) (proton) (acetate ion)

This ionization occurs to only a slight extent, however, most of the acetic acid remains in the form of $HC_2H_3O_2$; only a small amount of hydrogen and acetate ions is formed.

2 Thus, acetic acid is said to be a weak acid.

3 In the case of other acids, ionization into the component ions occurs to a large degree, and the term strong acid is applied; e.g., hydrochloric acid.

$$HCl = H^+ + Cl^-$$

(hydrochloric acid) (hydrogen ion) (proton) (chloride ion)

4 The terms "strong" and "weak" are also applied to bases. In water solutions, those which break down into their component ions to a large extent are termed "strong", and those which do not are "weak". Sodium hydroxide is a relatively strong base, while ammonium hydroxide is realtively weak.

5 Sodium acetate (a salt of acetic acid) dissociates or "breaks down" into sodium ions and acetate ions when placed in water.

$$NaC_2H_3O_2 = Na^+ + C_2H_3O_2^-$$

(sodium acetate) (sodium ion) (acetate ion)

This dissociation occurs to a large extent, and practically all of the sodium acetate is in the form of sodium ions and acetate ions.

D It would be difficult and expensive to prepare large quantities of buffers for use in a treatment plant. However, certain naturally occurring buffers may be available (carbon dioxide is an example). It dissolves in water to form the species indicated below.

$$CO_2 + H_2O = H_2CO_3$$

(carbon dioxide) (Water) (carbonic acid)

$$H_2CO_3 = H^+ + HCO_3^-$$

(hydrogen ion) (proton) (hydrogen carbonate ion) (bicarbonate)

The hydrogen ions react with hydroxide ions which might appear in the water as the result of an alkali "spill".

$$H^+ + OH^- = H_2O$$

(in the buffer) (hydroxide ion "spilled")

The hydrogen carbonate ions react with hydrogen ions which might appear in the water as the result of an acid "spill".

$$H^+ + HCO_3^- = H_2CO_3$$

(hydrogen ion) (proton) "spilled" (in the buffer)

This buffering action will be in effect as long as there is carbonic acid present.

E Buffering action is not identical with a process in which acid wastes are "neutralized" with alkali wastes, or conversely. The desired effect is achieved in both cases, however (i.e., the pH is maintained within a desired range.)

This outline was prepared by C. R. Feldmann, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268

Descriptors: Acids, Acidity, Alkalis, Alkalinity, Analytical Techniques, Buffers, Buffering Capacity, Chemical Analysis, Hydrogen Ion Concentration, Indicators, Neutralization, Water Analysis.

OPERATING CHARACTERISTICS AND USE OF THE pH METER

I INTRODUCTION

pH is a term used to describe the intensity of the acid or alkaline condition of a solution. The concept of pH evolved from a series of developments that led to a fuller understanding of acids and alkaline solutions (bases). Acids and bases were originally distinguished by their difference in physical characteristics (acids-sour, bases-soapy feel). In the 18th century it was recognized that acids have a sour taste (vinegar-acetic acid), that they react with limestone with the liberation of a gaseous substance (carbon dioxide) and that neutral substances result from their interaction with alkaline solutions.

Acids are also described as compounds that yield hydrogen ions when dissolved in water. And that bases yield hydroxide ions when dissolved in water. The process of neutralization is then considered to be the union of hydrogen (H^+) ions and hydroxyl (OH^-) ions to form neutral water ($H^+ + OH \longrightarrow H_2O$).

It has been determined that there are 1/10,000,000 grams of hydrogen ions and 17/10,000,000 grams of hydroxyl ions in one liter of pure water. The product of the H^+ and OH^- ions equal a constant value. Therefore, if the concentration of the H^+ ions is increased there is a corresponding decrease in OH^- ions. The acidity or alkalinity, hydrogen ion concentration of a solution is given in terms of pH. The pH scale extends from 0 to 14 with the neutral point at 7.0.

II INSTRUMENTATION

A General

Because of the differences between the many makes and model of pH meters which are available commercially, it is impossible to provide detailed instructions for the correct operation of every instrument. In each case, follow the manufacturer's instructions. Thoroughly wet the glass electrode and the calomel electrode and prepare for use in accordance with the instructions given. Standardize the instrument against a buffer solution with a pH approaching that of the sample, and then check the linearity of electrode response against at least one additional buffer of a different pH. The readings with the additional buffers will afford a rough idea of the limits of accuracy to be expected of the instrument and the technic of operation.

B Electrode Design

About 1925 it was discovered that an electrode could be constructed of glass which would develop a potential related to the hydrogen-ion concentration without interference from most other ions. The glass pH electrode is the nearest approach to a universal pH indicator known at present. It works on the principle of establishing a potential across a pH-sensitive, glass membrane whose magnitude is proportional to the difference in pH of the solution separated by this membrane.

All glass pH indicating electrodes have a similar basic design. Contained on one side of an appropriate glass membrane is a solution of constant pH. In contact with the other side of this pH sensitive glass is the solution of unknown pH. Between the surfaces of the glass membrane, a potential is established which is proportional to the pH difference of these solutions. As the pH of one solution is constant, this developed potential is a measure of the pH of the other.

To measure this potential, a half-cell is introduced into both the constant, internal solution and into the unknown, external solution. These half-cells are in turn connected to your pH meter. The internal reversible half-cell sealed within the chamber of constant pH is almost exclusively a wire of silver-silver chloride. The external reversible half-cell is often silver-silver chloride. If both the internal and external electrodes are combined in a common pH measuring device, the electrode is a combination pH electrode.

As the function of these half-cells is to provide a steady reference voltage against which voltage changes at the glass pH sensitive membrane can be referred, they must be protected from contamination and dilution by the unknown solutions. This is accomplished by permanently sealing the internal half-cell in a separate chamber which makes electrical contact to the unknown solution through a porous ceramic plug. This ceramic plug allows current to flow, but does not permit exchange of solution to this chamber. Gradually the KC1 solution is slowly lost, therefore a filling port is placed in this electrode so that additional saturated potassium chloride can be added.

C Instrument Calibration

The pH balance control, by adding a voltage in series with the pH electrode system, allows the operator to adjust the meter readout to conform to the pH of the calibrating buffer. In general, calibrate the meter in the general range of the unknown solution. Appropriate buffers can be selected (pH 4.0, 6.8, 7.4 and 10.0). Always set the temperature compensator on the instrument to the temperature of the standard buffer solution.

For most accurate analysis the pH of the sample should be determined, and then buffered solutions of a pH above and below the determined pH should be selected to re-calibrate the instrument and the determination of the pH of the sample repeated for a final reading.

D Precision and Accuracy

The precision and accuracy attainable with a given pH meter will depend upon the type and condition of the instrument employed and the technique of standardization and operation. With the proper care, a precision of $\pm$ 0.02 pH unit and an accuracy of $\pm$ 0.05 pH unit can be achieved with many of the new and improved models. However, $\pm$ 0.1 pH unit represents the limit of accuracy under normal conditions. For this reason, pH values generally should be reported to the nearest 0.1 pH unit.

E Maintenance Practices

The reference chamber of the pH electrode system should always be kept nearly full of saturated KC1 solution. Routinely check the level and saturation of potassium chloride in this reference chamber and add saturated KC1 if necessary.

The pH sensitive glass membrane dehydrates when removed from water, and thus it is imperative that dry electrodes be soaked in buffer or water for several hours before use. To avoid this break-in period always keep the glass pH sensitive membrane wet between periods of use.

The buffers are pH standards; do not contaminate them.

If the meter is a battery operated instrument. To conserve the battery life, the instrument should be turned off when not in use.

III PROCEDURE

In the measurement of pH values of industrial wastes, effluents, sludges and similar

samples, the electrodes must be thoroughly rinsed with buffer solution between samples and after calibrating. Buffer solutions can be prepared by use of the formulations shown in Table I.

The electrodes should be kept free of oil and grease and stored in water when not in use. In testing samples containing gaseous or volatile components which affect the pH value, any handling technic such as stirring or heating may cause loss of such components and thereby introduce error. For example, the loss of carbon dioxide from an anaerobic sludge digester sample due to stirring will result in an observed pH value which is too

Table I

Preparation of pH Standard Solutions

Standard Solution (Molality)	pH at 25°C	Weight of Chemicals Needed per 1,000 ml of Aqueous Solution at 25°C
Primary standards		
Potassium hydrogen tartrate (saturated at 25°C)	3.557	6.4g$KHC_4H_4O_6$*
0.05 potassium dihydrogen citrate	3.776	11.41g$KH_2C_6H_5O_7$
0.05 potassium hydrogen phthalate	4.008	10.12g$KHC_8H_4O_4$
0.025 potassium dihydrogen phosphate + 0.025 disodium hydrogen phosphate	6.865	3.388gKH_2PO_4† + 3.533gNa_2HPO_4†‡
0.008695 potassium dihydrogen phosphate + 0.03043 disodium hydrogen phosphate	7.413	1.179gKH_2PO_4† + 4.302gNa_2HPO_4†‡
0.01 sodium borate decahydrate (borax)	9.180	3.80g$Na_2B_4O_7 \cdot 10H_2O$‡
0.025 sodium bicarbonate + 0.025 sodium carbonate	10.012	2.092g$NaHCO_3$ + 2.640gNa_2CO_3
Secondary Standards		
0.05 potassium tetroxalate dihydrate	1.679	12.61g$KH_3C_4O_8 \cdot 2H_2O$
Calcium hydroxide (saturated at 25°C)	12.454	1.5g$Ca(OH)_2$*

*Approximate solubility

†Dry chemical at 110-130 C for 2 hr.

‡Prepare with freshly boiled and cooled distilled water (carbon dioxide-free)

high. If a sample of sludge or mud is highly buffered, a small amount of water may be added but the result cannot be considered valid unless further dilutions yield the same pH value. All dilutions should be reported along with the result.

REFERENCES

1 Nebergall, W.H., Schmidt, F. C. and Holtzclaw, Jr., H.F. College Chem., 2nd Ed. Heath and Co., Boston, 1963.

2 Sawyer, C.N., and McCarty, P.L. Chem. for San. Eng. 2nd Ed. McGraw-Hill, New York 1967

3 Instruction - Manual IL 175 Porto-matic pH meter, Instrumentation Laboratory, Inc. Lexington, Massachusetts, Standard Methods, APHA-AWWA-WPCF., 13th Ed., 1971.

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Descriptors: Hydrogen Ion Concentration, Instrumentation, Chemical Analysis

SPECIFIC CONDUCTANCE

I INTRODUCTION

An electrical conductivity measurement of a solution determines the ability of the solution to conduct an electrical current. Very concentrated solutions have a large population of ions and transmit current easily or with small resistance. Since resistance is inversely related to conductivity $K = \frac{1}{R}$, a very concentrated solution has a very high electrical conductivity.

Electrical conductivity is determined by transmitting an electrical current through a given solution, using two electrodes. The resistance measured is dependent principally upon the ionic concentration, ionic charge, and temperature of the solution although electrode characteristics (surface area and spacing of electrodes) is also critical. Early experiments in standardizing the measurement led to construction of a "standard cell" in which the electrodes were spaced exactly 1 cm and each had a surface area of 1 cm^2. Using this cell, electrical conductivity is expressed as "Specific Conductance". Modern specific conductance cells do not have the same electrode dimensions as the early standard cell but have a characteristic electrode spacing/area ratio known as the "cell constant".

$$K_{sp} = \frac{1}{R} \times \frac{\text{distance (cm)}}{\text{area (cm}^2)}, \quad K_{sp} = \frac{1}{R} \times k$$

$$k = \text{cell constant}$$

Specific conductance units are Mhos/cm or reciprocal ohms/cm. Most natural, fresh waters in the United States have specific conductances ranging from 10 to 1,000 micromhos/cm. (1 micromho = 10^{-6} mho).

II CONDUCTIVITY INSTRUMENTS

Nearly all of the commercial specific conductance instruments are of a bridge circuit design, similar to a Wheatstone Bridge. Null or balance is detected either by meter movement, electron "ray eye" tubes, or headphones. Since resistance is directly related to temperature, some instruments have automatic temperature compensators, although inexpensive models generally have manual temperature compensation.

Conductivity instruments offer direct specific conductance readout when used with a cell "matched" to that particular instrument.

Electrodes within the cell may become damaged or dirty and accuracy may be affected; therefore, it is advisable to frequently check the instrument readings with a standard KCl solution having a known specific conductance.

III CONDUCTIVITY CELLS

Several types of conductivity cells are available, each having general applications. Dip cells are generally used for field measurement, flow cells for measurement within a closed system, and pipet cells for laboratory use. Many modifications of the above types are available for specialized laboratory applications; the Jones cells and inductive capacitance cells are perhaps the most common.

Examples of various cell ranges for the RB3 - Industrial Instruments model (0-50 micromhos/cm scale range) are in Table 1.

Cell Number	Relative Conductivity Value	Maximum range micromhos/cm	Most accurate range micromhos/cm
Cell VSO2	1	0 - 50	2 - 30
Cell VS2	10	0 - 500	20 - 300
Cell VS20	100	0 - 5000	200 - 3000

Table 1

IV Computation of Calibration Constant

A calibration constant is a factor to which scale readings must be multiplied by compute specific conductance.

$K_{sp} = cM$

where K_{sp} = actual specific conductance

c = calibration constant

M = meter reading

For example, a 0.001 N KCl solution (147 micromhos/cm standard) may show a scale reading of 147.

$$147 = c\ 147,\ c = \frac{147}{147} = 1.00$$

In this case the cell is perfectly "matched" to the instrument, the calibration constant is 1.00, and the scale reading represents actual specific conductance. A variety of cells, each covering a specific range, may be used with any one instrument. However, a calibration constant for each cell must be computed before solutions of unknown specific conductance can be determined.

V RELATIONSHIP OF SPECIFIC CONDUCTANCE TO IONIC CONCENTRATION

Natural water consists of many chemical constituents, each of which may differ widely in ionic size, mobility, and solubility. Also, total constituent concentration and proportions of certain ions in various natural waters range considerably. However, it is surprising that for most natural waters having less than 2,000 mg/l. dissolved solids, dissolved solids values are closely related to specific conductance values, ranging in a ratio of .62 to .70. Of course this does not hold true for certain waters having considerable amounts of nonionized soluble materials, such as organic compounds and nonionized, colloidal inorganics.

Properties of some inorganic ions in regard to electrical conductivity are shown below:

Ion	Micromhos/cm per meq/l conc.
Calcium	52.0
Magnesium	46.6
Sodium	48.9
Potassium	72.0
Bicarbonate	43.6
Carbonate	84.6
Chloride	75.9

VI ESTIMATION OF CONSTITUENT CONCENTRATIONS

Generally speaking, for waters having a dissolved solids concentration of less than 1,000 mg/l, calcium and magnesium (total hardness), sodium, bicarbonate and carbonate (total alkalinity), and sulfate are the principal or most abundant ions, representing perhaps 90-99% of the total ionic concentration of the water. Specific conductance, total hardness and total alkalinity are all simple and expedient measurements which can be performed in the field. Therefore, the remaining principal ions are sodium and sulfate, and concentrations of these can be estimated by empirical methods. For example, we find that a certain water has:

K_{sp} = 500 micromhos/cm
Total Hardness = 160 mg/l or 3.20 meq/l
Total Alkalinity = 200 mg/l or 3.28 meq/l, as bicarbonate.

Next we multiply the specific conductance by *0.011 (500 × 0.011 = 5.50) to estimate the total ionic concentration in meq/l.

* This factor may vary slightly for different waters

Cations (meq/l)		Anions (meq/l)
Calcium	3.20	Carbonate 0.00
Magnesium		Bicarbonate 3.28
Sodium 5.50-3.20 = 2.30		Sulfate 5.50-3.28 = 2.22
Total Cations 5.50		Total Anions 5.50

Realizing that several variables are involved in empirical analysis, application rests entirely upon testing the formula with previous complete laboratory analyses for that particular water. If correlation is within acceptable limits, analytical costs may be substantially reduced. Empirical analysis can also be used in determination of proper aliquots (dilution factor) necessary for laboratory analysis.

Records of laboratory chemical analysis may indicate that a particular stream or lake shows a characteristic response to various streamflow rates or lake water levels. If the water's environment has not been altered and water composition responds solely to natural causes, a specific conductivity measurement may be occasionally used in substitution for laboratory analyses to determine water quality. Concentration of individual constituents can thus be estimated from a specific conductance value.

VII APPLICATIONS FOR SPECIFIC CONDUCTANCE MEASUREMENTS

A Laboratory Operations[2]

1 Checking purity of distilled and de-ionized water

2 Estimation of dilution factors for samples

3 Quality control check on analytical accuracy

4 An electrical indicator

B Agriculture

1 Evaluating salinity

2 Estimating Sodium Adsorption Ratio

C Industry[3]

1 Estimating corrosiveness of water in steam boilers

2 Efficiency check of boiler operation

D Geology

1 Stratigraphic identification and characterization

a geological mapping
b oil explorations

E Oceanography

1 Mapping ocean currents

2 Estuary studies

F Hydrology

1 Locating new water supplies

a buried stream channels (See Fig. 1)
b springs in lakes and streams (See Fig. 2)

2 Detection and regulation of sea water encroachment on shore wells

G Water Quality Studies

1 Estimation of dissolved solids[2] (See Section V, also Fig. 3)

2 Empirical analysis of constituent concentrations (See Section VI, also reference 2)

3 Quality control check for salt water conversion studies

4 Determination of mixing efficiency of streams (See Fig. 4)

5 Determination of flow pattern of polluted currents (See Fig. 4)

6 Identification of significant fluctuations in industrial wastewater effluents

7 Signal of significant changes in the composition of influents to waste treatment plants

FIGURE 1

DETECTION OF BURIED STREAM CHANNELS

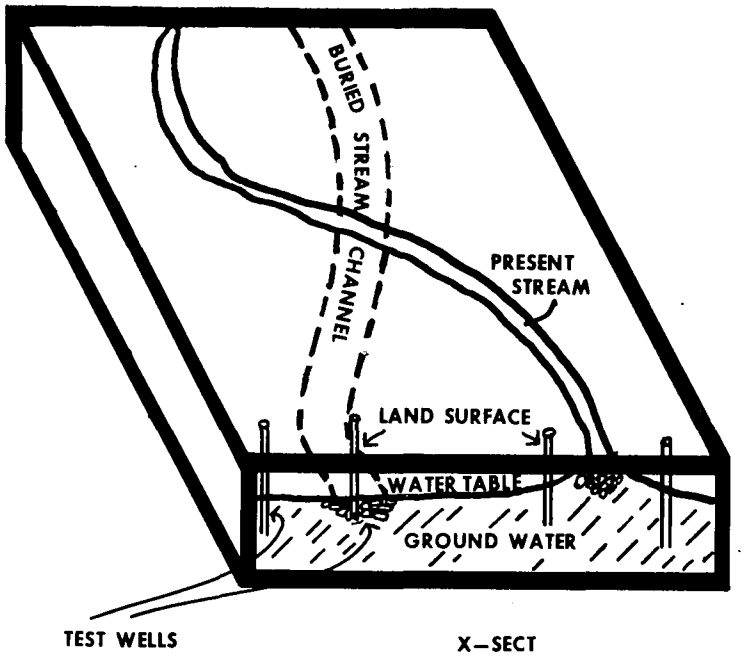


FIGURE 2

DETECTION OF SPRINGS IN LAKES AND STREAMS

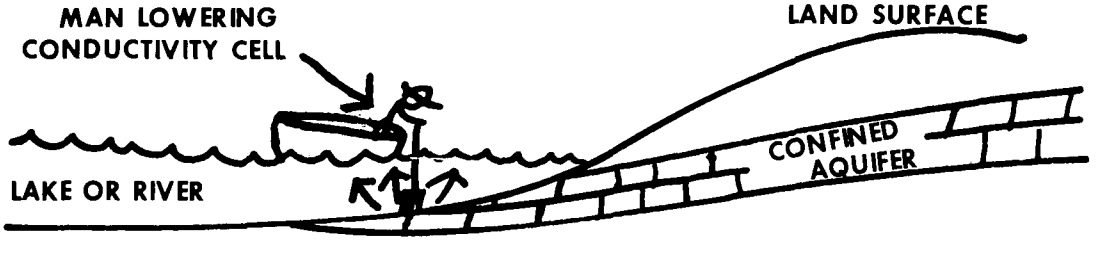
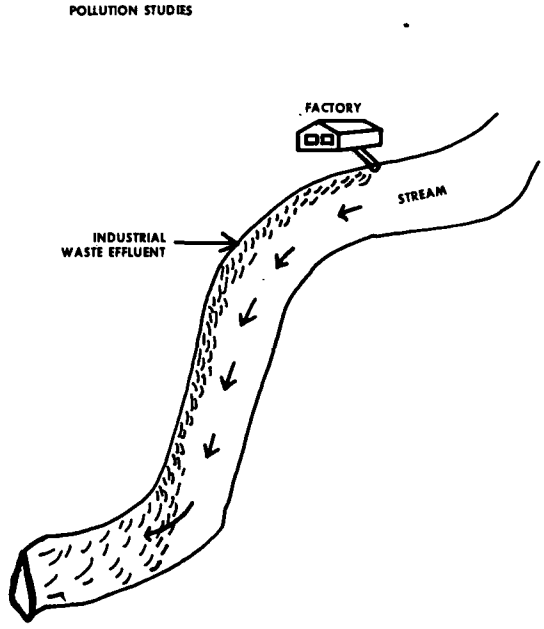


FIGURE 3



VIII EPA METHODOLOGY

A The current EPA Methods Manual[1] specifies that specific conductance be measured with a self-contained conductivity meter, Wheatstone bridge-type or equivalent.

Samples should preferably be analyzed at 25°C. If not, temperature corrections should be made and results reported at 25°C.

1 The instrument should be standardized using KCl solutions

2 It is essential to keep the conductivity cell clean

B The EPA manual specifies using the procedure as described in Standard Methods[2] or in ASTM Standards[3]. These are approved in 40 CFR 136 for NPDES Report purposes.

C Precision and Accuracy[1]

Forty-one analysts in 17 laboratories analyzed 6 synthetic water samples containing the following K_{sp} increments of inorganic salts: 100, 106, 808, 848, 1640 and 1710 micromhos/cm.

The standard deviation of the reported values was 7.55, 8.14, 66.1, 79.6, 106 and 119μ mhos/cm respectively.

The accuracy of the reported values was -2.0, -0.8, -29.3, -38.5, -87.9 and -86.9μ mhos/cm bias respectively.

REFERENCES

1 Methods for Chemical Analysis of Water and Wastes, MDQARL, Cincinnati, OH 45268. 1974.

2 Standard Methods for the Examination of Water and Wastewater, APHA-AWWA-WPCF, 13th edition, 1971.

3 ASTM Standards, Part 23, 1973.

This outline was prepared by John R. Tilstra, Chemist, National Eutrophication Research Program, Corvallis, Oregon with additions by Audrey E. Donahue, Chemist, MPOD, WPO, EPA, Cincinnati, OH 45268.

CALIBRATION AND USE OF A CONDUCTIVITY METER

I EQUIPMENT AND REAGENTS

A Equipment

1 Solu Bridge conductivity meters

2 Probes

a Cell VSO2

b Cell VS2

c Cell VS2O

3 Thermometers

4 400 ml beakers

B Reagents

1 Standard KC1 solutions

Normality of KC1 Solution	Specific Conductance micromhos/cm.
0.0001	14.9
0.001	147.0
0.01	1413.0
0.1	12900.0

2 Distilled water

II CHECKING THE INSTRUMENT

A The measurement of specific conductivity as presented in sections II and III is written for one type of conductivity meter and probe.

B A battery check is made by depressing the Battery Check switch, and at the same time pressing the on-off button. The meter needle should deflect to the right (positive) and come to rest in the green zone.

C Place a 10,000 ohm resistor in the holes of the electrical contacts on the meter. Turn the temperature knob to read 25°C. Depress the on-off button and bring the meter needle to a reading of 0 by turning the specific conductance switch. The specific conductance reading should be approximately 200 micromhos/cm.

III DETERMINATION OF THE CALIBRATION CONSTANT

A Determine the temperature of the standard KC1 solutions and move the temperature knob to that value.

B Connect probe Cell VS02 to the conductivity meter.

C Rinse the probe in the beaker of distilled water, wipe the excess water with a kimwipe and place probe in the first beaker of KC1 solution (0.0001 N).

D Make certain the cell is submerged to a point at least 1/2 inch above the air hole and that no entrapped air remains. The cell should also be at least 1/2 inch from the inside walls of the flask.

E Press and hold down the ON-OFF button, simultaneously rotating the main scale knob until the meter reads zero. Release the button. (If the meter needle remains off scale or cannot be nulled, discontinue testing in that solution.)

F Record the scale reading in Table 1 and proceed to KC1 solutions 0.001N, 0.01N, 0.1 N using Steps C, D and E.

G Repeat steps C through F using the VS2, then the VS20 probe.

H Compute the cell calibration constant-a factor by which scale readings must be multiplied to compute specific conductance:

$K_{sp} = cM$

where K_{sp} = actual specific conductance,
c = calibration constant
M= meter reading

(continued next page)

TABLE 1 DATA FOR CALIBRATION CONSTANTS

Probe	Cell VSO2				Cell VS2				Cell VS20			
KCl Solutions	0.0001N	0.001N	0.01N	0.1N	0.0001N	0.001N	0.01N	0.1N	0.0001N	0.001N	0.01N	0.1N
Test # 1												
Cell Constant												

For each cell, calculate the cell constant by using the meter reading closest to the 400 - 600 range. The known specific conductance for the corresponding KCl solution can be found in IB Reagents. Record the cell constants on Table I.

IV DETERMINATION OF K_{sp} FOR SAMPLES

Obtain meter readings, M, for samples A, B and C using Section III C, D and E. Record M in Table 2. See Table I for the appropriate cell constant, c, to calculate K_{sp} for each sample where K_{sp} = cM. Record results in Table 2.

V EPA METHODOLOGY

The current EPA Manual[1] specifies using the procedures found in References 2 and 3. These procedures have been adapted for this laboratory session and all are approved in 40CFR136 for NPDES report purposes.

ACKNOWLEDGMENT

This outline contains certain portions of previous outlines by Messrs. J. W. Mandia, and J. R. Tilstra.

REFERENCES

1 Methods for Chemical Analysis of Water and Wastes, EPA-MDQARL Cincinnati, OH 45268, 1974.

2 Standard Methods for the Examination of Water and Wastewater, 13th Edition. 1971.

3 Book of ASTM Standards, Part 23, 1973

This outline was prepared by C. R. Feldmann, Chemist, National Training Center, and revised by Audrey E. Donahue, Chemist, National Training Center, MPOD, WPO, USEPA, Cincinnati, OH 45268.

Descriptors: Analytical Techniques, Conductivity, Electrical Conductance, Specific Conductivity, Water Analysis

TABLE 2. SPECIFIC CONDUCTIVITY TESTS

Sample	A			B			C		
Probe	Cell VSO2	Cell VS2	Cell VS2O	Cell VSO2	Cell VS2	Cell VS2O	Cell VSO2	Cell VS2	Cell VS2O
1									
Cell Constant									
Sp. Cond. µmhos/cm									

CHLORINE DETERMINATIONS AND THEIR INTERPRETATION

INTRODUCTION

Chlorine normally is applied to water as a bactericidal agent; it reacts with water contaminants to form a variety of products containing chlorine. The difference between applied and residual chlorine represents the chlorine demand of the water under conditions specified. Wastewater chlorination is particularly difficult because the concentration of organisms and components susceptible to interaction with chlorine are high and variable. Interferences with the chlorine determination in wastewater confuse interpretation with respect to the chlorine residual at a given time and condition, its bactericidal potency, or its future behavior.

II CHEMISTRY OF CHLORINATION

A Chlorine compounds (Cl_2) dissolve in water, and hydrolyze immediately according to the reaction.

$$Cl_2 + H_2O \leftrightarrows HOCl + H^+ + Cl^-$$

The products of this reaction are hypochlorous and hydrochloric acid. The reaction is reversible, but at pH values above 3.0 and concentrations of chlorine below 1000 mg/l the shift is predominantly to the right leading to hypochlorous acid (HOCl).

Hypochlorous acid is a weak acid and consequently ionizes in water according to the equation:

$$HOCl \leftrightarrows H^+ + OCl^-$$

This reaction is reversible. At a pH value of 5.0 or below almost all of the chlorine is present as hypochlorous acid (HOCl) whereas above pH 10.0 nearly all of it exists as hypochlorite ion (OCl^-). The pH value that will control is the pH value reached after the addition of chlorine. Chlorine addition tends to lower the pH and the addition of alkali hypochlorites tends to raise the pH.

B The initial reactions on adding chlorine to wastewaters may be assumed to be fundamentally the same as when chlorine is added to water except for the additional complications due to contaminants and their concentration.

Hypochlorous acid (HOCl) reacts with ammonia and with many other complex derivatives of ammonia to produce compounds known as chloramines. Formation of the simple ammonia chloramines includes:

1 $NH_3 + HOCl \rightarrow NH_2Cl + H_2O$

monochloramine

2 $NH_2Cl + HOCl \rightarrow NHCl_2 + H_2O$

dichloramine

3 $NH_2Cl + NHCl_2 \rightarrow N_2 + 3\ HCl$

The distribution of the ammonia chloramines is dependent on pH, as illustrated below:

ph	Percentage of Chlorine Present as Monochloramine	Dichloramine
5	16	84
6	38	62
7	65	35
8	85	15
9	94	6

The formation of the ammonia chloramines are dependent on pH, temperature, and chlorine-ammonia ratio. Chlorine reactions with amino acids are likely; product disinfecting powers are lower than those of chlorine or of ammonia chloramines.

III TERMINOLOGY

A Terms used with Respect to Application Site

1 Pre-chlorination - chlorine added prior to any other treatment.

2 Post-chlorination - chlorine added after other treatment.

3 Split chlorination - chlorine added at different points in the plant - may include pre- and post-chlorination.

B Terms Used in Designating Chlorine Fractions

1 Free available residual chlorine - the residual chlorine present as hypochlorous acid and hypochlorite ion.

2 Combined available residual chlorine - the residual chlorine present as chloramines and organic chlorine containing compounds.

3 Total available residual chlorine - the free available residual chlorine + the combined available residual chlorine - may represent total amount of chlorine residual present without regard to type.

In ordinary usage these terms are shortened to free residual chlorine, combined residual chlorine and total residual chlorine. In the chlorination of wastewaters only combined residual chlorine is ordinarily present and is often improperly termed chlorine residual.

C Breakpoint chlorination specifically refers to the ammonia-chlorine reaction where applied chlorine hydrolyzes and reacts to form chloramines and HCl with the chloramines eventually forming N_2 + HCl as in II. B. 3. Assuming no other chlorine demand, the total chlorine residual will rise, decrease to zero and rise again with increasing increments of applied chlorine. Other substances may produce humps in the applied chlorine vs residual chlorine plot due to oxidation of materials other than ammonia. Sometimes these are erroneously considered as a breakpoint.

IV ANALYTICAL METHODS

Iodometric and amperometric titration methods are the basis for determining chlorine residuals in water. The relative advantages of a specific determination depend upon the form in which the reactable chlorine exists and the amount and nature of interferences in the water.

A Amperometric Titration - Direct Method

1 Scope and application

This method is applicable to the determination of free, combined or total residual chlorine in all types of water and wastewaters that do not contain substantial amounts of organic matter.

2 Summary of Method

When the cell of the titrator is immersed in a sample at pH 7.0, the cell unit produces a small direct current if free chlorine (an oxidizing agent) is present. As phenylarsine oxide (PAO) solution is added, it reduces the free chlorine. When all the chlorine is neutralized, the generation of current ceases. At this end point, the microammeter pointer on the apparatus no longer deflects down-scale.

To determine combined chlorine, pH 4.0 buffer and potassium iodide are then added to the sample. Free iodine released by the combined chlorine also causes the cell to produce a small direct current. Addition of PAO reduces the free iodine and the generation of current ceases. Again, the end point occurs

when the microammeter pointer no longer deflects down-scale.

In either titration, the amount of PAO reducing agent used to reach the end point is ultimately stoichiometrically proportional to chlorine present in the sample. The sum of the free and combined chlorine is the total residual chlorine in the sample.

Total residual chlorine can be determined directly by adding pH 4.0 buffer and potassium iodide to the sample before beginning the titration. Any free or combined chlorine present will stoichiometrically liberate free iodine which is then reduced with PAO titrant. The amount of PAO titrant used measures the total amount of free and combined chlorine originally present in the sample.

3 Interferences

a Organic matter reacts with liberated iodine.

b Cupric ions may cause erratic behavior of the apparatus.

c Cuprous and silver ions tend to poison the electrode by plating out on it.

B Amperometric Titration - Indirect Method

1 Scope and Application

This method is applicable to the determination of total chlorine residual in all types of water and wastewaters. In contrast to the direct amperometric titration, this back-titration procedure minimizes interferences in waters containing substantial amounts of organic matter.

2 Summary of Method

A sample is treated with a measured excess of standard phenylarsine oxide (PAO) solution followed by addition of potassium iodide and a buffer to maintain the pH between 3.5 and 4.2.

Any form of chlorine present will stoichiometrically liberate iodine which immediately reacts with the PAO before significant amounts are lost to reactions with organic matter in the sample.

When the cell of the amperometric titrator is immersed in a sample so treated, no current is generated since neither free chlorine nor free iodine is present.

The amount of PAO used to reduce the liberated iodine is then determined by titrating the excess with a standard iodine solution. No current is generated until all the excess PAO has been oxidized by the iodine. At this end point the next small addition of iodine causes current to be generated and the microammeter pointer permanently deflects up-scale.

The excess PAO thus measured is subtracted from the original amount of PAO added. The difference is the PAO used to reduce the liberated iodine and is ultimately a measure of the total chlorine originally present in the sample.

NOTE: Sodium thiosulfate solution may be used instead of PAO, but PAO is more stable and is to be preferred.

3 Interferences

a Cupric ions may cause erratic behavior of the apparatus.

b Cuprous and silver ions tend to poison the electrode by plating out on it.

C Colorimetric Starch-Iodide Titration - Indirect Method

1 Scope and Application

This method is applicable to the determination of total chlorine residual in all types of water and wastewater. A back-titration procedure is used to minimize interferences in waters containing substantial amounts of organic matter.

2 Summary of Method

A sample is treated with a measured excess of standard phenylarsine oxide (PAO) solution followed by addition of potassium iodide and a buffer to maintain the pH between 3.5 and 4.2. Any form of chlorine present will stoichiometrically liberate iodine which immediately reacts with PAO before significant amounts are lost to reactions with organic matter in the sample.

The amount of PAO used to reduce the liberated iodine is then determined by titrating the excess with a standard iodine solution in the presence of starch until the PAO is completely oxidized. At this end point, the next small addition of iodine causes a faint blue color to persist in the sample.

The excess PAO thus measured is subtracted from the original amount of PAO added. The difference is the PAO used to reduce the liberated iodine and is ultimately a measure of the total chlorine originally present in the sample.

NOTE: Sodium thiosulfate solution may be used instead of PAO, but PAO is more stable and is to be preferred.

3 Interferences

a An unusually high content of organic matter may cause some uncertainty in the end point. If manganese, iron and nitrite are definitely absent, this uncertainty can be reduced by acidification to pH 1.0.

b Color and turbidity in the sample cause difficulty with end-point detection.

V EVALUATION OF METHODS

Iodometric titration using amperometric end point detection appears to be the most accurate residual chlorine method available.[1,2] Besides being inherently more accurate than color detection methods, this electrical end point is free of interference from color and turbidity.

The accuracy of the colorimetric starch-iodide end point is improved by employing the indirect titration method described above. By adding an excess of the standard reducing agent and back-titrating, contact between the liberated iodine and organic matter in the sample is minimized.

1 Standard Methods for the Examination of Water and Wastewaters, 13th Ed., APHA, AWWA, WPCF. 1971.

2 Book of ASTM Standards, Part 23 - Industrial Water; Atmospheric Analysis. American Society for Testing and Materials. Philadelphia, PA. 1972.

3 Sawyer, C. N. Chemistry for Sanitary Engineers. McGraw-Hill Book Company. New York. 1960.

4 Moore, E. W. Fundamentals of Chlorination of Sewage and Wastes. Water and Sewage Works. Vol. 98. No. 3. March 1951.

5 Day, R. V., Horchler, D. H., and Marks, H. C. Residual Chlorine Methods and Disinfection of Sewage. Industrial and Engineering Chemistry. May 1953.

6 Marks, H. C., Joiner, R. R., and Strandskov, F. B. Amperometric Titration of Residual Chlorine in Sewage. Water and Sewage Works. May 1948.

This outline was prepared by J. L. Holdaway, Chemist, Technical Program, EPA, Region III, Charlottesville, VA 22901 (1971); and revised by Audrey E. Donahue, Chemist, National Training Center, MPOD, WPO, USEPA, Cincinnati, OH 45268.

Descriptors: Chemical Analysis, Chlorination, Chlorine, Disinfection, Sewage Treatment, Wastewater Treatment, Water Analysis.

PHOSPHORUS IN THE AQUEOUS ENVIRONMENT

I Phosphorus is closely associated with water quality because of (a) its role in aquatic productivity such as algal blooms, (b) its sequestering action, which causes interference in coagulation, (c) the difficulty of removing phosphorus from water to some desirable low concentration, and (d) its characteristic of converting from one to another of many possible forms.

A Phosphorus is one of the primary nutrients such as hydrogen (H), carbon (C), nitrogen (N), sulfur (S) and phosphorus (P).

1 Phosphorus is unique among nutrients in that its oxidation does not contribute significant energy because it commonly exists in oxidized form.

2 Phosphorus is intimately involved in oxidative energy release from and synthesis of other nutrients into cell mass via:

a Transport of nutrients across membranes into cell protoplasm is likely to include phosphorylation.

b The release of energy for metabolic purposes is likely to include a triphosphate exchange mechanism.

B Most natural waters contain relatively low levels of P (0.01 to 0.05 mg/l) in the soluble state during periods of significant productivity.

1 Metabolic activity tends to convert soluble P into cell mass (organic P) as a part of the protoplasm, intermediate products, or sorbed material.

2 Degradation of cell mass and incidental P compounds results in a feedback of lysed P to the water at rates governed by the type of P and the environment. Aquatic metabolic kinetics show marked influences of this feedback.

3 The concentrations of P in hydrosoils, sludges, treatment plant samples and soils may range from 10^2 to 10^6 times that in stabilized surface water. Both concentration and interfering components affect applicability of analytical techniques.

II The primary source of phosphorus in the aqueous system is of geological origin. Indirect sources are the processed mineral products for use in agriculture, household, industry or other activities.

A Agricultural fertilizer run-off is related to chemicals applied, farming practice and soil exchange capacity.

B Wastewaters primarily of domestic origin contain major amounts of P from:

1 Human, animal and plant residues

2 Surfactants (cleaning agent) discharge

3 Microbial and other cell masses

C Wastewaters primarily of industrial origin contain P related to:

1 Corrosion control

2 Scale control additives

3 Surfactants or dispersants

4 Chemical processing of materials including P

5 Liquors from clean-up operations of dusts, fumes, stack gases, or other discharges

III Phosphorus terminology is commonly confused because of the interrelations among biological, chemical, engineering, physical, and analytical factors.

A Biologically, phosphorus may be available as a nutrient, synthesized into living mass, stored in living or dead cells, agglomerates, or mineral complexes, or converted to degraded materials.

B Chemically, P exists in several mineral and organic forms that may be converted from one to another under favorable conditions. Analytical estimates are based upon physical or chemical techniques necessary to convert various forms of P into ortho phosphates which alone can be quantitated in terms of the molybdenum blue colorimetric test.

C Engineering interest in phosphorus is related to the prediction, treatment, or control of aqueous systems to favor acceptable water quality objectives. Phosphorus removal is associated with solids removal.

D Solubility and temperature are major physical factors in phosphorus behavior. Soluble P is much more available than insoluble P for chemical or biological transformations and the rate of conversion from one to another is strongly influenced by temperature.

E Table 1 includes a classification of the four main types of chemical P and some of the relationships controlling solubility of each group. It is apparent that no clear-cut separation can be made on a solubility basis as molecular weight, substituent and other factors affect solubility.

F Table 2 includes a scheme of analytical differentiation of various forms of P based upon:

1 The technique required to convert an unknown variety of phosphorus into ortho P which is the only one quantitated by the colorimetric test.

2 Solubility characteristics of the sample P or more precisely the means required to clarify the sample.

a Any clarification method is subject to incomplete separation. Therefore, it is essential to specify the method used to interpret the yield factor of the separation technique. The degree of separation of solubles and insolubles will be significantly different for:

1 Membrane filter separation (0.5 micron pore size)

2 Centrifugation (at some specified rpm and time)

3 Paper filtration (specify paper identification)

4 Subsidence (specify time and conditions)

G Analytical separations (Table 2) like those in Table 1, do not give a precise separation of the various forms of P which may be included quantitatively with ortho or poly P. Conversely some of the poly and organic P will be included with ortho P if they have been partially hydrolyzed during storage or analysis. Insolubles may likewise be included as a result of poor separation and analytical conditions.

1 The separation methods provide an operational type of definition adequate in most situations if the "operation" is known. Table 2 indicates the nature of incidental P that may appear along with the type sought.

Table 1

PHOSPHORUS COMPOUNDS CLASSIFIED BY CHEMICAL AND SOLUBILITY RELATIONS

Form	Water Soluble(1)	Insoluble(1)
1. Ortho phosphates $(PO_4)^{-3}$	Combined with monovalent cations such as H^+, Na^+, K^+, NH_4^+	Combined with multi valent cations such as Ca^{+2} Al^{+3} Fe^{+3}
2. Poly phosphates $(P_2O_7)^{-4}$, $(P_3O_{10})^{-5}$ $(P_3O_9)^{-3}$ and others depending upon the degree of dehydration.	as in 1 above Increasing dehydration decreases solubility	(a) as in 1 above (b) multi P polyphosphates (high mol. wt.) including the "glassy" phosphates
3. Organic phosphorus R-P, R-P-R (2) (unusually varied nature)	(a) certain chemicals (b) degradation products (c) enzyme P (d) phosphorylated nutrients	(a) certain chemicals (b) cell mass, may be colloidal or agglomerated (c) soluble P sorbed by insoluble residues
4. Mineral phosphorus	(a) as in 1 above	(a) as in 1 above (b) as in 2 above (c) geological P such as phosphosilicates (d) certain mineral complexes.

(1) Used in reference to predominance under common conditions.

(2) R represents an organic radical, P represents P, PO_4^{-3}, or its derivatives.

---

2 Total P in Table 2 includes liquid and separated residue P that may exist in the whole sample including silt, organic sludge, or hydrosoils. This recognizes that the feedback of soluble P from deposited or suspended material has a real effect upon the kinetics of the aqueous environment.

Table 2

PHOSPHORUS COMPOUNDS CLASSIFIED BY ANALYTICAL METHODOLOGY

Desired P Components	Technique[1]	Incidental P Included[2]
1. Ortho phosphates	No treatment on clear samples	Easily hydrolyzed (a) poly phosphates - (b) organic -P, - (c) Mineral -P, + or -
2. Polyphosphates (2)-(1) = poly P (hydrolyzable)	acid hydrolysis on clear samples, dilute (a) H_2SO_4 (b) HCl heated	(a) ortho-P + (b) organic -P + or - (c) mineral -P + or -
3. Organic phosphorus (3) - (2) + org P (hydrolyzable)	acid + oxidizing hydrolysis on whole sample. dilute (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + (NH_4)_2S_2O_8$ heated	(a) ortho P + (b) poly P + (c) mineral P + or -
4. Soluble phosphorus (preferably classified by clarification method)	clarified liquid following filtration, centrifugation or subsidence	generally includes (a) 1, 2, or 3 (b) particulates not completely separated
5. Insoluble phosphorus (residue from clarification)	Retained residues separated during clarification See (6)	(a) generally includes sorbed or complexed solubles.
6. Total phosphorus	Strong acid + oxidant digestion (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + HNO_3 + HClO_4$ (c) $H_2O_2 + Mg(NO_3)_2$ fusion	all components in 1, 2, 3, 4, 5 in the whole sample

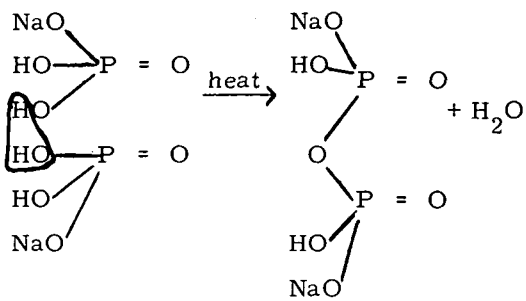
(1) Determinative step by phospho molybdate colorimetric method.

(2) Coding: + quantitative yield
- a small fraction of the amount present
+ or - depends upon the individual chemical and sample history

IV Polyphosphates are of major interest in cleaning agent formulation, as dispersants, and for corrosion control.

A They are prepared by dehydration of ortho phosphates to form products having two or more phosphate derivatives per molecule.

1 The simplest polyphosphate may be prepared as follows:



mono sodium ortho phosphate (2) — disodium dihydrogen polyphosphate

2 The general form for producing polyphosphates from mono substituted orthophosphates is:

$$n\ (NaH_2PO_4) \xrightarrow[200\text{-}400^{\circ}C]{heat} (NaPO_3)_n + n\ H_2O$$

3 Di-substituted ortho phosphates or mixtures of substituted ortho phosphates lead to other polyphosphates:

$$\underset{\text{disodium hydrogen ortho phosphate}}{2Na_2HPO_4} + \underset{\text{mono sodium di hydrogen ortho phosphate}}{NaH_2PO_4} \xrightarrow{heat} \underset{\text{pentasodium tri-phosphate}}{Na_5P_3O_{10}} + \underset{\text{water}}{2H_2O}$$

4 The polyphosphate series usually consist of the polyphosphate anion with a negative charge of 2 to 5. Hydrogen or metals commonly occupy these sites. The polyphosphate can be further dehydrated by heat as long as hydrogen remains. Di or trivalent cations generally produce a more insoluble polyphosphate than the same cation in the form of insoluble ortho phosphate. Insolubility increases with the number of P atoms in the polyphosphate. The "glassy" polyphosphates are a special group with limited solubility that are used to aid corrosion resistance in pipe distribution systems and similar uses.

B Polyphosphates tend to hydrolyze or "revert" to the ortho P form by addition of water. This occurs whenever polyphosphates are found in the aqueous environment.

1 The major factors affecting the rate of reversion of poly to orthophosphates include:

a) Temperature, increased T increases rate

b) pH, lower pH increases rate

c) Enzymes, hydrolase enzymes increase rate

d) Colloidal gels, increase rate

e) Complexing cations and ionic concentration increase rate

f) Concentration of the polyphosphate increases rate

2 Items a, b and c have a large effect upon reversion rate compared with other factors listed. The actual reversion rate is a combination of listed items and other conditions or characteristics.

3 The differences among ortho and ortho + polyphosphates commonly are close to experimental error of the colorimetric test in stabilized surface water samples. A significant difference generally indicates that the sample was obtained relatively close to a source of polyphosphates and was promptly analyzed. This implies that the reversion rate of polyphosphates is much higher than generally believed.

V SAMPLING AND PRESERVATION TECHNIQUES

A Sampling

1 Great care should be exercised to exclude any benthic deposits from water samples.

2 Glass containers should be acid rinsed before use.

3 Certain plastic containers may be used. Possible adsorption of low concentrations of phosphorus should be checked.

4 If a differentiation of phosphorus forms is to be made, filtration should be carried out immediately upon sample collection. A membrane filter of 0.45 μ pore size is recommended for reproducible separations.

B Preservation

1 If at all possible, samples should be analyzed on the day of collection. At best, preservation measures only retard possible changes in the sample.

a Possible physical changes include solubilization, precipitation, absorption on or desorption from suspended matter.

b Possible chemical changes include reversion of poly to ortho P and decomposition of organic or mineral P.

c Possible biological changes include microbial decomposition of organic P and algal or bacterial growth forming organic P.

2 Refrigeration at 4°C is recommended if samples are to be stored up to 24 hours. This decreases hydrolysis and reaction rates and also losses due to volatility.

3 Addition of 40 mg $HgCl_2$/liter is recommended for longer storage periods. This chemical limits biological changes.

a $HgCl_2$ is an interference in the analytical procedure if the chloride level is low (less than 50 mg. Cl/l). See Part VI B 3 below.

VI THE EPA ANALYTICAL PROCEDURE[6]

A This is a colorimetric determination, specific for orthophosphate. Depending on the nature of the sample and on the type of data sought, the procedure involves two general operations:

1 Conversion of phosphorus forms to soluble orthophosphate (See Fig. 1):

a sulfuric acid-hydrolysis for polyphosphates, and some organic P compounds,

b persulfate digestion for organic P compounds.

2 The color determination involves reacting dilute solutions of phosphorus with ammonium molybdate and potassium antimonyl tartrate in an acid medium to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the orthophosphate concentration.

Color absorbance is measured at 880 nm or 650 nm and a concentration value obtained using a standard curve.

Reagent preparation and the detailed procedure can be found in the EPA manual.

The methods described there are usable in the 0.01 to 0.5 mg/liter phosphorus range.

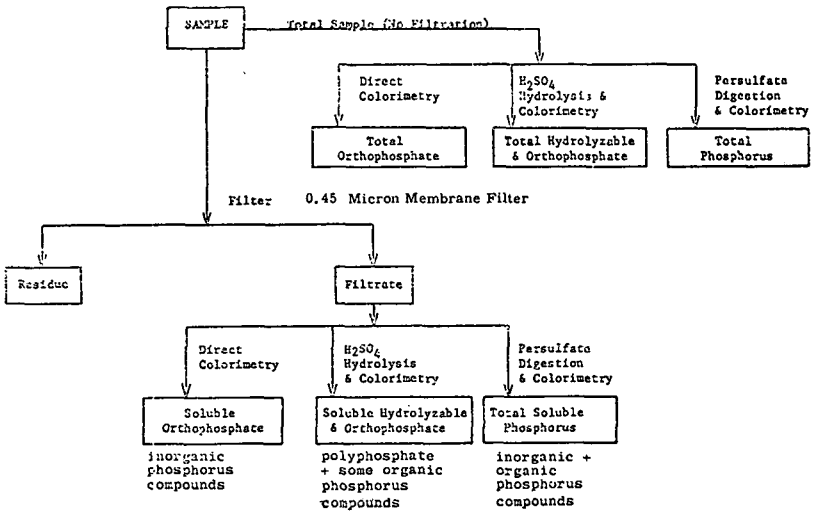


FIGURE 1

ANALYTICAL SCHEME FOR DIFFERENTIATION OF PHOSPHORUS FORMS

B Interferences

1 Erroneous results from contaminated glassware is avoided by cleaning it with hot 1:1 HCl, treating it with procedure reagents and rinsings with distilled water. Preferably this glassware should be used only for the determination of phosphorus and protected from dust during storage. Commercial detergents should never be used.

2 High iron concentrations in samples can precipitate phosphorus.

3 If $HgCl_2$ is used as a preservative, it interferes if the chloride level of the sample is less than 50 mg Cl/liter. Spiking with NaCl is then recommended.

4 Others have reported interference from chlorine, chromium, sulfides, nitrite, tannins, lignin and other minerals and organics at high concentrations.

C Precision and Accuracy[6]

1 Organic phosphate - 33 analysts in 19 laboratories analyzed natural water samples containing exact increments of organic phosphate of 0.110, 0.132, 0.772, and 0.882 mg P/liter.

Standard deviations obtained were 0.033, 0.051, 0.130 and 0.128 respectively.

Accuracy results as bias, mg P/liter were: +0.003, +0.016, +0.023 and - 0.008, respectively.

2 Orthophosphate was determined by 26 analysts in 16 laboratories using samples containing orthophosphate in amounts of 0.029, 0.038, 0.335 and 0.383 mg P/liter.

Standard deviations obtained were 0.010, 0.008, 0.018 and 0.023 respectively.

Accuracy results as bias, mg P/liter were -0.001, -0.002, -0.009 and -0.007 respectively.

D Automated Methods

The EPA Manual also contains a procedure for an automated colorimetry method using the ascorbic acid reduction method.

VII VARIABLES IN THE COLORIMETRIC PROCEDURE

Several important variables affect formation of the yellow heteropoly acid and its reduced form, molybdenum blue, in the colorimetric test for P.

A Acid Concentration during color development is critical. Figure 2 shows that color will appear in a sample containing no phosphate if the acid concentration is low. Interfering color is negligible when the normality with respect to H_2SO_4 approaches 0.4.

1 Acid normality during color development of 0.3 to slightly more than 0.4 is feasible for use. It is preferable to control acidity carefully and to seek a normality closer to the higher limits of the acceptable range.

2 It is essential to add the acid and molybdate as one solution.

3 The aliquot of sample must be neutralized prior to adding the color reagent.

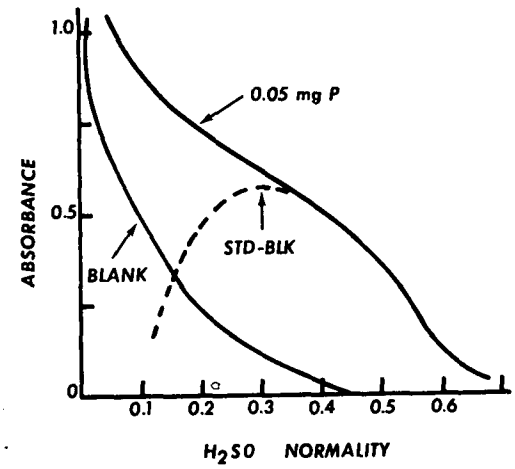


Figure 2

0-PHOSPHATE COLOR VS ACIDITY

B Choice of Reductant - Reagent stability, effective reduction and freedom from deleterious side effects are the bases for reductant selection. Several reductants have been used effectively. Ascorbic acid reduction is highly effective in both marine and fresh water. It is the reductant specified in the EPA method.

C Temperature affects the rate of color formation. Blank, standards, and samples must be adjusted to the same temperature (± 1°C), (preferably room temperature), before addition of the acid molybdate reagent.

D Time for Color Development must be specified and consistent. After addition of reductant, the blue color develops rapidly for 10 minutes then fades gradually after 12 minutes.

VIII DETERMINATION OF TOTAL PHOSPHORUS

A Determination of total phosphorus content involves omission of any filtration procedure and using the acid-hydrolysis and persulfate treatments to convert all phosphorus forms to the test-sensitive orthophosphate form.

B Determining total phosphorus content yields the most meaningful data since the various forms of phosphorus may change from one form to another in a short period of time. (See part V, B1)

IX DEVELOPMENT OF A STANDARD PROCEDURE

Phosphorus analysis received intensive investigation; coordination and validation of methods is more difficult than changing technique.

A Part of the problem in methods arose because of changes in analytical objectives such as:

1 Methods suitable to gather "survey" information may not be adequate for "standards".

2 Methods acceptable for water are not necessarily effective in the presence of significant mineral and organic interference characteristic of hydro-soils, marine samples, organic sludges and benthic deposits.

3 Interest has been centered on "fresh" water. It was essential to extend them for marine waters.

4 Instrumentation and automation have required adaptation of methodology.

B Analysts have tended to work on their own special problems. If the method apparently served their situations, it was used. Each has a "favorite" scheme that may be quite effective but progress toward widespread application of "one" method has been slow. Consequently, many methods are available. Reagent acidity, Mo content, reductant and separation techniques are the major variables.

C At the present time there is not sufficient data to warrant EPA endorsement of the P procedure for sediment-type samples, sludges, algal blooms, etc. Following is a procedure (not included in the EPA manual) which is useful when solids are present in samples:

1 If sample contains large particles, grind and emulsify solids in a blender.

2 Transfer 50 ml sample, or aliquot diluted to 50 ml, into a 250 ml Erlenmeyer flask.

3 Add 6.0 ml of 18N H_2SO_4, 5 ml concentrated HNO_3, 2 berl saddles and digest on hot plate.

4 Digest until the disappearance of nitric acid fumes and the appearance of white SO_3 fumes. Continue digestion for approximately 5 minutes. Cool before proceeding with Step 5.

5 Add 2 ml of HNO_3-$HClO_4$ mixture and 5 ml concentrated HNO_3. Continue digestion until all of the nitric acid is driven off and dense fumes of perchloric evolve. Perchloric acid requires dilution with sulfuric acid and prior destruction of most organics for safety.

6 Cool. Add approximately 40 ml distilled water and transfer to 100 ml volumetric flask.

7 Add 2-3 drops of phenolphthalein and concentrated ammonium hydroxide until a pink color is seen. Then discharge the pink color with the strong sulfuric acid. It is advisable to add an equivalent amount of salt formed during neutralization of digested samples to the calibration standards to equalize salt content during color development.

8 Determine orthophosphate according to the usual color procedure.

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REFERENCES

1 Jenkins, David, A Study of Methods Suitable for the Analysis and Preservation of Phosphorus Forms in the Estuarine Environment. DHEW, Central Pacific River Basins Project. SERL Report No. 65-18, University of California, Berkeley, Calif. November 1965.

2 Gales, Morris E., Jr., Julian, Elmo C., and Kroner, Robert C., Method for Quantitative Determination of Total Phosphorus in Water. JAWWA 58: (10) 1363. October 1966.

3 Lee, G. Fred, Clesceri, Nicholas L. and Fitzgerald, George P., Studies on the Analysis of Phosphates in Algal Cultures. Int. J. Air & Water Poll. 9:715. 1965.

4 Barth, E. F. and Salotto, V. V., Procedure for Total Phosphorus in Sewage and Sludge, Unpublished Memo, Cincinnati Water Research Laboratory, FWQA. April 1966.

5 Moss, H. V., (Chairman, AASGP Committee) Determination of Ortho Phosphate, Hydrolyzable Phosphate and Total Phosphate in Surface Water, JAWWA 56:1563. December 1958.

6 Methods for Chemical Analysis of Water & Wastes, EPA, MDQARL, Cincinnati,Ohio 45268, 1974.

This outline was prepared by Ferdinand J. Ludzack, Chemist, Audrey E. Donahue, Chemist, both with National Training Center, MPOD, WPO, EPA, Cincinnati, Ohio 45268.

Descriptors:
Chemical Analysis, Nutrients, Phosphates, Phosphorus, Phosphorus Compounds, Pollutant Identification, Sampling, Water Analysis, Water Pollution Sources.

CHEMICAL TESTS, OBSERVATIONS, AND MEASUREMENTS IN THE FIELD

I INTRODUCTION

A Laboratory determinations with approved equipment and convenient facilities by experienced specialists generally are easier, faster, and more reliable. It is advisable to transport the samples to the laboratory whenever it is feasible to do so.

B Field tests are essential because:

1 Certain sample components are inherently unstable with respect to biological, chemical, or physical changes. Any test result performed in the laboratory may not represent true conditions on site at the time because of delay, displacement, or changed conditions.

2 Subsequent operations generally may be coordinated and made more meaningful by preliminary on site field investigation to identify and evaluate problems, locate critical areas, and minimize surprises.

II FIELD TEST CONDITIONAL CONSIDERATIONS

A Moving the laboratory into the field means improvisation and adaptation to more "primitive" conditions.

1 Rugged construction of field equipment is a first consideration. Sturdy and convenient cases are required; the case often may be the only available work-bench. Polyethylene bottles, beakers, burets, and pipets generally are necessary to eliminate breakage of fragile glass.

2 Portability is essential, particularly when the site is not accessible by boat or car. Ideally, the field kit should be small enough and light enough to be carried by one man for extended periods.

3 Procedures for field use are restricted in equipment and manipulation. In general, it is not possible to use long, tedious routines or highly precise measurements. Numerous reagents generally cannot be carried. Quick positive reactions are essential. Small visual comparators, test papers or spot tests are popular. Titration assemblies must be modified for field use.

4 Instrumentation increases objectivity of the measurements but the instruments must be adapted to the items outlined in II. A. 1, 2 and 3.

The instruments commonly used must be simplified yet rugged enough to keep working in spite of temperature changes, moisture, bumps, etc. A meaningful validation procedure must be worked out for calibration on site with simple adjustment or repair for emergencies.

5 Personnel engaged in field testing often are unaccustomed to laboratory procedures. Advance training is essential in procedures and instrumentation used. Field testing is a multi-disciplinarian operation:

a The individuals must be good observers and recognize what may be significant later.

b They must be good interpreters of a variety of observed and derived test information.

c They must be good technicians to follow prescribed procedures diligently and recognize anomalous behavior when it occurs.

d They must be good reporters to describe what happened, where it happened, and when. Failure to report anomalous behavior

and related events in an understandable and consistent fashion may render the entire effort meaningless because of some doubt or inconsistency. Subsequent operations also may be mislead by the early observer.

B Use of field data imposes certain constraints relatable to the item, the methods, and to the personnel involved:

1 How valid is it?

2 How consistent is it in line with other observed or recorded information?

3 What backup information is required to verify suggested information?

C Decisions must be made on all field data applications:

1 Where "in-place" and "now" measurements are required how much of the laboratory must be moved out to the site to obtain data reliable enough to meet objectives?

2 Which items are to be determined on site? Which items in a central laboratory?

3 What use is to be made of the preliminary field estimates?

4 What do you "need" to know?

5 What would be "nice" to know?

6 In line with facilities, time, cost, manpower and objectives, what can you determine?

III COMPONENT CHARACTERISTICS FAVORING FIELD TESTING:

A Any item that changes rapidly during a holding period as a result of temperature or pressure variations, or is highly reactive from a biological, chemical, or physical standpoint, usually means that it must be determined on site and in place with minimum time lapse and manipulation.

1 Dissolved gases, such as O_2, CO_2, H_2S, Cl_2, are sensitive to pressure or temperature changes and may react with other sample components readily. These require in place and now readout.

2 The collective analysis of all forms of a given item may be preserved and analyzed later. Estimates of different forms of the same substance are not subject to delay unless they can be separated promptly. Examples of this include the relative ratios of oxidized or reduced substances, such as Cu^{+1} and Cu^{+2}, Fe^{+2} and Fe^{+3}, Cr^{+3} and Cr^{+6}; hydrolysis is the principal factor in the ratios of organic and NH_3-N and in changes among organic, poly and ortho-P.

3 Reactive substances contributing to "oxygen demand" tests, such as BOD or other respiratory tests generally require a minimum time delay before testing and are not amenable to preservation without altering results.

4 Soluble/insoluble ratios commonly change with time and conditions due to complexation, hydrolysis precipitation, agglomeration, or other factors. Particulate size may increase or decrease. Solubles may be sorbed on or desorbed from solid surfaces. Settleability or turbidity are transient phenomena subject to change with time or conditions.

5 Biological progressions are dynamic entities that shift among "critters" in relation to predominence in variety, numbers, growth, and decay. Whatever is, will change in response to nutrition and conditions.

IV SITUATIONS FAVORING FIELD TESTING

A Preliminary information often is needed to guide situation evaluation, problem identification, on site variation with respect to cross section, depth, or time. These data are useful to determine whether there is a problem or not and for planning of subsequent operations.

1 It may be necessary to locate suspected inflows, channels, or sources of items affecting water quality.

2 Definition of mixing zones often is required.

3 Laboratory time may be reduced if approximate concentrations of items sought are known.

4 Field tests or observations may suggest other tests that are more critical in evaluation of the given situation.

5 Stratification may be evaluated for guidance of subsequent operations.

6 Knowledge of the distribution of components is useful for selecting meaningful future sampling sites.

B The field test may be used to check compliance with regulations prior to more rigorous backup testing.

1 An established operation may be in control or out of control.

2 The field test may reveal which of multiple discharges are in conformance.

3 Undisclosed discharges may become apparent.

4 It may be necessary to "track" some hazardous discharge down stream to guide subsequent users on the choice of intake or storage water.

5 A complaint or inquiry may be evaluated by field tests.

C In-plant field type tests are essential to guide operations toward the production of continuous high quality effluents. Record tests commonly are provided too late to do anything about the situation; quick test results are emphasized for process control because time, manpower, and change are critical.

1 Surprises in the form of wastewater changes in flow, concentration, or significant components are common for treatment process operators. They need quick numbers rather than impressions to distinguish real from apparent problems.

2 Process upsets are minimized or prevented by regular and meaningful tests from which trends may be established. Small imbalances may be corrected before they become major upsets.

3 Backup or supplementary treatment such as coagulation, adsorption, neutralization, may prevent serious process disruption if tests indicate the problem and its magnitude in time to do something about it.

V TYPICAL FIELD TESTS - INSTRUMENTAL

A pH is one of the simplest and most valuable field tests. If the result is due to mineral acids or alkalies in stable form, color indicators or comparators, impregnated paper or other devices will serve. When free CO_2 in solution is a major variable such as in the vicinity of benthic deposits or in active biological systems, the application of an electronic sensor in-place and extension wire to an indicator instrument is essential. Any delay, pressure change or manipulation will change free CO_2 and alter pH response. Electronic pH instruments are produced for field use by many apparatus supply firms. With care, they will function effectively.

B Conductivity is a very useful index of ionic materials and usually may be correlated with wet chemical data to give a reasonable correlation with total dissolved solids for a given mixture. This test is very useful to detect saline intrustions or discharges, springs, hidden channels of different salinity from the main body of water. Several reliable instruments are marketed.

C DO analyzers are available for field use application from several manufacturers. They differ in portability accessory equipment and in versatility so that it is possible to obtain one or more fitting almost any requirement. These units are based upon reduction of oxygen at the cathode surface to convert chemical to electrical energy. Direct measurement of electrical energy produced or the change in some carrier current or voltage may be used for readout. The signal may be amplified in line with sensitivity to give direct concentration readout. Temperature compensation is useful and available. Membrane covered sensors selective for dissolved gases protect sensor surfaces from most interfering components. The analyst must learn to use his particular instrument effectively to enable him to obtain valid results under conditions in which a wet chemical procedure would be misleading.

VI TYPICAL FIELD TESTS - WET CHEMICAL

A DO titrations for clean water samples may be adapted for field use by shifting to dry reagents encased in plastic pillows, substituting phenyl arsene oxide for thio and using plastic burets, sample containers, etc. Powdered starch substitutes also are available. Sample sizes may be altered along with reagent concentration to maintain the 1 to 1 ratio of DO to titrant volume. Hach, La Motte and probably others, provide DO test kits packaged for field use. "Home made" kits are common.

B Alkalinity and hardness titrations are commonly sought under field conditions using standard acid and EDTA solutions. Methyl purple or mixed indicators commonly are used in place of methyl orange indicator because of better visibility in the field. Powder pillows for buffer and indicator are available for the hardness test.

C Chlorine tests using ortho tolidine in a comparator, such as those by Hilger or Taylor and others, frequently are used for rapid on site measurements. The comparators, reagents and comparator tubes commonly are available from apparatus, equipment, or chlorine suppliers.

VII SUMMARY

Simple and rapid field test kits are available from several manufacturers, suppliers and scientific apparatus houses for individual tests, as well as combination test kits for the items mentioned and others.

It is possible for the analyst to devise one suited to his particular requirements with available or improvised materials. Effectiveness of the field operation depends on the knowledge and care exercised in using the equipment and procedure.

CAUTION: Field test results are useful, but not always valid enough for the record.

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This outline was prepared by F. J. Ludzack, Chemist, National Training Center, WPO, Cincinnati, OH 45268.

Descriptors: Chemical Analysis, On-Site Tests, Water Analysis

BACTERIOLOGICAL INDICATORS OF WATER POLLUTION

Part 1. General Concepts

I INTRODUCTION

A Bacterial Indication of Pollution

1 In the broadest sense, a bacterial indicator of pollution is any organism which, by its presence, would demonstrate that pollution has occurred, and often suggest the source of the pollution.

2 In a more restrictive sense, bacterial indicators of pollution are associated primarily with demonstration of contamination of water, originating from excreta of warm-blooded animals (including man, domestic and wild animals, and birds).

B Implications of Pollution of Intestinal Origin

1 Intestinal wastes from warm-blooded animals regularly include a wide variety of genera and species of bacteria. Among these the coliform group may be listed, and species of the genera Streptococcus, Lactobacillus, Staphylococcus, Proteus, Pseudomonas, certain spore-forming bacteria, and others.

2 In addition, many kinds of pathogenic bacteria and other microorganisms may be released in wastes on an intermittent basis, varying with the geographic area, state of community health, nature and degree of waste treatment, and other factors. These may include the following:

a Bacteria: Species of Salmonella, Shigella, Leptospira, Brucella, Mycobacterium, and Vibrio comma.

b Viruses: A wide variety, including that of infectious hepatitis, Polioviruses, Coxsackie virus, ECHO viruses (enteric cytopathogenic human orphan -- "viruses in search of a disease"), and unspecified viruses postulated to account for outbreaks of diarrheal and upper respiratory diseases of unknown etiology, apparently infective by the water-borne route.

c Protozoa: Endamoeba histolytica

3 As routinely practiced, bacterial evidence of water pollution is a test for the presence and numbers of bacteria in wastes which, by their presence, indicate that intestinal pollution has occurred. In this context, indicator groups discussed in subsequent parts of this outline are as follows:

a Coliform group and certain subgroupings

b Fecal streptococci and certain subgroupings

c Miscellaneous indicators of pollution

4 Evidence of water contamination by intestinal wastes of warm-blooded animals is regarded as evidence of health hazard in the water being tested.

II PROPERTIES OF AN IDEAL INDICATOR OF POLLUTION

A An "ideal" bacterial indicator of pollution should:

1 Be applicable in all types of water

2 Always be present in water when pathogenic bacterial constituents of fecal contamination are present. Ramifications of this include --
   a Its density should have some direct relationship to the degree of fecal pollution.
   b It should have greater survival time in water than enteric pathogens, throughout its course of natural disappearance from the water body.
   c It should disappear rapidly from water following the disappearance of pathogens, either through natural or man-made processes.
   d It always should be absent in a bacteriologically safe water.

3 Lend itself to routine quantitative testing procedures without interference or confusion of results due to extraneous bacteria

4 Be harmless to man and other animals

B In all probability, an "ideal" bacterial indicator does not exist. The discussion of bacterial indicators of pollution in the following parts of this outline include consideration of the merits and limitations of each group, with their applications in evaluating bacterial quality of water.

III APPLICATIONS OF TESTS FOR POLLUTION INDICATORS

A Tests for Compliance with Bacterial Water Quality Standards

1 Potability tests on drinking water to meet Interstate Quarantine or other standards of regulatory agencies.

2 Determination of bacterial quality of environmental water for which quality standards may exist, such as shellfish waters, recreational waters, water resources for municipal or other supplies.

3 Tests for compliance with established standards in cases involving the protection or prosecution of municipalities, industries, etc.

B Treatment Plant Process Control

1 Water treatment plants

2 Wastewater treatment plants

C Water Quality and Pollutant Source Monitoring

1 Determination of intestinal pollution in surface water to determine type and extent of treatment required for compliance with standards.

2 Tracing sources of pollution

3 Determination of effects on bacterial flora, due to addition of organic or other wastes

D Special Studies, such as

1 Tracing sources of intestinal pathogens in epidemiological investigations

2 Investigations of problems due to the *Sphaerotilus* group

3 Investigations of bacterial interference to certain industrial processes, with respect to such organisms as *Pseudomonas*, *Achromobacter*, or others

IV SANITARY SURVEY

The laboratory bacteriologist is not alone in evaluation of indication of water pollution of intestinal origin. On-site study (Sanitary Survey) of the aquatic environment and adjacent areas, by a qualified person, is a necessary collateral study with the laboratory work and frequently will reveal information regarding potential bacteriological hazard which may or may not be demonstrated through laboratory findings from a single sample or short series of samples.

Part 2. The Coliform Group and Its Constituents

I ORIGINS AND DEFINITION

A Background

1 In 1885, Escherich, a pioneer bacteriologist, recovered certain bacteria from human feces, which he found in such numbers and consistency as to lead him to term these organisms "the characteristic organism of human feces."

He named these organisms Bacterium coli-commune and B. lactis aerogenes. In 1895, another bacteriologist, Migula, renamed B. coli commune as Escherichia coli, which today is the official name for the type species.

2 Later work has substantiated much of the original concept of Escherich, but has shown that the above species are in fact a heterogeneous complex of bacterial species and species variants.

a This heterogeneous group occurs not only in human feces but representatives also are to be found in many environmental media, including sewage, surface freshwaters of all categories, in and on soils, vegetation, etc.

b The group may be subdivided into various categories on the basis of numerous biochemical and other differential tests that may be applied.

B Composition of the Coliform Group

1 Current definition

As defined in "Standard Methods for the Examination of Water and Wastewater" (13th ed): "The coliform group includes all of the aerobic and facultative anaerobic, Gram-negative, nonspore-forming rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35° C."

2 The term "coliforms" or "coliform group" is an inclusive one, including the following bacteria which may meet the definition above:

a Escherichia coli, E. aurescens, E. freundii, E. intermedia

b Aerobacter aerogenes, A. cloacae

c Biochemical intermediates between the genera Escherichia and Aerobacter

* The above terminology is in accordance with the current editions of Standard Methods and Bergey's Manual of Determinative Bacteriology and will be consistent throughout this manual until these sources are modified.

3 There is no provision in the definition of coliform bacteria for "atypical" or "aberrant" coliform strains.

a An individual strain of any of the above species may fail to meet one of the criteria of the coliform group.

b Such an organism, by definition, is not a member of the coliform group, even though a taxonomic bacteriologist may be perfectly correct in classifying the strain in one of the above species.

II SUBDIVISION OF COLIFORMS INTO "FECAL" AND "NONFECAL" CATEGORIES

A Need

Single-test differentiations between coliforms of "fecal" origin and those of "nonfecal" origin are based on the assumption that typical E. coli and closely related strains are of fecal origin while A. aerogenes and its close relatives are not of direct fecal origin. (The latter assumption is not fully borne out by investigations at this Center. See Table 1, IMViC Type --++). A number of single differential tests have been proposed to differentiate between "fecal" and "nonfecal" coliforms.

Without discussion of their relative merits, several may be cited here:

B Types of Single-Test Differentiation

1 Determination of gas ratio

Fermentation of glucose by E. coli results in gas production, with hydrogen and carbon dioxide being produced in equal amounts. Fermentation of glucose by A. aerogenes results in generation of twice as much carbon dioxide as hydrogen.

Further studies suggested absolute correlation between H_2/CO_2 ratios and the terminal pH resulting from glucose fermentation. This led to the substitution of the methyl red test.

2 Methyl red test

Glucose fermentation by E. coli typically results in a culture medium having terminal pH in the range 4.2 - 4.6 (red color a positive test with the addition of methyl red indicator). A. aerogenes typically results in a culture medium having pH 5.6 or greater (yellow color, a negative test).

3 Indole

When tryptophane, an amino acid, is incorporated in a nutrient broth, typical E. coli strains are capable of producing indole (positive test) among the end products, whereas A. aerogenes does not (negative test).

In reviewing technical literature, the worker should be alert to the method used to detect indole formation, as the results may be greatly influenced by the analytical procedure.

4 Voges-Proskauer test (acetylmethyl carbinol test)

The test is for detection of acetylmethyl carbinol, a derivative of 2, 3, butylene-glycol, as a result of glucose fermentation in the presence of peptone. A. aerogenes produces this end product (positive test) whereas E. coli gives a negative test.

a Experience with coliform cultures giving a positive test has shown a loss of this ability with storage on laboratory media for 6 months to 2½ years, in 20 - 25% of cultures (105 out of 458 cultures).

b Some workers consider that all coliform bacteria produce acetylmethyl carbinol in glucose metabolism. These workers regard acetylmethyl carbinol-negative cultures as those which have enzyme systems capable of further degradation of acetylmethyl carbinol to other end products which do not give a positive test with the analytical procedure. Cultures giving a positive test for acetylmethyl carbinol lack this enzyme system.

c This reasoning leads to a hypothesis (not experimentally proven) that the change of reaction noted in certain cultures in 4.a above is due to the activation of a latent enzyme system.

5 Citrate utilization

Cultures of E. coli are unable to use the carbon of citrates (negative test) in their metabolism, whereas cultures of A. aerogenes are capable of using the carbon of citrates in their metabolism (positive test).

Some workers (using Simmons Citrate Agar) incorporate a pH indicator (brom thymol blue) in the culture medium in order to demonstrate the typical alkaline reaction (pH 8.4 - 9.0) resulting with citrate utilization.

6 Elevated temperature (Eijkman) test

a The test is based on evidence that E. coli and other coliforms of fecal

origin are capable of growing and fermenting carbohydrates (glucose or lactose) at temperatures significantly higher than the body temperature of warm-blooded animals. Organisms not associated with direct fecal origin would give a negative test result, through their inability to grow at the elevated temperature.

b While many media and techniques have been proposed, EC Broth, a medium developed by Perry and Hajna, used as a confirmatory medium for 24 hours at 44.5 ± 0.2°C are the current standard medium and method.
While the "EC" terminology of the medium suggests "E. coli" the worker should not regard this as a specific procedure for isolation of E. coli.

c A similar medium, Boric Acid Lactose Broth, has developed by Levine and his associates. This medium gives results virtually identical with those obtained from EC Broth, but requires 48 hours of incubation.

d Elevated temperature tests require incubation in a water bath. Standard Methods 13th Ed. requires this temperature to be 44.5 ± 0.2°C. Various workers have urged use of temperatures ranging between 43.0°C and 46.0°C. Most of these recommendations have provided a tolerance of ± 0.5°C from the recommended levels. However, some workers, notably in the Shellfish Program of the Public Health Service, stipulate a temperature of 44.5 ± 0.2°C. This requires use of a water bath with forced circulation to maintain this close tolerance. This tolerance range was instituted in the 13th Edition of Standard Methods and the laboratory worker should conform to these new limits.

e The reliability of elevated temperature tests is influenced by the time required for the newly-inoculated cultures to reach the designated incubation temperature. Critical workers insist on placement of the cultures in the water bath within 30 minutes, at most, after inoculation.

7 Other tests

Numerous other tests for differentiation between coliforms of fecal vs. nonfecal origin have been proposed. Current studies suggest little promise for the following tests in this application: uric acid test, cellobiose fermentation, gelatin liquefaction, production of hydrogen sulfide, sucrose fermentation, and others.

C IMViC Classification

1 In 1938, Parr reported on a review of a literature survey on biochemical tests used to differentiate between coliforms of fecal vs. nonfecal origin. A summary follows:

Test	No. of times used for differentiation
Voges-Proskauer reaction	22
Methyl red test	20
Citrate utilization	20
Indole test	15
Uric acid test	6
Cellobiose fermentation	4
Gelatin liquefaction	3
Eijkman test	2
Hydrogen sulfide production	1
Sucrose fermentation	1
a-Methyl-d-glucoside fermentation	1

2 Based on this summary and on his own studies, Parr recommended utilization of a combination of tests, the indole, methyl red, Voges-Proskauer, and the citrate utilization tests for this differentiation. This series of reactions is designated by the mnemonic "IMViC". Using this scheme, any coliform culture can be described by an "IMViC Code" according to the reactions for each culture. Thus, a typical culture of E. coli would have a code ++--, and a typical A. aerogenes culture would have a code --++.

3 Groupings of coliforms into fecal, non-fecal, and intermediate groups, as shown in "Standard Methods for the Examination of Water and Wastewater" are shown at the bottom of this page.

D Need for Study of Multiple Cultures

All the systems used for differentiation between coliforms of fecal vs. those of nonfecal origin require isolation and study of numerous pure cultures. Many workers prefer to study at least 100 cultures from any environmental source before attempting to categorize the probable source of the coliforms.

III NATURAL DISTRIBUTION OF COLIFORM BACTERIA

A Sources of Background Information

Details of the voluminous background of technical information on coliform bacteria recovered from one or more environmental media are beyond the scope of this discussion. References of this outline are suggested routes of entry for workers seeking to explore this topic.

B Studies on Coliform Distribution

1 Since 1960 numerous workers have engaged in a continuing study of the natural distribution of coliform bacteria and an evaluation of procedures for differentiation between coliforms of fecal vs. probable nonfecal origin. Results of this work have special significance because:

a Rigid uniformity of laboratory methods have been applied throughout the series of studies

b Studies are based on massive numbers of cultures, far beyond any similar studies heretofore reported

Groupings of Coliforms into Fecal, Nonfecal and Intermediate Groups

Organism	Indole	Methyl red	Voges-Proskauer	Citrate
E. coli, Variety I	+	+	-	-
Variety II	-	+	-	-
E. freundii (Intermediates)				
Variety I	-	+	-	±
Variety II	+	+	-	+
A. aerogenes				
Variety I	-	-	+	±
Variety II	±	-	+	+

c A wider variety of environmental and biological sources is being studied than in any previous series of reports.

d All studies are based on freshly recovered pure culture isolates from the designated sources.

e All studies are based on cultures recovered from the widest feasible geographic range, collected at all seasons of the year. It is believed that no more representative series of studies has been made or is in progress.

2 Distribution of coliform types

Table 1 shows the consolidated results of coliform distributions from various biological and environmental sources.

a The results of these studies show a high order of correlation between known or probable fecal origin and the typical E. coli IMViC code (++--). On the other hand, human feces also includes numbers of A. aerogenes and other IMViC types, which some regard as "nonfecal" segments of the coliform group. (Figure 1)

b The majority of coliforms attributable to excretal origin tend to be limited to a relatively small number of the possible IMViC codes; on the other hand, coliform bacteria recovered from undisturbed soil, vegetation, and insect life represent a wider range of IMViC codes than fecal sources, without clear dominance of any one type. (Figure 2)

c The most prominant IMViC code from nonfecal sources is the intermediate type, -+-+, which accounts for almost half the coliform cultures recovered from soils, and a high percentage of those recovered from vegetation and from insects. It would appear that if any coliform segment could be termed a "soil type" it would be IMViC code -+-+.

d It should not be surprising that cultures of typical E. coli are recovered in relatively smaller numbers from sources judged, on the basis of sanitary survey, to be unpolluted. There is no known way to exclude the influence of limited fecal pollution from small animals and birds in such environments.

e The distribution of coliform types from human sources should be regarded as a representative value for large numbers of sources. Investigations have shown that there can be large differences in the distribution of IMViC types from person to person, or even from an individual.

3 Differentiation between coliforms of fecal vs. nonfecal origin

Table 2 is a summary of findings based on a number of different criteria for differentiating between coliforms of fecal origin and those from other sources.

a IMViC type ++-- is a measurement of E. coli, Variety I, and appears to give reasonably good correlation between known or highly probable fecal origin and doubtful fecal origin.

b The combination of IMViC types, ++--, +---, and -+--, gives improved identification of probable fecal origin, and appears also to exclude most of the coliforms not found in excreta of warm-blooded animals in large numbers.

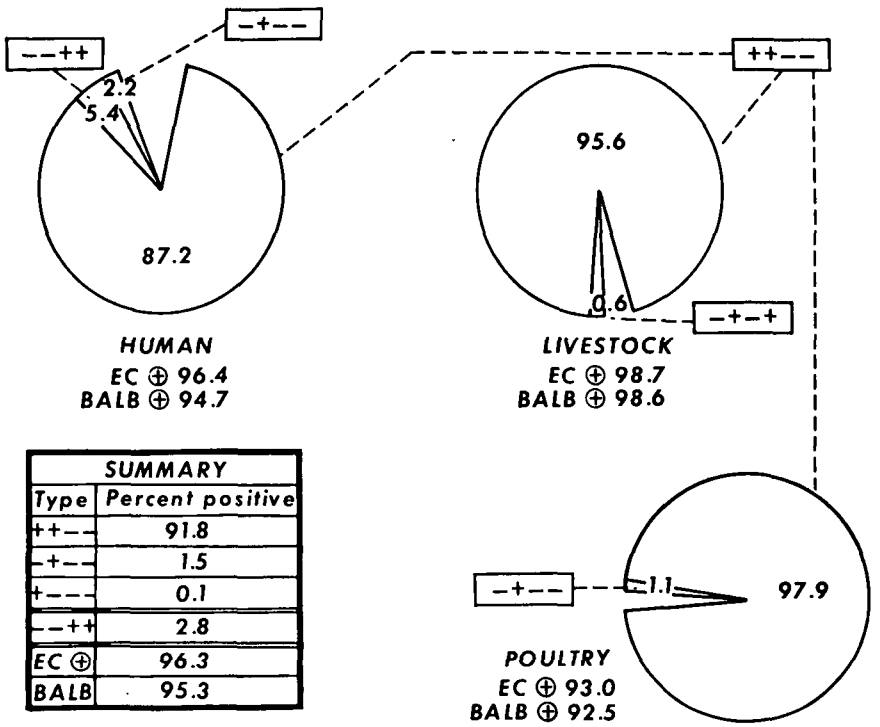
c While the indole, methyl red, Voges Proskauer, and citrate utilization tests, each used alone, appear to give useful answers when applied only to samples of known pollution from fecal sources, the interpretation is not as clear when applied to coliforms from sources believed to be remote from direct fecal pollution.

Table 1. COLIFORM DISTRIBUTION BY IMViC TYPES AND ELEVATED TEMPERATURE TEST FROM ENVIRONMENTAL AND BIOLOGICAL SOURCES

IMViC type	Vegetation		Insects		Soil				Fecal sources				Poultry	
					Undisturbed		Polluted		Human		Livestock			
	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total
++--	128	10.6	134	12.4	131	5.6	536	80.6	3932	87.2	2237	95.6	1857	97.9
--++	237	19.7	113	10.4	443	18.8	13	2.0	245	5.4	0	<0.1	1	0.1
-+--	23	1.9	0	<0.1	78	3.3	1	0.2	99	2.2	14	0.6	20	1.1
+++-	2	0.2	0	<0.1	7	0.3	0	<0.1	106	2.4	59	2.5	0	<0.1
-+-+	168	14.0	332	30.6	1131	48.1	87	13.0	50	1.1	1	<0.1	5	0.3
++-+	116	9.6	118	10.9	87	3.7	22	3.3	35	0.8	27	1.2	11	0.6
-+++	32	2.7	28	2.6	181	7.7	5	0.7	21	0.5	0	<0.1	0	<0.1
++++	291	24.2	254	23.4	159	6.8	0	<0.1	6	0.1	0	<0.1	0	<0.1
+-++	88	7.3	46	4.2	67	2.9	0	<0.1	14	0.2	0	<0.1	0	<0.1
---+	87	7.2	42	3.9	4	0.2	1	0.2	2	<0.1	0	<0.1	0	<0.1
-++-	5	0.4	0	<0.1	1	<0.1	0	<0.1	0	<0.1	0	<0.1	0	<0.1
--+-	19	1.6	0	<0.1	53	2.3	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+-+-	2	0.2	0	<0.1	6	0.3	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+--+	5	0.4	8	0.7	0	<0.1	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+---	0	<0.1	9	0.8	0	<0.1	0	<0.1	2	<0.1	0	<0.1	2	<0.1
Total	1203		1084		2348		665		4512		2339		1896	
No. EC +	169*		162*		216		551		4349		2309		1765	
% EC +	14.1*		14.9*		9.2		82.9		96.4		98.7		93.0	

*120 of these were ++--, 15 --++, 11 -+--

*129 of these were ++--, 27 -+-+, 5 ++-+



SUMMARY	
Type	Percent positive
++--	91.8
-+--	1.5
+---	0.1
--++	2.8
EC ⊕	96.3
BALB	95.3

FIGURE 1

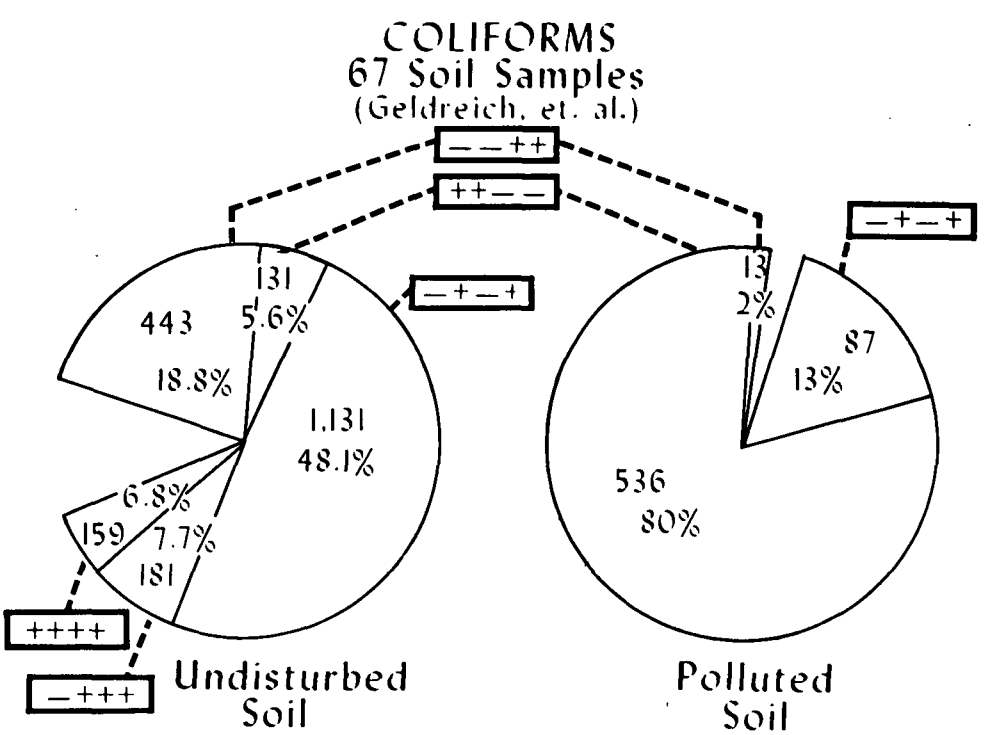


FIGURE 2

Table 2. COMPARISON OF COLIFORM STRAINS ISOLATED FROM WARM-BLOODED ANIMAL FECES, FROM UNPOLLUTED SOILS AND POLLUTED SOILS WITH USE OF THE IMViC REACTIONS AND THE ELEVATED TEMPERATURE TEST IN EC MEDIUM AT 44.5° C (±0.5°) (12th ed. 1965; Standard Methods for the Examination of Water and Wastewater)

Test	Warm-blooded animal feces	Soil: Unpolluted	Soil: Polluted	Vegetation	Insects
+ + - -	91.8%	5.6%	80.6%	10.6%	12.4%
+ + - -, + - - - and - + - -	93.3%	8.9%	80.7%	12.5%	13.2%
Indole positive	94.0%	19.4%	82.7%	52.5%	52.4%
Methyl red positive	96.9%	75.6%	97.9%	63.6%	79.9%
Voges-Proskauer positive	5.1%	40.7%	97.3%	56.3%	40.6%
Citrate utilizers	3.6%	88.2%	19.2%	85.1%	86.7%
Elevated temperature (EC) positive	96.4%	9.2%	82.9%	14.1%	14.9%
Number of cultures studied	8,747	2,348	665	1,203	1,084

Total Pure Cultures Studied: 14,047

d The elevated temperature test gives excellent correlation with samples of known or highly probable fecal origin. The presence of smaller, but demonstrable, percentages of such organisms in environmental sources not interpreted as being polluted could be attributed largely to the warm-blooded wildlife in the area, including birds, rodents, and other small mammals.

e The elevated temperature test yields results equal to those obtained from the total IMViC code. It has marked advantages in speed, ease and simplicity of performance, and yields quantitative results for each water sample. Therefore, it is the official standard method for differentiation coliforms of probable direct fecal origin from those which may have become established in the bacterial flora of the aquatic or terrestrial habitat.

IV EVALUATION OF COLIFORMS AS POLLUTION INDICATORS

A The Coliform Group as a Whole

1 Merits

a The absence of coliform bacteria is evidence of a bacteriologically safe water.

b The density of coliforms is roughly proportional to the amount of excretal pollution present.

c If pathogenic bacteria of intestinal origin are present, coliform bacteria also are present, in much greater numbers.

d Coliforms are always present in the intestines of humans and other warm-blooded animals, and are eliminated in large numbers in fecal wastes.

e Coliforms are more persistent in the aquatic environment than are pathogenic bacteria of intestinal origin.

f Coliforms are generally harmless to humans and can be determined quantitatively by routine laboratory procedures.

2 Limitations

a Some of the constituents of the coliform group have a wide environmental distribution in addition to their occurrence in the intestines of warm-blooded animals.

b Some strains of the coliform group may multiply in certain polluted waters ("aftergrowth"), of high nutritive values thereby adding to the difficulty of evaluating a pollution situation in the aquatic environment. Members of the A. aerogenes section of the coliform are commonly involved in this kind of problem.

c Because of occasional aftergrowth problems, the age of the pollution may be difficult to evaluate under some circumstances.

d Tests for coliforms are subject to interferences due to other kinds of bacteria. False negative results sometimes occur when species of Pseudomonas are present. False positive results sometimes occur when two or more kinds of non-coliforms produce gas from lactose, when neither can do so alone (synergism).

B The Fecal Coliform Component of the Coliform Group (as determined by elevated temperature test)

1 Merits

a The majority (over 95% of the coliform bacteria from intestines of warm-blooded animals grow at the elevated temperature.

b These organisms are of relatively infrequent occurrence except in association with fecal pollution.

c Survival of the fecal coliform group is shorter in environmental waters than for the coliform group as whole. It follows, then, that high densities of fecal coliforms is indicative of relatively recent pollution.

d Fecal coliforms generally do not multiply outside the intestines of warm-blooded animals. In certain high-carbohydrate wastes, such as from the sugar beet refineries, exceptions have been noted.

e In some wastes, notably those from pulp and paper mills, Klebsiella has been found in large numbers utilizing the elevated temperature test. There has been much controversy about whether the occurrence of Klebsiella is due to aftergrowth due to soluble carbohydrates in such wastes. The significance of Klebsiella as an indicator of direct discharge of intestinal wastes thus is under challenge. The issue is still further complicated by questions over whether Klebsiella is in and of itself a pathogenic organism or is potentially pathogenic. This is a serious problem which is the subject of current intensive research by this Agency.

2 Limitations

a Feces from warm-blooded animals include some (though proportionately low) numbers of coliforms which do not yield a positive fecal coliform test when the elevated temperature test is used as the criterion of differentiation. These organisms are E. coli varieties by present taxonomic classification.

b There is at present no established and consistent correlation between

ratios of total coliforms/fecal coliforms in interpreting sanitary quality of environmental waters.

In domestic sewage, the fecal coliform density commonly is greater than 90% of the total coliform density. In environmental waters relatively free from recent pollution, the fecal coliform density may range from 10-30% of the total coliforms. There are, however, too many variables relating to water-borne wastes and surface water runoff to permit sweeping generalization on the numerical relationships between fecal- and total coliforms.

c Studies have been made regarding the survival of fecal coliforms in polluted waters compared with that of enteric pathogenic bacteria. In recent pollution studies, species of Salmonella have been found in the presence of 220 fecal coliforms per 100 ml (Spino), and 110 fecal coliforms per 100 ml (Brezenski, Raritan Bay Project).

3 The issue of the Klebsiella problem described in an earlier paragraph may ultimately be resolved as a merit or as a limitation of the value of the fecal coliform test.

V APPLICATIONS OF COLIFORM TESTS

A Current Status in Official Tests

1 The coliform group is designated, in "Standard Methods for the Examination of Water and Wastewater" (13th ed., 1971), through the Completed Test MPN procedure as the official test for bacteriological potability of water. The Confirmed Test MPN procedure is accepted where it has been demonstrated, through comparative tests, to yield results equivalent to the Completed Test. The membrane filter method also is accepted for examination of waters subject to interstate regulation.

2 The 12th edition of Standard Methods introduced a standard test for fecal coliform bacteria. It is emphasized that this is to be used in pollution studies, and does not apply to the evaluation of water for potability. This procedure has been continued in the 13th Edition.

B Applications

1 Tests for the coliform group as a whole are used in official tests to comply with interstate drinking water standards, state standards for shellfish waters, and in most, if not all, cases where bacterial standards of water quality have been established for such use as in recreational or bathing waters, water supplies, or industrial supplies. Laboratory personnel should be aware of possible implementation of the fecal coliform group as the official test for recreational and bathing waters.

2 The fecal coliform test has application in water quality surveys, as an adjunct to determination of total coliform density. The fecal coliform test is being used increasingly in all water quality surveys.

3 It is emphasized that no responsible worker advocates substitution of a fecal coliform test for total coliforms in evaluating drinking water quality.

Part 3. The Fecal Streptococci

I INTRODUCTION

Investigations regarding streptococci progressed from the streptococci of medical concern to those which were distributed in differing environmental conditions which, again, related to the welfare of man. The streptococci were originally reported by Laws and Andrews (1894), and Houston (1899, 1900) considered those streptococci, which we now call "fecal streptococci," as ... "indicative of dangerous pollution, since they are readily demonstrable in waters recently polluted and seemingly altogether absent from waters above suspicion of contamination.

From their discovery to the present time the fecal streptococci appear characteristic of fecal pollution, being consistently present in both the feces of all warm-blooded animals and in the environment associated with animal discharges. As early as 1910 fecal streptococci were proposed as indicators to the Metropolitan Water Board of London. However, little progress resulted in the United States until improved methods of detection and enumeration appeared after World War II.

Renewed interest in the group as indicators began with the introduction of azide dextrose broth in 1950, (Mallmann & Seligmann, 1950). The method which is in the current edition of Standard Methods appeared soon after. (Litsky, et al. 1955).

With the advent of improved methods for detection and enumeration of fecal streptococci, significant body of technical literature has appeared.

This outline will consider the findings of various investigators regarding the fecal streptococci and the significance of discharges of these organisms into the aquatic environment.

II FECAL MATERIALS

A Definition

The terms "enterococci," "fecal streptococci," "Group D streptococci," "Streptococcus fecalis," and even "streptococci" have been used in a loose and interchangeable manner to indicate the streptococci present in the enteric tract of warm-blooded animals or of the fresh fecal material excreted therefrom.

Enterococci are characterized by specific taxonomic biochemistry. Serological procedures differentiate the Group D streptococci from the various groups. Although they overlap, the three groups, fecal streptococcus, enterococcus, and Group D streptococcus, are not synonymous. Because our emphasis is on indicators of unsanitary origin, fecal streptococcus is the more appropriate term and will include the enterococcus as well as other groups.

A rigid definition of the fecal streptococcus group is not possible with our present knowledge. The British Ministry of Health (1956) defines the organisms as "Gram-positive" cocci, generally occurring in pairs or short chains, growing in the presence of bile salt, usually capable of development at 45° C, producing acid but not gas in mannitol and lactose, failing to attack raffinose, failing to reduce nitrate to nitrite, producing acid in litmus milk and precipitating the casein in the form of a loose, but solid curd, and exhibiting a greater resistance to heat, to alkaline conditions and to high concentrations of salt than most vegetative bacteria." However, it is pointed out that "streptococci departing in one or more particulars from the type species cannot be disregarded in water."

For the proposes of this outline, and in line with the consensus of most water microbiologists in this country, the definition of the fecal streptococci is:

> . . . "The group composed of Group D species consistently present in significant numbers in fresh fecal excreta of warm-blooded animals, which includes all of the enterococcus group in addition to other groups of streptococci."

B Species Isolated

1 Findings

a Human feces

Examination of human fecal specimens yields a high percentage of the enterococcus group and usually demonstration of the S. salivarius which is generally considered a member of the human throat flora and to be surviving in human fecal materials rather than actively multiplying in the enteric tract. Also present would be a small percentage of variants or biotypes of the enterococcus group.

b Nonhuman Feces

1) Fecal material which are from nonhuman or not from fowl will yield high percentages of the S. bovis and/or S. equinus organisms with a concomitantly reduced percentage of the enterococcus group.

2) Fowl excreta

Excrement from fowl characteristically yields a large percentage of enterococcal biotypes as well as a significant percentage of enterococcus group.

2 Significance

Species associations with particular animal hosts is an established fact and leads to the important laboratory technique of partition counting of colonies from the membrane filter or agar pour plates in order to establish or confirm the source of excretal pollution in certain aquatic investigations.

It is important to realize that a suitable medium is necessary in order to allow all of the streptococci which we consider to be fecal streptococci to grow in order to give credence to the derived opinions. Use of liquid growth media into which direct inoculations from the sample are made have not proven to be successful for partition counting due to the differing growth rates of the various species of streptococci altering the original percentage relationships. Due to the limited survival capabilities of some of the fecal streptococci it is necessary to sample fresh fecal material or water samples in close proximity to the pollution source especially when multiple sources are contributing to a reach of water. Also the pH range must be within the range of 4.0-9.0.

III FECAL STREPTOCOCCI IN THE AQUATIC ENVIRONMENT

A General

From the foregoing it is appears that the preponderant human fecal streptococci are composed of the enterococcus group and, as this is the case, several media are presently available which will detect only the enterococcal group will be suitable for use with aquatic samples which are known to be contaminated or potentially contaminated with purely domestic (human) wastes. On the other hand, when it is known or suspected that other-than-human wastes have potential egress to the aquatic environment under investigation, it is necessary to utilize those media which are capable of quantitating the whole of the fecal streptococci group.

B Stormwaters and Combined Sewers

1 General

Storm sewers are a series of pipes and conduits which receive surface runoffs from the action of rainstorms and do not include sewage which are borne by a system of sanitary sewers. Combined sewers receive both the storm runoff and the water-borne wastes of the sanitary system.

Both storm water and combined sewer flows have been found to usually contain large quantities of fecal streptococci in numbers which generally are larger than those of the fecal coliform indicator organisms.

2 Bacteriological Findings

Table 1 represents, in a modified form, some of the findings of Geldreich and Kenner (1969) with respect to the densities of fecal streptococci when considering Domestic sewage in contrast to Stormwaters:

Table 1

DISTRIBUTION OF FECAL STREPTOCOCCI IN DOMESTIC SEWAGES AND STORMWATER RUNOFFS

Water Source	Fecal Streptococci per 100 ml median values	Ratio FC/FS
Domestic Sewage		
Preston, ID	64,000	5.3
Fargo, ND	290,000	4.5
Moorehead, MN	330,000	4.9
Cincinnati, OH	2,470,000	4.4
Lawrence, MA	4,500,000	4.0
Monroe, MI	700,000	27.9
Denver, CO	2,900,000	16.9
Stormwater		
Business District	51,000	0.26
Residential	150,000	0.04
Rural	58,000	0.05

The Ratio FC/FS is that of the Fecal coliform and Fecal streptococci and it will be noted that in each case, when considering the Domestic Sewage, it is 4.0 or greater while it is less than 0.7 for stormwaters. The use of this ratio is useful to identify the source of pollution as

Table 2. ESTIMATED PER CAPITA CONTRIBUTION OF INDICATOR MICROORGANISMS FROM SOME ANIMALS*

		Average indicator density per gram of feces		Average contribution per capita per 24 hr		
Animals	Avg wt of Feces/24 hr, wet wt, g	Fecal coliform, million	Fecal streptococci, million	Fecal coliform, million	Fecal streptococci, million	Ratio FC/FS
Man	150	13.0	3.0	2,000	450	4.4
Duck	336	33.0	54.0	11,000	18,000	0.6
Sheep	1,130	16.0	38.0	18,000	43,000	0.4
Chicken	182	1.3	3.4	240	620	0.4
Cow	23,600	0.23	1.3	5,400	31,000	0.2
Turkey	448	0.29	2.8	130	1,300	0.1
Pig	2,700	3.3	84.0	8,900	230,000	0.04

*Publication WP-20-3, P. 102

being human or nonhuman warm-blooded animal polluted. When the ratio is greater than 4.0 it is considered to be human waste contaminated while a ratio of less than 0.7 is considered to be nonhuman. It is evident that the storm-waters have been primarily polluted by excreta of rats and other rodents and possibly domestic and/or farm animals.

Species differences are the main cause of different fecal coliform-fecal streptococci ratios. Table 2 compares fecal streptococcus and fecal coliform counts for different species. Even though individuals vary widely, masses of individuals in a species have characteristic proportion of indicators.

C Surface Waters

In general, the occurrence of fecal streptococci indicates fecal pollution and its absence indicates that little or no warm-blooded fecal contribution. In studies of remote surface waters the fecal streptococci are infrequently isolated and occurrences of small numbers can be attributed to wild life and/or snow melts and resultant drainage flows.

Various examples of fecal streptococcal occurrences are shown in Table 3 in relation to surface waters of widely varying quality. (Geldreich and Kenner 1969)

Table 3

INDICATOR ORGANISMS IN SURFACE WATERS

Water Source	Densities/100 ml Fecal coliform	Densities/100 ml Fecal streptococci
Prairie Watersheds		
Cherry Creek, WY	90	83
Saline River, KS	95	180
Cub River, ID	110	160
Clear Creek, CO	170	110
Recreational Waters		
Lake Mead	2	444
Lake Moovalaya	9	170
Colorado River	4	256
Whitman River	32	88
Merrimack River	100	96
Public Water Intakes		
Missouri River (1959)		
Mile 470.5	11,500	39,500
Mile 434.5	22,000	79,000
Mile 408.8	14,000	59,000

IV FECAL STREPTOCOCCI: ADVANTAGES AND LIMITATIONS

A General

Serious studies concerning the streptococci were instituted when it became apparent that they were the agents responsible or suspected for a wide variety of human diseases. Natural priority then focused itself to the taxonomy of these organisms and this study is still causing consternation as more and more microbiological techniques have been brought to bear on these questions. The sanitary microbiologist is concerned with those streptococci which inhabit the enteric tract of warm-blooded animals, their detection, and utilization in developing a criterium for water quality standards.

Kabler (1962) discussed the slow acceptance of the fecal streptococci as indicators of pollution resulting from:

1 Multiplicity and difficulty of laboratory procedures

2 Poor agreement between methods of quantitative enumeration

3 Lack of systematic studies of . . . .

a sources

b survival, and

c interpretations, and

4 Undue attention to the S. faecalis group.

Increased attention to the fecal streptococci, especially during the last decade, have clarified many of the earlier cloudy issues and have elevated the stature of these organisms as indicators of pollution. Court precedents establishing legal status and recommendations of various technical advisory boards have placed the fecal coliform group in a position of primacy in many water quality applications. The fecal streptococci have evolved from a position of a theoretically useful indicator to one which was ancillary to the coliforms to one which was useful when discrepancies or questions evolved as to the validity of the coliform data to one where an equality status was achieved in certain applications. In the future it is anticipated that, for certain applications, the fecal streptococci will achieve a position of primacy for useful data, and, as indicated by Litsky (1955) "be taken out of the realm of step-children and given their legitimate place in the field of santiary bacteriology as indicators of sewage pollution."

B Advantages and Limitations

1 Survival

In general, the fecal streptococci have been observed to have a more limited survival time in the aquatic environment when compared to the coliform group. They are rivaled in this respect only by the fecal coliforms. Except for cases of persistence in waters of high electrolytic content, as may be common to irrigation waters, the fecal streptococci have not been observed to multiply in polluted waters as may sometimes be observed for some of the coliforms. Fecal streptococci usually require a greater abundance of nutrients for survival as compared to the coliforms and the coliforms are more dependent upon the oxygen tension in the waterbody. In a number of situations it was concluded that the fecal streptococci reached an extinction point more rapidly in warmer waters while the reverse was true in the colder situations as the coliforms now were totally eliminated sooner.

2 Resistance to Disinfection

In artificial pools the source of contamination by the bathers is usually limited to throat and skin flora and thus increasing attention has been paid to indicators other than those traditionally from the enteric tract. Thus, one of the organisms considered to be a fecal streptococci, namely, S. salivarius, can be a more reliable indicator when detected along with the other fecal streptococci especially since studies have confirmed the greater resistance of the fecal streptococci to chlorination. This greater resistance to chlorination, when compared to the fecal coliforms, is important since the dieoff curve differences are insignificant when the curves of the fecal coliforms are compared to various Gram negative pathogenic bacteria which reduces their effectiveness as indicators.

3 Ubiquitous Strains

Among the fecal streptococcus are two organisms, one a biotype and the other a variety of the S. faecalis, which, being ubiquitous (omnipresent) have limited sanitary significance. The biotype, or atypical, S. faecalis is characterized by its ability to hydrolyze starch while the varietal form, liquefaciens, is nonbeta haemolytic and capable of liquefying gelatin. Quantitation of these organisms in anomalous conditions is due to their capability of survival in soil or high electrolytic waters and in waters with a temperature of less than 12 Degrees C.

Samples have been encountered which have been devoid of fecal coliforms and yet contain a substantial number of "fecal streptococci" of which these ubiquitous strains constitute the majority or all of the isolations when analyzed biochemically.

V STANDARDS AND CRITERIA

Acceptance and utilization of Total Coliform criteria, which must now be considered a pioneering effort, has largely been supplanted in concept and in fact by the fecal coliforms in establishing standards for recreational waters.

The first significant approach to the utilization of the fecal streptococci as a criterium for recreational water standards occurred in 1966 when a technical committee recommended the utilization of the fecal streptococci with the total coliforms as criteria for standards pertaining to the Calumet River and lower Lake Michigan waters. Several sets of criteria were established to fit the intended uses for this area. The use of the fecal streptococci as a criterium is indicated to be tentative pending the accumulation of existing densities and could be modified in future standards.

With the existing state-of-the-art knowledge of the presence of the fecal streptococci in waters containing low numbers of fecal coliforms it is difficult to establish a specific fecal streptococcus density limit of below 100 organisms/100 ml when used alone or in conjunction with the total coliforms.

Part 4. Other Bacterial Indicators of Pollution

I TOTAL BACTERIAL COUNTS

A Historical

1 The early studies of Robert Koch led him to develop tentative standards of water quality based on a limitation of not more than 100 bacterial colonies per ml on a gelatin plating medium incubated 3 days at 20° C.

2 Later developments led to inoculation of samples on duplicate plating media, with one set incubated at 37° C and the other at 20° C.

a Results were used to develop a ratio between the 37° C counts and the 20° C counts.

b Waters having a predominant count at 37° C were regarded as being of probable sanitary significance, while those giving predominant counts at 20° C were considered to be of probable soil origin, or natural inhabitants of the water being examined.

B Groups Tested

There is no such thing as "total" bacterial count in terms of a laboratory determination.

1 Direct microscopic counts do not differentiate between living and dead cells.

2 Plate counting methods enumerate only the bacteria which are capable of using the culture medium provided, under the temperature and other growth conditions used as a standard procedure. No one culture medium and set of growth conditions can provide, simultaneously, an acceptable environment for all the heterogeneous, often conflicting, requirements of the total range of bacteria which may be recovered from waters.

C Utilization of Total Counts

1 Total bacterial counts, using plating methods, are useful for:

a Detection of changes in the bacterial composition of a water source

b Process control procedures in treatment plant operations

c Determination of sanitary conditions in plant equipment or distributional systems

2 Serious limitations in total bacterial counts exist because:

a No information is given regarding possible or probable fecal origin of bacterial changes. Large numbers of bacteria can sometimes be cultivated from waters known to be free of fecal pollution.

b No information of any kind is given about the species of bacteria cultivated.

c There is no differentiation between harmless or potentially dangerous forms.

3 Status of total counts

a There is no total bacterial count standard for any of the following:

Interstate Quarantine Drinking Water Standards

PHS regulations for water potability (as shown in "Standard Methods" Public Health Service Drinking Water Standards of 1962.)

b The most widely used current application of total bacterial counts in water bacteriology today is in

water treatment plants, where some workers use standard plate counts for process control and for determination of the bacterial quality of distribution systems and equipment.

c Total bacterial counts are not used in PHS water quality studies, though extensively used until the 1940's.

B Spore-Forming Bacteria (*Clostridium perfringens*, or *C. welchii*)

1 Distribution

This is one of the most widely distributed species of bacteria. It is regularly present in the intestinal tract of warm-blooded animals.

2 Nature of organism

C. perfringens is a Gram-positive, spore-forming rod. The spores cause a distinct swelling of the cell when formed. The organism is extremely active in fermentation of carbohydrates, and produces the well-known "stormy fermentation" of milk.

3 Status

The organism, when present, indicates that pollution has occurred at some time. However, because of the extremely extended viability of the spores, it is impossible to obtain even an approximation of the recency of pollution based only on the presence of *C. perfringens*.

The presence of the organism does not necessarily indicate an unsafe water.

C Tests for Pathogenic Bacteria of Intestinal Origin

1 Groups considered include *Salmonella* sp, *Shigella* sp, *Vibrio comma*, *Mycobacterium* sp, *Pasteurella* sp, *Leptospira* sp, and others.

2 Merits of direct tests:

Demonstration of any pathogenic species would demonstrate an unsatisfactory water quality, hazardous to persons consuming or coming into contact with that water.

3 Limitations

a There is no available routine procedure for detection of the full range of pathogenic bacteria cited above.

b Quantitative methods are not available for routine application to any of the above.

c The intermittent release of these pathogens makes it impossible to regard water as safe, even in the absence of pathogens.

d After detection, the public already would have been exposed to the organism; thus, there is no built-in margin of safety, as exists with tests for the coliform group.

4 Applications

a In tracing the source of pathogenic bacteria in epidemiological investigations

b In special research projects

c In water quality studies concerned with enforcement actions against pollution, increasing attention is being given to the demonstration of enteric pathogenic bacteria in the presence of the bacterial indicators of pollution.

D Miscellaneous Indicators

It is beyond this discussion to explore the total range of microbiological indicators of pollution that have been proposed and

investigated to some extent. Mention can be made, however, of consideration of tests for the following.

1 Bacteriophages specific for any of a number of kinds of bacteria

2 Serological procedures for detection of coliforms and other indicators; a certain amount of recent attention has been given to applications of fluorescent antibodies in such tests

3 Tests for Pseudomonas aeruginosa

4 Tests for viruses, which may persist in waters even longer than members of the coliform group.

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater, 13th ed., APHA, AWWA, WPCF. Published by American Public Health Association, 1790 Broadway, New York, N.Y. 1971.

2 Prescott, S.C., Winslow, C.E.A., and McCrady, M. Water Bacteriology. John Wiley & Sons, Inc. 1946.

3 Parr, L.W. Coliform Intermediates in Human Feces. Jour. Bact. 36:1. 1938.

4 Clark, H.F. and Kabler, P.W. The Physiology of the Coliform Group. Proceedings of the Rudolfs Research Conference on Principles and Applications in Aquatic Microbiology. 1963.

5 Geldreich, E.E., Bordner, R.H., Huff, C.B., Clark, H.F., and Kabler, P.W. Type Distribution of Coliform Bacteria in the Feces of Warm-Blooded Animals. JWPCF. 34:295-301. 1962.

6 Geldreich et al. The Fecal Coli-Aerogenes Flora of Soils from Various Geographic Areas. Journal of Applied Bacteriology 25:87-93. 1962.

7 Geldreich, E.E., Kenner, B.A., and Kabler, P.W. Occurrence of Coliforms, Fecal Coliforms, and Streptococci on Vegetation and Insects. Applied Microbiology. 12:63-69. 1964.

8 Kabler, P.W., Clark, H.F., and Geldreich, E.E. Sanitary Significance of Coliform and Fecal Coliform Organisms in Surface Water. Public Health Reports. 79:58-60. 1964.

9 Clark, H.F. and Kabler, P.W. Re-evaluation of the Significance of the Coliform Bacteria. Journal AWWA. 56:931-936. 1964.

10 Kenner, B.S., Clark, H.F., and Kabler, P.W. Fecal Streptococci. II. Quantification in Feces. Am. J. Public Health. 50:1553-59. 1960.

11 Litsky, W., Mallman, W.L., and Fifield, C.W. Comparison of MPN of Escherichia coli and Enterococci in River Water. Am. Jour. Public Health. 45:1949. 1955.

12 Medrek, T.F. and Litsky, W. Comparative Incidence of Coliform Bacteria and Enterococci in Undisturbed Soil. Applied Microbiology. 8:60-63. 1960.

13 Mallman, W.L., and Litsky, W. Survival of Selected Enteric Organisms in Various Types of Soil. Am. J. Public Health. 41:38-44. 1950.

14 Mallman, W.L., and Seligman, E.B., Jr. A Comparative Study of Media for Detection of Streptococci in Water and Sewage. Am. J. Public Health. 40:286-89. 1950.

15 Ministry of Health (London). The Bacterial Examination of Water Supplies. Reports on Public Health and Medical Subjects. 71:34.

16 Morris, W. and Weaver, R.H. Streptococci as Indices of Pollution in Well Water. Applied Microbiology, 2:282-285. 1954.

17 Mundt, J.O., Coggin, J.H., Jr., and Johnson, L.F. Growth of Streptococcus fecalis var. liquefaciens on Plants. AppliedMicrobiology. 10:552-555. 1962.

18 Geldreich, E.E. Sanitary Significance of Fecal Coliforms in the Environment. U. S. Department of the Interior. FWPCA Publ. WP-20-3. 1966.

19 Geldreich, E.E. and Kenner, B.A. Concepts of Fecal Streptococci in Stream Pollution. J. WPCF. 41:R336. 1969.

20 Kabler, P.W. Purification and Sanitary Control of Water (Potable and Waste) Ann. Rev. of Microbiol. 16:127. 1962.

21 Litsky, W., Mallman, W.L., and Fifield, C.W. Comparison of the Most Probable Numbers of Escherichia coli and Enterococci in River Waters. A.J.P.H. 45:1049. 1955.

22 Geldreich, E.E. Applying Bacteriological Parameters to Recreational Water Quality. J. AWWA. 62:113. 1970.

23 Geldreich, E.E., Best, L.C., Kenner, B.A. and Van Donsel, D.J. The Bacteriological Aspects of Stormwater Pollution. J. WPCF. 40:1860. 1968.

24 FWPCA Report of Water Quality Criteria Calumet Area - Lower Lake Michigan, Chicago, IL. Jan. 1966.

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Descriptors: Coliforms, Escherichia coli, Fecal Coliforms, Fecal Streptococci, Indicator Bacteria, Microbiology, Sewage Bacteria, Water Pollution

EXAMINATION OF WATER FOR COLIFORM AND FECAL STREPTOCOCCUS GROUPS
(Multiple Dilution Tube [MPN] Methods)

I INTRODUCTION

The subject matter of this outline is contained in three parts, as follows:

A Part 1

1 Fundamental aspects of multiple dilution tube ("most probable numbers") tests, both from a qualitative and a quantitative viewpoint.

2 Laboratory bench records.

3 Useful techniques in multiple dilution tube methods.

4 Standard supplies, equipment, and media in multiple dilution tube tests.

B Part 2

Detailed, day-by-day, procedures in tests for the coliform group and subgroups within the coliform group.

C Part 3

Detailed, day-by-day, procedures in tests for members of the fecal streptococci.

D Application of Tests to Routine Examinations

The following considerations (Table 1) apply to the selection of the Presumptive Test, the Confirmed Test, and the Completed Test. Termination of testing at the Presumptive Test level is not practiced by laboratories of this agency. It must be realized that the Presumptive Test alone has limited use when water quality is to be determined.

TABLE 1

	Examination Terminated at -		
Type of Receiving Water	Presumptive Test	Confirmed Test	Completed Test
Sewage Receiving	Applicable	Applicable	Important where results are to be used for control of raw or finished water. Application to a statistically valid number of samples from the Confirmed Test to establish its validity in determining the sanitary quality.
Treatment Plant - Raw	Applicable	Applicable	
Chlorinated	Not Done	Applicable	
Bathing	Not Done	Applicable	
Drinking	Not Done	Applicable	
Other Information		Applicable in all cases where Presumptive Test alone is unreliable.	

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Environmental Protection Agency.

II BASIS OF MULTIPLE TUBE TESTS

A Qualitative Aspects

1 For purely qualitative aspects of testing for indicator organisms, it is convenient to consider the tests applied to one sample portion, inoculated into a tube of culture medium, and the follow-up examinations and tests on results of the original inoculation. Results of testing procedures are definite: positive (presence of the organism-group is demonstrated) or negative (presence of the organism-group is not demonstrated.)

2 Test procedures are based on certain fundamental assumptions:

a First, even if only one living cell of the test organism is present in the sample, it will be able to grow when introduced into the primary inoculation medium;

b Second, growth of the test organism in the culture medium will produce a result which indicates presence of the test organism; and,

c Third, extraneous organisms will not grow, or if they do grow, they will not limit growth of the test organism; nor will they produce growth effects that will be confused with those of the bacterial group for which the test is designed.

3 Meeting these assumptions usually makes it necessary to conduct the tests in a series of stages (for example, the Presumptive, Confirmed, and Completed Test stages, respectively, of standard tests for the coliform group).

4 Features of a full, multi-stage test

a First stage: The culture medium usually serves primarily as an enrichment medium for the group tested. A good first-stage growth medium should support growth of all the living cells of the group tested, and it should include provision for indicating the presence of the test organism being studied. A first-stage medium may include some component which inhibits growth of extraneous bacteria, but this feature never should be included if it also inhibits growth of any cells of the group for which the test is designed. The Presumptive Test for the coliform group is a good example. The medium supports growth, presumably, of all living cells of the coliform group; the culture container has a fermentation vial for demonstration of gas production resulting from lactose fermentation by coliform bacteria, if present; and sodium lauryl sulfate may be included in one of the approved media for suppression of growth of certain noncoliform bacteria. This additive apparently has no adverse effect on growth of members of the coliform group in the concentration used. If the result of the first-stage test is negative, the study of the culture is terminated, and the result is recorded as a negative test. No further study is made of negative tests. If the result of the first-stage test is positive, the culture may be subjected to further study to verify the findings of the first stage.

b Second stage: A transfer is made from positive cultures of the first-stage test to a second culture medium. This test stage emphasizes provision to reduce confusion of results due to growth effects of extraneous bacteria, commonly achieved by addition of selective inhibitory agents. (The Confirmed Test for coliforms meets these requirements. Lactose and fermentation vials are provided for demonstration of coliforms in the medium. Brilliant green dye and bile salts are included as inhibitory agents which tend to suppress growth of practically all kinds of noncoliform bacteria, but do not suppress growth of coliform bacteria when used as directed).

If result of the second-stange test is negative, the study of the culture is terminated, and the result is recorded as a negative test. A negative test here means that the positive results of the first-stage test were "false positive," due to one or more kinds of extraneous bacteria. A positive second-stage test is partial confirmation of the positive results obtained in the first-stage test; the culture may be subjected to final identification through application of still further testing procedures. In routine practice, most sample examinations are terminated at the end of the second stage, on the assumption that the result would be positive if carried to the third, and final stage. This practice should be followed only if adequate testing is done to demonstrate that the assumption is valid. Some workers recommend continuing at least 5% of all sample examinations to the third stage to demonstrate the reliability of the second-stage results.

B Quantitative Aspects of Tests

1 These methods for determining bacterial numbers are based on the assumption that the bacteria can be separated from one another (by shaking or other means) resulting in a suspension of individual bacterial cells, uniformly distributed through the original sample when the primary inoculation is made.

2 Multiple dilution tube tests for quantitative determinations apply a Most Probable Number (MPN) technique. In this procedure one or more measured portions of each of a stipulated series of decreasing sample volumes is inoculated into the first-stage culture medium. Through decreasing the sample increments, eventually a volume is reached where only one cell is introduced into some tubes, and no cells are introduced into other tubes. Each of the several tubes of sample-inoculated first-stage medium is tested independently, according to the principles previously described, in the qualitative aspects of testing procedures.

3 The combination of positive and negative results is used in an application of probability mathematics to secure a single MPN value for the sample.

4 To obtain MPN values, the following conditions must be met:

a The testing procedure must result in one or more tubes in which the test organism is demonstrated to be present; and

b The testing procedure must result in one or more tubes in which the test organism is not demonstrated to be present.

5 The MPN value for a given sample is obtained through the use of MPN Tables. It is emphasized that the precision of an individual MPN value is not great when compared with most physical or chemical determinations.

6 Standard practice in water pollution surveys conducted by this organization, is to plant five tubes in each of a series of sample increments, in sample volumes decreasing at decimal intervals. For example, in testing known polluted waters, the initial sample inoculations might consist of 5 tubes each in volumes of 0.1, 0.01, 0.001, and 0.0001 ml, respectively. This series of sample volumes will yield determinate results from a low of 200 to a high of 1,600,000 organisms per 100 ml.

III LABORATORY BENCH RECORDS

A Features of a Good Bench Record Sheet

1 Provides complete identification of the sample.

2 Provides for full, day-by-day information about all tests performed on the sample.

3 Provides easy step-by-step record applicable to any portion of the sample.

4 Provides for recording of the quantitative result which will be transcribed to subsequent reports.

5 Minimizes the amount of writing by the analyst.

6 Identifies the analyst(s).

B There is no such thing as "standard" bench sheet for multiple tube tests; there are many versions of bench sheets. Some are prescribed by administrative authority (such as the Office of a State Sanitary Engineer); others are devised by laboratory or project personnel to meet specific needs.

C It is not the purpose of this discussion to recommend an "ideal" bench form; however, the form used in this training course manual is essentially similar to that used in certain research laboratories of this organization. The student enrolled in the course for which this manual is written should make himself thoroughly familiar with the bench sheet and its proper use. See Figure 1.

IV NOTES ABOUT WORKING PROCEDURES IN THE LABORATORY

A Each bacteriological examination of water by multiple dilution tube methods requires a considerable amount of manipulation; much is quite repetitious. Laboratory workers must develop and maintain good routine working habits, with constant alertness to guard against lapses into careless, slip-shod laboratory procedures and "short cuts" which only can lead to lowered quality of laboratory work.

The student reader is urged to review the form for laboratory surveys (PHS-875, Rev. 1966) used by Public Health Service personnel charged with responsibility for accreditation of laboratories for examination of water under Interstate Quarantine regulations.

B Specific attention is brought to the following by no means exhaustive, critical aspects of laboratory procedures in multiple dilution tube tests:

1 Original sample

a Follow prescribed care and handling procedures before testing.

b Maintain absolute identification of sample at all stages in testing.

c Vigorously shake samples (and sample dilutions) before planting in culture media.

2 Sample measurement into primary culture medium

a Sample portions must be measured accurately into the culture medium for reliable quantitative tests to be made. Standard Methods prescribes that calibration errors should not exceed $\pm$ 2.5%.

Project Ohio River Survey

Sample Station Broadway Landing

Collection Data

Date 2/6/67 Time 8:50 By KJ
Temperature 8 °C pH 7.3
Other Observations

Analytical Record

Bench Number of Sample 2
Analyst Zahel-Jeter
Test started at 11:45 By HLJ

ml sample	Coliform Test								Fecal coliform	Fecal Streptococcus				Remarks
	LTB		BGLB		EMB	LSTB		Gram stain		A - D		EVA		
	24	48	24	48	24	24	48		24	24	48	24	48	
10														
1.0														
0.1														
0.01														
0.001														
0.001														

Coliform MPN/100 ml
Confirmed:
Completed:
Fecal Coliform MPN:

Fecal Streptococcus MPN/100 ml
A - D - EVA:

Figure 1. SAMPLE BENCH SHEET

Suggested sample measuring practices are as follows: Mohr measuring pipets are recommended. 10 ml samples are delivered at the top of the culture tube, using 10 ml pipets. 1.0 ml samples are delivered down into the culture tube, near the surface of the medium, and "touched off" at the side of the tube when the desired amount of sample has been delivered. 1.0 ml or 2.0 ml pipets are used for measurement of this volume. 0.1 ml samples are delivered in the same manner as 1.0 ml samples, using great care that the sample actually gets into the culture medium. Only 1.0 ml pipets are used for this sample volume. After delivery of all sample increments into the culture tubes, the entire rack of culture tubes may be shaken gently to carry down any of the sample adhering to the wall of the tube above the medium.

Workers should demonstrate by actual tests that the pipets and the technique in use actually delivers the rated volumes within the prescribed limits of error.

b Volumes as small as 0.1 ml routinely can be delivered directly from the sample with suitable pipets. Lesser sample volumes first should be diluted, with subsequent delivery of suitable volumes of diluted sample into the culture medium. A diagrammatic scheme for making dilutions is shown in Figure 2.

3 Reading of culture tubes for gas production

a On removal from the incubator, shake culture rack gently, to encourage release of gas which may be supersaturated in the culture medium.

b Gas in any quantity is a positive test. It is necessary to work in conditions of suitable lighting for easy recognition of the extremely small amounts of gas inside the tops of some fermentation vials.

4 Reading of liquid culture tubes for growth as indication of a positive test requires good lighting. Growth is shown by any amount of increased turbidity or opalescence in the culture medium, with or without deposit of sediment at the bottom of the tube.

5 Transfer of cultures with inoculation loops and needles

a Always sterilize inoculation loops and needles in flame immediately before transfer of culture; do not lay it down or touch it to any nonsterile object before making the transfer.

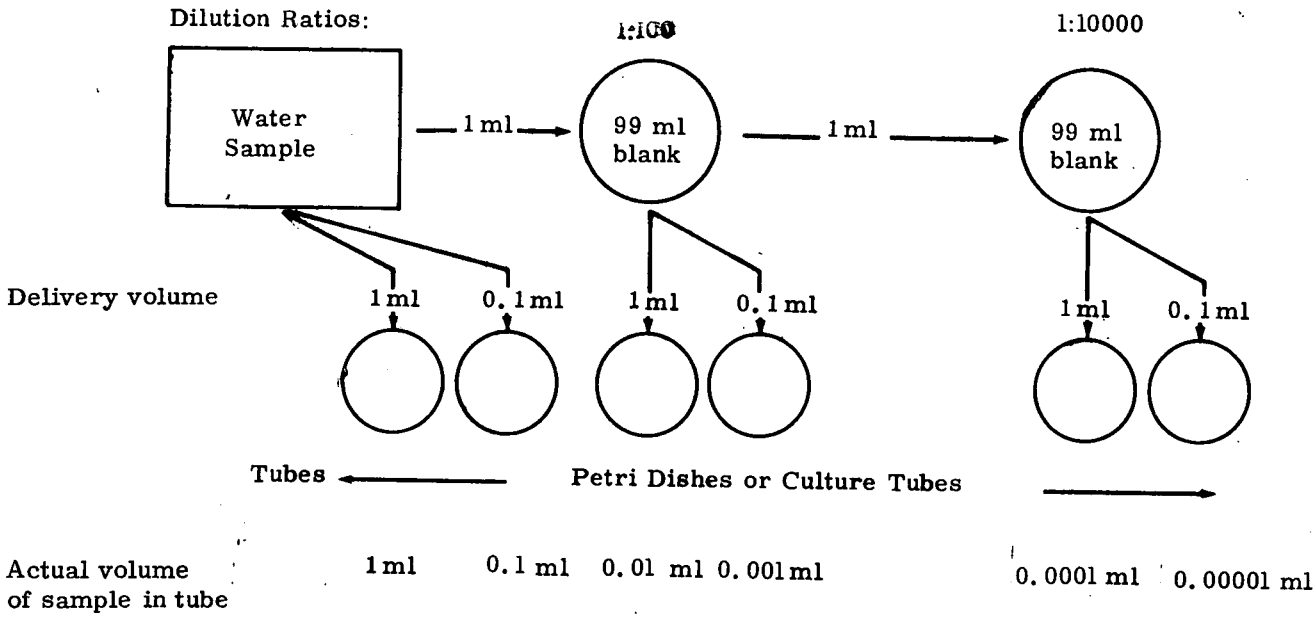
b After sterilization, allow sufficient time for cooling, in the air, to avoid heat-killing bacterial cells on the hot wire.

c Loops should be 3 mm in inside diameter, with a capability of holding a drop of water or culture.

For routine standard transfers requiring transfer of 3 loopsful of culture, many workers form three 3-mm loops on the same length of wire.

6 As an alternative to use of standard inoculation loops, the use of "applicator sticks" have now been sanctioned by the 13th Edition of Standard Methods.

Figure 2. PREPARATION OF DILUTIONS



The applicator sticks are dry heat sterilized (autoclave sterilization is not acceptable because of possible release of phenols if the wood is steamed) and are used on a single-service basis. Thus, for every culture tube transferred, a new applicator stick is used.

This use of applicator sticks is particularly attractive in field situations where it is inconvenient or impossible to provide a gas burner suitable for sterilization of the inoculation loop. In addition, use of applicator sticks is favored in laboratories where room temperatures are significantly elevated by use of gas burners.

7 Streaking cultures on agar surfaces

a All streak-inoculations should be made without breaking the surface of the agar. Learn to use a light touch with the needle; however, many inoculation needles are so sharp that they are virtually useless in this respect. When the needle is platinum or platinum-iridium wire, it sometimes is beneficial to fuse the working tip into a small sphere. This can be done by momentary insertion of a well-insulated (against electricity) wire into a carbon arc, or some other extremely hot environment. The sphere should not be more than twice the diameter of the wire from which it is formed, otherwise it will be entirely too heat-retentive to be useful.

When the needle is nichrome resistance wire, it cannot be heat-fused; the writer prefers to bend the terminal 1/16 - 1/8" of the wire at a slight angle to the overall axis of the needle. The side of the terminal bent portion of the needle then is used for inoculation of agar surfaces.

b When streaking for colony isolation, avoid using too much inoculum. The streaking pattern is somewhat variable according to individual preference. The procedure favored by the writer is shown in the accompanying figures. Note particularly that when going from any one stage of the streaking to the next, the inoculation needle is heat-sterilized.

8 Preparation of cultures for Gram stain

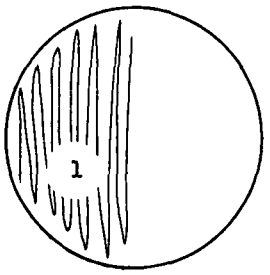
a The Gram stain always should be made from a culture grown on a nutrient agar surface (nutrient agar slants are used here) or from nutrient broth.

b The culture should be young, and should be actively growing. Many workers doubt the validity of the Gram stain made on a culture more than 24 hours old.

c Prepare a thin smear for the staining procedure. Most beginning workers tend to use too much bacterial suspension in preparing the dried smear for staining. The amount of bacteria should be so small that the dried film is barely visible to the naked eye.

V EQUIPMENT AND SUPPLIES

Consolidated lists of equipment, supplies, and culture media required for all multiple dilution tube tests described in this outline are shown in Table 2. Quantitative information is not presented; this is variable—according to the extent of the testing procedure, the number of dilutions used, and the number of replicate tubes per dilution. It is noted that requirements for alternate procedures are fully listed and choices are made in accordance to laboratory preference.

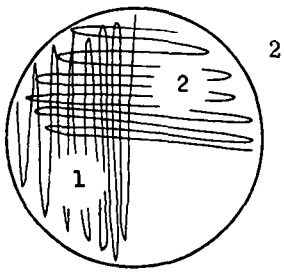


1 a Flame-sterilize an inoculation needle and air-cool.

b Dip the tip of the inoculation needle into the bacterial culture being studied.

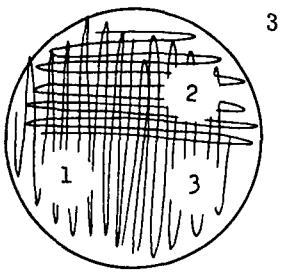
c Streak the inoculation needle tip lightly back and forth over half the agar surface, as in (1), avoiding scratching or breaking the agar surface.

d Flame-sterilize the inoculation needle and air-cool.



2 a Turn the Petri dish one-quarter-turn and streak the inoculation needle tip lightly back and forth over one-half the agar surface, working from area (1) into one-half the unstreaked area of the agar.

b Flame-sterilize the inoculation needle and air-cool.



3 a Turn the Petri dish one-quarter-turn and streak the inoculation needle tip lightly back and forth over one-half the agar surface, working from area (2) into area (3), the remaining unstreaked area.

b Flame-sterilize the inoculation needle and set it aside.

c Close the culture container and incubate as prescribed.

Figure 3. A SUGGESTED PROCEDURE FOR COLONY ISOLATION BY A STREAK-PLATE TECHNIQUE

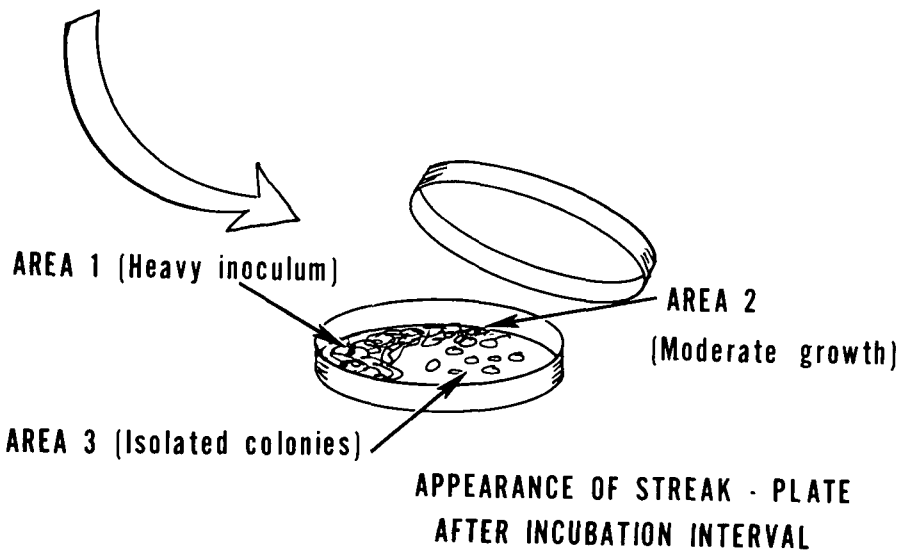


TABLE 2. APPARATUS AND SUPPLIES FOR STANDARD FERMENTATION TUBE TESTS

Description of Item	Total Coliform Group			Fecal Coliforms	
	Presumptive Test	Confirmed Test	Completed Test	(BALB)	(EC broth)
Lauryl tryptose broth or Lactose broth. 20 ml amounts of 1.5 X concentration medium, in 25 X 150 mm culture tubes with inverted fermentation vials, suitable caps.	X				
Lauryl tryptose broth or Lactose broth. 10 ml amounts of single strength medium in 20 X 150 mm culture tubes with inverted fermentation vials, suitable caps.	X		X		
Brilliant green lactose bile broth, 2% in 10 ml amounts, single strength, in 20 X 150 mm culture tubes with inverted fermentation vials, suitable caps.		X	X		
Eosin methylene blue agar, poured in 100 X 15 mm Petri dishes		X	X		
Endo Agar, poured in 100 X 15 mm dishes		X			
Nutrient agar slant, screw cap tube			X		
Boric acid lactose broth, 10 ml amounts of single strength medium in fermentation tubes.				X	
EC Broth, 10 ml amounts of single strength medium in fermentation tubes					X
Formate ricinoleate broth (provisional)			X		
Culture tube racks, 10 X 5 openings; each opening to accept 25 mm diameter tubes.	X	X	X	X	X
Pipettes, 10 ml, Mohr type, sterile, in suitable cans.	X				
Pipettes, 2 ml (optional), Morh type, sterile, in suitable cans	X				
Pipettes, 1 ml, Mohr type, sterile in metal suitable cans	X				
Standard buffered dilution water, sterile, 99-ml amounts in screw-capped bottles.	X				
Gas burner, Bunsen type		X	X	X	X
Inoculation loop, loop 3mm diameter, of nichrome or platinum-iridium wire, 26 B & S gauge, in suitable holder. (or sterile applicator stick)		X	X	X	X
Inoculation needle, nichrome, or platinum-iridium wire, 26 B & S gauge, in suitable holder.		X	X		
Incubator, adjusted to 35 ± 0.5° C	X	X	X		
Waterbath incubator, adjusted to 43 ± 0.2°C				X	
Waterbath incubator, adjusted to 44.5 ± 0.2°C.					X
Glass microscopic slides, 1" X 3"			X		
Slide racks (optional)			X		
Gram-stain solutions, complete set			X		
Compound microscope, oil immersion lens, Abbe' condenser			X		
Basket for discarded cultures	X	X	X	X	X
Container for discarded pipettes	X				

Part 2

DETAILED TESTING PROCEDURES FOR MEMBERS OF THE COLIFORM GROUP BY MULTIPLE DILUTION TUBE METHODS

I SCOPE

A Tests Described

1 Presumptive Test

2 Confirmed Test

3 Completed Test

4 Fecal Coliform Test

B Form of Presentation

The Presumptive, Confirmed, and Completed Tests are presented as total, independent procedures. It is recognized that this form of presentation is somewhat repetitious, inasmuch as the Presumptive Test is preliminary to the Confirmed Test, and both the Presumptive Test and the Confirmed Test are preliminary to the Completed Test for total coliforms.

In using these procedures, the worker must know at the outset what is to be the stage at which the test is to be ended, and the details of the procedures throughout, in order to prevent the possibility of discarding gas-positive tubes before proper transfer procedures have been followed.

Thus, if the worker knows that the test will be ended at the Confirmed Test, he will turn at once to Section III, TESTING TO THE CONFIRMED TEST STAGE, and will ignore Sections II and IV.

The Fecal Coliform Test is described separately, in Section V, as an adjunct to the Confirmed Test and to the Completed Test.

II TESTING TO PRESUMPTIVE TEST STAGE

A First-Day Procedures

1 Prepare a laboratory data sheet for the sample. Record the following information: assigned laboratory number, source of sample, date and time of collection, temperature of the source, name of sample collector, date and time of receipt of sample in the laboratory. Also show the date and time of starting tests in the laboratory, name(s) of worker(s) performing the laboratory tests, and the sample volumes planted.

2 Label the tubes of lauryl tryptose broth required for the initial planting of the sample (Table 3). The label should bear three identifying marks. The upper number is the identification of the worker(s) performing the test (applicable to personnel in training courses), the number immediately below is the assigned laboratory number, corresponding with the laboratory record sheet. The lower number is the code to designate the sample volume and which tube of a replicate series is indicated.

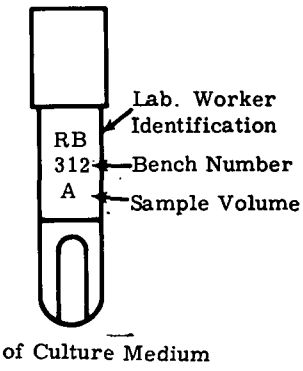
NOTE: Be sure to use tubes containing the correct concentrations of culture medium for the inoculum/tube volumes. (See the chapter on media and solutions for multiple dilution tube methods or refer to the current edition of Standard Methods for Water and Wastewater).

Table 3. SUGGESTED LABELING SCHEME FOR ORIGINAL CULTURES AND SUBCULTURES IN MULTIPLE DILUTION TUBE TESTS

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Sample volume represented
Bench number Volume & tube	312 A	312 B	312 C	312 D	312 E	Tubes with 10 ml of sample
Bench number Volume & tube	312 a	312 b	312 c	312 d	312 e	Tubes with 1 ml of sample
Bench number Volume & tube	312 a	312 b	312 c	312 d	312 e	Tubes with 0.1 ml of sample
Bench number Volume & tube	312 1a	312 1b	312 1c	312 1d	312 1e	Tubes with 0.01 ml of sample
Bench number Volume & tube	312 2a	312 2b	312 2c	312 2d	312 2e	Tubes with 0.001 ml of sample

The labeling of cultures can be reduced by labeling only the first tube of each series of identical sample volumes in the initial planting of the sample. All subcultures from initial plantings should be labeled completely.

Typical Example



3 Place the labeled culture tubes in an orderly arrangement in a culture tube rack, with the tubes intended for the largest sample volumes in the front row, and those intended for smaller volumes in the succeeding rows.

4 Shake the sample vigorously, approximately 25 times, in an arc of one foot within seven seconds and withdraw the sample portion at once.

5 Measure the predetermined sample volumes into the labeled tubes of lauryl tryptose broth, using care to avoid introduction of any bacteria into the culture medium except those in the sample.

a Use a 10 ml pipet for 10 ml sample portions, and 1 ml pipets for portions of 1 ml or less. Handle sterile pipets only near the mouthpiece, and protect the delivery end from external contamination. Do not remove the cotton plug in the mouthpiece as this is intended to protect the user from ingesting any sample.

b When using the pipet to withdraw sample portions, do not dip the pipet more than 1/2 inch into the sample; otherwise sample running down the outside of the pipet will make measurements inaccurate.

6 After measuring all portions of the sample into their respective tubes of medium, gently shake the rack of inoculated tubes to insure good mixing of sample with the culture medium. Avoid vigorous shaking, as air bubbles may be shaken into the fermentation vials and thereby invalidate the test.

7 Place the rack of inoculated tubes in the incubator at 35° ± 0.5°C for 24 ± 2 hours.

B 24-hour Procedures

1 Remove the rack of lauryl tryptose broth cultures from the incubator, and shake gently. If gas is about to appear in the fermentation vials, the shaking will speed the process.

2 Examine each tube carefully. Record, in the column "24" under LST on the laboratory data sheet, each tube showing gas in the fermentation vial as a positive (+) test and each tube not showing gas as a negative (-) test. GAS IN ANY QUANTITY IS A POSITIVE TEST.

3 Discard all gas-positive tubes of lauryl tryptose broth, and return all the gas-negative tubes to the 35°C incubator for an additional 24 ± 2 hours.

C 48-hour Procedures

1 Remove the rack of culture tubes from the incubator, read and record gas production for each tube.

2 Be sure to record all results under the 48-hour LTB column on the data sheet. Discard all tubes. The Presumptive Test is concluded at this point, and Presumptive coliforms per 100 ml can be computed according to the methods described elsewhere in this manual.

III TESTING TO CONFIRMED TEST STAGE

Note that the description starts with the sample inoculation and includes the Presumptive Test stage. The Confirmed Test preferred in Laboratories of this agency is accomplished by means of the brilliant green lactose bile broth (BGLB) and the acceptable alternate tests are mentioned in III F. In addition, the Fecal Coliform Test is included as an optional adjunct to the procedure.

A First-Day Procedures

1 Prepare a laboratory data sheet for the sample. Record the following information: assigned laboratory number, source of sample, date and time of collection, temperature of the source, name of sample collector, date and time of receipt of sample in the laboratory. Also show the date and time of starting tests in the laboratory. name(s) of worker(s) performing the laboratory tests, and the sample volumes planted.

2 Label the tubes of lauryl tryptose broth required for the initial planting of the sample. The label should bear three identifying marks. The upper number is the identification of the worker(s) performing the test (applicable to personnel in training courses), the number immediately below is the assigned laboratory number, corresponding with the laboratory record sheet. The lower number is the code to designate the sample volume and which tube of a replicate series is indicated.

NOTE: If 10-ml samples are being planted, it is necessary to use tubes containing the correct concentration of culture medium. This has previously been noted in II A-2.

3 Place the labeled culture tubes in an orderly arrangement in a culture tube rack, with the tubes intended for the largest sample volumes in the front row, and those intended for smaller volumes in the succeeding rows.

4 Shake the sample vigorously, approximately 25 times, in an up-and-down motion.

5 Measure the predetermined sample volumes into the labeled tubes of lauryl tryptose broth, using care to avoid introduction of any bacteria into the culture medium except those in the sample.

a Use a 10-ml pipet for 10 ml sample portions, and 1-ml pipets for portions of 1 ml or less. Handle sterile pipets only near the mouthpiece, and protect the delivery end from external contamination. Do not remove the cotton plug in the mouthpiece as this is intended to protect the user from ingesting any sample.

b When using the pipet to withdraw sample portions, do not dip the pipet more than 1/2 inch into the sample; otherwise sample running down the outside of the pipet will make measurements inaccurate.

c When delivering the sample into the culture medium, deliver sample portions of 1 ml or less down into the culture tube near the surface of the medium. Do not deliver small sample volumes at the top of the tube and allow them to run down inside the tube; too much of the sample will fail to reach the culture medium.

d Prepare preliminary dilutions of samples for portions of 0.01 ml or less before delivery into the culture medium. See Table 1 for preparation of dilutions. NOTE: Always deliver diluted sample portions into the culture medium as soon as possible after preparation. The interval between preparation of dilution and introduction of sample into the medium never should be as much as 30 minutes.

6 After measuring all portions of the sample into their respective tubes of medium, gently shake the rack of inoculated tubes to insure good mixing of sample with the culture medium. Avoid vigorous shaking, as air bubbles may be shaken into the fermentation vials and thereby invalidate the test.

7 Place the rack of inoculated tubes in the incubator at 35° ± 0.5° C for 24 ± 2 hours.

B 24-hour Procedures

1 Remove the rack of lauryl tryptose broth cultures from the incubator, and shake gently. If gas is about to appear in the fermentation vials, the shaking will speed the process.

2 Examine each tube carefully. Record, in the column "24" under LST on the laboratory data sheet, each tube showing gas in the fermentation vial as a positive (+) test and each tube not showing gas as a negative (-) test. GAS IN ANY QUANTITY IS A POSITIVE TEST.

3 Retain all gas-positive tubes of lauryl tryptose broth culture in their place in the rack, and proceed.

4 Select the gas-positive tubes of lauryl tryptose broth culture for Confirmed Test procedures. Confirmed Test procedures may not be required for all gas-positive cultures. If, after 24-hours of incubation, all five replicate cultures are gas-positive for two or more consecutive sample volumes, then select the set of five cultures representing the smallest volume of sample in which all tubes were gas-positive. Apply Confirmed Test procedures to all these cultures and to any other gas-positive cultures representing smaller volumes of sample, in which some tubes were gas-positive and some were gas-negative.

5 Label one tube of brilliant green lactose bile broth (BGLB) to correspond with each tube of lauryl tryptose broth selected for Confirmed Test procedures.

6 Gently shake the rack of Presumptive Test cultures. With a flame-sterilized inoculation loop transfer one loopful of culture from each gas-positive tube to the corresponding tube of BGLB. Place each newly inoculated culture into BGLB in the position of the original gas-positive tube.

7 After making the transfers, the rack should contain some 24-hour gas-negative tubes of lauryl tryptose broth and the newly inoculated BGLB.

8 If the Fecal Coliform Test is included in the testing procedures, consult Section V of this part of the outline of testing procedures.

9 Incubate the 24-hour gas-negative BGLB tubes and any newly-inoculated tubes of BGLB an additional 24 ± 2 hours at 35° ± 0.5°C.

C 48-hour Procedures

1 Remove the rack of culture tubes from the incubator, read and record gas production for each tube.

2 Some tubes will be lauryl tryptose broth and some will be brilliant green lactose bile broth (BGLB). Be sure to record results from LTB under the 48-hour LTB column and the BGLB results under the 24-hour column of the data sheet.

3 Label tubes of BGLB to correspond with all (if any) 48-hour gas-positive cultures in lauryl tryptose broth. Transfer one loopful of culture from each gas-positive LTB culture to the correspondingly-labeled tube of BGLB. NOTE: All tubes of LTB culture which were negative at 24 hours and became positive at 48 hours are to be transferred. The option described above for 24-hour cultures does not apply at 48 hours.

4 If the Fecal Coliform Test is included in the testing procedure, consult Section V of the part of the outline of testing procedures.

5 Incubate the 24-hour gas-negative BGLB tubes and any newly-inoculated tubes of BGLB 24 ± 2 hours at 35° ± 0.5°C.

6 Discard all tubes of LTB and all 24-hour gas-positive BGLB cultures.

D 72-hour Procedures

1 If any cultures remain to be examined, all will be BGLB. Some may be 24 hours old and some may be 48 hours old. Remove such cultures from the incubator, examine each tube for gas production, and record results on the data sheet.

2 Be sure to record the results of 24-hour BGLB cultures in the "24" column under BGLB and the 48-hour results under the "48" column of the data sheet.

3 Return any 24-hour gas-negative cultures for incubation 24 ± 2 hours at 35 ± 0.5°C.

4 Discard all gas-positive BGLB cultures and all 48-hour gas-negative cultures from BGLB.

5 It is possible that all cultural work and results for the Confirmed Test have been finished at this point. If so, codify results and determine Confirmed Test coliforms per 100 ml as described in the outline on use of MPN Tables.

E 96-hour Procedures

At most only a few 48-hour cultures in BGLB may be present. Read and record gas production of such cultures in the "48" column under BGLB on the data sheet. Codify results and determine Confirmed Test coliforms per 100 ml.

F Streak-plate methods for the Confirmed Test, using eosin methylene blue agar or Endo agar plates, are accepted procedures in Standard Methods. The worker who prefers to use one of these media in preference to BGLB (also approved in Standard Methods) is advised to refer to the current edition of "Standard Methods for the Examination of Water and Wastewater" for procedures.

IV TESTING TO COMPLETED TEST STAGE

(Note that this description starts with the sample inoculation and proceeds through the Presumptive and the Confirmed Test stages. In addition, the Fecal Coliform Test is referred to as an optional adjunct to the procedure.)

A First-Day Procedures

1 Prepare a laboratory data sheet for the sample. Record the following information: assigned laboratory number, source of sample, date and time of collection, temperature of the source, name of sample collector, date and time of receipt of sample in the laboratory. Also show the date and time of starting tests in the laboratory, name(s) of worker(s) performing the laboratory tests, and the sample volumes planted.

2 Label the tubes of lauryl tryptose broth required for the initial planting of the sample. The label should bear three identifying marks. The upper number is the identification of the worker(s) performing the test (applicable to personnel in training courses), the number immediately below is the assigned laboratory number, corresponding with the laboratory record sheet. The lower number is the code to designate the sample volume and which tube of a replicate series is indicated. Guidance on labeling for laboratory data number and identification of individual tubes is described elsewhere in this outline.

NOTE: If 10-ml samples are being plated, it is necessary to use tubes containing the correct concentration of culture medium. This has previously been noted elsewhere in this outline and referral is made to tables.

3 Place the labeled culture tubes in an orderly arrangement in a culture tube rack, with the tubes intended for the largest sample volumes in the front row, and those intended for smaller volumes in the succeeding rows.

4 Shake the sample vigorously, approximately 25 times, in an up-and-down motion.

5 Measure the predetermined sample volumes into the labeled tubes of lauryl tryptose broth, using care to avoid introduction of any bacteria into the culture medium except those in the sample.

a Use a 10-ml pipet for 10 ml sample portions, and 1-ml pipets for portions of 1 ml or less. Handle sterile pipets only near the mouthpiece, and protect the delivery end from external contamination. Do not move the cotton plug in the mouthpiece as this is intended to protect the user from ingesting any sample.

b When using the pipet to withdraw sample portions, do not dip the pipet more than 1/2 inch into the sample; otherwise sample running down the outside of the pipet will make measurements inaccurate.

c When delivering the sample into the culture medium, deliver sample portions of 1 ml or less down into

the culture tube near the surface of the medium. Do not deliver small sample volumes at the top of the tube and allow them to run down inside the tube; too much of the sample will fail to reach the culture medium.

d Prepare preliminary dilutions of samples for portions of 0.01 ml or less before delivery into the culture medium. See Table 2 for preparation of dilutions. NOTE: Always deliver diluted sample portions into the culture medium as soon as possible after preparation. The interval between preparation of dilution and introduction of sample into the medium never should be as much as 30 minutes.

6 After measuring all portions of the sample into their respective tubes of medium, gently shake the rack of inoculated tubes to insure good mixing of sample with the culture medium. Avoid vigorous shaking, as air bubbles may be shaken into the fermentation vials and thereby invalidate the test.

7 Place the rack of inoculated tubes in the incubator at 35° ± 0.5° C for 24 ± 2 hours.

B 24-hour Procedures

1 Remove the rack of lauryl tryptose broth cultures from the incubator, and shake gently. If gas is about to appear in the fermentation vials, the shaking will speed the process.

2 Examine each tube carefully. Record, in the column "24" under LST on the laboratory data sheet, each tube showing gas in the fermentation vial as a positive (+) test and each tube not showing gas as a negative (-) test. GAS IN ANY QUANTITY IS A POSITIVE TEST.

3 Retain all gas-positive tubes of lauryl tryptose broth culture in their place in the rack, and proceed.

4 Select the gas-positive tubes of lauryl tryptose broth culture for the Confirmed Test procedures. Confirmed Test procedures may not be required for all gas-positive cultures. If, after 24-hours of incubation, all five replicate cultures are gas-positive for two or more consecutive sample volumes, then select the set of five cultures representing the smallest volume of sample in which all tubes were gas-positive. Apply Confirmed Test procedures to all these cultures and to any other gas-positive cultures representing smaller volumes of sample, in which some tubes were gas-positive and some were gas-negative.

5 Label one tube of brilliant green lactose bile broth (BGLB) to correspond with each tube of lauryl tryptose broth selected for Confirmed Test procedures.

6 Gently shake the rack of Presumptive Test cultures. With a flame-sterilized inoculation loop transfer one loopful of culture from each gas-positive tube to the corresponding tube of BGLB. Place each newly inoculated culture into BGLB in the position of the original gas-positive tube.

7 If the Fecal Coliform Test is included in the testing procedure, consult Section V of this outline for details of the testing procedure.

8 After making the transfer, the rack should contain some 24-hour gas-negative tubes of lauryl tryptose borth and the newly inoculated BGLB. Incubate the rack of cultures at 35° C ± 0.5° C for 24 ± 2 hours.

C 48-hour Procedures

1 Remove the rack of culture tubes from the incubator, read and record gas production for each tube.

2 Some tubes will be lauryl tryptose broth and some will be brilliant green lactose

bile broth (BGLB). Be sure to record results from LTB under the 48-hour LTB column and the BGLB results under the 24-hour column of the data sheet.

3 Label tubes of BGLB to correspond with all (if any) 48-hour gas-positive cultures in lauryl tryptose broth. Transfer one loopful of culture from each gas-positive LTB culture to the correspondingly-labeled tube of BGLB. NOTE: All tubes of LTB culture which were negative at 24 hours and became positive at 48 hours are to be transferred. The Option described above for 24-hour LTB cultures does not apply at 48 hours.

4 Incubate the 24-hour gas-negative BGLB tubes and any newly-inoculated tubes of BGLB 24 ± 2 hours at 35° ± 0.5° C. Retain all 24-hour gas-positive cultures in BGLB for further test procedures.

5 Label a Petri dish preparation of eosin methylene blue agar (EMB agar) to correspond with each gas-positive culture in BGLB.

6 Prepare a streak plate for colony isolation from each gas-positive culture in BGLB on the correspondingly-labeled EMB agar plate.

Incubate the EMB agar plates 24 ± 2 hours at 35° ± 0.5° C.

D 72-hour Procedures

1 Remove the cultures from the incubator. Some may be on BGLB; several EMB agar plates also can be expected.

2 Examine and record gas production results for any cultures in BGLB.

3 Retain any gas-positive BGLB cultures and prepare streak plate inoculations for colony isolation in EMB agar. Incubate the EMB agar plates 24 ± 2 hours at 35 ± 0.5° C. Discard the gas-positive BGLB cultures after transfer.

4 Reincubate any gas-negative BGLB cultures 24 ± 2 hours at 35° ± 0.5° C.

5 Discard all 48-hour gas-negative BGLB cultures.

6 Examine the EMB agar plates for the type of colonies developed thereon. Well-isolated colonies having a dark center (when viewed from the lower side, held toward a light) are termed "nucleated or fisheye" colonies, and are regarded as "typical" coliform colonies. A surface sheen may or may not be present on "typical" colonies. Colonies which are pink or opaque but are not nucleated are regarded as "atypical colonies." Other colony types are considered "noncoliform." Read and record results as + for "typical" (nucleated) colonies ± for "atypical" (non-nucleated pink or opaque colonies), and - for other types of colonies which might develop.

7 With plates bearing "typical" colonies, select at least one well-isolated colony and transfer it to a correspondingly-labeled tube of lactose broth and to an agar slant. As a second choice, select at least two "atypical" colonies (if typical colonies are not present) and transfer them to labeled tubes of lactose broth and to agar slants. As a third choice, in the absence of typical or atypical coliform-like colonies, select at least two well-isolated colonies representative of those appearing on the EMB plate, and transfer them to lactose broth and to agar slants.

8 Incubate all cultures transfered from EMB agar plates 24 ± 2 hours at 35 ± 0.5° C.

E 96-hour Procedures

1 Subcultures from the samples being studied may include: 48-hour tubes of BGLB, EMB agar plates, lactose broth tubes, and agar slant cultures.

2 If any 48-hour tubes of BGLB are present, read and record gas production in the "48" column under BGLB. From any gas-positive BGLB cultures prepare streak plate inoculations for colony isolation on EMB agar. Discard all tubes of BGLB, and incubate EMB agar plates 24 ± 2 hours at 35 ± 0.5° C.

3 If any EMB plates are present, examine and record results in the "EMB" column of the data sheet. Make transfers to agar slants and to lactose broth from all EMB agar plate cultures. In decreasing order of preference, transfer at least one typical colony, or at least two atypical colonies, or at least two colonies representative of those on the plate.

4 Examine and record results from the lactose broth cultures.

5 Prepare a Gram-stained smear from each of the agar slant cultures, as follows:

NOTE: Always prepare Gram stain from an actively growing culture, preferably about 18 hours old, and never more than 24 hours old. Failure to observe this precaution often results in irregular staining reactions.

a Thoroughly clean a glass slide to free it of any trace of oily film.

b Place one drop of distilled water on the slide.

c Use the inoculation needle to suspend a tiny amount of growth from the nutrient agar slant culture in the drop of water.

d Mix the thin suspension of cells with the tip of the inoculation needle, and allow the water to evaporate.

e "Fix" the smear by gently warming the slide over a flame.

f Stain the smear by flooding it for 1 minute with crystal violet solution.

g Flush the excess crystal violet solution off in gently running water, and gently blot dry with filter paper or with other clean absorbent paper.

h Flood the smear with Lugol's iodine for 1 minute.

i Wash the slide in gently running water and blot dry with filter paper.

j Decolorize the smear with 95% alcohol solution with gentle agitation for 10-30 seconds, depending upon extent of removal of crystal violet dye, then blot dry.

k Counterstain for 10 seconds with safranin solution, then wash in running water and blot dry.

l Examine the slide under the microscope, using the oil immersion lens. Coliform bacteria are Gram-negative, nonspore-forming, rod-shaped cells, occurring singly, in pairs, or rarely in short chains.

m If typical coliform staining reaction and morphology are observed, record + in the appropriate space under the "Gram Stain" column of the data sheet. If typical morphology and staining reaction are not observed, then mark it ± or -, and make suitable comment in the "remarks" column at the right-hand side of the data sheet.

n If spore-forming bacteria are observed, it will be necessary to repurify the culture from which the observations were made. Consult the instructor, or refer to Standard Methods, for procedures.

At this point, it is possible that all cultural work for the Completed Test has been finished. If so, codify results and determine Completed Test coliforms per 100 ml.

F 120-hour Procedures and following:

1 Any procedures to be undertaken from this point are "straggler" cultures on media already described, and requiring step-by-step methodology already given in detail. Such cultures may be on: EMB plates, agar slants, or lactose broth. The same time-and-temperature of incubation required for earlier studies applies to the "stragglers" as do the observations, staining reactions, and interpretation of results. On conclusion of all cultural procedures, codify results and determine Completed Test coliforms per 100 ml.

V FECAL COLIFORM TEST

A General Information

1 The procedure described is an elevated temperature test for fecal coliform bacteria.

2 Equipment required for the tests are those required for the Presumptive Test of Standard Methods, a water-bath incubator, and the appropriate culture media.

B Fecal Coliform Test with EC Broth

1 Sample: The test is applied to gas-positive tubes from the Standard Methods Presumptive Test (lauryl tryptose broth), in parallel with Confirmed Test procedures.

2 24-hour Operations. Initial procedures are the planting procedures described for the Standard Methods Presumptive Coliform test.

a After reading and recording gas-production on lauryl tryptose broth, temporarily retain all gas-positive tubes.

b Label a tube of EC broth to correspond with each gas-positive tube of lauryl tryptose broth. The option regarding transfer of only a limited number of tubes to the Confirmed Test sometimes can be applied here. However, the worker is urged to avoid exercise of this option until he has assured the applicability of the option by preliminary tests on the sample source.

c Transfer one loopful of culture from each gas-positive culture in lauryl tryptose broth to the correspondingly labeled tube of EC broth.

d Incubate EC broth tubes 24 ± 2 hours at 44.5 ± 0.2°C in a waterbath with water depth sufficient to come up at least as high as the top of the culture medium in the tubes. Place in waterbath as soon as possible after inoculation and always within 30 minutes after inoculation.

3 48-hour operations

a Remove the rack of EC cultures from the waterbath, shake gently, and record gas production for each tube. Gas in any quantity is a positive test.

b As soon as results are recorded, discard all tubes. (This is a 24-hour test for EC broth inoculations and not a 48-hour test.)

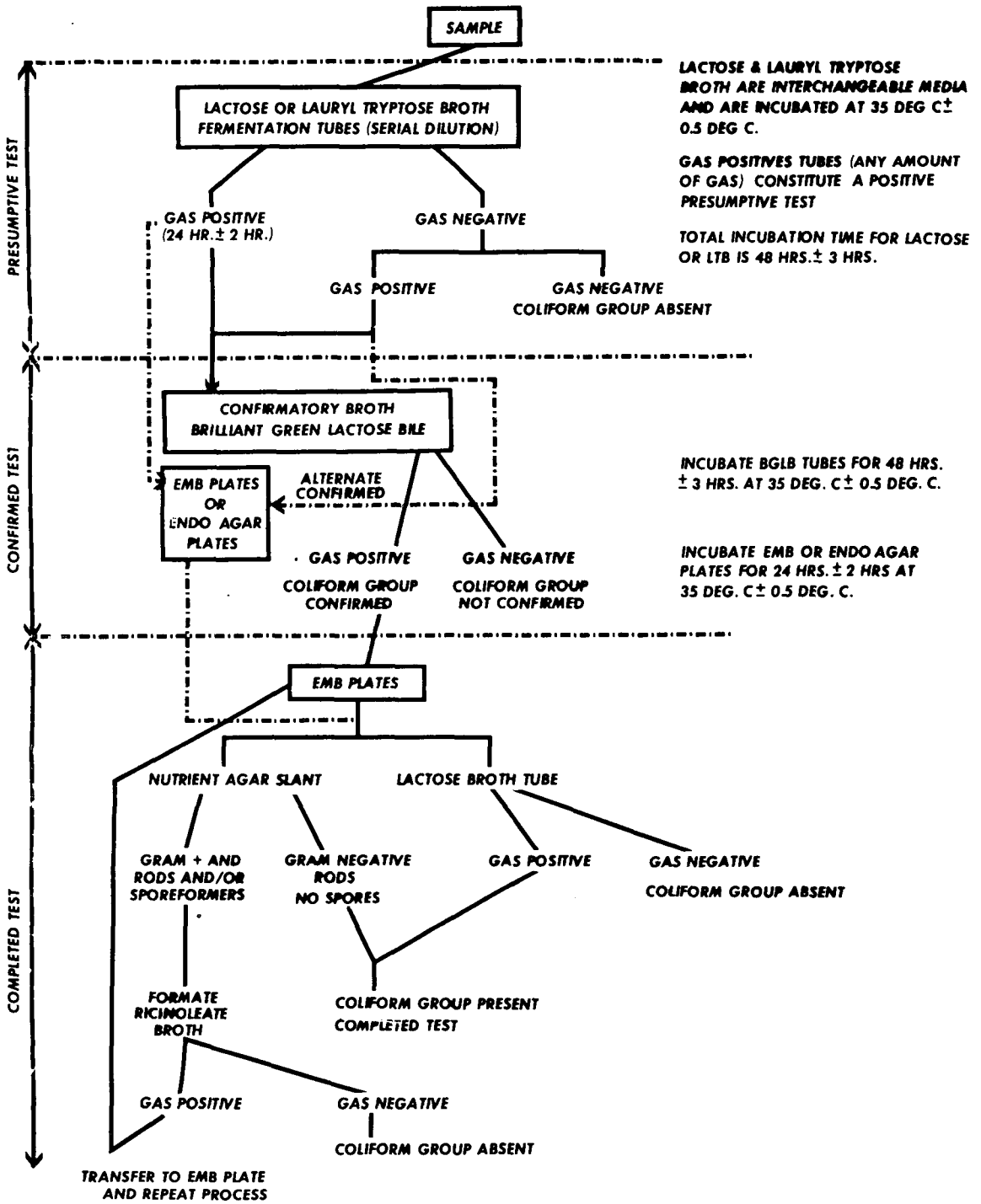
c Transfer any additional 48-hour gas-positive tubes of lauryl tryptose broth to correspondingly labeled tubes of EC broth. Incubate 24 ± 2 hours at 44.5 ± 0.2°C.

4 72-hour operations

a Read and record gas production for each tube. Discard all cultures.

b Codify results and determine fecal coliform count per 100 ml of sample.

TESTS FOR COLIFORM GROUP



Part 3
LABORATORY METHODS FOR FECAL STREPTOCOCCUS
(Day-By-Day Procedures)

I GENERAL INFORMATION

A The same sampling and holding procedures apply as for the coliform test.

B The number of fecal streptococci in water generally is lower than the number of coliform bacteria. It is good practice in multiple dilution tube tests to start the sample planting series with one sample increment larger than for the coliform test. For example: If a sample planting series of 1.0, 0.1, 0.01, and 0.001 ml is planned for the coliform test, it is suggested that a series of 10, 1.0, 0.1, and 0.01 ml be planted for the fecal streptococcus test.

C Equipment required for the test is the same as required for the Standard Methods Presumptive and Confirmed Tests, except for the differences in culture media.

II STANDARD METHODS (Tentative) PROCEDURES

A First-Day Operations

1 Prepare the sample data sheet and labeled tubes of azide dextrose broth in the same manner as for the Presumptive Test. NOTE: If 10-ml samples are included in the series, be sure to use a special concentration (ordinarily double-strength) of azide dextrose broth for these sample portions.

2 Shake the sample vigorously, approximately 25 times, in an up-and-down motion.

3 Measure the predetermined sample volumes into the labeled tubes of azide dextrose broth, using the sample measurement and delivery techniques used for the Presumptive Test.

4 Shake the rack of tubes of inoculated culture media, to insure good mixing of sample with medium.

5 Place the rack of inoculated tubes in the incubator at 35° ± 0.5° C for 24 ± 2 hours.

B 24-hour Operations

1 Remove the rack of tubes from the incubator. Read and record the results from each tube. Growth is a positive test with this test. Evidence of growth consists either of turbidity of the medium, a "button" of sediment at the bottom of the culture tube, or both.

2 Label a tube of ethyl violet azide broth to correspond with each positive culture of azide dextrose broth. It may be permissible to use the same confirmatory option as described for the coliform Confirmed Test, in this outline.

3 Shake the rack of cultures gently, to resuspend any living cells which have settled out to the bottom of the culture tubes.

4 Transfer three loopfuls of culture from each growth-positive tube of azide dextrose broth to the correspondingly labeled tube of ethyl violet azide broth.

5 As transfers are made, place the newly inoculated tubes of ethyl violet azide broth in the positions in the rack formerly occupied by the growth-positive tubes of azide dextrose broth. Discard the tubes of azide dextrose broth culture.

6 Return the rack, containing 24-hour growth-negative azide dextrose broth tubes and newly-inoculated tubes of ethyl violet azide broth, to the incubator. Incubate 24 ± 2 hours at 35° ± 0.5° C.

C 48-hour Operations

1 Remove the rack of tubes from the incubator. Read and report results. Growth, either in azide dextrose broth or in ethyl violet azide broth, is a positive test. Be sure to report the results of the azide dextrose broth medium under the "48" column for that medium and the results of the ethyl violet azide broth cultures under the "24" column for that medium.

2 Any 48-hour growth-positive cultures of azide dextrose broth are to be transferred (three loopfulls) to ethyl violet azide broth. Discard all 48-hour growth-negative tubes of azide dextrose broth and all 24-hour growth-positive tubes of ethyl violet azide broth.

3 Incubate the 24-hour growth-negative and the newly-inoculated tubes of ethyl violet azide broth 24 ± 2 hours at 35° ± 0.5°C.

D 72-hour Operations

1 Read and report growth results of all tubes of ethyl violet azide broth.

2 Discard all growth-positive cultures and all 48-hour growth-negative cultures.

3 Reincubate any 24-hour growth-negative cultures in ethyl violet azide broth 24 ± 2 hours at 35° ± 0.5°C.

E 96-hour Operations

1 Read and report growth results of any remaining tubes of ethyl violet azide broth.

2 Codify results and determine fecal streptococci per 100 ml.

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater (13th Ed). Prepared and published jointly by American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1971.

2 Geldreich, E.E., Clark, H.F., Kabler, P.W., Huff, C.B. and Bordner, R.H. The Coliform Group. II. Reactions in EC Medium at 45°C. Appl. Microbiol. 8:347-348. 1958.

3 Geldreich, E.E., Bordner, R.H., Huff, C.B., Clark, H.F. and Kabler, P.W. Type Distribution of Coliform Bacteria in the Feces of Warm-Blooded Animals. J. Water Pollution Control Federation. 34:295-301. 1962.

4 Recommend Proc. for the Bacteriological Examination of Sea Water and Shellfish. 3rd Edition, American Public Health Association. 1962.

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Descriptors: Coliforms, Fecal Coliforms, Fecal Streptococci, Indicator Bacteria, Laboratory Equipment, Laboratory Tests, Microbiology, Most Probable Number, MPN, Sewage Bacteria, Water Analysis

EXAMINATION OF WATER FOR COLIFORM AND FECAL STREPTOCOCCUS GROUPS
(Membrane Filter Methods)
Part 1 - Overview

I INTRODUCTION

A Scope

1 The membrane filter consists of a porous disk of cellulose esters 47 mm in diameter and approximately 0.15 mm thick. Filters used in water bacteriology have pores 0.45 microns in diameter. The basic principles of use in water bacteriology are as follows: Currently, active research is underway to reevaluate the pore size in an effort thereby to improve colony productivity.

a A sterile membrane filter is fastened into a suitable filter holding device and a predetermined volume of a water sample is filtered through the membrane filter.

b The filter is placed in a culture container either on a paper pad impregnated with moist culture medium or upon an agar medium.

c The inoculated filter is incubated under prescribed conditions of temperature and humidity for a designated time.

d After incubation, the resulting culture is examined, and the necessary interpretations and/or additional tests are made.

2 Through variations in such factors as the composition of the culture medium, incubation time and temperature, and combinations with other cultural and biochemical tests, several kinds of bacterial determinations are possible.

B Basic Principles and Origins of Membrane Filter Techniques

The purpose of this discussion is to provide orientation in the types and amount of equipment and supplies needed in membrane filter analyses in water quality surveys, the basic methodology, limitations of membrane filter procedures, and the status of membrane filter methods in water quality testing. Finally, it considers the level of technical skill and the workloads of personnel assigned to perform tests by this method.

II REQUIREMENTS FOR EQUIPMENT AND SUPPLIES

A Laboratory Supplies and Equipment

1 Membrane filters

a Membrane filters are available from several different commercial sources, representing the United States, Britain, and Germany. Each source applies a distinctive tradename to its MF product; such as:

"Millipore Filters" - Millipore Filter Corp., U.S.A.

"Bac-T-Flex Filters" - Schleicher and Schuell, U.S.A. representatives for Sartorius - Werke, Germany.

"Oxoid Filter" - Oxo Limited, Britain

b Membrane filters are available in a wide variety of controlled pore

sizes. Each commercial source will designate the filter most suitable for water bacteriological tests upon request.

2 Absorbent pads for nutrients

Absorbent paper disks for mechanical support of the filters during incubation and for maintaining an adequate supply of culture medium in contact with the filters, ordinarily are sold with the membrane filters. Additional supplies are obtainable from filter manufacturers. These pads may be the source of cultural problems due to chemical impurities.

3 Filter-holding units

a The filter-holding unit is a device for supporting the membrane filter and for holding the sample until it passes through the filter. During the filtration, the sample passes through a circular area, usually about 35 mm in diameter, in the center of the filter. The outer part of the filter disk is clamped between the two essential components of the filter-holding unit.

1) The lower element, called the filter base, or receptable, supports the membrane filter on a plate about 50 mm in diameter. The central part of this plate is porous, to allow free passage of liquid. The outer part of the plate is a smooth nonporous surface. The lower element includes fittings for mounting the unit in a suction flask or other container suitable for filtration with vacuum.

2) The upper element, usually called the funnel, holds the sample until it is drawn through the filter. Its lower portion is a flat ring that rests firectly over the nonporous part of the filter support plate.

3) When assembled, the two elements of the filter-holding unit are joined by a locking ring or by one or more clamps or springs.

b Filter-holding units are either metal or glass. Metal units are available from at least six commercial sources, and glass units are available from at least two sources. Most workers prefer to use metal filter-holding units, due to their greater durability. Most of the use of glass units seems to be dictated by economic considerations, as these are much lower in initial cost.

4 Culture media

a Culture media are commercially available for use with membrane filters in the determination of bacterial plate counts, for coliform counts, fecal coliform counts, fecal streptococcus counts and, in addition, for several special applications.

b These media can be obtained in several forms, including:

1) Dehydrated media, requiring weighing, addition of a suitable amount of distilled water, and some form of sterilization prior to use. This form of media is preferred as it appears to give the most satisfactory combination of performance quality, stability and economy.

2) Ready-to-use culture media, sealed in glass ampoules.

5 Culture containers

Several forms of culture containers are suitable for membrane filter works. These include small glass Petri dishes, and plastic Petri dishes. The plastic Petri dishes may be preferred, as they are sufficiently cheap to encourage single-service use.

6 Vacuum sources

In the fixed laboratory, central vacuum service, laboratory-scale electric vacuum pumps, or water pumps ("aspirators") are suitable for vacuum filtration of samples.

7 Glassware

Conventional laboratory glassware is used in fixed laboratories for measurement and delivery of samples for filtration, sample dilutions, and the like. Under field conditions it sometimes is necessary to use a limited amount of laboratory glassware, with some form of field application of sterilization methods.

8 General laboratory facilities

Such facilities as autoclave, incubators, weighing equipment, and the like are required in conventional bacteriology laboratories, and are needed equally when membrane filter methods are used.

B Field Equipment

Field operations cannot be carried on indefinitely without support from a central laboratory. Functions such as preparation of distilled water, pre-weighing of culture media, sterilization of membrane filters and culture containers, and other general preparations are best performed in a central laboratory facility.

1 With the introduction of membrane filters to the bacteriological analysis of water, a number of investigators gave attention to the development of membrane filter equipment which can be used in the field in easily portable, self-contained water laboratories. Such portable laboratory kits would have three major fields of usefulness:

a They would be useful in certain routine water quality control operations. Examples include such places as on board ships, in some national parks, on airlines, etc.

b They could be used in certain types of stream surveys, where there is an excessive delay between sample collection and the time when examination can be started, due to time required for transporation of samples.

c Finally, such units would be invaluable in emergencies, when existing laboratories are overburdened or inoperative. Portable kits already have proven extremely useful in testing many small water supplies in a short period of time. Further, there is a predictable need for such equipment in the event of natural or wartime civil defense disaster.

2 Availability of field equipment

Commercially developed field equipment is currently available from only one source.

Equipment of this type includes provision for sample filtration, field sterilization (where necessary) of filtration equipment, limited capacity for reserves of culture media and other supplies, and provision for incubation of cultures. Such equipment does not include satisfactory visual aid equipment (a low powered microscope is needed for streptococcus counts, and at least a simple lens is needed for coliform work), and provision for many of the supplies is not included.

III MEMBRANE FILTER PROCEDURES IN WATER QUALITY AND POLLUTANT SOURCE MONITORING

A Standard Coliform Test

1 Basic procedures

a Sample filtration volumes are selected (usually 3 volumes) to give at least one volume which will contain 20-80 typical coliform colonies.

b The sample volumes are filtered through membrane filters, and incubated at 35°C for 20-22 hours in an atmosphere at or near saturation.

c Following incubation, the typical coliform colonies are counted and reported as coliforms per 100 ml of sample.

2 Applications

The total coliform count has been used most widely of all procedures when bacteriological data are collected in a survey. Applications of membrane filter methods in surveys necessarily have a short history. The method has been used in certain studies of the Potomac River System, in the Illinois-Great Lakes studies, and in certain surveys in the West.

The Standard MF coliform test is applicable when it can be initiated within the recommended period between sample collection and receipt at the laboratory. The procedure is applicable with MF field units.

B "Fecal Coliform" Test with Membrane Filters

1 Procedures

a Sample filtration procedures are the same as for the Standard Membrane Filter coliform test. Sample filtration volumes should be selected so that at least one filter will produce 20-60 fecal coliform colonies.

b The culture medium is M-FC Broth. The medium requires addition of a rosolic acid solution prior to use.

c Within 20 minutes after sample filtration, the membrane filters are placed in small plastic bags, which are sealed, and immersed in a forced circulation water bath at 44.5°C ± 0.2°C for a 24 hour incubation time.

d Fecal coliform colonies are blue or blue-hues, generally 1 - 3 mm in diameter. The fecal coliform density is reported as fecal coliforms per 100 ml of sample.

2 Application

The fecal coliform test is being used increasingly in water quality studies. Fecal coliform criteria are being established for compliance with effluent limits for NPDES Permit Compliance.

C Fecal Streptococcus Tests

1 Basic procedures

a The filtration procedures are the same as with the coliform tests. The sample volumes should be such that at least one membrane filter will produce 20 - 100 fecal streptococcus colonies.

b The culture medium can be the M-Enterococcus Agar developed by Slanetz and his associates, or it can be KF-Broth, developed by Kenner and his associates. In either case, incubation is 48 hours at 35°C.

c With both media, fecal streptococcus colonies are pale pink to deep red, up to 2 mm in diameter.

d Because the density of fecal streptococci in most waters is lower than coliform density, it is necessary to filter greater volumes of water. Further, the number of colonies that can be counted effectively is greater than the number of coliform colonies. Sample volumes often can be 10 to 100-fold greater for fecal streptococcus counts than for coliform counts.

2 Application

Fecal streptococcus counts are especially useful when supplementary data will support information from the coliform counts. Fecal streptococcus counts are used in many states in addition to coliform tests in examination of bathing waters as well as in stream surveys.

IV LIMITATIONS OF MEMBRANE FILTER METHODS

Most of the recognized limitations of the membrane filter method have been related to tests for members of the coliform group. Extension of these recognized limitations to tests for other pollution indicators requires special interpretation.

A Need for Selection of Suitable Filtration Volume

1 In tests for coliform bacteria, the sample filtration volumes should be so selected that one of volumes yields 20-80 coliform colonies. With fewer than 20 colonies, the random variation of colony counts becomes great enough to cause some difficulty in statistical analysis; with more than 80 coliform colonies, the proportionality between number of coliform colonies differentiated and sample volume begins to fail. This is especially troublesome in waters containing large numbers of noncoliform bacteria capable of growing on the filters.

2 In tests for the fecal streptococci, fewer nonstreptococcus interference colonies are capable of growing on the medium. For this reason and because fecal streptococcus colonies are small, the sample filtration volumes are so selected as to yield 20-100 colonies on a membrane filter.

B Influence of Turbidity

When the water being studied contains large amounts of suspended matter, such as clay, and has relatively low counts of the indicator organisms being studied, cultural difficulties often result. The particulate matter suspended in the water is deposited on the surface of the membrane filter in sample filtration, with any bacteria contained in the sample. Culture medium diffusing through the membrane filter forms a capillary layer around all the particles of matter deposited on the filter. This results in development of spreading, poorly defined bacterial colonies.

The solutions to problems of this type are: 1) to filter the sample in several smaller increments so that the combined sample filtration volume is spread over several membrane filters, each of which has a lesser amount of particulate matter deposited on the surface; or, 2) perform the analysis by means of the dilution tube (MPN) procedure.

C Influence of large numbers of bacteria representing forms other than the indicator group being studied.

1 In coliform testing, this can be a serious problem. The difficulty arises when appreciable numbers of noncoliform bacteria are present and are capable of growing on the Endo-type culture medium used. When large numbers of noncoliform bacteria grow on the medium, and few coliform bacteria are present, the sheen-producing capacity of many of the coliform colonies is impaired, and the result indicates the presence of fewer coliform bacteria than actually are present.

A solution for this type of problem is to filter several smaller sample increments, or else to use the MPN method.

2 In tests for fecal streptococci, the presence of large numbers of bacteria of other groups has not proven troublesome. The medium is extremely selective, and the few forms that do seem to grow on the medium produce extremely small colonies which have not been shown to have any adverse effect on the qualitative results obtained in tests for fecal streptococci.

D Increasingly, problems in sensitivity of the test are being recognized in tests on chlorinated effluents from wastewater treatment facilities.

V OFFICIAL STATUS OF MEMBRANE FILTER METHODS

A American Public Health Association, American Water Works Association, and Water Pollution Control Federation, in "Standard Methods for the Examination of Water and Wastewater." (13th Edition, 1971)

1 A single-stage procedure, using M-Endo Broth MF for tests for members of the coliform group, is described as an official testing procedure.

2 A new two-stage procedure has been added.

3 A delayed incubation procedure, using benzoated M-Endo Broth MF, is designated a Tentative Method.

4 A test for enterococci (fecal streptococci), using M Enterococcus Agar or KF broth, is designated a Tentative Method.

B The Technical Committee on Methods for the Bacteriological Examination of Boundary Waters Recommended use of membrane filter methods for the examination of boundary waters. The Advisory Committee adopted the recommendation in January, 1957.

C The U. S. Government, in Interstate Quarantine Drinking Water Standards

1 (Federal Register, October 23, 1956, pp. 8110-11 and March 1, 1957, Vol. 22, p. 1271). Preliminary and final authorization was granted to use membrane filter coliform tests for the examination of potable waters at interstate carrier water points and other waters under water quality control regulations of the Public Health Service or other agencies of the U. S. Government.

D The U. S. Government, in test procedures for compliance with water quality criteria. Federal Register, October 16, 1973, pp. 28758-60, Volume 38, No. 199. The same information also appears in the Code of Federal Regulations, Title 40, Part 136, pp. 111 ff, state July 1, 1974.

DETAILED MEMBRANE FILTER METHODS
Part 2

I BASIC PROCEDURES

A Introduction

Successful application of membrane filter methods requires development of good routine operational practices. The detailed basic procedures described in this Section are applicable to all membrane filter methods in water bacteriology for filtration, incubation, colony counting, and reporting of results. In addition, equipment and supplies used in membrane filter procedures described here are not repeated elsewhere in this text in such detail.

Workers using membrane filter methods for the first time are urged to become thoroughly familiar with these basic procedures and precautions.

B General Supplies and Equipment List

Table 1 is a check list of materials.

C "Sterilizing" Media

Set tubes of freshly prepared medium in a boiling waterbath for 10 minutes. This method suffices for medium in tubes up to 25 × 150 mm. Frequent agitation is needed with media containing agar.

Alternately, coliform media can be directly heated on a hotplate to the first bubble of boiling. Stir the medium frequently if direct heat is used, to avoid charring the medium.

Do not sterilize in the autoclave.

D General Laboratory Procedures with Membrane Filters

1 Prepare data sheet

Minimum data required are: sample identification, test performed including media and methods, sample filtration volumes, and the bench numbers assigned to individual membrane filters.

2 Disinfect the laboratory bench surface.

Use a suitable disinfectant solution and allow the surface to dry before proceeding.

3 Set out sterile culture containers in an orderly arrangement.

4 Label the culture containers.

Numbers correspond with the filter numbers shown on the data sheet.

5 Place one sterile absorbent pad* in each culture container, unless an agar medium is being used.

Use sterile forceps for all manipulations of absorbent pads and membrane filters. Forceps sterility is maintained by storing the working tips in about 1 inch of methanol or ethanol. Because the alcohol deteriorates the filter, dissipate it by burning before using the forceps. Avoid heating the forceps in the burner as hot metal chars the filter.

*When an agar medium is used, absorbent pads are not used. The amount of medium should be sufficient to make a layer approximately 1/8" deep in the culture container. In the 50 mm plastic culture containers this corresponds to approximately 6-8 ml of culture medium.

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Office Of Water Programs, Environmental Protection Agency.

Table 1. EQUIPMENT, SUPPLIES AND MEDIA

Item	Total Coliforms					
	M-Endo Broth	L. E. S. Coliform	Delayed Coliform	Fecal Coliform	Fecal Streptococcus	Verified Tests
Funnel unit assemblies	X	X	X	X	X	
Ring stand, with about a 3" split ring, to support the filtration funnel	X	X	X	X	X	
Forceps, smooth tips, type for MF work	X	X	X	X	X	
Methanol, in small wide-mouthed bottles, about 20 ml for sterilizing forceps	X	X	X	X	X	
Suction flasks, glass, 1 liter, mouth to fit No. 8 stopper	X	X	X	X	X	
Rubber tubing, 2-3 feet, to connect suction flask to vacuum services, latex rubber 3/16" I.D. by 3/32" wall	X	X	X	X	X	
Pinch clamps strong enough for tight compression of rubber tubing above	X	X	X	X	X	
Pipettes, 10 ml, graduated, Mohr type, sterile, dispense 10 per can per working space per day. (Resterilize daily to meet need).	X	X	X	X	X	
Pipettes, 1 ml, graduated, Mohr type, sterile, dispense 24 per can per working space per day. (Resterilize daily to meet need).	X	X	X	X	X	
Pipette boxes, sterile, for 1 ml and 10 ml pipettes (sterilize above pipettes in these boxes).	X	X	X	X	X	
Cylinders, 100 ml graduated, sterile, (resterilize daily to meet need).	X	X	X	X	X	
Jars, to receive used pipettes	X	X	X	X	X	
Gas burner, Bunsen or similar laboratory type	X	X	X	X	X	X
Wax pencils, red, suitable for writing on glass	X	X	X	X	X	
Sponge in dilute iodine, to disinfect the desk tops	X	X	X	X	X	
Membrane filters (white, grid marked, sterile,and suitable pore size for microbiological analysis of water)	X	X	X	X	X	
Absorbent pads for nutrient, (47 mm in diameter), sterile, in units of 10 pads per package. Not required if medium contains agar.	X	X	X	X	X	
Petri dishes, disposable, plastic, 50 X 12 mm, sterile	X	X	X	X	X	
Waterbath incubator 44.5 ± 0.2°C				X		
Vegetable crispers, or cake boxes, plastic, with tight fitting covers, for membrane filter incubations	X	X	X		X	
Fluorescent lamp, with extension cord.	X	X	X	X	X	X
Ring stand, with clamps, utility type	X	X	X	X	X	

Table 1. EQUIPMENT, SUPPLIES AND MEDIA (Cont'd)

Item	Total Coliforms					
	M-Endo Broth	L. E. S. Coliform	Delayed Coliform	Fecal Coliform	Fecal Streptococcus	Verified Tests
Half-round glass paper weights for colony counting, with lower half of a 2-oz metal ointment box	X	X	X	X	X	
Hand tally, single unit acceptable, hand or desk type	X	X	X	X	X	
Stereoscopic (dissection) microscope, magnification of 10X or 15X, preferable binocular wide field type	X	X	X	X	X	X
Bacteriological inoculating needle						X
Wire racks for culture tubes, 10 openings by five openings preferred, dimensions overall approximately 6" × 12"						X
Phenol Red Lactose Broth in 16 X 150 mm fermentation tubes with metal caps, 10 ml per tube						X
Eosin Methylene Blue Agar (Levine) in petri plates, prepared ready for use						X
Nutrient agar slants, in screw capped tubes, 16 × 126 mm						X
Gram stain solutions, 4 solutions per complete set						X
Microscope, compound, binocular, with oil immersion lens, microscope lamp and immersion oil						X
Microscope slides, new, clean, 1" × 3" size						X
Water proof plastic bags for fecal coliform culture dish incubation				X		
M-Endo medium, MF dehydrated medium in 25 × 95 mm flat bottomed screw-capped glass vials, 1.44 g per tube, sufficient for 30 ml of medium	X		X			
Ethanol, 95% in small bottles or screw-capped tubes, about 20 ml per tube	X	X	X			
Sodium benzoate solution, 12% aqueous, in 25 × 150 mm screw-capped tubes, about 10 ml per tube			X			
L. E. S. Endo Agar MF, dehydrated M-Endo medium, 0.36 g per 25 × 95 mm flat bottomed screw-capped glass vial, plus 0.45 g agar, for 30 ml		X				
Lactose Lauryl Sulfate Tryptose Broth in 25 × 150 mm test tube without included gas tube, about 25 ml, for enrichment in L. E. S. method		X				

Table 1. EQUIPMENT, SUPPLIES AND MEDIA (Cont'd)

Item	Total Coliforms					
	M-Endo Broth	L. E. S. Coliform	Delayed Coliform	Fecal Coliform	Fecal Streptococcus	Verified Test
M-FC Broth for fecal coliform, dehydrated medium in 25 × 95 mm flat bottomed screw-capped glass vials, 1.11 g per tube, sufficient for 30 ml of culture medium				X		
Rosolic acid, 1% solution, in 0.2N NaOH, in 25 × 150 mm flat bottomed screw-capped tubes, about 5 ml per tube, freshly prepared				X		
M-Enterococcus Agar, dehydrated medium in 25 × 150 mm screw-capped tubes, sufficient for 30 ml, 1.26 g per tube					X	
Dilution bottles, 6-oz, preferable boro-silicate glass, with screw-cap (or rubber stopper protected by paper), each containing 99 ml of sterile phosphate buffered distilled water	X	X	X	X	X	
Electric hot plate surface	X	X	X	X	X	
Beakers, 400 - 600 ml (for water-bath in preparation of membrane filter culture media)	X	X	X	X	X	
Crucible tongs, to be used at electric hot plates, for removal of hot tubes of culture media for boiling waterbath	X	X	X	X	X	

6 Deliver enough culture medium to saturate each absorbent pad, using a sterile pipette.

Exact quantities cannot be stated because pads and culture containers vary. Sufficient medium should be applied so that when the culture container is tipped, a good-sized drop of culture medium freely drains out of the absorbent pad.

7 Organize supplies and equipment for convenient sample filtration. In training courses, laboratory instructors will suggest useful arrangements; eventually the individual will select a system of bench-top organization most suited to his own needs. The important point in any arrangement is to have all needed equipment and supplies conveniently at hand, in such a pattern as to minimize lost time in useless motions.

8 Lay a sterile membrane filter on the filter holder, grid-side up, centered over the porous part of the filter support plate.

Membrane filters are extremely delicate and easily damaged. For manipulation, the sterile forceps should always grasp the outer part of the filter disk, outside the part of the filter through which the sample passes.

9 Attach the funnel element to the base of the filtration unit.

To avoid damage to the membrane filter, locking forces should only be applied at the locking arrangement. The funnel element never should be turned or twisted while being seated and locked to the lower element of the filter holding unit. Filter holding units featuring a bayonet joint and locking ring to join the upper element to the lower element require special care on the part of the operator. The locking ring should be turned sufficiently to give a snug fit, but should not be tightened excessively.

10 Shake the sample thoroughly.

11 Measure sample into the funnel with vacuum turned off.

The primary objectives here are: 1) accurate measurement of sample; and 2) optimum distribution of colonies on the filter after incubation. To meet these objectives, methods of measurement and dispensation to the filtration assembly are varied with different sample filtration volumes.

a With samples greater than 20 ml, measure the sample with a sterile graduated cylinder and pour it into the funnel. It is important to rinse this graduate with sterile buffered distilled water to preclude the loss of excessive sample volume. This should be poured into the funnel.

b With samples of 10 ml to 20 ml, measure the sample with a sterile 10 ml or 20 ml pipette, and pipette on a dry membrane in the filtration assembly.

c With samples of 2 ml to 10 ml, pour about 20 ml of sterile dilution water into the filtration assembly, then measure the sample into the sterile buffered dilution water with a 10 ml sterile pipette.

d With samples of 0.5 to 2 ml, pour about 20 ml of sterile dilution water into the funnel assembly, then measure the sample into the sterile dilution water in the funnel with a 1 ml or a 2 ml pipette.

e If a sample of less than 0.5 ml is to be filtered, prepare appropriate dilutions in sterile dilution water, and proceed as applicable in item c or d above.

When dilutions of samples are needed, always make the filtrations as soon as possible after dilution of the sample; this never should exceed

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30 minutes. Always shake sample dilutions thoroughly before delivering measured volumes.

12 Turn on the vacuum.

Open the appropriate spring clamp or valve, and filter the sample.

After sample filtration a few droplets of sample usually remain adhered to the funnel walls. Unless these droplets are removed, the bacteria contained in them will be a source of contamination of later samples. (In laboratory practice the funnel unit is not routinely sterilized between successive filtrations of a series). The purpose of the funnel rinse is to flush all droplets of a sample from the funnel walls to the membrane filter. Extensive tests have shown that with proper rinsing technique, bacterial retention on the funnel walls is negligible.

13 Rinse the sample through the filter.

After all the sample has passed through the membrane filter, rinse down the sides of the funnel walls with at least 20 ml of sterile dilution water. Repeat the rinse twice after all the first rinse has passed through the filter. Cut off suction on the filtration assembly.

14 Remove the funnel element of the filter holding unit.

If a ring stand with split ring is used, hang the funnel element on the ring; otherwise, place the inverted funnel element on the inner surface of the wrapping material. This requires care in opening the sterilized package, but it is effective as a protection of the funnel ring from contamination.

15 Take the membrane filter from the filter holder and carefully place it, grid-side up on the medium.

Check that no air bubbles have been trapped between the membrane filter and the underlying absorbent pad or agar. Relay the membrane if necessary.

16 Place in incubator after finishing filtration series.

Invert the containers. The immediate atmosphere of the incubating membrane filter must be at or very near 100% relative humidity.

17 Count colonies which have appeared after incubating for the prescribed time.

A stereoscopic microscope magnifying 10-15 times and careful illumination give best counts.

For reporting results, the computation is:

bacteria/100 ml =

$$\frac{\text{No. colonies counted} \times 100}{\text{Sample volume filtered in ml}}$$

Example:

A total of 36 colonies grew after filtering a 10 ml sample. The number reported is:

$$\frac{36 \text{ colonies}}{10 \text{ ml}} \times 100 = 360 \text{ per } 100 \text{ ml}$$

Report results to two significant figures.

Example:

A total of 40 colonies grew after filtering a 3 ml sample.

This calculation gives:

$$\frac{40 \text{ colonies}}{3 \text{ ml}} \times 100 = 1333.33 \text{ per } 100 \text{ ml}$$

But the number reported should be 1300 per 100 ml.

II MF LABORATORY TESTS FOR COLIFORM GROUP

A Standard Coliform Test (Based on M-Endo Broth MF)

1 Culture medium

a M-Endo Broth MF Difco 0749-02 or the equivalent BBL M-Coliform Broth 01-494

Preparation of Culture Medium (M-Endo Broth) for Standard MF Coliform Test

Yeast extract	1.5	g
Casitone or equivalent	5.0	g
Thiopeptone or equivalent	5.0	g
Tryptose	10.0	g
Lactose	12.5	g
Sodium desoxycholate	0.1	g
Dipotassium phosphate	4.375	g
Monopotassium phosphate	1.375	g
Sodium chloride	5.0	g
Sodium lauryl sulfate	0.05	g
Basic fuchsin (bacteriological)	1.05	g
Sodium sulfite	2.1	g
Distilled water (containing 20.0 ml ethanol)	1000	ml

This medium is available in dehydrated form and it is recommended that the commercially available medium be used in preference to compounding the medium of its individual constituents.

To prepare the medium for use, suspend the dehydrated medium at the rate of 48 grams per liter of water containing ethyl alcohol at the rate of 20 ml per liter.

As a time-saving convenience, it is recommended that the laboratory worker preweigh the dehydrated medium in closed tubes for several days, or even weeks, at one operation.

With this system, a large number of increments of dehydrated medium (e.g., 1.44 grams), sufficient for some convenient (e.g., 30 ml) volume of finished culture medium are weighed and dispensed into screw-capped culture tubes, and stored until needed. Storage should preferably be in a darkened disiccator.

A supply of distilled water containing 20 ml stock ethanol per liter is maintained.

When the medium is to be used, it is reconstituted by adding 30 ml of the distilled water-ethanol mixture per tube of pre-weighed dehydrated culture medium.

b Medium is "sterilized" as directed in I, C.

c Finished medium can be retained up to 96 hours if kept in a cool, dark place. Many workers prefer to reconstitute fresh medium daily.

2 Filtration and incubation procedures are as given in I, D.

Special instructions:

a For counting, use the wide field binocular dissecting microscope, or simple lens. For illumination, use a light source perpendicular to the plane of the membrane filter. A small fluorescent lamp is ideal for the purpose.

b Coliform colonies have a "metallic" surface sheen under reflected light which may cover the entire colony, or it may appear only in the center. Non-coliform colonies range from colorless to pink, but do not have the characteristic sheen.

c Record the colony counts on the data sheet, and compute the coliform count per 100 ml of sample.

B Standard Coliform Tests (Based on L. E. S. Endo Agar)

The distinction of the L. E. S. count is a two hour enrichment incubation on LST broth. M-Endo L. E. S. medium is used as agar rather than the broth.

1 Preparation of culture medium (L. E. S. Endo Agar) for L. E. S. coliform test

a Formula from McCarthy, Delaney, and Grasso [2]

Bacto-Yeast Extract	1.2 g
Bacto-Casitone	3.7 g
Bacto-Thiopeptone	3.7 g
Bacto-Tryptose	7.5 g
Bacto-Lactose	9.4 g
Dipotassium phosphate	3.3 g
Monopotassium phosphate	1.0 g
Sodium chloride	3.7 g
Sodium desoxycholate	0.1 g
Sodium lauryl sulfate	0.05 g
Sodium sulfite	1.6 g
Bacto-Basic fuchsin	0.8 g
Agar	15 g
Distilled water (containing 20 ml ethyl alcohol)	1000 ml

b To rehydrate the medium, suspend 51 grams in the water-ethyl alcohol solution.

c Medium is "sterilized" as directed in I, C.

d Pour 4-6 ml of freshly prepared Agar into the smaller half of the container. Allow the medium to cool and solidify.

2 Procedures for filtration and incubation

a Lay out the culture dishes in a row or series of rows as usual. Place these with the upper (lid) or top side down.

b Place one sterile absorbent pad in the larger half of each container (lid). Use sterile forceps for all manipulations of the pads. (Agar occupies smaller half or bottom).

c Using a sterile pipette, deliver enough single strength lauryl sulfate tryptose broth to saturate the pad only. Avoid excess medium.

d Follow general procedures for filtering in I, D. Place filters on pad with lauryl sulfate tryptose broth.

e Upon completion of the filtrations, invert the culture containers and incubate at 35° C for 1 1/2 to 2 hours.

3 2-hour procedures

a Transfer the membrane filter from the enrichment pad in the upper half to the agar medium in the lower half of the container. Carefully roll the membrane onto the agar surface to avoid trapping air bubbles beneath the membrane.

b Removal of the used absorbent pad is optional.

c The container is inverted and incubated 22 hours ± 2 hours ± 0.5°C.

4 Counting procedures are as in I, D.

5 L. E. S. Endo Agar may be used as a single-stage medium (no enrichment step) in the same manner as M-Endo Broth, MF.

C Delayed Incubation Coliform Test

This technique is applicable in situations where there is an excessive delay between sample collection and plating. The procedure is unnecessary when the interval between sample collection and plating is within acceptable limits.

1 Preparation of culture media for delayed incubation coliform test

a Preservative media M-Endo Broth base

To 30 ml of M-Endo Broth MF prepared in accordance with directions in II, A, 1 of this outline, add 1.0 ml of a sterile 12% aqueous solution of sodium benzoate.

L.E.S. MF Holding Medium-Coliform: Dissolve 12.7 grams in 1 liter of distilled water. No heating is necessary. Final pH 7.1 ± 0.1. This medium contains sodium benzoate.

b Growth media

M-Endo Broth MF is used, prepared as described in II, A, 1 earlier in this outline. Alternately, L.E.S. Endo Medium may be used.

2 General filtration followed is in I, D.

Special procedures are:

a Transfer the membrane filter from the filtration apparatus to a pad saturated with benzoated M-Endo Broth.

b Close the culture dishes and hold in a container at ambient temperature. This may be mailed or transported to a central laboratory. The mailing or transporting tube should contain accurate transmittal data sheets which correspond to properly labeled dishes.

Transportation time, in the case of mailed containers, should not exceed three days to the time of reception by the testing laboratory.

c On receipt in the central laboratory, unpack mailing carton, and lay out the culture containers on the laboratory bench.

d Remove the tops from the culture containers. Using sterile forceps, remove each membrane and its absorbent pad to the other half of the culture container.

e With a sterile pipette or sterile absorbent pad, remove preservative medium from the culture container.

f Place a sterile absorbent pad in each culture container, and deliver enough freshly prepared M-Endo Broth to saturate each pad.

g Using sterile forceps, transfer the membrane to the new absorbent pad containing M-Endo Broth. Place the membrane carefully to avoid entrapment of air between the membrane and the underlying absorbent pad. Discard the absorbent pad containing preservative medium.

h After incubation of 20 ± 2 hours at 35°C, count colonies as in the above section A, 2.

i If L.E.S. Endo Agar is used, the steps beginning with (e) above are omitted; and the membrane filter is removed from the preservative medium and transferred to a fresh culture container with L.E.S. Endo Agar, incubated, and colonies counted in the usual way.

D Verified Membrane Filter Coliform Test

This procedure applies to identification of colonies growing on Endo-type media used for determination of total coliform counts. Isolates from these colonies are studied for gas production from lactose and typical coliform morphology. In effect, the procedure corresponds with the Completed Test stage of the multiple fermentation tube test for coliforms.

Procedure:

1 Select a membrane filter bearing several well-isolated coliform-type colonies.

2 Using sterile technique, pick all colonies in a selected area with the inoculation needle, making transfers into tubes of phenol red lactose broth (or lauryl sulfate tryptose lactose

broth). Using an appropriate data sheet record the interpretation of each colony, using, for instance, "C" for colonies having the typical color and sheen of coliforms; "NC" for colonies not conforming to coliform colony appearance on Endotype media.

3 Incubate the broth tubes at 35° C ± 0.5°C.

4 At 24 hours:

a Read and record the results from the lactose broth fermentation tubes. The following code is suggested:

Code	
O	No indication of acid or gas production, either with or without evidence of growth.
A	Evidence of acid but not gas (applies only when a pH indicator is included in the broth medium)
G	Growth with production of gas. If pH indicator is used, use symbol AG to show evidence of acid. Gas in any quantity is a positive test.

b Tubes not showing gas production are returned to the 35° C incubator.

c Gas-positive tubes are transferred as follows:

1) Prepare a streak inoculation on EMB agar for colony isolation, and using the same culture.

2) Inoculate a nutrient agar slant.

3) Incubate the EMB agar plates and slants at 35° C ± 0.5°C.

5 At 48 hours:

a Read and record results of lactose broth tubes which were negative at 24 hours and were returned for further incubation.

b Gas-positive cultures are subjected to further transfers as in 4c. Gas-negative cultures are discarded without further study; they are coliform- negative.

c Examine the cultures transferred to EMB agar plates and to nutrient agar slants, as follows:

1) Examine the EMB agar plate for evidence of purity of culture; if the culture represents more than one colony type, discard the nutrient agar culture and reisolate each of the representative colonial types on the EMB plate and resume as with 4c for each isolation. If purity of culture appears evident, continue with c (2) below.

2) Prepare a smear and Gram stain from each nutrient agar slant culture. The Gram stain should be made on a culture not more than 24 hours old. Examine under oil immersion for typical coliform morphology, and record results.

6 At 72 hours:

Perform procedures described in 5c above, and record results.

7 Coliform colonies are considered verified if the procedures demonstrate a pure culture of bacteria which are gram negative nonspore-forming rods and produce gas from lactose at 35° C within 48 hours.

E Fecal Coliform Count (Based on M-FC Broth Base)

The count depends upon growth on a special medium at 44.5 ± 0.2°C.

1 Preparation of Culture Medium (M-FC Broth Base) for Fecal Coliform Count

a Composition

Tryptose	10.0 g
Proteose Peptone No. 3	5.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose	12.5 g
Bile salts No. 3	1.5 g
Rosolic acid* (Allied Chemical)	10.0 ml
Aniline blue (Allied Chemical)	0.1 g
Distilled water	1000 ml

b To prepare the medium dissolve 37.1 grams in a liter of distilled water which contains 10 ml of 1% rosolic acid (prepared in 0.2 N NaOH).

Fresh solutions of rosolic acid give best results. Discard solutions which have changed from dark red to orange.

c To sterilize, heat to boiling as directed in I, C.

d Prepared medium may be retained up to 4 days in the dark at 2-8° C.

2 Special supplies

Small water proof plastic sacks capable of being sealed against water with capacity of 3 to 6 culture containers.

3 Filtration procedures are as given in I, D.

4 Elevated temperature incubation

a Place fecal coliform count membranes at 44.5 ± 0.2°C as rapidly as possible.

Filter membranes for fecal coliform counts consecutively and immediately place them in their culture containers. Insert as many as six culture containers all oriented in the same way (i.e., all grid sides facing the same direction) into the sacks and seal. Tear off the perforated top, grasp the side wires, and twirl the sack to roll the open end inside the folds of sack. Then submerge the sacks with culture containers inverted beneath the surface of a 44.5 ± 0.2°C waterbath.

b Incubate for 22 ± 2 hours.

5 Counting procedures

Examine and count colonies as follows:

a Use a wide field binocular dissecting microscope with 5 - 10X magnification.

b Low angle lighting from the side is advantageous.

c Fecal coliform colonies are blue, generally 1-3 mm in diameter.

d Record the colony counts on the data sheet, and report the fecal coliform count per 100 ml of sample. (I, D, 17 illustrates method)

III TESTS FOR FECAL STREPTOCOCCAL GROUP-MEMBRANE FILTER METHOD

A 48 hour incubation period on a choice of two different media, giving high selectivity for fecal streptococci, are the distinctive features of the tests.

*Prepare 1% solution of rosolic acid in 0.2 N NaOH. This dye is practically insoluble in water.

A Test for Members of Fecal Streptococcal Group (Tentative, Standard Methods) M-Enterococcus Agar Medium

1 Preparation of the culture medium

a Formula (The Difco formula is shown, but equivalent constituents from other sources are equally acceptable).

Bacto tryptose	20.0 g
Bacto yeast extract	5.0 g
Bacto dextrose	2.0 g
Dipotassium phosphate	4.0 g
Sodium Azide	0.4 g
Bacto agar	10.0 g
2, 3, 5, Triphenyl tetrazolium chloride	0.1 g

b The medium is prepared by rehydration at the rate of 42 grams per 1000 ml of distilled water. It is recommended that the medium in dehydrated form be preweighed and dispensed into culture containers (about 25 × 150 mm) in quantities sufficient for preparation of 30 ml of culture medium (1.26 g per tube).

c Follow I, C, for "sterilizing" medium and dispense while hot into culture containers. Allow plates to harden before use.

2 List of apparatus, materials, as given in Table I.

3 Procedure, in general, as given in I.

Special instructions

a Incubate for 48 hours, inverted, with 100% relative humidity, after filtrations are completed. If the entire incubator does not have saturated humidity, acceptable conditions can be secured by placing the cultures in a tightly closed container with wet paper, towels, or other moist material.

b After incubation, remove the cultures from the incubator, and count all colonies under wide field binocular dissecting microscope with magnification set at 10X or 20X. Fecal streptococcus colonies are 0.5 - 2 mm in diameter, and flat to raised smooth, and vary from pale pink to dark red in color.

c Report count per 100 ml of sample. This is conveniently computed:

No. fecal streptococci per 100 ml =

$$\frac{\text{No. fecal streptococcus colonies counted}}{\text{Sample filtration volume in ml}} \times 100$$

B Test for Members of Fecal Streptococcal Group based on KF-Agar

1 Preparation of the culture medium

a Formula: (The dehydrated formula of Bacto 0496 is shown, but equivalent constituents from other sources are acceptable). Formula is in grams per liter of reconstituted medium.

Bacto proteose peptone #3	10.0	g
Bacto yeast extract	10.0	g
Sodium chloride (reagent grade)	5.0	g
Sodium glycerphosphate	10.0	g
Maltose (CP)	20.0	g
Lactose (CP)	1.0	g
Sodium azide (Eastman)	0.4	g
Sodium carbonate (Na_2CO_3 reagent grade)	0.636	g
Brom cresol purple (water soluble)	0.015	g
Bacto agar	20.0	g

b Reagent

2, 3, 5-Triphenyl tetrazolium chloride reagent (TPTC)

This reagent is prepared by making a 1% aqueous solution of the above chemical passing it through a Seitz filter or membrane filter. It can

be kept in the refrigerator in a screw-capped tube until used.

c The dehydrated medium described above is prepared for laboratory use as follows:

Suspend 7.64 grams of the dehydrated medium in 100 ml of distilled water in a flask with an aluminum foil cover.

Place the flask in a boiling waterbath, melt the dehydrated medium, and leave in the boiling waterbath an addional 5 minutes.

Cool the medium to 50°-60° C, add 1.0 ml of the TPTC reagent, and mix.

For membrane filter studies, pour 5-8 ml in each 50 mm glass or plastic culture dish or enough to make a layer approximately 1/8" thick. Be sure to pour plates before agar cools and solidifies.

For plate counts, pour as for standard agar plate counts.

NOTE: Plastic dishes containing media may be stored in a dark, cool place up to 30 days without change in productivity of the medium, provided that no dehydration occurs. Plastic dishes may be incubated in an ordinary air incubator. Glass dishes must be incubated in an atmosphere with saturated humidity.

2 Apparatus, and materials as given in Table 1.

3 General procedure is as given in I.

Special instructions

a Incubate 48 hours, inverted with 100% relative humidity after filtration.

b After incubation, remove the cultures from the incubator, and count colonies under wide field binocular dissecting microscope, with magnification set at 10X or 20X. Fecal streptococcus colonies are pale pink to dark wine-color. In size they range from barely visible to approximately 2mm in diameter. Colorless colonies are not counted.

c Report fecal streptococcus count per 100 ml of sample. This is computed as follows:

No. fecal streptococci per 100 ml =

$$\frac{\text{No. fecal streptococcus colonies}}{\text{Sample filtration volume in ml}} \times 100$$

C Verification of Streptococcus Colonies

1 Verification of colony identification may be required in waters containing large numbers of Micrococcus organisms. This has been noted particularly with bathing waters, but the problem is by no means limited to such waters.

2 A verification procedure is described in "Standard Methods for the Examination of Water and Wastewater," 13th ed, (1971). The worker should use this reference for the step-by-step procedure.

IV PROCEDURES FOR USE OF MEMBRANE FILTER FIELD UNITS

A Culture Media

1 The standard coliform media used with laboratory tests are used.

2 To simplify field operations, it is suggested that the medium be sent to the field, preweighed, in vials or capped culture tubes. The medium then requires only the addition of a suitable volume of distilled water-ethanol prior to sterilization.

3 Sterilization procedures in the field are the same as for laboratory methods.

4 Laboratory preparation of the media, ready for use, would be permissible provided that the required limitations on time and conditions of storage are met.

B Operation of Millipore Water Testing Kit, Bacteriological

1 Supporting supplies and equipment are the same as for the laboratory procedures.

2 Set the incubator voltage selector switch to the voltage of the available supply, turn on the unit and adjust as necessary to establish operating incubator temperature at 35 $\pm$ 0.5°C.

3 Sterilize the funnel unit assembly by exposure to formaldehyde or by immersion in boiling water. If a laboratory autoclave is available, this is preferred.

Formaldehyde is produced by soaking an asbestos ring (in the funnel base) with methanol, igniting, and after a few seconds of burning, closing the unit by placing the stainless steel flask over the funnel and base. This results in incomplete combustion of the methanol, whereby formaldehyde is produced. Leave the unit closed for 15 minutes to allow adequate exposure to formaldehyde.

4 Filtration and incubation procedures correspond with laboratory methods.

5 The unit is supplied with a booklet containing detailed step-by-step operational procedures. The worker using the equipment should become completely versed in its contents and application.

C Other commercially available field kits should be used according to manufacturer's instructions. It is emphasized that the standards of performance are required for field devices as for laboratory equipment.

D Counting of Colonies on Membrane Filters

1 Equipment and materials

Membrane filter cultures to be examined

Illumination source

Simple lens, 2X to 6X magnification

Hand tally (optional)

2 Procedure

a Remove the cultures from the incubator and arrange them in numerical sequence.

b Set up illumination source as that light will originate from an area perpendicular to the plane of membrane filters being examined. A small fluorescent lamp is ideal for the purpose. It is highly desirable that a simple lens be attached to the light source.

c Examine results. Count all coliform and noncoliform colonies. Coliform and noncoliform colonies. Coliform colonies have a "metallic" surface sheen under reflected light, which may cover the entire colony or may appear only on the center.

Noncoliform colonies range from colorless to pink or red, but do not have the characteristic "metallic" sheen.

d Enter the colony counts in the data sheets.

e Enter the coliform count per 100 ml of sample for each membrane having a countable number of coliform colonies. Computation is as follows:

No. coliform per 100 ml =

$$\frac{\text{No. coliform colonies on MF}}{\text{No. milliliters sample filtered}} \times 100$$

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WPCF. 12th Edition. 1965.

2 McCarthy, J.A., Delaney, J.E. and Grasso, R.J. Measuring Coliforms in Water. Water and Sewage Works. 1961: R-426-31. 1961.

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