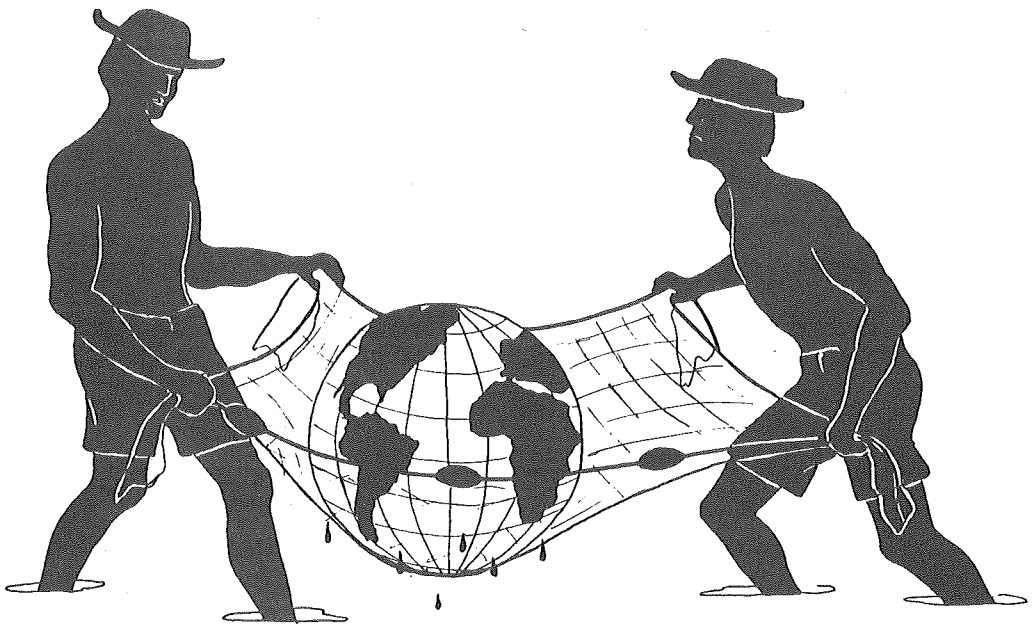
TITLE XII
COLLABORATIVE RESEARCH SUPPORT PROGRAM

POND DYNAMICS/AQUACULTURE

CRSP WORK PLAN: SECOND

EXPERIMENTAL CYCLE

JULY 1984



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1. INTRODUCTION

It is the policy of the Pond Dynamics/Aquaculture CRSP to develop annual work plans for the standardized experiments conducted at the host country research sites. The CRSP Technical Advisory Committee (TAC) formulates a generalized research plan each year. This generalized plan is transformed into a more detailed experimental plan by the CRSP Research Team composed of Host Country and U.S. Principal Investigators of the several Collaborative Research Projects.

The TAC and CRSP Research Team met in Atlanta, Georgia on April 10-12, 1984 to develop a work plan for the second cycle of CRSP experiments. The meeting participants reviewed accomplishments and discussed problems encountered during the first cycle of experiments. As a result of these discussions, substantial improvements were suggested for the continuing baseline studies proposed by the TAC for the second year. Additionally, several site specific problems were identified that will ultimately confound comparison of data between sites. It was decided to include a series of pilot experiments intended to circumvent site specific problems at these locations.

The resulting work plan is detailed in the following chapters. Included are Description of the Experiments (Chapter 2), Materials and Methods (Chapter 3), Reporting Requirements (Chapter 4), and Appendices which expand upon materials, methods, and procedures. Chapter 3 and the Appendices have been edited extensively and represent a substantial improvement over the previous year's plan.

2. DESCRIPTION OF EXPERIMENTS

The second cycle of CRSP experiments will focus mainly on continuing a set of standardized pond dynamics experiments. Special site specific studies also will be carried out at some research locations. The standardized pond dynamics experiments will involve the culture of tilapia at five research sites and penaeid shrimp at two sites. This chapter describes the experimental design for the standardized experiments and identifies the special research projects that may be undertaken at selected research locations.

STANDARDIZED POND DYNAMICS EXPERIMENTS

The second cycle of pond dynamics experiments is intended to expand the quantitative baselines initiated during the first year of research. In addition, the second cycle of experiments is designed to test the working hypothesis that organic fertilization will improve water quality and will produce higher fish yields than inorganic fertilization. In statistical terms, our primary hypothesis would be expressed as: Primary H_o = Organic and inorganic fertilization result in the same fish production.

During the second cycle, common culture organisms will be used to the extent possible. *Tilapia nilotica* will be used at the CRSP brackish water research site in the Philippines and at freshwater research stations in four other host countries: Honduras, Indonesia, Rwanda and Thailand. The CRSP research team in the Philippines also will carry out a set of experiments using penaeid shrimp. A second brackish water station in Panama will conduct its pond dynamics experiments with penaeid shrimp exclusively. As detailed below, experimental design is different for the two types of organisms.

Tilapia Experiments

A minimum of 12 earthen ponds will be used for second cycle experiments. Numbers of replicates per treatment will be dictated by variation among ponds observed in the first cycle.

Pond Treatments	Recommended pond replicates	Treatment description
I	4 - 6	Organic fertilizer
II	4 - 6	Inorganic fertilizer
III	4	Site specific plans

Nutrient Inputs

Detailed methods of fertilizer application are outlined in Appendix A. Fertilizer treatments for the second cycle are:

I. Organic Fertilizers - Chicken manure or other locally available animal wastes will be added at a rate of approximately 500 kg/ha/wk.

II. Inorganic Fertilizers in the form of triple super phosphate (0-46-0) and urea will be added at levels of total P and N equivalent to the levels of total P and N in the organic fertilizers used in the experiment.

Pond Preparation

Preparation of ponds for experiments, including the method for satisfying lime requirement of pond muds is outlined in Appendix A.

Stocking Procedures

All ponds are to be stocked with all male *Tilapia nilotica* of an average weight betwen 25 and 50 grams as described in Appendix B. The standard stocking density is to be one fish per square meter (10,000 fish per hectare). The recommended method for production of *T. nilotica* fingerlings is outlined in Appendix B.

Duration of Experiments

Two experiments will be accomplished during the second cycle. Each experiment should run for a period of five months (150 days). The experimental cycle will be established so that one experiment is run during the wet season and the other during the dry season. In some instances it may be difficult to complete an entire experiment in a particular climatic season. In this case, it is essential that the final 90 days of the culture period occur within a single climatic season.

Penaeid Shrimp Experiments

The CRSP project teams in Panama and the Philippines have proposed to devote a minimum of 16 earthen ponds to the common collaborative research plan. Ponds will be divided into the following treatments.

Pond treatment	Recommended pond reps.	Treatment description
I	4	Manure
II	4	Manure + inorganic fertilizer
III	4	Manure + feed
IV	4	Manure + fertilizer + feed

Nutrient Inputs

I. Manures: dried chicken manure will be applied as a pretreatment to ponds at a rate of 2000 kg/ha, broadcast over the pond bottom.

II. Inorganic Fertilizers: 200 kg/ha of 16-20-0 (N-P-K) will be added, broadcast over the pond bottom prior to filling.

III. Feed: a 25% protein feed will be added to the pond beginning on day 31.

Stocking

Approximately 4 juvenile penaeid shrimp will be stocked per square meter. Species selection will depend on local availability of stocks.

Duration of Experiments

The experimental periods will be 90 to 120 days.

Variables to be Measured

Sampling methods, frequencies, and methods of analysis are detailed in a later section. The variables to be measured in the second cycle are:

I. Physical Environment Measurements

A. Required measurements for all sitex

1. Solar Radiation
2. Rainfall
3. Wind Speed
4. Air Temperature
5. Pond Soil Characteristics
6. Pond Temperature Extremes
7. Pond Depth
8. Hydrologic Characteristics

II. Water Analysis

A. Required analysis during second cycle fish production experiments

1. Dissolved Oxygen
2. Temperature
3. pH
4. Alkalinity
5. Total Hardness
6. Water Quality Characteristics
7. Total Nitrogen
8. Ammonia
9. Nitrate
10. Total Phosphorus
11. Dissolved Orthophosphate (Filterable Reactive Phosphorus)

III. Fish Production

A. Required measurements during fish production experiments

1. Growth
2. Reproduction
3. Survival

IV. Biological Limnology

A. Required measurements during fish production experiments

1. Secchi Disk Visibility
2. Chlorophyll a
3. Chlorophyll b, c (Brackish Water Sites Only)

B. Recommended observations during fish production experiments (not required during second cycle)

1. Primary Productivity
2. Qualitative Identification of Phytoplankton, Zooplankton and Benthos

SPECIAL RESEARCH TOPICS

After carrying out an initial set of CRSP experiments, program participants were able to identify a number of research topics that may have special relevance at particular host country sites. Project teams have proposed to undertake research in addition to that required as part of the standardized work plan in order to address these special topics. As briefly described below, five special topic activities have been proposed.

Effects of Turbidity on Fish Production
Honduras-Auburn University Project

Results of an initial set of pond dynamics experiments at the CRSP research site in the Comayagua Valley of Honduras suggested that clay turbidity may be suppressing fish production in experimental ponds. As noted by Boyd (1979), a number of investigators have reported that applications of organic matter are effective in reducing clay turbidity in ponds. CRSP researchers in Honduras plan to test the effects of organic fertilization on levels of total suspended solids, total volatile solids, and fish production during the second cyle of experimentation.

Effects of Detergents and Pesticides on Fish Production
Indonesia-Michigan State University Project

The CRSP research site in Bogor, Indonesia may be affected by regional water quality problems mainly related to agricultural practices. During the second cycle, the water supply for selected ponds will be filtered to remove contaminants. Levels of detergents, selected pesticides, suspended and dissolved solids, turbidity and conductivity will be monitored in treated and control ponds. Effects on fish production will be measured.

Effects of Various Forms of Nutrient Input on Shrimp Production
Panama-Auburn University Project
Philippines-University of Hawaii Project

CRSP research at the Aquaculture Fish Culture Station in Panama indicated that penaeid shrimp production is strongly affected by the type of fertilization-feed regime employed. During the second cycle of CRSP experiments, researchers

in Panama will work with the CRSP team in Iloilo Province in the Philippines to explore the production efficiency of various feeding strategies. Ponds will be treated with various combinations of organic fertilizer, inorganic fertilizer and formula feed. In addition to measurements made as part of the standardized CRSP work plan, chlorophyll (a, b, c) and various forms of nitrogen and phosphorus will be measured frequently.

Effects of Pond Depth on Water Quality in Shrimp Ponds
Philippines-University of Hawaii Project

Researchers at the University of the Philippines in the Visayas and the University of Hawaii have observed that average depth affects numerous aspects of pond environments, including heat budget, chemical stratification, and minimum oxygen concentration. During the second cycle of experiments, CRSP researchers in Iloilo will initiate a special project to measure water quality in replicated shrimp ponds with depths of 0.3 to 0.5 m; 1.0 m and 2.0 m. One set of ponds at each depth will be circulated artificially.

Adjustment of Fertilization Rates by Monitoring Phosphate Levels in Ponds
Thailand-University of Michigan Project

The CRSP research plan involves the application of fertilizers at a constant rate. CRSP researchers at Thailand's National Inland Fisheries Institute noted that conditions such as inorganic turbidity, soil chemistry and pH may limit the availability of nutrients contained in fertilizers for phytoplankton production. During the second cycle of experiments, ponds will be treated either with organic or inorganic fertilizers. Delivery levels of PO_4-P will be carefully monitored and delivery rates will be adjusted to mainain levels of at least 200 µg/l.

3. MATERIALS AND METHODS

This chapter outlines the materials and methods required to accomplish the second year of CRSP experiments. In content, this section is similar to the "Sampling Protocol" Chapter of the first work plan. However, some important changes have been made and CRSP researchers should read this chapter carefully to note modifications in sampling procedures, frequencies of measurement and analytical methods.

Readers also will note that a new tabular format has been used to describe materials and methods. Parameters have been grouped according to frequency of measurement rather than the four topical categories used in Chapter 2. As shown in Tables 1-4, the new classification scheme is as follows:

- Daily Measurements: Weather variables and monitoring of pond depth (Table 1).
- Biweekly and Weekly Measurements: Primary set of limnological variables, including dissolved oxygen, temperature and pH (Table 2).
- Monthly Measurements: A variety of parameters representing four categories: Secondary set of limnological variables; diurnal study measurements; variables for monitoring fish growth, reproduction and survival; and optional measurements of primary productivity and characterization of phytoplankton, zooplankton and benthos (Table 3).
- Occasional Measurements: Five sets of variables to be examined at quarterly or less frequent intervals. Some measurements are part of baseline characterizations (e.g., morphology, hydrology). A section on fish/shrimp production refers to measurements at the initiation and termination of each experiment.

More detailed information on specific procedures, instrumentation and analytical methods is presented in Appendix F.

TABLE 1
DAILY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
Solar Radiation	Install solar monitor and quantum sensor at study site and read at 24 hour intervals	LI-COR Solar Monitor Model LI-1776 and Quantum Sensor Model LI-190SB (Appendix F)	---	$E/m^2/day$
Rainfall	Install three rain gauges at study site; read and empty at 24 hour intervals; report average of 3 readings.	No type specified.	---	cm/day
Wind Speed	If instantaneous windspeed and direction meter already in use, read at appropriate intervals to correlate with thermal and oxygen stratification of ponds. With preferred totalizing anemometer, read between 8:00-9:00 AM and calculate average hourly wind speed.	Instantaneous wind speed and direction meter comparable to Taylor Model 110930 acceptable if already in use. For new purchase, recommend totalizing anemometer comparable to WEATHERtronics Model 2510 (Appendix F).	---	km/hour
Air Temperature	Install 3 maximum-minimum thermometers in the shade near ponds; read at 24 hour intervals and report average maximum and average minimum.	Maximum-Minimum thermometer comparable to Taylor Model 5460 (Appendix F).	---	Max: °C Min: °C

TABLE 1 (Continued)
DAILY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
Pond Depth	Install staff gauge in each pond and read to nearest 0.5 cm at same time each day. (Maintain 0.9 m average depth on daily basis.)	No type specified.	---	m

TABLE 2
BIWEEKLY AND WEEKLY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
Dissolved Oxygen*	Near center of each pond at 25 cm below water surface. midwater and 25 cm above the bottom. Take readings weekly at dawn and as part of monthly diurnal study at 4 hour intervals beginning 30 minutes before sunrise until after sunrise.	YSI Model 57 Dissolved Oxygen Meter. Calibrate meter each month using the Winkler Method or HACH Digital Titrator kit/Dissolved Oxygen. (Appendix F).	Winkler or Iodometric Method (American Public Health Association, 1980) (Appendix F).	mg/l
Pond Temperature Extremes	In 3 ponds, place one maximum-minimum thermometer at 25 cm below the water surface and one at 25 cm above the bottom. Take weekly readings.	No type specified.	---	max: °C min: °C
Pond Temperature*	Near center of each pond, take readings at 25 cm below the water surface and 25 cm above the bottom. Take readings once per week, and as part of monthly diurnal study at four hour intervals beginning 30 minutes before sunrise until after sunset. If a probe is used, calibrate using a precision thermometer.	YSI Model 57 Dissolved Oxygen Meter with Temperature Indicator (Appendix F).	---	°C

* Indicates parameters to be measured as part of monthly diurnal studies.

TABLE 2 (Continued)
BIWEEKLY AND WEEKLY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
pH*	Measurements taken from three pooled 90 cm column samples per pond. Once per week, and as part of dirunal study at 4 hour intervals. Pooled samples can be taken to the laboratory and measured within one hour. Meter should be calibrated with standard buffers at pH 7 and pH 4.	pH Meter with Combination Electrode comparable to Orion 200 Series with Ross Model 81-55 Electrode (Appendix F).	---	pH Units
Total Kjeldahl Nitrogen*	Weekly, starting 2 days after fertilizer application, and once per month as part of diurnal study. For each pond, pool three 90 cm column samples. Composite samples should be refrigerated and analyzed within 24 hours.	Kontes or comparable Kjeldahl Nitrogen apparatus (Appendix F).	Semi-Micro-Kjeldahl Method (Michigan State University Limnological Research Laboratory, 1984) (Appendix F); or in-country analysis by qualified laboratory	mg/l
Secchi Disk Visibility*	Twice each week (same days as chlorophyll analyses with one sampling period coinciding with monthly diurnal study), at 2 locations in each pond, calculate Secchi Disk Visibility using procedure described by Lind (1974). (Appendix F).		---	cm

* Indicates parameters to be measured as part of monthly diurnal studies.

TABLE 2 (Continued)
BIWEEKLY AND WEEKLY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
Chlorophyll *a**	Collect one sample per pond by pooling three 90 cm column samples. Take samples twice each week with one sampling period coinciding with monthly diurnal study.		Spectrophotometric Determination (American Public Health Association, 1980) (Appendix F).	mg/m^3

* Indicates parameters to be measured as part of monthly diurnal studies.

TABLE 3
MONTHLY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNITS
Alkalinity*	As part of monthly diurnal study, collect one sample (by pooling three 90 cm column samples) from each pond. Keep samples cool in refrigeration unit or ice chest, and analyze within 24 hours. (The special water chemistry analyses carried out at the beginning and end of experiments can be used to determine CA^{++} contribution to total hardness, see Table 4).	Hach Digital Titrator Test Kit/Alkalinity (optional) (Appendix F).	Low or High Standard Alkalinity Method (as appropriate) (American Public Health Association, 1980), or Hach Test Kit (Appendix F).	mg $CaCO_3$/l
Total Hardness*	As part of monthly diurnal study, collect one sample (by pooling three 90 cm column samples) from each pond. Samples should be refrigerated and analyzed within 7 days.	Hach Digital Titrator Test Kit/Total Hardness (optional) (Appendix F).	EDTA Titrimetric Method (American Public Health Association, 1980); or using Hach Test Kit (Appendix F).	mg $CaCO_3$/l
Ammonia*	As part of monthly diurnal study, collect one sample (by pooling three 90 cm column samples) from each pond. Samples should be refrigerated and analyzed within 24 hours.	Kontes or comparable Kjeldahl Nitrogen apparatus	Nesslerization Method (Michigan State University Limnological Research Laboratory, 1984). (See Total Kjeldahl Nitrogen/ Ammonia, Appendix F).	mg/l

* Indicates parameters to be measured as part of monthly diurnal studies.

TABLE 3 (Continued)
MONTHLY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNITS
Nitrate*	As part of monthly diurnal study, collect one sample (by pooling three 90 cm column samples) from each pond. Samples should be refrigerated and analyzed within 24 hours.	---	Cadmium Reduction Method (Michigan State University Limnological Research Laboratory, 1984) (Appendix F).	mg/l
Total Phosphorus*	As part of monthly diurnal study, collect one sample (by pooling three 90 cm column samples) from each pond. Samples should be refrigerated and analyzed within 24 hours. Optional: Three additional sampling periods during weeks 1, 10, and 19 - samples taken just prior to fertilizer application, 12 hours after application and at 1, 2, 3, 6 and 14 days after application.	---	Persulfate digestion and Ascorbic Acid/Colorimetric Method (American Public Health Association, 1980) (Appendix F).	mg/l
Dissolved Orthophosphate* (Filterable Reactive Phosphorus)	Same as for total phosphorus (shown above).	---	Preliminary filtration and Ascorbic Acid/ Colorimetric Method (American Public Health Association, 1980) (Appendix F).	mg/l

* Indicates parameters to be measured as part of monthly diurnal studies.

TABLE 3 (Continued)
MONTHLY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNITS
Fish/Shrimp Group Weight	At 30 day intervals throughout each experimental cycle, collect grab sample equivalent to 10% of initial stock from each pond and weigh as a group. Indicate number of arrivals in grab sample.†	---	---	kg/# individuals
Fish/Shrimp Mean Weight per Individual	For a representative 10% subsample of the grab sample referenced above, weigh and count individuals. Express as mean weight per individual.	---	---	g
Fish/Shrimp Mean Length per Individual	For the representative 10% subsample referenced above, determine "total length" of each individual and express as mean length per individual.	---	---	cm
Tilapia Reproduction	Concurrent with measurement of fish growth, note the number and collective weight of any fry collected during monthly sampling.	---	---	g/# individual.

† Note: If substantial variation is observed or if reproduction is suspected, divide sample into centimeter groups; count and weigh each group. Any female tilapia observed should be removed and replaced with a male of similar weight. Any animals collected other than those stocked should be counted, weighed, measured and discarded. Record observations on reproduction and fish health.

TABLE 3 (Continued)
MONTHLY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNITS
Fish/Shrimp Health	During monthly sampling, record observations regarding fish/shrimp health. If disease/disorder is noted, estimate incidence.	---	---	text
Primary Productivity§	Monthly, take water samples and incubate for four hours in paired light-dark bottles suspended at mid-depth in ponds. Use solar monitor data to extrapolate results to entire photoperiod.	LI-COR Solar Monitor Model LI-1776 and Quantum Sensor Model LI-190SB (Appendix F).	Oxygen Method, adapted from the American Public Health Association (1980) (Appendix F).	mg carbon fixed/m^3/ day.
Phytoplankton Composition§	Monthly and when changes in the community are observed, collect samples using a plankton net with an attached collection bottle. Use a compound microscope and appropriate references to identify major groups (green, blue-green, or diatom) and relative abundance of each group (abundant, common, rare).	---	---	group/ relative abundance

§ Indicates analyses that are recommended, but not required.

TABLE 3 (Continued)
MONTHLY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNITS
Zooplankton Composition[§]	Monthly and when changes in the community are observed, collect at least three 90 cm column samples per pond or use trap or zooplankton net, as appropriate. Use a microscope to identify at the order level and note relative abundance (abundant, common, rare).	---	---	order/ relative abundance
Benthos Composition[§]	Monthly and when changes in the community are observed, collect at least three cores of mud per pond. Process samples through a No. 30 sieve, sort organisms and fix in 10% formalin or a 70% ethanol solution. Identify at the order level and note relative abundance (abundant, common, rare).	---	---	order/ relative abundance

[§] Indicates analyses that are recommended, but not required.

TABLE 4
OCCASIONAL MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	REPORTING UNITS
Pond Soil Characteristics: pH, Phosphorus, Extractable Bases (Ca, Mg, K, Na), Organic Matter, Total Nitrogen, Nitrate Nitrogen, Ammonium Nitrogen, Cation Exchange Capacity, Soluble Salts, Metals (Al, Fe, Zn, Mn, Cu), Sulfate Sulfur, Lime Requirement, Free $CaCO_3$ or $CaCO_3$ Equivalent, Exchangeable H, Exchangeable Na.	At the end of an experiment and before beginning another, collect twelve 15 cm core samples from each pond, combine and dry as described in Appendix D. Take an appropriate subsample for each pond and analyze using either a qualified local laboratory or the Oregon State University Soil Testing Laboratory.	As appropriate
Morphometric Characteristics: Maximum Length, Maximum Width, Area, Depth, Volume.	At project initiation and subsequently whenever pond facilities are altered, map ponds as described in Appendix F. Note inflow and outflow locations, pertinent surrounding elevations and buildings and structures on the site. Measure or calculate the listed morphometric parameters.	m, m^2, m^3 (as appropriate)
Hydrologic Characteristics: Surface Inflow Precipitation Outflow Evaporation Seepage (calculated)	In the course of each pond experiment, a water budget will be determined for each pond. Surface Inflow/Outflow and Evaporation should be determined using procedures described in Appendix F or comparable approaches. The contribution of precipitation should be calculated using rainfall data, while seepage must be estimated based on measurement of the other parameters.	m^3/day

TABLE 4 (Continued)
OCCASIONAL MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	REPORTING UNITS
Water Quality Characteristics: Alkalinity, Total Hardness, pH, Ammonia, Nitrates, Orthophosphate, Total Phosphorus, Chlorides,* Sulfates,* Boron, Calcium,* Copper, Iron, Magnesium,* Potassium,* Sodium* and Zinc.	At the end of an experiment and before starting another, collect a pooled sample of three 90 cm columns of water from each pond and water supply source. Samples should be analyzed on-site, by local laboratories or by the Michigan State University Limnological Laboratory (Appendix E).	As appropriate
Fish/Shrimp Production:		
Initial Stocking		
- group weight	Initial stock will be weighed as a group and counted. Tilapia will be sexed individually (Appendix F). A 10% sample will be weighed and measured (use total length for tilapia measurements). Refer to sections on stocking in Chapter 2.	kg/# individual
- mean weight per individual		g
- mean length per individual		cm
Termination of Experiments	All fish/shrimp will be removed from each pond 150 days (90 - 120 days for shrimp) after stocking. A random sample equivalent to 10% of the initial stocking will be weighed and measured. The total number of fish/shrimp from each pond will be determined and the total biomass per pond will be calculated. Any fish other than tilapia will be counted by species, weighed and measured.	
- mean weight per individual		g
- total number harvested		# individuals
- group weight (calculated)		kg
- survival (% of initial number stocked)		%

* Listed by the Technical Advisory Committee as being of greatest importance.

APPENDIX A

POND MANAGEMENT PROCEDURES

APPENDIX A

Pond Management Procedures

1. Pond Preparation

   A. Water inlets should be screened with saran filter cloth (Memphis Net & Twine) or nylon hose covering (Domestic Fabrics, Inc.) to prevent the introduction of fish and eggs into the pond. Where the water inlet is a pipe the saran screen can be sewn into a bag form and attached to the water inlet using a screw clamp or twine.

   B. Water outlets should be screened with coarse screen to prevent the escape of fish.

   C. If possible, the pond should be thoroughly dried so as to insure no residual fish/fish eggs are present upon filling.

      1) If the ponds can't be thoroughly dried, rotenone (1-2 mg/l) should be applied to the filled pond or applied to puddles. Rotenone will generally degrade within 1 to 2 weeks at warm water temperatures.

   D. Prior to filling the pond, bottom weeds should be removed manually. Herbicides should not be used.

      1) If weeds occur in a pond while the experimental cycle is in progress, an attempt should be made to manually remove all that is possible. Obtain a wet weight on weeds removed.

2. Pond liming and fertilization

   A. Agricultural limestone, $CaCO_3$ or $CaMg(CO_3)_2$, is the liming material of choice for fish ponds. Lime can be applied to either dry or full ponds. Even distribution of the lime is important.

      1) Lime should be applied at least two weeks prior to fertilization.

      2) The lime requirement of each pond should be determined as part of routine soil analysis (see Appendix D).

      3) Lime should be analyzed to determine its neutralizing value prior to use so that proper amounts of lime can be later added to the ponds.

   B. When possible, inorganic fertilizer for each complete experimental cycle will be purchased in one lot.

      1) A random sample of inorganic fertilizer will be collected from each lot for nutrient analysis. Samples will be analyzed for N, P and K.

      2) Fertilizer should be stored in a locked storage area, protected from rain and sunlight, and not in contact with the ground.

3) Each inorganic fertilizer dose will be weighed out and placed in a porous bag or container in the middle of the pond. This device will be anchored in place such that it is within the top 25 cm of the water column. A burlap bag is not recommended as it rots quickly; a woven plastic fertilizer bag is satisfactory. Prior to introducing the new fertilizer into the bag, mix/crumble any residual in the bag aid in nutrient dispersion. Fertilizer should not be broadcast over the pond surface.

4) Provide a detailed explanation in reports if alternate method of fertilizer application is used.

C. Organic fertilizer for each complete experimental cycle must be of one type, preferably dried chicken manure. If possible, manures should be obtained from a single facility.

1) A random sample of organic fertilizer will be collected from each lot for nutrient analysis. Samples will be analyzed for N, P, K and C.

2) Fertilizer should be stored as described above for inorganic fertilizer.

3) Each organic fertilizer dose will be weighed out and broadcast over the pond using a slurry method if required to facilitate uniform distribution.

4) Report any excursions from the work plan as required above for inorganic fertilizer.

3. Water and water quality management are outlined in detail in the work plan.

4. Fish health

A. While sampling fish for growth observe fish for any clinical signs indicating a disease problem.

B. In daily observation of pond/fish, note any fish which might indicate that a disease problem exists.

C. If more than 10% of fish die, eliminate the pond as a replicate. Do not treat the fish for controlling the disease.

D. If possible, accomplish appropriate disease diagnostic procedures; include in reports.

5. General

A. Control pond dike erosion by establishing and maintaining a good grass cover.

B. Keep grass on pond dikes trimmed.

C. Every attempt should be made to minimize fish predation.

APPENDIX B

PRODUCTION OF *TILAPIA NILOTICA* FINGERLINGS

APPENDIX B

Production of *Tilapia nilotica* Fingerlings

Assumed objective is to produce all-male (hand-sexed) fingerlings at least 25 g in size, of approximately the same age (± one month).

A. Brooder Spawning Ponds (SP)

1. Stock with adults (50 + g) at a density of 7500-10,000/Ha (= 0.75 - 1.0/m^2), at a sex ratio of 1 male to 3 females.

2. Feed broodfish daily with a good quality supplemental ration, beginning with a rate of about 20 kg/ha and increasing to 25 kg/ha by the sixth week.

3. Begin partial harvesting of seed at week 3 by passing a 1/4" mesh seine across the pond.

4. Transfer fry and fingerlings to the first of a nest of grader boxes described as follows:

   a) All seed to a wire cage, 3/4 to 1" mesh: retains sexable size fingerlings, allows smaller seed to pass through to next grader.

   b) Nylon mesh hapa, 1/2" mesh: retains fish equal in size to those reared for 2-3 weeks in the fingerling nursery pond, allows smaller seed to pass through to next grader.

   c) Nylon mesh hapa, 1/4" mesh: retains fish for stocking into initial phase of fingerling nursery pond.

5. Completely harvest all seed and drain pond at the end of 6 weeks.

6. Estimated seed production = 80,000 +/ha/week.

B. Fingerling Nursery Ponds (FP-1, FP-2 and FP-3)

1. All seed removed from the SP pond in week 3 and in subsequent weeks all seed from the SP, FP-1 and FP-2 ponds are graded as indicated below. Small fingerlings graded into the 1/4" mesh hapa (< 1 g in size) are stocked into pond FP-1 at a density of 150,000-175,000/ha. Large fingerlings graded into the 1/2" mesh hapa (1-25 g in size, not yet sexable) are stocked into pond FP-2 at a density of 50,000-75,000/ha. Sexable male fingerlings (> 25 g in size) are stocked into pond FP-3 at a density of 30,000-50,000/ha. All females found should be eliminated from the nursery ponds.

   Male tilapias of at least 50 g can be distinguished either by the size or shape of the genital papilla (Figure B-1). The male genital papilla contains two orifices. The female genital papilla is usually smaller and contains two orificies. Sex identification can be facilitated by lightly staining urogenital papilla with crystal violet on a cotton swab (Anderson and Smitherman, 1978).

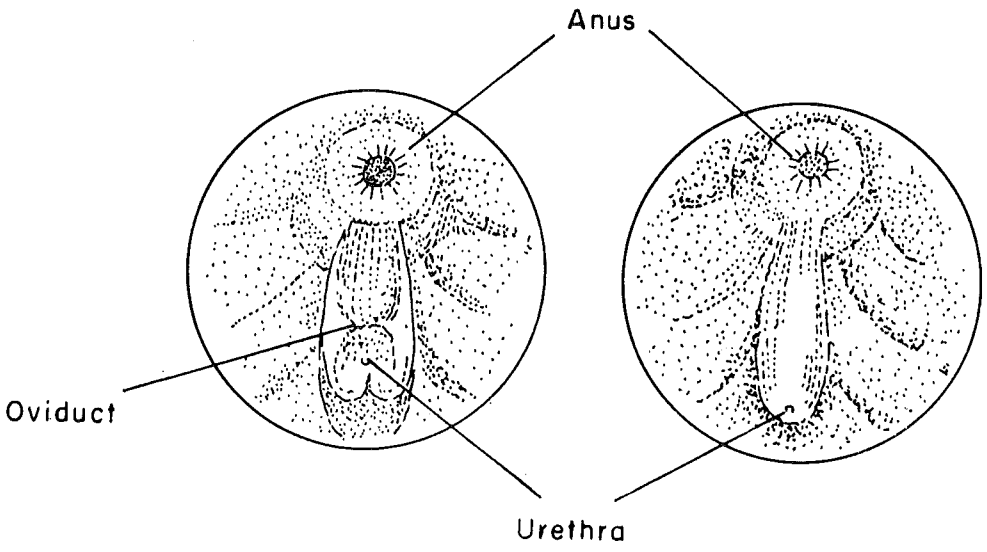


FIGURE B-1

GENITAL ORIFICES OF THE FEMALE (LEFT) AND MALE (RIGHT) *TILAPIA NILOTICA*.

Source: Lovshin, L. L. and A. B. DaSilva, 1975. Culture of monosex and hybrid tilapias. Paper presented at the FAO/CIFA Symposium on Aquaculture in Africa, 30 September - 6 October 1975 in Accra, Ghana. CIFA/75/SR 9, Food and Agricultural Organization of the United Nations, Rome, Italy. 14 pp.

2. Due to differential sizes of seed graded over weeks 3-7 an attempt will be made to hasten growth of seed in the FP-1 and FP-2 ponds by providing a supplemental feed, and to halt growth of sexed males in FP-3. Feed the same feed as fed to the brooders in the SP pond, in a ground form, at the daily rate of 5% body weight for fingerlings in both FP-1 and FP-2. All-male fingerlings in FP-3 should not continue to growth further, hence the manure and inorganic fertilizer applications are expected to provide an adequate nutrition for maintenance.

3. Expected mortality in the nursery phase = 50%.

C. Pond Preparation

1. Prior to stocking brooders or seed into their respectively assigned ponds the following treatments should be carried out in each pond:

   a) Air dry pond for at least two weeks prior to filling with water.

   b) Fill pond with water filtered through a saran sock.

   c) Fertilize each pond two weeks before stocking fish with the following:

      1) Inorganic fertilizer, at the rate of 120 kg/ha of 0-20-0, suspended in a porous bag in the upper half of the water column,

      2) Animal manure (fresh, if possible - and of a good quality), at the rate of 1000 kg/ha spread distributed evenly from each pond bank.

Requirements for pond space and brood fish to produce 16,000 males (25 + g in size) in a 4-week period.

Assume 50% mortality of seed in nursery phase (30% of original number in FP-1, 15% of original in FP-2, 5% of original in FP-3).

Total No. seed needed from SP pond =

16,000 male = 32,000 mixed sex = 50% overall

mortality = $\frac{32,000}{.50}$ = 64,000 total seed,

64,000/4 weekly harvests = 16,000 seed/harvest

SP Pond Area

With productivity of 80,000 seed/ha spawning area/week

16,000 seed/week requires $\frac{16,000}{80,000}$ Ha $\frac{2000\ m^2}{}$

Total No. Brooders Needed

Stocked at 1 brooder/m^2, sex ratio of 1 male: 3 females,

2000 total required (500 male + 1500 female)

FP-1 Pond Area

16,000 seed (as a maximum)/wk, stocked at 175,000/ha

(assuming retention time for seed in FP-1 of 1 week)

requires $\frac{16{,}000}{175{,}000}$ Ha $\underline{1000\ m^2}$

No. surviving seed - 16,000 - (.30) (16,000) = 11,200

FP-2 Pond Area

11,200 seed (as a maximum)/week, stocked at 75,000/ha

(assuming retention time of 2 weeks in FP-2)

requires (2) $\frac{(11{,}200\ ha)}{75{,}000} \simeq \underline{3000\ m^2}$

No. surviving seed = 11,200 - (.15) (16,000) = 8,800

FP-3 Pond Area

8,800 seed (as a maximum)/week, stocked at 50,000/ha

less 50% discarded as females = 4,400/week,

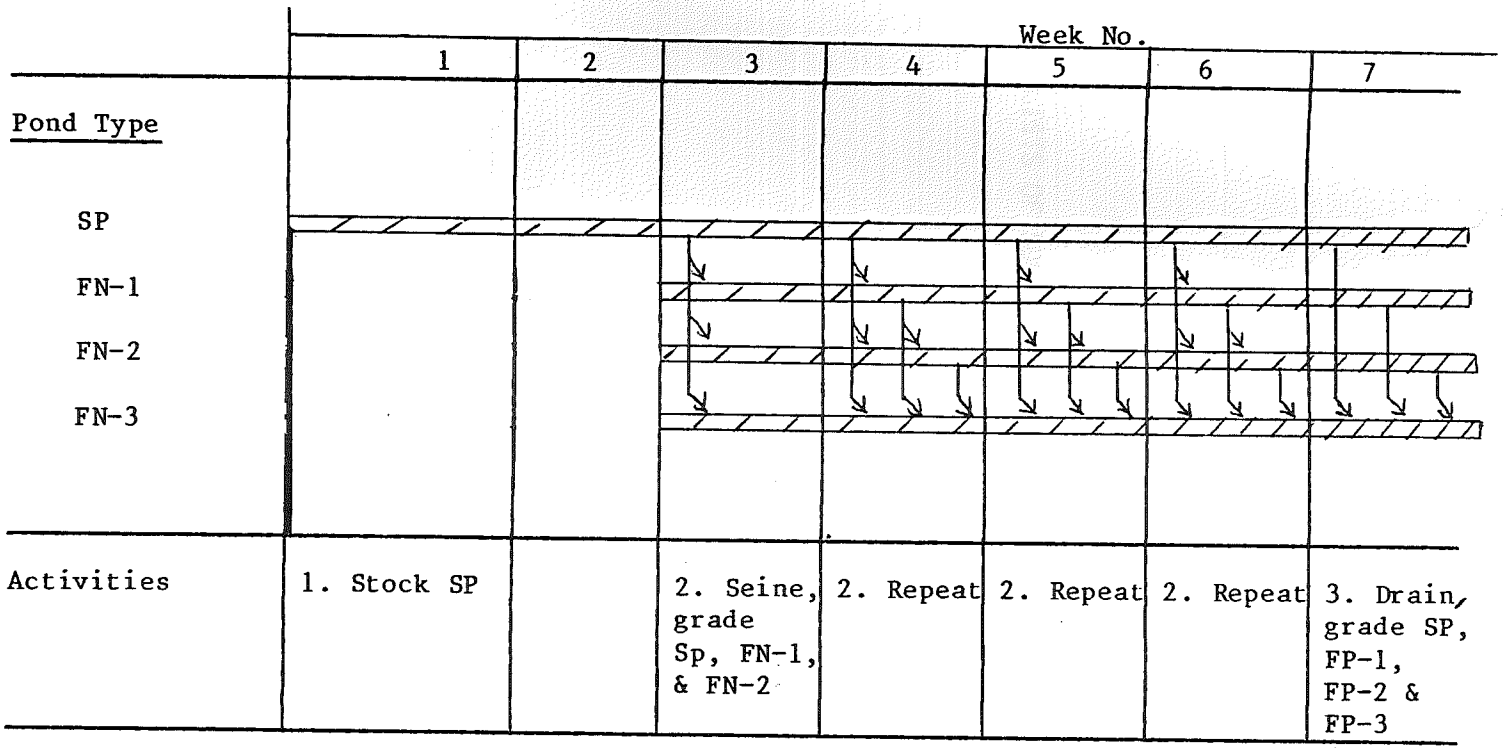
(assuming retention time of 4 weeks in FP-3)

requires (4) $\frac{(\ 4{,}400\ Ha)}{50{,}000} \simeq \underline{3500\ m^2}$

Total Pond Area Required = (2000 + 1000 + 3000 + 3500)m = $\underline{9{,}500\ m^2}$

TABLE B-1

FLOW CHART FOR PRODUCING ALL-MALE *TILAPIA NILOTICA* AT THE SAME AGE



Legend
SP = Brooder Spawning Pond
FN-1 = Fingerling Nursery Pond, < 1 g seed
FN-2 = Fingerling Nursery Pond, 1-25 g seed
FN-3 = Fingerling Nursery Pond, > 25 g seed

APPENDIX C

Lists of Suggested Equipment and Chemicals

The following list was supplied by Auburn University as a tentative list for their Honduras CRSP.

I. Fisheries Equipment and Chemicals

1. 125' x 6' x 1/2" seine with 6' x 6' x 6' bag, #9 knotted nylon
2. 75' x 6' x 1/4" Raschel nylon fingerling seine
3. 18' x 4' x 1/8" ace nylon fingerling seine
4. Dip nets a. #800 b. #802, 1/4" mesh
5. Spring scale, 20 kg x 50 gm
6. 5 gal plastic buckets
7. Saran filter screen
8. Stainless steel rulers, metric
9. Formaldehyde (formalin) 37%
10. 5% Rotenone powder
11. Quinaldine anesthetic, 90% Al, liquid

II. Laboratory and Field Equipment

The following equipment has been recommended in the preceeding discussion of sampling protocol and includes other essential materials to carry out the analyses.

	Equipment	Purpose
1.	Solar Monitor LI-COR Model LI-1776 Quantum Sensor LI-COR Model LI-190SB	measure solar radiation (PAR)
2.	Rain Gauges	estimate rainfall
3.	WEATHERTRONICS Totalizing Anemometer, WEATHERTRONICS Model 2510	wind speed
4.	Maximum-Minimum thermometers, Taylor Co.	air and water temperatures

	Equipment	Purpose
5.	YSI Dissolved Oxygen Meter	dissolved oxygen
6.	Corning or Orion pH meter and electrode	pH
7.	Hach Alkalinity Test Kit AL-DT	alkalinity
8.	Hach Total Calcium Hardness Test Kit HAC-DT	hardness
9.	Balance, Metler, Model AC100	analytical balance
10.	Spectrophotometer - Spectronic 21, by Bausch and Lomb, or Sequoia-Turner Model 340 and cuvettes	phosphorus, nitrogen and chlorophyll *a* analysis
11.	Kontes distillation apparatus (K-551100-0000) with Digestion/distillation Flasks 100 ml (K-551600)	preliminary distillation for ammonia procedure
12.	Tissue grinder, 10 ml chamber, pestile	chlorophyll *a*
13.	Membrane filter funnell, Gelman or Millipore	TDP, TDRP
14.	Dessicator, Pyrex, Fisher	storage of filters
15.	Hot plate, thermix, 300 M, Fisher or Thermolyne	digestion of TP
16.	BOD bottles, 300 ml (3 cases, 24/case)	light-dark bottle procedure
17.	Magnetic Stirrer, Thermix, 120 m model Fisher	alkalinity, phosphorus
18.	Precision Bore burets, 50 ml x 0.1 ml, teflon stop cocks, Fisher	alkalinity
19.	Double buret support, Fisher	alkalinity
20.	Still, Automatic, Barnstead or Wheaton	provides good quality distilled water for standards and washing
21.	Deionizing filtration system, Gelman Water-I with replacement capsules	provides deionized water for standards and phosphorus glassware
22.	Centrifuge, Table top, Model HN-S 11 (IEC 2355) with horizontal rotor ring and flanged carrier	chlorophyll *a* procedure

Equipment	Purpose
23. Microscope, compound with up to 100x oil immersion lens and illumination	qualitative analysis of biota
24. Microscope, dissecting, 1-6x power with illumination	qualitative analysis of biota

III. General Lab Equipment and Glassware

ITEM	QUANTITY	APPROX. PRICE
1. Beakers, Griffin, graduated, heavy duty, pyrex		
a. 150 ml	1 pkg. (12)	24.00
b. 250 ml	1 pkg. (12)	24.00
2. Beakers, Griffin, graduated, pyrex		
a. 50 ml	1 pkg. (12)	13.00
3. Beaker, Griffin, plastic, S/P		
a. 150 ml	1 pkg.	11.00
4. Bottles, narrow mouth, screw neck amber, 8 oz.	1 pkg. (12)	13.00
a. caps, size 24-400	1 pkg. (12)	2.00
5. Bottles, aspirator with spigot, conventional, polyethylene, naglene, 5.5 gal.	3	109.00
6. Wash bottle, polypropylene, naglene, nalge, 125 ml	1 pkg. (6)	7.00
7. Bottle, dropping, flint glass, 2 oz.	1 pkg. (12)	9.00
8. Detergent, liqui-nox, alconox	1 Cse (4 gal.)	50.00
9. Cylinder, graduated, single scale, red stripe, Kimax		
a. 25 ml	1 pkg. (2)	18.00
b. 100 ml	2 pkg. (4)	46.00
c. 250 ml	2 pkg. (4)	62.00
d. 500 ml	1 pkg. (1)	18.00
e. 1000 ml	1 pkg. (1)	26.00

ITEM	QUANTITY	APPROX. PRICE
10. Dish, evaporating, porcelain, coors		
a. 70 ml	25	55.00
11. Glass fiber filter, type A/e, Gelman		
a. 47 mm	10 pkg. (100)	152.00
12. Whatman filler paper, #42		
a. 12.5 cm diameter	4 pkg.	24.00
13. Flask, erlenmeyer, narrow mouth, graduated Kimax		
a. 50 ml	1 pkg. (12)	16.00
b. 125 ml	1 pkg. (12)	16.00
c. 250 ml	1 pkg. (12)	16.00
d. 500 ml	1 pkg. (6)	10.00
e. 1000 ml	1 pkg. (6)	17.00
14. Flask, filtering, heavy wall, graduated, pyrex brand		
a. 250 ml	1 pkg. (6)	32.00
b. 500 ml	1 pkg. (6)	41.00
15. Pump, filter, Chapman-type (for suction)	2	15.00
16. Flask, volumetric, T stopper, pyrex brand		
a. 250 ml	1 pkg. (6)	67.00
b. 500 ml	1 pkg. (6)	85.00
c. 1000 ml	6	104.00
17. Funnel, polypropylene, Nalgene		
a. top 1.0, mm = 75	1 pkg. (6)	6.00
18. Funnel, powder, polypropylene, Nalgene		
a. top 10, mm = 65	1 pkg. (12)	9.00
b. top 10, mm = 100	1 pkg. (6)	7.00
19. Gloves, Sol-vex, Edmont-Wilson		
a. size 7	2 pr.	7.00
b. size 8	2 pr.	7.00

ITEM		QUANTITY	APPROX. PRICE
20.	Gloves, asbestos	1 pr.	8.00
21.	Goggles, splash	3 pr.	10.00
22.	Microscope slide, Gold Seal, Clay-Adams, plain	2 gross	15.00
23.	Cover glass, rectangular, No. 1-1/2, Corning		
	a. 25 x 25 mm	1 oz.	5.00
24.	S/P lens paper, 4" x 6"	1 pkg. (12 books)	5.00
25.	Pipet, volumetric, TO, Class A, color-coded, Kimax		
	a. 0.5 ml	6	23.00
	b. 1.0 ml	6	23.00
	c. 2.0 ml	6	23.00
	d. 5.0 ml	6	23.00
	e. 10.0 ml	6	25.00
	f. 25.0 ml	1 pkg. (6)	36.00
26.	Pipet, measuring, TD, Mohr, long tip, color coded, Kimax		
	a. 0.5 ml	4	19.00
	b. 1.0 ml	6	25.00
	c. 5.0 ml	6	30.00
	d. 10.0 ml	6	35.00
27.	Pipet filler for caustic solns	2	34.00
28.	First aid kit, industrial-type	1	77.00
29.	Eye wash station, double (Bel-art)	1	22.00
30.	Spatula, 4"	2	6.00
31.	Spatula, micro	2	3.00
32.	S/P stirring bar, 5/16" x 1-1/2"	5	25.00
33.	Stirring bar retriever, Magnetic rigid, tekpro 3/8 x 12	1	5.00
34.	Stopper, flask, Polyetheylene (Hach)		
	a. size 16	1 pkg. (6)	4.00
	b. size 19	1 pkg. (6)	4.00
	c. size 22	1 pkg. (6)	5.00

ITEM		QUANTITY	APPROX. PRICE
35.	Stirring rod, Kimax brand 5 mm x 150 mm	1 pkg.	13.00
36.	Test tube, Kimax 13 x 100 mm	2 pkgs. (72)	24.00
37.	Tongs, Beaker	1	4.00
38.	Tongs, crucible, 9", steel	2	4.00
39.	Brushes		
	a. large beaker	1	5.00
	b. pipet (17")	1 pkg.	17.00
	c. cylinder (13")	1	1.00
	d. volumetric flask 50 ml + 100 ml	1 ea.	6.00
	e. volumetric flask 250 + 500 ml	1	3.00
	f. flask	1	3.00
	g. test tube (8")	1 pkg. (12)	7.00

IV. General Chemicals Needed for Analyses

This list represents the major chemicals needed for the methods that are listed in this document. The list was suggested as the tentative supply for Honduras CRSP and was supplemented to meet the changes in methods determined at the CRSP workshop. All chemicals are available from American Scientific Products and many may be available at the overseas CRSP location.

1. phenolphthalein
2. ethyl alcohol
3. methyl orange
4. sodium carbonate (primary std.)
5. sulfuric acid
6. sodium hydroxide (AR pellets)
7. sodium chloride
8. EDTA
9. ammonium chloride
10. ammonium hydroxide
11. eriochrome black T, Indicator grade

12. calcium carbonate, anhydrous
13. magnesium chloride, 6-hydrate
14. hydrochloric acid
15. manganous sulfate, monohydrate
16. phenoldisulfonic acid
17. sodium nitrate
18. ammonium molybdate
19. glycerol
20. potassium phosphate, monobasic
21. acetone
22. magnesium carbonate (powder)
23. potassium iodide, crystal
24. sodium azide
25. sodium thiosulfate
26. chloroform
27. potassium dichromate crystals
28. starch, soluble
29. formalin (formaldehyde soln.)
30. phenol, crystal
31. pH 6 buffer
32. pH 9 buffer
33. antimony potassium tartrate
34. ascorbic acid
35. silica gel, dissicant
36. potassium permanganate
37. sodium iodide

38. bromcresol green-methyl red
39. 5% NaOCl - commercial bleach
40. hydrogen peroxide
41. potassium fluoride
42. ammonium persulfate
43. sodium tetraborate
44. boric acid
45. chromerge (chromic-sulfuric acid)
46. mercuric oxide
47. potassium sulfate
48. mercuric iodide
49. potassium iodide
50. cadmium coarse powder
51. sulfanilamide
52. ethylenediamine dihydrochloride, reagent ACS grade
53. concentrated phosphoric acid
54. cupric sulfate penta-hydrate
55. potassium nitrate

APPENDIX D

PROCEDURE FOR POND SOIL SAMPLING AND ANALYSIS

APPENDIX D

PROCEDURE FOR POND SOIL SAMPLING AND ANALYSIS

Pond mud will be analyzed for particle size distribution and concentration of a number of constituents. Samples should be taken from each pond prior to initiating each of the two experiments during the second cycle. Analyses may be carried out by qualified laboratories within host countries or the U.S.

Researchers should allow from three to five months for receiving results of analyses carried out in the U.S. Sufficient time should be allowed for analysis by local laboratories such that results will be received in time to apply lime if necessary. About two weeks should be allowed between application of lime and the first fertilizer application (see Appendix A).

Soil Sampling

1. Begin at the shallow end of each of the twelve empty ponds, opposite the drain.

2. Proceed in an 'S' shape toward the deep end of each pond, collecting 12 core samples of the top 15 cm of bottom material.

3. For each pond, combine the 12 subsamples and mix the composite thoroughly. Note that wet mud mixes more easily than dry mud.

4. Spread each mud sample in a thin layer on a plastic sheet to dry.

Analysis in the U.S.

Samples may be sent to a qualified laboratory in the U.S. The Oregon State University Soil Testing Laboratory is one facility that is authorized by the U.S. Department of Agriculture (USDA) to receive soil samples from outside the U.S.

1. Select 200 g of rock-free dried mud from each pond and package each in a plastic bag, identified and recorded as to origin.

2. Send all samples, preferably in one sturdy, leak-proof container, labeled with a USDA authorization sticker.

3. Specify the analyses required for each sample. The particle size distribution analysis and all 22 tests listed on Table D-1 are required. Note that the Oregon State University Soil Testing Laboratory does not offer tests for aluminum and iron. These analyses will have to be carried out by another facility. (Researchers planning to ship samples to another laboratory within the U.S. are responsible for ensuring that the facility is authorized by the USDA to receive soil samples from outside the U.S.)

TABLE D-1

FEE SCHEDULE FOR RESEARCH SOIL ANALYSIS
BY THE OREGON STATE UNIVERSITY SOIL TESTING LABORATORY[1]

Analysis	Fee (per Sample)
Determination of clay, silt, and sand fractions (by pipette method and including removal of organic matter)[2]	$ 33.00
Sample preparation (grinding, handling, storage)	1.00
pH	1.25
Phosphorus	3.75
Extractable Bases (Ca, Mg, K and Na)	6.00
Organic Matter	6.00
Total Nitrogen	9.75
Nitrate Nitrogen (NO_3-N) and Ammonium Nitrogen (NH_4-N)	8.50
Cation Exchange Capacity	8.50
Soluble Salts	2.75
Heavy Metals (Zn, Mn and Cu)	10.00
Sulfate-Sulfur (SO_4-S)	9.75
SMP Lime Requirement	1.25
Free $CaCO_3$ or $CaCO_3$ Equivalent[3]	6.75
Exchangeable H	6.75
Exchangeable Na	6.75
Aluminum	Not Offered
Iron	Not Offered
TOTAL per sample cost, excluding analyses for aluminum and iron.	$121.75

[1] Oregon State University Soil Testing Laboratory, Agriculture Hall 114, Corvallis, OR 97331.
[2] Analysis performed by the Oregon State University Soil Physics Laboratory, Agriculture Hall, Corvallis, OR 97331.
[3] If it is known that soil pH is below 7, then no free $CaCO_3$ will be detected and this analysis is unnecessary.

Analysis within the Host Country

Researchers should identify a qualified laboratory or laboratories, capable of carrying out the particle size distribution analysis and the 22 tests identified on Table D-1. The amount of each sample required and handling and labeling procedures should be determined in each case.

APPENDIX E

MICHIGAN STATE UNIVERSITY WATER ANALYSIS SERVICE

APPENDIX E

MICHIGAN STATE UNIVERSITY WATER ANALYSIS SERVICE

The Limnology Laboratory at Michigan State University (MSU) has offered to carry out analyses of chlorides, sulfates, boron, copper, iron, magnesium manganese, potassium, sodium and zinc at a charge to the individual country budgets of $55.00 per water sample. If you use this service, a payment made out to Michigan State University should be submitted by the U.S. Principal Investigator in correspondence alerting the Laboratory that a shipment is on the way. This correspondence should be sent to:

John R. Craig
Department of Fisheries and Wildlife
Michigan State University
East Lansing, MI 48824

Samples should be shipped from CRSP sites to:

V. G. Nahrgang Company
155 West Congress St.
Detroit, MI 48826
USA

Nahrgang is MSU's Customs Broker and their charge of $5.00 per sample to clear customs is included above the total charge of $55.00 per sample. Shipments should be marked (1) Crude Mineral Substances, (2) No Commercial Value (3) For Testing Purposes Only, and (4) Consigned to Limnology Laboratory, Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824, USA. The return address of the CRSP site should appear on the shipment as well. Correspondence should be sent from the CRSP site to John R. Craig at the above address alerting him of the shipment's arrival. Copies of the data will be returned to U.S. Principal Investigators and personnel on the CRSP site.

One liter of water is required for the above analyses. Each sample should be composed of a pooled sample of three 90 cm columns of water from each pond, and a sample representative of the supply water. Enough concentrated HNO_3 (analytical or reagent grade) should be added to each one liter sample shipped to take pH to less than 2.0 (H_2SO_4 and HCl are of course not suitable). Since pH probes lose Cl^- to surrounding water, the amount of HNO_3 required should be estimated from companion samples to those shipped. Generally, 2 ml of HNO_3 should be sufficient. The amount added should appear on the sample label along with your code identifying the sample. Samples must be packed for shipping in Nalgene plastic bottles that have been cleaned to prevent contamination. Avoid including particles from the sediments in your samples.

APPENDIX F

MATERIALS AND METHODS REFERENCE

APPENDIX F

MATERIALS AND METHODS REFERENCE

As previously discussed in Chapter 2, the second cycle of CRSP pond dynamics experiments involve a number of daily, biweekly and weekly, monthly and occasional measurements. The measurement of some parameters require the use of specific instrumentation, procedures or analytical methods. To the extent possible, catalog references and standard methods for selected procedures and analyses have been reproduced for use by researchers at host country locations. Researchers should read these references carefully to confirm that the described materials and methods are applicable given site specific conditions. After reviewing these materials, any planned modifications should be reported to the Program Management Office before implementation.

This appendix is presented in three sections: Instruments, Procedures, and Analytical Methods. The contents of each section are:

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INSTRUMENT	UNIT OF MEASURE
SOLAR MONITOR WITH QUANTUM SENSOR	E m^{-2} day^{-1}

SUGGESTED SUPPLIER

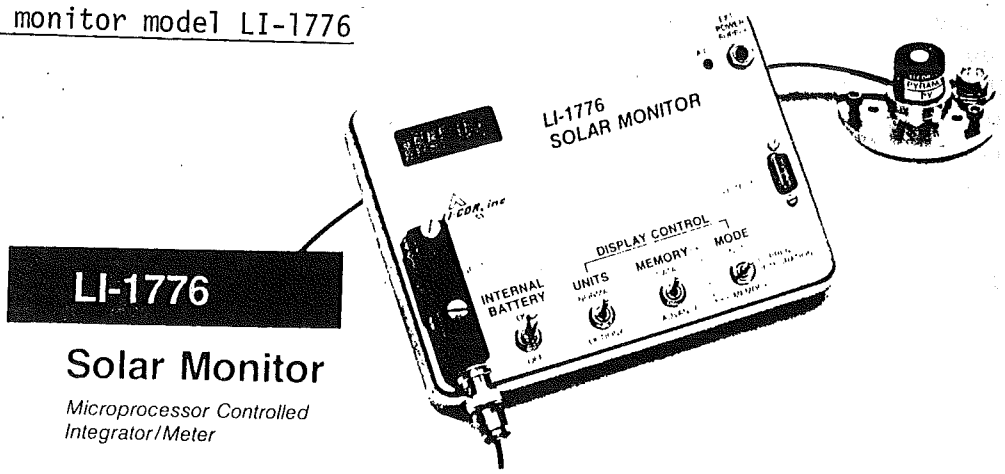
LI-COR
4421 Superior Street/P.O. Box 4425
Lincoln, Nebraska 68504 (402) 467-3576

COST (MAY 1984)

monitor: $750
sensor: $260

DESCRIPTIVE INFORMATION

Solar monitor model LI-1776



LI-1776

Solar Monitor

Microprocessor Controlled Integrator/Meter

- Integrated and instantaneous measurements
- Memory storage of integrated values for unattended monitoring
- Direct readout in engineering units
- Compatible with all LI-COR sensors (with Calconnectors)
- Module for mV signal sensors*
- Weatherproof enclosure for environmental monitoring*
- Cassette tape or RS-232C output*
- Analog output of instantaneous values

Totalized Measurements

The LI-1776 Solar Monitor averages the effects due to changing atmospheric conditions, solar elevation, etc. Meter readings, point measurements and strip chart plots are difficult to interpret in such situations. Instead of an erratic strip chart plot, the LI-1776 displays an integrated value. Dividing this total by the integration time provides the average value.

Typical Applications

When used with the LI-200SB Pyranometer, the LI-1776 provides a sophisticated system for solar energy assessment and site evaluation, performance evaluation, meteorological studies, and irrigation scheduling. For various applications in plant science, a quantum sensor can be used to measure photosynthetically active radiation (PAR). A photometric sensor is used for illuminance measurements.

optional

COMPUTER COMPATABILITY

Expanded datalogging capability is provided with any one of these variations of the LI-1776 Solar Monitor: LI-1776/02A, LI-1776/02M, or LI-1776/07.

- **Nine user-selectable integration periods** (7.5, 15, 30 minutes, 1, 2, 4, 8, 12, and 24 hours)
- **Real time operation** (set in 7.5 minute intervals)
- **Compatible with any RS-232C peripheral** (computer, terminal, modem, printer, etc.)

Data is transferred in 7 bit plus parity ASCII characters through the LI-1776 Output. For either the LI-1776/02A or LI-1776/02M, the 1600-05 Cassette Tape Reader and a tape recorder are needed to transfer data to a computer system.

LI-1776/02A

Integrated data that is stored in the memory can be transferred directly onto cassette tape. Data is transferred ***automatically*** at the end of each integration period. This is especially useful for long-term monitoring, where up to 900 integration values can be recorded on one side of a 60 minute tape without user attention

LI-1776/02M

With the LI-1776/02M, the user initiates the transfer of data onto cassette tape. All of the values in memory are transferred onto tape each time a transfer is initiated.

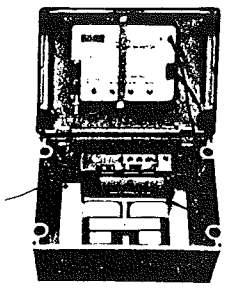
LI-1776/07

Through the use of a standard RS-232C serial interface, the LI-1776/07 can be connected ***directly*** to a terminal, computer or modem for data transfer. The LI-1776/07 can function as either the DCE (Data Communication Equipment) or the DTE (Data Terminal Equipment). The factory-set Baud rate is 300 (600 or 1200 optional).

1600-05 Cassette Tape Reader

Integrated data stored in the memory of an LI-1776/02A or LI-1776/02M can be stored on cassette tape using the 1600-04 Cassette Tape Recorder. Battery life has been increased by internal modifications to allow extended operation in the field.

The 1600-05 Cassette Tape Reader is used to interface the tape-recorded data to a computer system (see page 6). The 1600-05 is compatible with any computer system that utilizes the EIA (Electronic Institute of America) RS-232C interface. Tape recorded data can also be interfaced using either a Campbell Scientific or Omnidata International cassette tape reader.



1776-06 Weatherproof Case

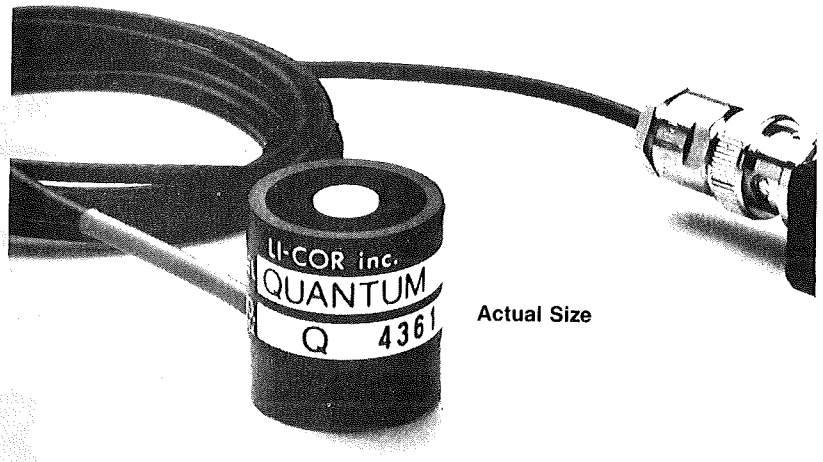
For field operation, this fiberglass case provides a weatherproof enclosure for the LI-1776 and a cassette tape recorder (optional). Included with the 1776-06 is a 12 volt battery pack and external battery leads.

Battery Life: 2 months at 25 C. Size: 29.9 x 20.8 x 18.0 cm deep (11.8" x 8.2" x 7.1"). Weight: 4.8 kg (10.6 lbs).

SOLAR MONITOR WITH QUANTUM SENSOR (continued)

Quantum sensor model LI-190SB

Measure Photosynthetically Active Radiation (PAR): 400 to 700 nm waveband



Actual Size

Plants use the 400 to 700 nm waveband of the light spectrum for photosynthesis (3,9). A simple integral relationship exists between the number of molecules changed photochemically and the number of photons absorbed within the requisite waveband regardless of photon energy (12). The preferred measurement for PAR is *Photosynthetic Photon Flux Density* (PPFD) (9,14). This is the number of photons in the 400 to 700 nm waveband incident per unit time on a unit surface.*

LI-190SB Quantum Sensor

Plant scientists, meteorologists, horticulturists, ecological survey groups and other environmental scientists are using this sensor to measure PPFD in the atmosphere, growth chamber and greenhouse.

Accurate measurements are obtained under ***natural and artificial light conditions*** because of the computer-tailored spectral response of the LI-190SB. This sensor, which has been developed from earlier work (1), was pioneered by LI-COR and has become a standard for PPFD measurement in most photosynthesis related studies.

The LI-190SB is also used in oceanography, limnology, and marine science as a reference sensor for comparison to underwater PPFD measured by the LI-192SB Underwater Quantum Sensor.

*Units currently in use are einsteins, moles, photons and quanta (6, 9, 14). 1 μE s^{-1} m^{-2} ≡ 1 μmol s^{-1} m^{-2} ≡ $6.02 \cdot 10^{17}$ photons s^{-1} m^{-2} ≡ $6.02 \cdot 10^{17}$ quanta s^{-1} m^{-2}.

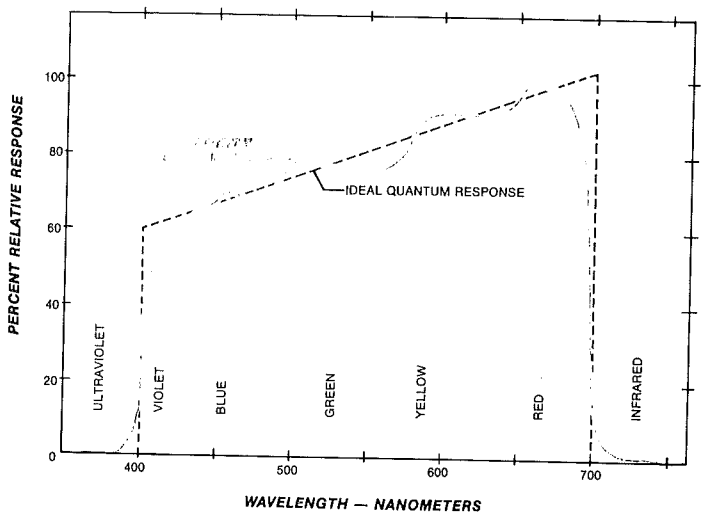


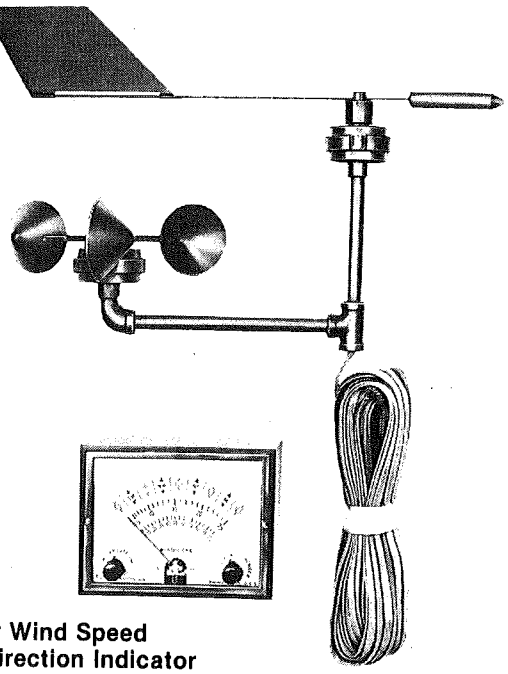
Figure 1. Typical spectral response of LI-COR Quantum Sensors vs. Wavelength and the Ideal Quantum Response (equal response to all photons in the 400-700 nm waveband). An interference filter (for sharp near infrared cutoff at 700 nm), and colored glass filters, tailor the silicon photodiode response to the desired quantum response.

INSTRUMENT	UNIT OF MEASURE
WIND SPEED AND DIRECTION METER*	km/hr and direction

SUGGESTED SUPPLIER	COST (MAY 1984)
Ben Meadows Company 3589 Broad Street P.O. Box 80549 Atlanta(Chamblee), GA 30366 (800) 241-6401	$345

DESCRIPTIVE INFORMATION

Instantaneous TAYLOR Wind Speed and Direction Indicator



Taylor Wind Speed and Direction Indicator

Includes cup and vane assembly. Self-contained—no outside power source needed. Outdoor parts are corrosion-resistant. Mahogany-finished indoor indicating unit is 6½" wide x 5" high. Furnished with 60 ft. of lead-in wire and instructions.

No. 110930 Shipping Weight 9 lb. **$345.00**

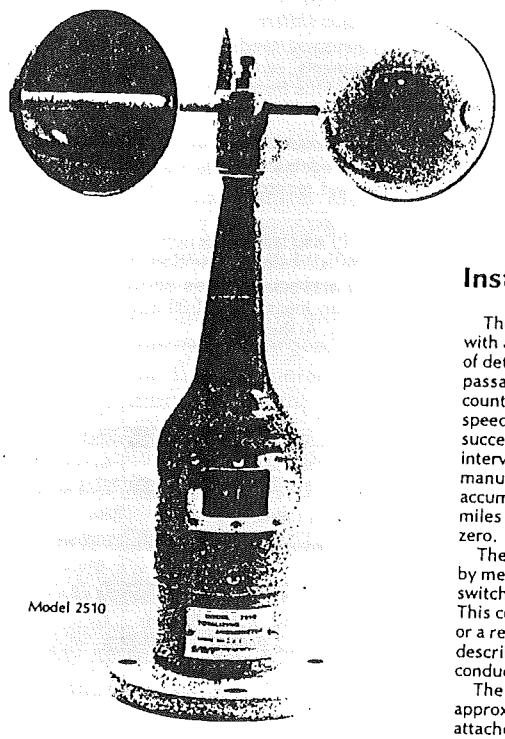
*Note: The totalizing anemometer described on the following page is the preferred instrument for CRSP research.

INSTRUMENT	UNIT OF MEASURE
TOTALIZING ANEMOMETER	km/elapsed time calculate km/hr

SUGGESTED SUPPLIER	COST (MAY 1984)
WEATHERtronics A Division of Qualimetrics, Inc. P.O. Box 41039 Sacramento, CA 95841 (916) 481-7750	$370

DESCRIPTIVE INFORMATION

Model 2511 Totalizing Anemometer



Model 2510

Features:

- 6-digit mechanical counter
- Electrical contact output
- Metric or English models
- Predrilled mounting base

Instrument Description:

The Model 2510 totalizing anemometer is equipped with a counter to provide a simple, yet precise, method of determining average wind speed and total air passage. An internal gear train converts cup rotation to counter input (917 revolutions per mile). Average wind speed can be calculated from the difference between successive counter readings divided by the time interval between readings. The 6-digit counter is not manually resettable. The anemometer can typically accumulate wind run for a year or longer (up to 99,999.9 miles or kilometers) before automatically resetting to zero.

The 2510 also provides an electrical contact output, by means of a magnet-activated reed switch. The switch furnishes one closure per 0.1 mile or kilometer. This contact can be used to advance an event recorder or a remote digital counter. (These instruments are described in detail on pages 160-162 and 166.) Two-conductor cable makes the necessary connections.

The 4-inch-diameter brass cups have a threshold of approximately 1 mph. The cups are beaded and are attached to the hub by sturdy arms. Turning radius is 6 inches. Self-lubricating stainless steel bearings support the anemometer shaft.

The flanged base of the instrument is predrilled, permitting mounting on a wooden, metal, or concrete support. In an evaporation station the anemometer is typically mounted on the platform supporting the evaporation pan. A mast adapter is available for mounting to a 1¼-inch O.D. pipe.

Specifications:

Type	3-cup anemometer
Cup size	4" diameter
Cup material	Brass
Cup design	Conical and beaded
Counter	Six digit-mechanical
Electrical contact	Reed switch
Contact current rating	0.4 A at 24 VDC, resistive load
Resolution (counter & contact)	0.1 mile or 0.1 kilometer
Cup constant	917 rev./mi. (570 rev./km)
Threshold	1-2 mph
Body	Cast aluminum
Flange	Four 3/8" dia. predrilled holes
Max. speed	100 mph (160 km/hr)
Size	12" dia. x 16" H (305 x 400 mm)
Weight/shipping	5 lbs./9 lbs. (2.3 kg./4 kg.)

Ordering Information:

Model	
2510	**Totalizing Anemometer** with 6 digit counter registering each 0.1 mile of wind; switch closure for remote recording included
2511	**Totalizing Anemometer** with 6 digit counter registering each 0.1 kilometer of wind; switch closure for remote recording included
25101	**Mounting Adapter** for mounting on 1¼" O.D. pipe
600502	**Two-conductor, 20 AWG, Shielded Cable**

INSTRUMENT	UNIT OF MEASURE
pH METER WITH COMBINATION ELECTRODE	pH units

SUGGESTED SUPPLIER

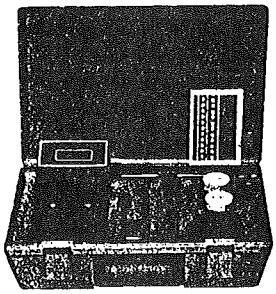
Orion Research
840 Memorial Drive
Cambridge, MA 02139 (800) 225-1480

COST (MAY 1984)

Model 201: $285
Model 211: $295
Model 221: $395
Ross Electr: $175

DESCRIPTIVE INFORMATION

200 Series pH meters



All models in the ORION 200 Series come in a foam-lined carrying case supplied with everything you need to make measurements in the field or lab: rinse water and buffer containers; an electrode support attachment; an unbreakable electrode that never needs refilling; and, a line adaptor for meter use without battery drainage.

Model 201 includes everything you need to make routine pH measurements, especially outside the laboratory. This meter reads over the entire pH range in 0.05 increments and displays results in large, high intensity LEDs.

Model 211 has features usually associated with more expensive meters - readability to 0.01 pH, slope adjustment and recorder output. And a large LCD display makes the instrument ideal for reading in sunlight.

Model 221 measures pH to 0.01 *and* temperature inside or outside the laboratory. The meter is supplied with a precision temperature probe so you can measure sample temperature accurately. At the lab bench Model 221 becomes an economical method for measuring biochemical oxygen demand when used with the ORION 97-08 dissolved oxygen electrode.

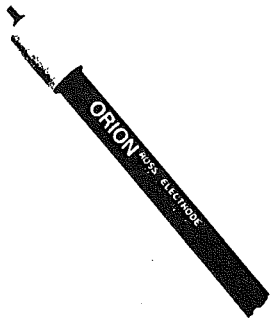
	Model 201	Model 211	Model 221
pH range/resolution	0 - 14/ ± 0.025	0 - 14/ ± 0.005	0 - 14/ ± 0.005
temperature compensation	manual, 0 to 100°C	manual, 0 to 100°C	manual, 0 to 100°C
display	7.5 mm high LED	1 cm high LCD	1 cm high LCD
battery complement	six size AA 1.5 volt batteries	six size AA 1.6 volt batteries	one size D 9 volt battery
battery life	3000 ten second measurements	3000 ten second measurements	3000 ten second measurements
temperature range	---	---	-5°C to 100°C, ± 0.1°C or 1%
size and weight	14 x 9 x 4.5 cm, 0.4 kg	14 x 9 x 4.5 cm, 0.4 kg	14 x 9 x 4.5 cm, 0.4 kg
line adaptor	115 or 230 V AC, ± 20%, 50/60 Hz	115 or 230 V AC, ± 20%, 50/60 Hz	115 or 230 V AC, ± 20%, 50/60 Hz
recorder output	---	± 100 mV, fixed	---

Specifications subject to change without notice

Ross Model 81-55 combination electrode

Superior to conventional ectrode that is supplied with the Orion 200 series meters.

Model	81-55/81-56	Temperature Range	0-100°C
Description	Ross combination with epoxy body, bulb guard.	Internal Reference	Ross
		Junction	Glass Fiber
Recommended Use	Use for ruggedness and durability in field pH measurements.	Dimensions	Length: 120 mm Diameter: 12 mm Cap Diameter: 16 mm
pH Range	0-14	Connector (100 cm cable)	U.S. Standard/BNC
Drift	Less than 0.002 pH per day	ORION Cat. No.	815500/815600



INSTRUMENT	UNIT OF MEASURE
DISSOLVED OXYGEN METER WITH TEMPERATURE INDICATOR	mg/l , °C

SUGGESTED SUPPLIER

Scientific Division
Yellow Springs Instrument Co., Inc.
Yellow Springs, Ohio 45387 (513) 767-7241

COST (MAY 1984)
meter: $795
probe: $157
cable: $ 94

DESCRIPTIVE INFORMATION

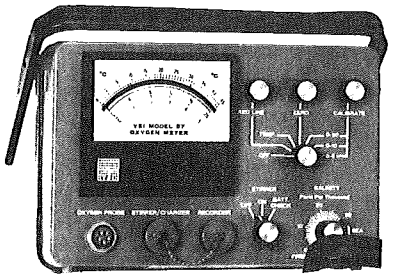
YSI Model 57 Dissolved Oxygen Meter

The YSI Model 57, an exceptionally rugged D.O. meter, is designed for extra hard duty in the field. It features shock resistant, water resistant construction, metal meter bezel and an ABS molded case. Accidentally knock the instrument overboard and it will float until you retrieve it.

This is also an extremely sensitive instrument, measuring dissolved oxygen in three different reading ranges of 0-5, 0-10 and 0-20, mg/l, with accuracy to ±0.1 mg/l. A -5° to +45°C scale indicates temperature. Membrane coefficient and changes in water temperature are compensated for automatically. Calibration is by the fast air calibration technique, which can be completed in seconds. Two flashlight batteries operate the Model 57 for up to 1,000 hours, and an internal rechargeable battery pack is available to operate the submersible stirrer. There's also a recorder output for making permanent records.

Corrections for ocean salinity can be made by direct-dialing the salinity concentration, 0 to 40 PPT, to cover the full range from fresh water to sea water. For frequent salt water measurements the YSI 33 S-C-T Meter (salinity, conductivity, temperature) is an ideal companion instrument for determining salinity.

These and other features provide maximum convenience and reliability for on-the-spot measurements just about anywhere under the toughest working conditions.



OXYGEN MEASUREMENT
RANGES: 0-5, 0-10 and 0-20 mg/l (0-2.5, 0-5 and 0-10 mg/l with YSI 5776 High Sensitivity Membrane.)

ACCURACY: ±1% full scale at calibration temperature, or ±0.1 mg/l, whichever is greater.

READABILITY: 0.025 mg/l on 0-5 scale; 0.05 mg/l on 0-10 scale; 0.1 mg/l on 0-20 scale.

TEMPERATURE MEASUREMENT
RANGE: -5° to +45°C

ACCURACY: ±0.5°C

READABILITY: 0.25°C

COMPENSATION
Automatic temperature compensation accurate to ±1% of D.O. readings made within ±5°C of calibration temperature, and accurate to ±3% of D.O. readings from -5° to +45°C. Manual, direct-dial salinity compensation from fresh water to sea water, 0 to 40 PPT, accurate to ±2.5% of compensated reading.

AMBIENT RANGE
Instrument and probe operating range is -5° to +45°C.

SYSTEM RESPONSE TIME
Typical response time for temperature and D.O. readings is 90% in 10 seconds at constant temperature of 30°C.

RECORDER OUTPUT
0 to 114-130mV. Recorder should have 50,000 ohms minimum input impedance.

POWER SUPPLY
Two disposable "C" size carbon-zinc batteries provide approximately 1,000 hours instrument operation. Optional internal rechargeable battery pack, YSI 5721, operates the submersible stirrer (see accessories).

DIMENSIONS
21.6 x 28 x 9.5cm, 1.9 kg (8.5 x 11 x 3.75 inches, 4.2 lbs.)

D.O. Field Probe with 3 Meter Cable

YSI 5739 Dissolved Oxygen Probe (without cable)
YSI 5740-10 Probe Cable — 3 meters (10')
YSI 5740-25 Probe Cable — 7.5 meters (25')
YSI 5740-50 Probe Cable — 15 meters (50')
YSI 5740-100 Probe Cable — 30.5 meters (100')
YSI 5740-150 Probe Cable — 46 meters (150')
YSI 5740-200 Probe Cable — 61 meters (200')
(For longer cables, order YSI 5740-X and specify length to 250'. All cables 25' or longer supplied with storage reel.)

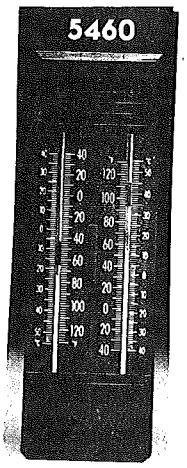
Note: BOD Bottle Probes are also available at a cost of $353 each.

INSTRUMENT	UNIT OF MEASURE
MAXIMUM-MINIMUM THERMOMETER (AIR TEMPERATURE)	^{o}C

SUGGESTED SUPPLIER	COST (MAY 1984)
Taylor Scientific Consumer Instruments Division of Sybron Corp. Glenn Bridge Road Arden, North Carolina 28704 (704) 684-8111	$22.98

DESCRIPTIVE INFORMATION

Model 5460 Maximum-Minimum Thermometer

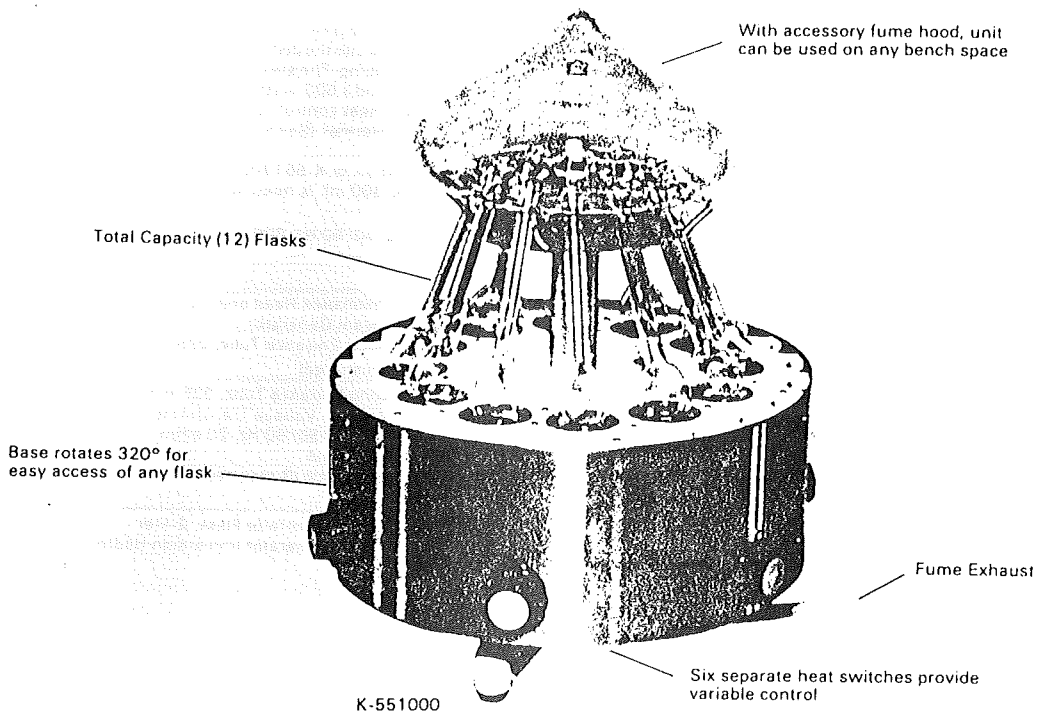


Thermometer registers high and low readings from last setting. Pushbutton reset. F° & C° scales. 8¾" x 2¾". Blister carded, 4 per carton. Weight: 6 ozs.

INSTRUMENT	UNIT OF MEASURE
KJELDAHL NITROGEN APPARATUS	mg/l

SUGGESTED SUPPLIER	COST (MAY 1984)
Kontes Spruce Street Vineland, New Jersey 08360 (609) 692-8500	$2,081.80 See itemization in this section

DESCRIPTIVE INFORMATION



K-551000 ROTARY KJELDAHL DIGESTION APPARATUS

Design of this apparatus positions all flasks an equal distance from adjacent flasks to assure uniform heat distribution. More uniform digestions are accomplished since cold-end flasks are eliminated as found in older straight line units. The entire apparatus can be rotated 320° on the base to allow easy access to every flask.

An accessory glass fume hood fits over the digestion apparatus, allowing it to be used on the laboratory bench. A connection to vacuum is made at the base. Heat generated by the apparatus prevents condensation. This accelerates exhaustion of SO_2 and SO_3 gases through the lower opening.

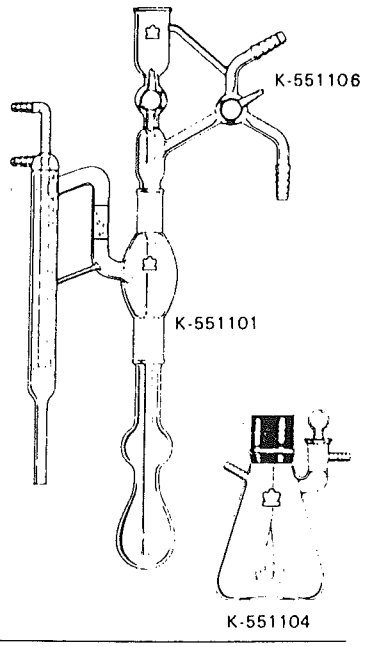
Heat control is maintained by six variable output switches, each controlling two adjacent heating units. For a wider range of temperature selection, an accessory variable-voltage transformer may be used.

Power Requirement: 115 VAC 50/60 Hz, 1320 watts
Dimensions: 18" (430 mm.)
Height: 12" (304 mm.)
Weight: 25 lbs. (11.5 Kg.)

Accessories

K-551001-0025	Fume Hood, 25 mm. dia. holes to accept K-551500 30 and 100 ml. flasks.
K-551001-0030	Fume Hood, 30 mm. dia. holes to accept K-551600 30 and 100 ml. flasks.
K-551002-0000	Replacement Adapter Rings to accommodate 30 ml. flasks.
K-552100-0000	Variable Voltage Transformer 115 vac 50/60 Hz, 20 amps. (Not illustrated)

KJELDAHL NITROGEN APPARATUS (Continued)



K-551100 KJELDAHL DISTILLATION APPARATUS

Design Features Include:

- Entrance tube for complete transfer to the distillation flask. No chance to introduce sample into the sidearm.
- Platinum gauze assures maximum distillation rates with negligible blank.
- Hopkins Condenser directly attached to distillation head. Removes possibility of atmospheric condensation and possible contamination.
- Pressurized steam entry into sample improves distilling efficiency
- Velocity of ammonia emerging from condenser is maximized prior to entrapment in a boric acid solution
- Sample can be based on the actual weight or the amount of distillable ammonia after digestion. Can be used for ultra-micro, macro, or semi-micro determinations.

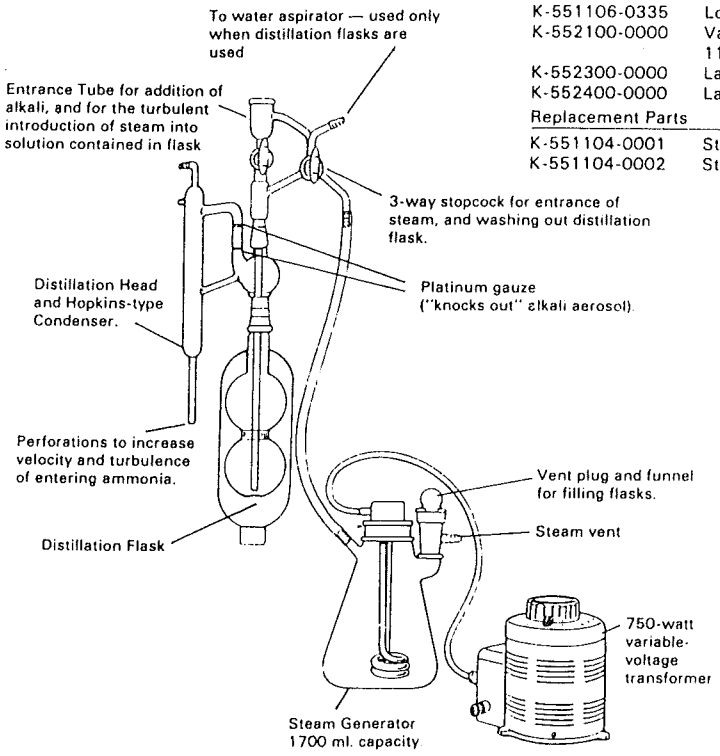
Assembly:

Insert digestion tube with sample into the distillation head and connect steam generator to unit with flexible tubing. The steam generator consists of a two-liter flask with a stopcock vent and a 600-watt immersion heating coil. It will not be damaged if it runs dry. Heat control can be maintained by using an accessory variable-voltage transformer. (See K-552100). Approximate overall height is 485 mm.

See K-551600 30 ml. and 100 ml. or K-551700 100 ml. flasks for use with short entry tube. If K-551700 300 ml. is needed, use accessory long entry tube.

Power Requirement. 115 VAC, 50/60 Hz, 600 watts.

Parts	
K-551101-0000	Distillation Head and Condenser
K-551104-0000	Steam Generator
K-551106-0285	Short Entrance Tube, 285 mm. Teflon®
Accessories	**(Not Illustrated)**
K-551106-0335	Long Entrance Tube, 335 mm., Teflon
K-552100-0000	Variable Voltage Transformer, 115 vac 50/60 Hz, 20 amps
K-552300-0000	Lab Stand
K-552400-0000	Lab Clamp (2 required)
Replacement Parts	
K-551104-0001	Steam Generator Flask, 2-liter
K-551104-0002	Steam Generator Immersion Heater, 600 watt



KJELDAHL NITROGEN APPARATUS (Continued)

K-551600

K-551600 KJELDAHL DIGESTION/DISTILLATION FLASK

Dual purpose flask which can be used in both the K-551000 Rotary Digestion Apparatus and K-551100 Distillation Apparatus. ȝ 24/40 joint allows connection to the distillation assembly following the digestion process, thus saving a transfer.

Flask design includes an expansion bulb as a guard against bumping and foaming during digestion. Also allows a more vigorous introduction of steam during distillation.

Capacity, ml.	Overall Height, mm.
30	210
100	210

Quantity	Catalog No.	Description	Price
1	K551000-0000	Digestion apparatus, Kjeldahl, rotary, 12-place, 115 V, 50/60 Hz, 1325 Watts	$1,249.30
1	K551001-0030	Fume hood	138.80
1*	K551100-0000	Distillation apparatus, Kjeldahl	529.30
12*	K551600-0100	Flask, Kjeldahl, 100 ml, @ 13.70	164.40
		Total	$2,081.80

* Number of flasks and distillation apparatus needed is dependent on number of samples to be collected and manpower available to clean glassware, etc.

INSTRUMENT	UNIT OF MEASURE
DIGITAL TITRATOR TEST KIT/DISSOLVED OXYGEN	mg/l

SUGGESTED SUPPLIER	COST (MAY 1984)
HACH Company P.O. Box 389 Loveland, CO 80539 (800) 525-5940	$139

DESCRIPTIVE INFORMATION

Model OX-DT with Digital Titrator

DISSOLVED OXYGEN TEST KIT
Model OX-DT with Digital Titrator

This kit uses the Winkler dissolved oxygen method with azide modification and a 200-mL sample size. All sample treatment reagents (alkaline iodide-azide, manganous sulfate and sulfamic acid) are packaged in unit-dose powder pillows for convenience. Titrations are performed with Hach's Digital Titrator and a prestandardized PAO titration cartridge. A glass-stoppered sample bottle is included.

Range: 0-10 mg/L as DO
Smallest Increment: 0.01 mg/L
Sample: 300 mL
Analysis Method: Titrimetric
Number of Tests: 50 (average)
Case: 41 × 20 × 18 cm (16 × 8 × 7″)
Shipping Weight: 4.1 kg (9 lbs)
Order Cat. No. 20631-00 ..... $139.00

Note: If digital titrator is purchased for dissolved oxygen determinations, investigators need only purchase appropriate HACH reagents and titration cartridges to determine levels of 18 other parameters, including alkalinity and total hardness.

INSTRUMENT	UNIT OF MEASURE
DIGITAL TITRATOR TEST KIT/ALKALINITY	mg/l

SUGGESTED SUPPLIER	COST (MAY 1984)
HACH Company P.O. Box 389 Loveland, CO 80539 (800) 525-5940	$110

DESCRIPTIVE INFORMATION

High and Low Range
Model AL-DT with Digital Titrator

ALKALINITY TEST KIT
High and Low Range
Model AL-DT with Digital Titrator

Model AL-DT is a higher-accuracy version of our Model AL-AP Test Kit. This kit contains the precise Digital Titrator, two prestandardized sulfuric acid titration cartridges and premeasured indicator powder pillows. Hach's Model AL-DT allows measurement in two ranges, 0-100 and 0-1000 mg/L alkalinity as calcium carbonate, with results read directly in mg/L. Both phenolphthalein (P) and total (M) alkalinity are determined.

Ranges: 0-100 and 0-1000 mg/L as $CaCO_3$
Smallest Increment: 0.1 and 1 mg/L
Sample: 100 mL
Analysis Method: Titrimetric
Number of Tests: 200 (average) (100 phenolphthalein and 100 total)
Case: 23 × 18 × 13 cm (9 × 7 × 5")
Shipping Weight: 2.3 kg (5 lb)
Order Cat. No. 20637-00 ..... $110.00

Note: If digital titrator is purchased for alkalinity determinations, investigators need only purchase appropriate HACH reagents and titration cartridges to determine levels of 18 other parameters, including dissolved oxygen and total hardness.

INSTRUMENT	UNIT OF MEASURE
DIGITAL TITRATOR TEST KIT/TOTAL HARDNESS	mg/l

SUGGESTED SUPPLIER	COST (MAY 1984)
HACH Company P.O. Box 389 Loveland, CO 80539 (800) 525-5940	$123

DESCRIPTIVE INFORMATION

High and Low Range
Model HAC-DT with Digital Titrator

MAGNESIUM, CALCIUM AND TOTAL HARDNESS TEST KIT
High and Low Range
Model HAC-DT with Digital Titrator

Measure total hardness, calcium and magnesium (by difference) in two ranges with this kit. Tests are performed using Hach's Digital Titrator and two different EDTA titration cartridges. Determine total hardness using ManVer 2 Hardness Indicator Powder Pillows; the color change is from red to blue. Repeat the same titration using CalVer® 2 Calcium Indicator Powder Pillows to measure calcium content. All reagents and apparatus needed are included in the kit carrying case.

Ranges: 0-100 and 0-1000 total or calcium hardness as $CaCO_3$
Smallest Increment: 0.2 and 2 mg/L
Sample: 50 mL
Analysis Method: Titrimetric
Number of Tests: 200 (average) (100 total and 100 calcium)
Case: 23 × 18 × 13 cm (9 × 7 × 5″)
Shipping Weight: 2.3 kg (5 lb)
Order Cat. No. 20639-00 ..... $123.00

Note: If digital titrator is purchased for total hardness determinations, investigators need only purchase appropriate HACH reagents and titration cartridges to determine levels of 18 other parameters, including dissolved oxygen and alkalinity.

PROCEDURE

POND MAPPING

REFERENCE

Lind, O.T. 1974. Handbook of common methods in limnology. C.V. Mosby Company, Saint Louis, pp. 5-16.

F-17

CHAPTER ONE PHYSICAL LIMNOLOGY

MAPPING

Mapping a pond or small lake by plane table method

The mapping of lakes is usually beyond the capability of the limnologist, and he must rely on maps made by well-equipped survey teams. Often aerial photographs with known scale will suffice. However, small ponds (generally less than 10 acres) may be accurately mapped with a minimum of equipment. Whenever possible, mapping in winter on sound ice is preferable. Welch (1948) includes an extensive section on map methods from simple to complex.

Apparatus

Plane table and tripod
Alidade (a crude alidade may be made by setting pins in opposite ends of wooden ruler for "sights")
Compass
Ruler, graduated in tenths of inches
Map paper
Hard lead pencils
Round-headed map pins
100-foot steel measuring tape
2 steel stakes, approximately 2 feet long

Wooden stakes, approximately 3 feet long (25 to 100 depending on size of pond)
Sledgehammer
Stadia rod or other painted pole at least 6 feet long
Metal-core clothesline rope, of sufficient length to reach across pond, marked in 5-foot increments
Small boat or canoe
Depth sounding line or electronic depth sounder

Procedure

1. Select the longest relatively straight section of lakeshore for establishing a base line.

2. Drive steel stake near shore for one end of base line. Most of the pond should be visible from this point. Repeat for other end of base line at a point at least 100 feet from first stake and approximately the same distance from shore as the first. Make the base line as long as possible (Fig. 1).

3. Drive a wooden stake at water's edge at every major change in shoreline configuration. Maximum distance between any two wooden stakes should probably not exceed 50 feet. Drive wooden stakes just deep enough to stay in place for mapping. Steel stakes on base line are permanent and should be driven so only 4 to 6 inches protrude. Tie a cloth "flag" to top of every fifth stake to facilitate counting.

4. Attach map paper to plane table. Set up tripod directly over one metal stake at end of base line, and plumb table center to the stake. Level table and align one edge with true north-south line. Lock table in place. Draw true north arrow in corner of map; also indicate magnetic north (Fig. 2).

5. Judge shape of pond and length of base line, and determine appropriate scale. Record scale on map near north-south arrow.

6. Determine position on map that represents end of base line where table is set up. Mark this on map by firmly setting map pin in table. The pin must not move.

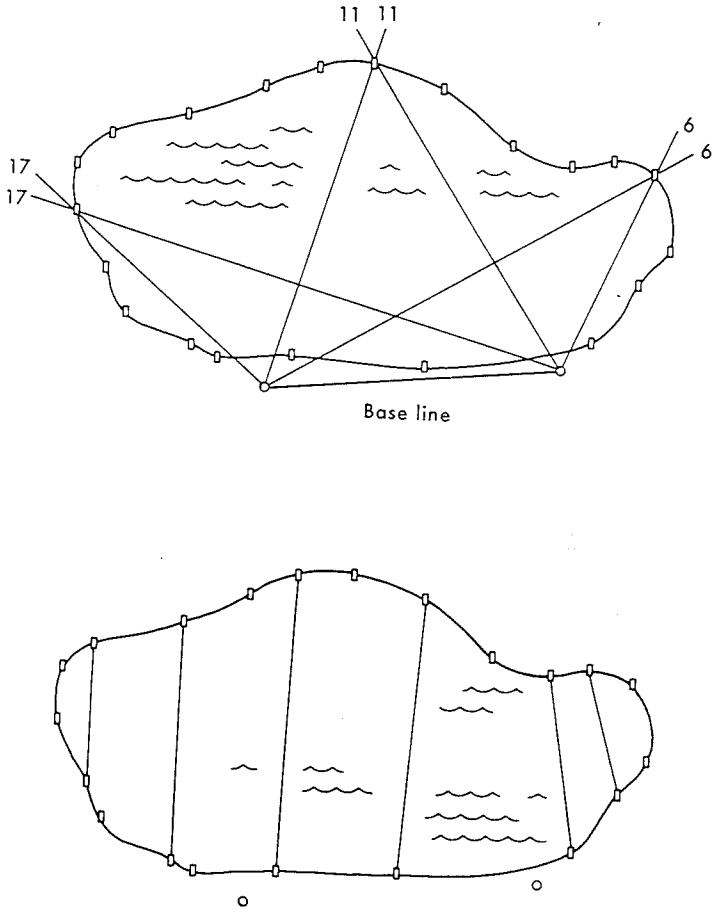


FIG. 1. Plane table map *(top)* showing position of a numbered shoreline stake and the base line. Distances for three shoreline points as determined by triangulation from the ends of the base line are shown. In a small lake or pond, bottom contours may be determined by sounding along a calibrated rope stretched between approximately opposite shoreline stakes *(bottom)*.

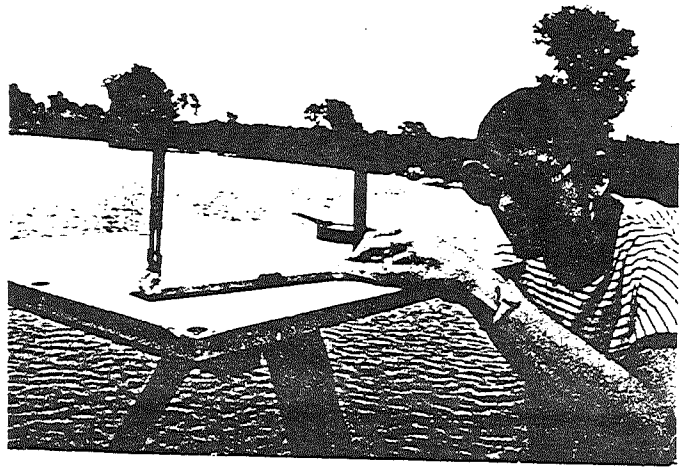


FIG. 2. Student using an open-sight alidade and a plane table to map a small pond. A compass is used to ascertain north orientation of the map. The edge of the alidade at the end nearest the student is always placed against a map pin firmly set in the table. This pin is the map position corresponding to the base line stake over which the table is centered.

Check again to be certain that all parts of pond will fall on map according to scale selected and position of pin.

7. Have flagman set stadia rod vertically on steel rod at other end of base line. Place zero mark on alidade against pin, and sight through alidade down base line to line up with stadia rod (Fig. 2). Draw base line on map along edge of alidade for the appropriate length as determined by scale. *Be careful not to bump the plane table at any time.*

8. Now in a similar manner, progressively sight alidade around the pond on each wooden stake (flagman holds stadia rod just behind stake), and draw line along edge of alidade. Number each line consecutively. The

fifth line and every multiple of 5 should be sighted on a stake with flag. Any deviation indicates a missed stake. After each flagged wooden stake has been sighted and the line drawn, resight on other end of base line. If this sighting does not coincide with original line, the table has been moved and another series must be taken.

9. After a complete circuit has been made, move and reset table over stake at other end of base line. Set pin in map at this end of base line and align table by sighting back down to other end of base line. Repeat step 8.

10. The point where corresponding numerical lines intersect is the position of each wooden stake marking the shoreline. Connect these points with a smooth line. By doing this in the field, you are able to include minor changes in shoreline. Also indicate position of obvious features in the water (logs, weed beds, and others).

11. It is more difficult to map bottom contours for the purpose of morphometric calculations. The problem is the accurate plotting on the map of the position of the sounding team. On smaller ponds, mapping bottom contours may be done as follows: starting at one end of the pond, stretch the calibrated rope between any two known wooden stake locations. Represent this by a light line on the map.

12a. The sounding team in the boat moves along this line, taking soundings at appropriate intervals from one shore to the other (intervals vary depending on amount of change in bottom contours). With the use of the scale, a point is placed at the proper position on the line, and the measured depth written in at that point (Fig. 1).

12b. An alternate procedure, more suitable for larger bodies of water but less precise so far as exact position on the lake is concerned, is the use of timed echo soundings. Echo sounders are becoming increasingly available, especially as "fish finders." The boat's pilot makes a line of sight between two stakes on opposite banks and sets the boat at a constant slow speed. It is

important that he make every effort to maintain a straight line and constant speed between the two points. A second person calls out regular time intervals—for example, every 30 seconds—and a third person reads and records the depth shown on the echo sounder at that time. The total time taken to transect the lake is recorded. Thus the total time for distance is known, and time for any timed increment can be calculated. Although this procedure is less precise, it has a compensating advantage in that it allows many more soundings to be taken, which more accurately gives the shape of the lake basin and allows for the plotting of more closely spaced bottom contours. This increases the accuracy of the lake volume calculation.

13. Repeat the chosen sounding procedure for a parallel series of lines across the pond.

14. Determine degree of bottom development to be shown (1-m intervals are often used, but intervals may be more or less). Draw in contour lines by connecting the appropriate points and the lines. For shape between points, parallel the shoreline.

15. Label map and include date and names of map crew. Roll up map (do not fold).

16. Return map to laboratory, retrace shoreline and contours in permanent ink. Clean up other working lines with soft eraser.

Direct measure modification of plane table method

The direct measure modification of the plane table method is suited for ponds of regular outline and open water. It is simple, and depth soundings may be taken simultaneously if the work party is of sufficient size.

Apparatus

Same as for plane table method plus the following
Good quality 18- to 24-inch ruler graduated in tenths of inches
Steel-core clothesline rope, of sufficient length to

reach across pond's longest dimension, marked in 5-foot increments

If approximate size of pond is known before going to the field, a previously prepared table of scale conversions of feet to inches will facilitate time spent in the field

A small boat or canoe may be necessary if obstructions are present

Procedure

1. Select a base point that is relatively open of vegetation and from which all parts of the pond may be seen. Drive iron stake and center drawing board over this stake. All work will be done from this location.
2. Prepare board for drawing as in plane table procedure.
3. Set out shoreline stakes as in plane table procedure.
4. Select point on paper to correspond with plane-table setup over iron stake, and set map pin.
5. Use alidade and take sightings on first stake to left. Draw light line along alidade from base point out to near edge of paper.
6. Place zero end of calibrated line on iron stake, and measure distance to the sighted stake to the nearest foot. Convert this distance to scale in inches; using ruler, measure out from base point the corresponding distance on the line, and mark point. Thus location and distance of first stake have been determined.
7. Repeat step 6 for all remaining stakes.
8. Connect the points, filling in detail from observation.
9. If the map party is of sufficient size, a boat sounding team may work alongside the measuring rope, taking soundings and distance between base point and stake. Depths called out may be marked on map at proper distance from base point (see following section, Morphometry).

MORPHOMETRY

Most limnological phenomena and productivity are directly related to the morphological features of the water basin. Therefore, certain morphometric features are of interest to a limnologist beginning a study of any water. Morphometric measurements are based on good hydrographic maps, and in general, the larger the map, the more reliable the morphometric data that may be obtained from them.

Area by polar planimeter

Whenever instrumentation is available, the polar planimeter method is preferred. The polar planimeter is a delicate instrument, and care must be taken whenever it is used. Read over the directions supplied by the manufacturer for the instrument in use.

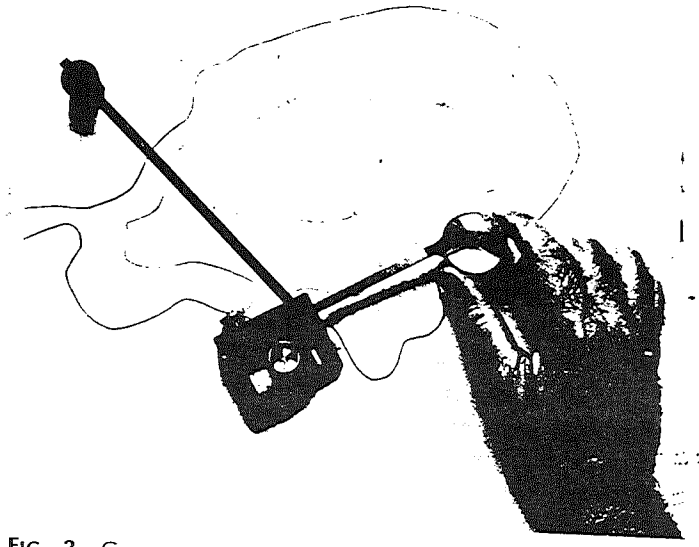


FIG. 3. Compensating polar planimeter used to determine areas. Here the planimeter is tracing the shoreline to determine the total area of a pond drawn by the plane table method.

1. Prepare map by placing it on hard, smooth surface. Tape it in place.

2. Most maps will be too large to be covered by one cycle of the planimeter. These maps must be ruled off into halves or smaller segments and the areas of each summed for the total.

3. Check the calibration of your instrument on the map paper being used. Use calibration device supplied by manufacturer, or carefully rule off a known area and trace with planimeter 3 times.

4. Proceed with actual planimetry by tracing outline of lake or bottom contours following carefully the manufacturer's directions.

Area by cut and weight

1. Lightly trace map outline with bottom contours onto a good grade of paper.

2. From an area outside traced area, cut out a square of known area (a 9-inch square is usable) and weigh this piece of paper. Calculate weight per square inch.

3. Cut out outline of entire lake and weigh. Calculate area by dividing weight of entire lake by weight of 1 square inch.

4. Repeat step 3 for each successive bottom contour.

Volume by calculation

If a lake basin is considered as a cone, then the volume may be calculated by the appropriate equation (cone volume = $1.047\ r^2h$). However, because the slopes of lake bottoms are rarely regular, a better approximation for volume may be obtained by calculating and then summing the volumes of conical segments (frustra), with upper and lower surfaces delimited by the areas of sequential depth contours. The calculation is then as given by Welch (1948):

$$\text{Lake volume} = \sum \text{frustrum volumes}$$

where

$$\text{frustrum volume} = \frac{h}{3}(a_1 + a_2 + \sqrt{a_1 a_2})$$

h = depth of frustrum
a_1 = area of frustrum surface
a_2 = area of frustrum bottom

Volume by planimetry

Lake volume may also be determined by planimetric integration.

1. Using linear graph paper, plot the area at a given depth against that depth. Make the plot with the horizontal axis for area at the top and the vertical axis for depth at the left of the page. This places the 0—0 ordinate in the upper left corner.

2. Integrate the area beneath the curve by using a polar planimeter or by counting squares. Include those squares that are more than half within, and disregard those that are more than half outside the line. If equal di-

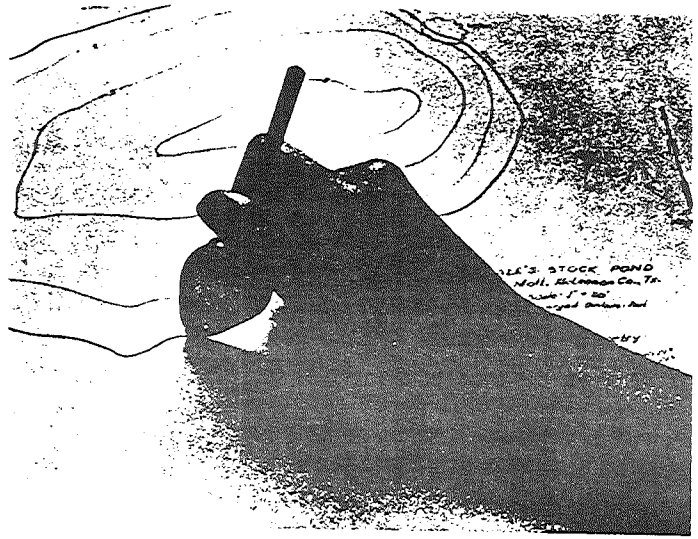


Fig. 4. Cartometer in use measuring the shoreline length of a plane table map.

mensions for each square of the grid are used (that is, each grid has dimensions of 1 m^2 in one direction versus 1 m in the other direction), that grid represents 1 m^3 volume. Summing the number of cubic meter volume grids under the curve will give the approximate total volume of the lake.

Shoreline length: cartometer method

The map measure (cartometer) is a convenient method for measuring lengths of shorelines (Fig. 4). It is also a delicate instrument and deserves care in handling.

1. Set dial by turning wheel to zero line. Draw a line of known length on the map paper, and trace three times with the instrument to check its accuracy of calibration.
2. Set instrument to zero line, and carefully trace the shoreline of the lake. Watch carefully to see if the dial revolves more than one time. Record number of inches or centimeters, and convert to feet or meters per scale.
3. Repeat for each of the submerged contours.

Shoreline development

Shoreline development is an index of the regularity of the shoreline. For a lake that is a perfect circle, the shoreline development is 1. As the value departs from unity, irregularity is indicated. This value is calculated as follows:

$$\text{Shoreline development (SLD)} = \frac{S}{2\sqrt{a\pi}}$$

where

S = length of shoreline
a = area of lake

Maximum length and orientation of main axis

These two factors are usually the same portion of the lake, but because of unusual irregularities, they may not be so. Maximum length is the longest straight line that may be drawn without intersecting any mainland.

Not all lakes will have a segment that may be considered to represent a maximum length. The orientation is expressed as opposing points on a 16-point compass: for example, SSE-NNW.

Maximum depth

The maximum depth is the deepest spot in the lake.

Mean depth

The mean depth is an important value, since it is used in certain other calculations, such as heat budgets. It is calculated as follows:

$$\text{Mean depth in meters} = \frac{\text{volume in } m^3}{\text{surface area in } m^2}$$

PROCEDURE

DETERMINING SECCHI DISK VISIBILITY

REFERENCE

Lind, O.T. 1974. Handbook of common methods in limnology. C.V. Mosby Company, Saint Louis, pp. 22-23.

VISIBILITY

Visibility is a measure of the depth to which one may see into the water. Obviously this is variable with the day conditions and the eyesight of the observer. The Secchi disk (Fig. 6) is a simple device used to estimate this depth. It consists of a weighted circular plate, 20 cm in diameter, with the surface painted with opposing black and white quarters. It is attached to a calibrated line by a ring at the center so that when held by the line, it hangs horizontally. To determine the Secchi disk visibility, slowly lower the disk into the water until it disappears, and note this depth. Lower the disk a few more feet, then slowly raise it until it reappears, and note this depth. The average of these two readings is taken for the final Secchi disk visibility depth.

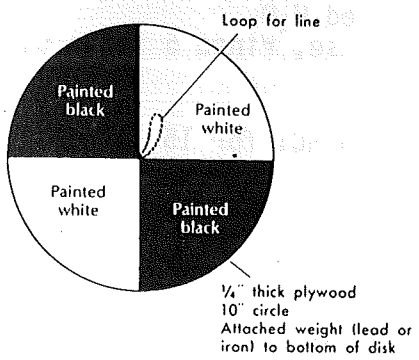


FIG. 6. The Secchi disk may be easily made from a 20-cm diameter metal or weighted wooden disk. Opposite quarters are painted gloss white and gloss black as shown. It is important that the calibrated line be attached so that the disk hangs horizontally in the water. (From Vivian, V. E.: Sourcebook for environmental education, St. Louis, 1973, The C. V. Mosby Co.)

The Secchi disk visibility is useful as a means of comparing the visibility of different waters, especially when measured by the same observer. Since clearness of the day, position of the sun, roughness of the water, and the observer all are significant considerations, they should be recorded along with the visibility depth data. Most important is for an observer to establish a standard set of operating conditions for himself; for example, always take readings while standing, with or without glasses or sunglasses, on the lee side of the boat with the sun to the observer's back, sometime between 9:00 AM and 3:00 PM.

Since one of the more frequently used optical relations in water studies is the photic depth, or depth of 1% surface illumination, some limnologists find it convenient to "calibrate their eye" to estimate photic depth by using only the simple Secchi disk. This is done by accurately determining the true photic depth by use of a submarine photometer and at the same time taking a series of Secchi disk readings to obtain an average. Dividing true photic depth by Secchi disk visibility depth will obtain a factor by which Secchi disk visibility depth is multiplied. This factor is used in the future to estimate photic depth when a submarine photometer is unavailable. If this procedure is used over a range of different water conditions, one general factor may be developed and a fair approximation of photic depth obtained.

PROCEDURE

GLASSWARE WASHING

REFERENCE

Michigan State University Limnological Research Laboratory, 1983. Unpublished memo on glassware washing procedures. East Lansing, MI. 2 pp.

PO_4 Glassware:

1) Rinse 3 times with distilled H_2O
2) Soak in 1:1 HCl (PO_4 ONLY) bath for at least 5 hours
3) Rinse 3 times with distilled H_2O, once with deionized
4) Dry (protect from dust) and cap with aluminum foil

NOTE: It may be necessary to periodically wash this glassware with HOT 1:1 HCl

NH_3-N and Kjeldahl-N Glassware:

1) Soak in Liquinox-H_2O solution, rinse with tap H_2O, drip dry
2) Soak in Chromic-Sulfuric Acid cleaning solution (Chromerge) for 15 minutes, rinse 3 times with distilled H_2O
3) Soak and store in 0.1N HCl until next use, rinse 5 times with deionized H_2O before use

NOTE: Soak glassware stored dry in the 0.1N HCl for 15 minutes before use

NO_2-N, NO_2-NO_3-N Glassware:

1) Soak in Liquinox-H_2O solution, rinse with tap H_2O, drip dry
2) Soak in Chromic-Sulfuric Acid cleaning solution (Chromerge) for 15 minutes, rinse 3 times with distilled H_2O
3) Soak in the 1 N HCl bath for 30 minutes, rinse 3 times with deionized H_2O
4) Dry and cap with aluminum foil

Nalge Polypropylene Sample Bottles and Other Plastic Labware:

1) Rinse 3 times with distilled H_2O
2) Soak in 1 N HNO_3 bath for 15 minutes and rinse 3 times with distilled H_2O
3) Soak in 1 N HCl bath for 15 minutes and rinse 3 times with deionized H_2O
4) Dry and replace cap or cap with aluminum foil for storage

GLASSWARE WASHING (continued)

Crucibles:

1) Soak in Liquinox-H_2O solution, rinse with tap H_2O, drip dry
2) Soak in Chromic-Sulfuric Acid cleaning solution (Chromerge) for 15 minutes, rinse 3 times with distilled H_2O
3) Use a crumpled paper towel to scrub residue out of crucible
4) Soak in 1 N HCl bath for 15 minutes, rinse 3 times with deionized H_2O, dry and store

General Analytical Glassware (including other glassware not listed above):

1) Soak in Liquinox-H_2O solution, rinse with tap H_2O, drip dry
2) Soak in Chromic-Sulfuric Acid cleaning solution (Chromerge) for 15 minutes, rinse 3 times with distilled H_2O
3) Soak in 1 N HNO_3 bath for 15 minutes, rinse 3 times with distilled H_2O
4) Soak in 1 N HCl bath for 15 minutes, rinse 3 times with deionized H_2O
5) Dry (protect from dust) and cap with aluminum foil

PROCEDURE

MEASURING HYDROLOGIC CHARACTERISTICS

REFERENCE

Wood, J. W., 1974. Diseases of Pacific salmon: their prevention and treatment. State of Washington, Department of Fisheries, Olympia, WA. pp. 71-77.

EVAPORATION: Each project team should purchase from a commercial source or have constructed evaporation pans (3) to be floated and tethered on the surface of the ponds. It is expected that this will provide the best estimate of the evaporation rate at the pond surface. The pans should be of clear or white plexiglas, lexan (polycarbonate) or polypropylene. The dimensions (ID) should be approximately 70 (L) x 70 (W) x 25 (D) cm with vertical sides. A volume of water should be added to each pan and the change in water depth monitored over time. From the change in depth of water in the pans, pan area and the rainfall measurements at the site, the evaporation rate per unit area can be calculated. The pans must not overflow or dry out between measurement intervals.

INFLOW:

There are several ways to calculate the inflow into a pond. The following represent several methods of determining the rate of water inflow.

Direct Measurement — This may be done by simply placing a container of known volume under the spout and determining the time it takes to fill the container.

Example: A 50-gallon drum is filled in 25 seconds. 50 gals/25 secs x 60 secs = 120 gpm

Measurement from Table "Flow from Pipes" — This table (Table 2) was prepared by our engineers for 3 inch and 4 inch diameter pipes running full of water. For efficient use of this table, you should make up a wooden square, much like a carpenters square, with one leg (the Y leg) being either 9 or 16 inches long; the inside edge of the other leg (the X leg) should be marked off in 1 or ½ inch increments and be long enough to measure any water flow that you encounter at your hatchery.

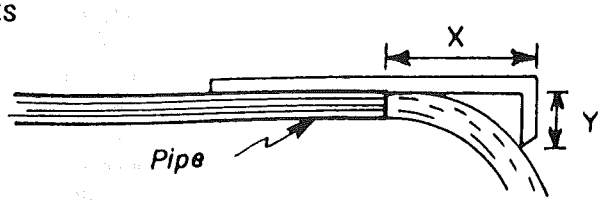
In use, the X leg of the square is placed on top of the pipe in a position where the end of the Y leg just touches the top of the extruding "jet" of water. Next, refer to the table under the correct column listing the diameter of pipe and length of Y that you used; go down the column until you find the X reading closest to the one that you measured; then read across to the appropriate gpm column to determine flow in gpm.

Example: The diameter of the pipe is 4 inches and the length of the Y leg of the square is 16 inches. When the end of the Y leg just touches the discharging water, the X leg extends out 24 inches. Referring to the appropriate column, it may be seen that the flow is between 240 and 250 gpm.

MEASURING HYDROLOGIC CHARACTERISTICS (Continued)

Table - 2

FLOW FROM PIPES



Formula – $G = \frac{2.56\ D^2\ X}{\sqrt{Y}}$ $X = \frac{G\sqrt{Y}}{2\ 56\ D^2}$

D = DIA. OF PIPE IN INCHES

G = GALLONS PER MINUTE (gpm)

4" Pipe Y = 16"		*3" Pipe Y = 16"*		*4" Pipe Y = 9"*		*3" Pipe Y = 9"*	
X"	*gpm*	*X"*	*gpm*	*X"*	*gpm*	*X"*	*gpm*
4.88	50	8.68	50	3.66	50	6.51	50
5.86	60	10.42	60	4.39	60	7.81	60
6.84	70	12.15	70	5.13	70	9.11	70
7.81	80	13.89	80	5.86	80	10.42	80
8.79	90	15.62	90	6.59	90	11.72	90
9.77	100	17.36	100	7.32	100	13.02	100
10.74	110	19.10	110	8.06	110	14.32	110
11.72	120	20.83	120	8.79	120	15.23	120
12.70	130	22.57	130	9.52	130	16.93	130
13.67	140	24.30	140	10.25	140	18.23	140
14.65	150	26.04	150	10.99	150	19.53	150
15.62	160	27.78	160	11.72	160	20.83	160
16.60	170	29.51	170	12.45	170	22.13	170
17.58	180	31.25	180	13.18	180	23.44	180
18.55	190	32.98	190	13.91	190	24.74	190
19.53	200	34.72	200	14.65	200	26.04	200
20.51	210	36.46	210	15.38	210	27.34	210
21.48	220	38.19	220	16.11	220	28.64	220
22.46	230	39.93	230	16.85	230	29.95	230
23.43	240	41.66	240	17.58	240	31.25	240
24.41	250	43.40	250	18.31	250	32.55	250
25.39	260	45.14	260	19.04	260	33.85	260
26.37	270	46.87	270	19.77	270	35.15	270
27.34	280	48.61	280	20.51	280	36.46	280
28.32	290	50.34	290	21.24	290	37.76	290
29.30	300	52.08	300	21.97	300	39.06	300
1" x = 10.2 gpm		1" x = 5.89 gpm		1" x = 13.7 gpm		1" x = 7.7 gpm	

X in every case equals inches and parts of inches.

MEASURING HYDROLOGIC CHARACTERISTICS (Continued)

Pipe Larger or Smaller than 3 or 4 Inches – The table is made up for only 3 inch and 4 inch diameter pipes but the formula at the top of the page may be used for calculating the discharge from any diameter pipe or for X measurements in excess of those listed in the table.

Example: A pipe is 6 inches in diameter and running full of water. A square with a Y leg 16 inches long just touches the top of the discharging water when the X leg extends out 30 inches.

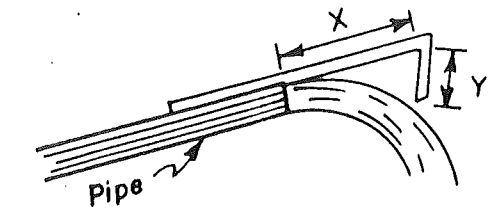
D = 6 inches
X = 30 inches
Y = 16 inches
G = Flow in gpm

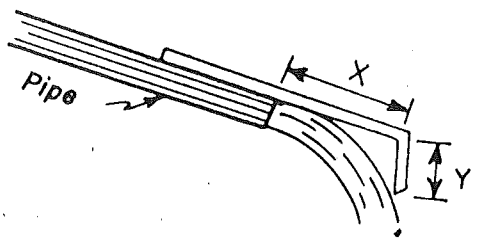
$$G = \frac{2.56\ D^2\ X}{\sqrt{Y}} = \frac{(2.56)\ (6 \times 6)\ (30)}{\sqrt{16}} = \frac{(2.56)\ (36)\ (30)}{4}$$

G = 691 gpm

Note: A 6 inch pipe running full will deliver 4 times the flow of a 3 inch pipe running full at the same X and Y.

Pipes not Level – The "Flow from Pipes" table (Table 2) may be used even if the pipe is not horizontal (level). To be used correctly, however, you cannot use a square; the X leg is placed on top of the pipe but the Y leg must extend directly down to the top of the water jet as in the diagrams below. The X reading is determined and the formula or table is used in the same manner as for a level discharge pipe.





Pipes not Full – The table (Table 2) and formula are only applicable for direct use if the pipe is running full of water. They may still be used with a pipe running partly full if a factor is used to compensate for the reduced flow resulting from the pipe only being partially filled. The following table, entitled "Effective Area Factor" table (Table 3), is used for any size pipe to find the effective area factor (EAF) after determining the ratio of the freeboard (F) to the diameter (D) of the pipe. Freeboard, in this case, is the measurement in inches of that portion of the diameter of the pipe that is not full of water. Determine X and Y in the usual manner; find the theoretical flow (as if the pipe was running full) from the "Flow from Pipes" table or formula; then multiply the theoretical flow by the effective area factor (EAF) to find the actual flow.

Example: A pipe is 4 inches in diameter and running with only 3 inches of water, Y is 16 inches and X is 24 inches. The free board (F) is 1 inch (4 inches minus 3 inches); therefore, the ratio of F to D (F/D) is 0.25. Looking at the "Effective Area Factor" table (Table 3) you can see that the EAF for an F/D of 0.25 is 9.805. In the "Flow from Pipes" table (Table 2), the flow for X = 24 inches and Y = 16 inches would be about 246 gpm if the pipe was full.

246 gpm X 0.805 = 198 gpm

Table 3
"Effective Area Factor" Table. For use in the calculation of flows thru pipes running partially full of water.

Ratio F/D	*EAF*	*Ratio F/D*	*EAF*	*Ratio F/D*	*EAF*
0.00	1.000	0.23	0.826	0.37	0.664
0.10	0.948	0.24	0.816	0.38	0.651
0.11	0.939	0.25	0.805	0.39	0.639
0.12	0.931	0.26	0.793	0.40	0.627
0.13	0.922	0.27	0.782	0.41	0.614
0.14	0.914	0.28	0.770	0.42	0.602
0.15	0.905	0.29	0.759	0.43	0.589
0.16	0.896	0.30	0.747	0.44	0.577
0.17	0.886	0.31	0.735	0.45	0.564
0.18	0.877	0.32	0.723	0.46	0.551
0.19	0.867	0.33	0.712	0.47	0.538
0.20	0.858	0.34	0.700	0.48	0.526
0.21	0.847	0.35	0.688	0.49	0.513
0.22	0.837	0.36	0.676	0.50	0.500

Note: For 6 inch pipes, the following factors may be used instead of Table 3 for obtaining flows from the 3 inch pipe table:

Full 6" pipe – 4 times 3" pipe full
5/6 Full 6" pipe – 3.5 times 3" pipe full
3/4 Full 6" pipe – 3.4 times 3" pipe full
2/3 Full 6" pipe – 2.8 times 3" pipe full
1/2 Full 6" pipe – 2.0 times 3" pipe full

MEASURING HYDROLOGIC CHARACTERISTICS (Continued)

Table 4 DISCHARGE IN CUBIC FEET PER SECOND (cfs) AND GALLONS PER MINUTE (gmp) OVER SHARP-CRESTED WEIRS, BY THE FRANCIS FORMULA: $Q = 3.33\ H^{3/2}$

(Adapted from King's "Handbook of Hydraulics", 4th ed., Table 36)

Depth on Crest *(inches)*	Discharge per Foot of Weir Crest *cfs*	*gpm*	Depth on Crest *(inches)*	Discharge per Foot of Weir Crest *cfs*	*gpm*
1/2	.03	13	6	1.18	528
3/4	.05	24	1/4	1.25	562
1	.08	36	1/2	1.33	596
1/4	.11	50	3/4	1.41	631
1/2	.15	66	7	1.48	665
3/4	.19	83	1/4	1.56	702
2	.23	102	1/2	1.65	738
1/4	.27	122	3/4	1.73	776
1/2	.32	142	8	1.81	814
3/4	.36	164	1/4	1.90	853
3	.42	187	1/2	1.98	890
1/4	.47	211	3/4	2.07	930
1/2	.53	236	9	2.16	971
3/4	.58	262	1/4	2.25	1,012
4	.64	287	1/2	2.35	1,053
1/4	.70	315	3/4	2.44	1,096
1/2	.77	343	10	2.53	1,136
3/4	.83	372	1/4	2.63	1,179
5	.90	402	1/2	2.73	1,223
1/4	.97	433	3/4	2.82	1,268
1/2	1.03	463	11	2.92	1,312
3/4	1.10	495	1/4	3.03	1,358
			1/2	3.12	1,401
			3/4	3.23	1,448
			12	3.33	1,495

The above table is to be used for measuring the discharge of water over damboards or other similar weirs. For practical purposes the width of a damboard fits the description of a sharp-crested weir. The discharge must be free falling to use this table. In practice the depth on the weir crest is measured to the top of the curl (see diagram) on the leading edge of a yardstick when the yardstick is placed on the leading edge of the top damboard. After measuring the depth on the crest, refer to the table and multiply the flow in gpm by the length of the weir in feet.

Example: A weir is 41 inches long and the depth on the crest is 1 3/4 in.
The flow is:
3.42 X 83 = 284 gpm

(41 inches = 3.42 feet)

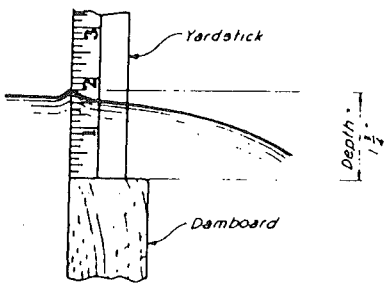


TABLE 4

MEASURING HYDROLOGIC CHARACTERISTICS (Continued)

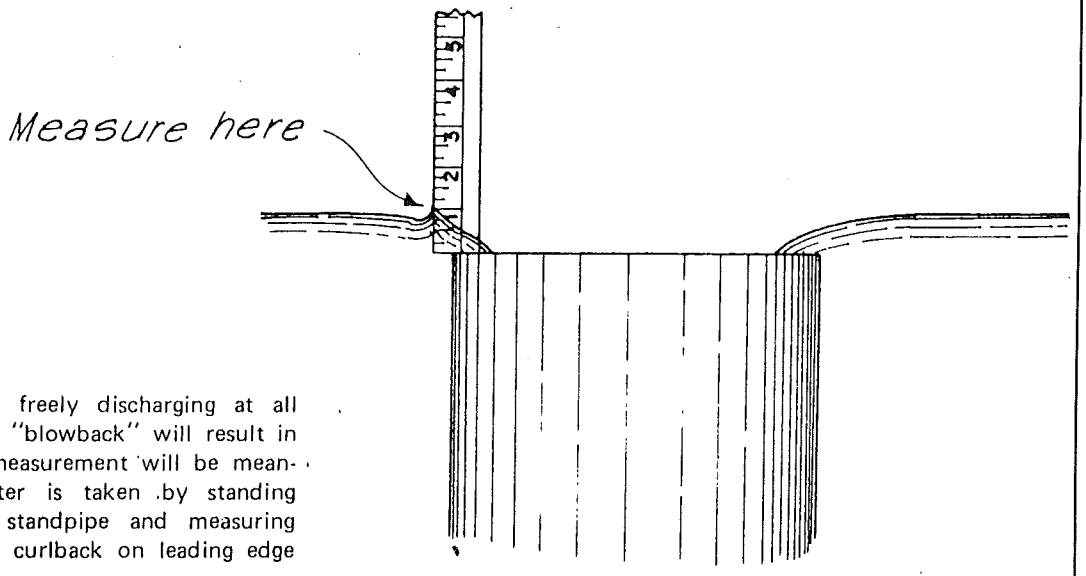
Table 5

WATER DISCHARGE DOWN DRAIN SUMPS

12" diam = 3.14' crest
10" diam = 2.62' crest
8" diam = 2.09' crest
6" diam = 1.57' crest

Flow in gallons per minute

Depth on Crest *(inches)*	*12" Sump*	*10" Sump*	*8" Sump*	*6" Sump*
1/2	42	35	28	21
3/4	70	59	47	35
1	113	94	75	57
1/8	141	118	94	70
1/4	155	129	103	78
3/8	183	153	122	92
1/2	211	176	141	106
5/8	240	200	160	120
3/4	268	224	179	134
7/8	296	247	197	148
2	324	271	216	162
1/8	352	294	235	176
1/4	381	317	254	190
3/8	423	353	282	211
1/2	451	376	301	225
5/8	479	400	320	240
3/4	522	435	349	261
7/8	564	470	376	282
3	592	494	394	296



Note: Water must be freely discharging at all times down the sump; "blowback" will result in increased depth and measurement will be meaningless. Depth of water is taken by standing yardstick on lip of standpipe and measuring depth to top of water curlback on leading edge of yardstick.

TABLE 5

MEASURING HYDROLOGIC CHARACTERISTICS (Continued)

Measurement in Open Channels and Streams — An approximation of fair accuracy of the flow in a channel or stream can be made with relatively little effort. You will need to measure off a length of the stream, preferable 100 ft; determine the average depth through this section (consider the entire length and width of the stream); and, determine the average width of this section of stream. You will then need to time the passage of a wood chip or float through the length of this section. The following formula is then used to calculate the volume of flow in cu ft/sec (cfs).

$$Q = \frac{WDal}{T}$$

Where: Q = volume of flow in cubic feet/sec (cfs)
W = average width of stream section in feet
D = average depth of stream section in feet
a = constant factor depending on type of stream bottom, use:
a - 0.9 for smooth sand, etc.
a = 0.8 for rough rocks, etc.
L = length of stream section measured
T = time in seconds for wood chip or float to travel the measured distance

Example: Suppose we find only a 50-foot length of stream that appears to have a uniform channel. By a series of measurements we determine the average depth to be 2 feet and the average width to be 15 feet. The bottom of the stream is mostly rocks and coarse gravel. We drop a wood chip at the upper end of the 50-foot section and find that it takes 65 seconds for the chip to reach the lower end of the section. Therefore:

W = 15 ft
D = 2 ft
a = 0.8
L = 50 ft
T = 65 secs

$$Q = \frac{15 \text{ ft} \times 2 \text{ ft} \times 0.8 \times 50 \text{ ft}}{65 \text{ secs}} = \frac{1200 \text{ cu ft}}{65 \text{ secs}}$$

= 18 cfs (approx)

Measurement from Pond Refill Time — The rate of water inflow to a pond may also be measured by determining the time necessary to refill a portion of the pond's volume. The method is valid providing no water leaves the pond during the refill period; if the pond leaks, you can't use this method. This method, however, may be the only one available to you if the water enters the pond through the upwelling chamber or through a flume or ditch at ground level.

It is necessary that the surface area of the pond be calculated. The pond is then drained down to a predetermined level below the top of the standpipe then allowed to refill. From the volume of water necessary to refill the discharged water, and the time necessary to do so, it is easy to calculate the rate of inflow.

Example: In a standard pond 80 ft long by 20 ft wide, the water is drained down 1 ft below the top of the standpipe. It takes 35 minutes to replace this top 1 ft of water.

Area of pond: 80 ft x 20 ft = 1,600 sq ft

Volume of top 1 ft: 1 ft x 1,600 sq ft = 1,600 cu ft; or, 7.48 gals/cu ft x 1,600 cu ft = 11,968 gals

Rate of inflow: 11,968 gals/35 mins = 342 gpm

ANALYTICAL METHOD

DISSOLVED OXYGEN

REFERENCE

American Public Health Association, 1980. Standard methods for the examination of water and waste water, 15th ed. APHA, Washington, D.C. pp. 388-399.

421 OXYGEN (DISSOLVED)

Dissolved oxygen (DO) levels in natural and wastewaters depend on the physical, chemical, and biochemical activities in the water body. The analysis for DO is a key test in water pollution and waste treatment process control.

Two methods for DO analysis are described: the Winkler or iodometric method and its modifications and the electrometric method using membrane electrodes. The iodometric method[1] is a titrimetric procedure based on the oxidizing property of DO while the membrane electrode procedure is based on the rate of diffusion of molecular oxygen across a membrane.[2] The choice of test procedure depends on the interferences present, the accuracy desired, and, in some cases, convenience or expedience.

421 A. Iodometric Methods

1. Principle

Improved by variations in technic and equipment and aided by instrumentation, the iodometric test remains the most precise and reliable titrimetric procedure for DO analysis. The test is based on the addition of divalent manganese solution, followed by strong alkali, to the sample in a glass-stoppered bottle. DO rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxides of higher valency states. In the presence of iodide ions and acidification, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent to the original DO content. The iodine is then titrated with a standard solution of thiosulfate.

The titration end point can be detected visually, with a starch indicator, or electrometrically, with potentiometric or dead-stop technics.[3] Experienced analysts can maintain a precision of $\pm 50\ \mu g/L$ with visual end-point detection and a precision of $\pm 5\ \mu g/L$ with electrometric end-point detection.[2,3]

The liberated iodine also can be determined directly by simple absorption spectrophotometers.[1] This method can be used on a routine basis to provide very accurate estimates for DO in the microgram-per-liter range provided that interfering particulate matter, color, and chemical interferences are absent.

2. Selection of Method

Before selecting a method consider the effect of interferences, oxidizing or reducing materials that may be present in the sample. Certain oxidizing agents liberate

DISSOLVED OXYGEN (continued)

iodine from iodides (positive interference) and some reducing agents reduce iodine to iodide (negative interference). Most organic matter is oxidized partially when the oxidized manganese precipitate is acidified, thus causing negative errors.

Several modifications of the iodometric method are given to minimize the effect of interfering materials.[2] Among the more commonly used procedures are the azide modification,[5] the permanganate modification,[6] the alum flocculation modification,[7] and the copper sulfate-sulfamic acid flocculation modification.[8,9] The azide modification (B) effectively removes interference caused by nitrite, which is the most common interference in biologically treated effluents and incubated BOD samples. Use the permanganate modification (C) in the presence of ferrous iron. When the sample contains 5 or more mg ferric iron salts/L, add potassium fluoride (KF) as the first reagent in the azide modification or after the permanganate treatment for ferrous iron. Alternately, eliminate Fe(III) interference by using 85–87% phosphoric acid (H_3PO_4) instead of sulfuric acid (H_2SO_4) for acidification. This pro-

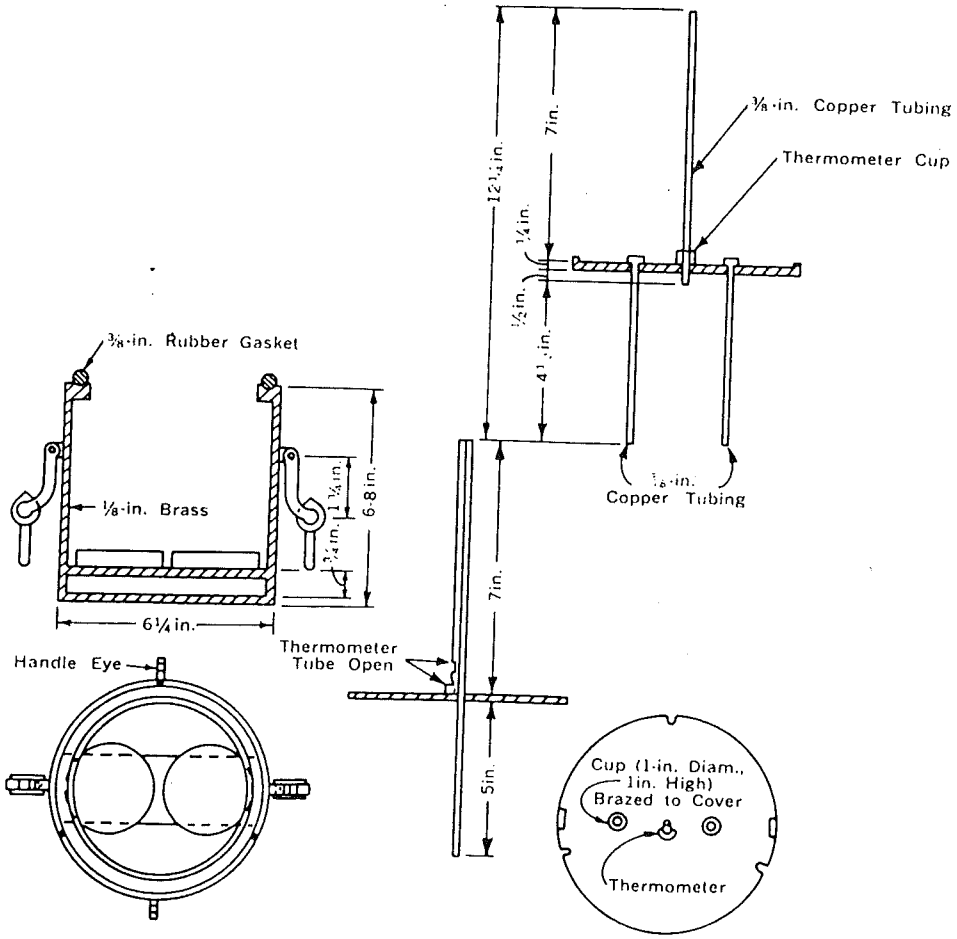


Figure 421:1. DO and BOD sampler assembly.

DISSOLVED OXYGEN (continued)

cedure has not been tested for Fe(III) concentrations above 20 mg/L.

Use the alum flocculation modification (D) in the presence of suspended solids that cause interference and the copper sulfate-sulfamic acid flocculation modification (E) on activated-sludge mixed liquor.

3. Collection of Samples

Collect samples very carefully. Methods of sampling are highly dependent on source to be sampled and, to a certain extent, on method of analysis. Do not let sample remain in contact with air or be agitated, because either condition causes a change in its gaseous content. Samples from any depth in streams, lakes, or reservoirs, and samples of boiler water, need special precautions to eliminate changes in pressure and temperature. Procedures and equipment have been developed for sampling waters under pressure and unconfined waters (e.g., streams, rivers, and reservoirs). Sampling procedures and equipment needed are described in American Society for Testing and Materials Special Technical Publication No. 148-I and in U.S. Geological Survey Water Supply Paper No. 1454.

Collect surface water samples in narrow-mouth glass-stoppered BOD bottles of 300-mL capacity with tapered and pointed ground-glass stoppers and flared mouths. Avoid entraining or dissolving atmospheric oxygen. In sampling from a line under pressure, attach a glass or rubber tube to the tap and extend to bottom of bottle. Let bottle overflow two or three times its volume and replace stopper so that no air bubbles are entrained.

Suitable samplers for streams, ponds, or tanks of moderate depth are of the APHA type shown in Figure 421:1. Use a Kemmerer-type sampler for samples collected from depths greater than 2 m. Bleed sample from bottom of sampler through a tube extending to bottom of a 250- to 300-mL BOD bottle. Fill bottle to overflowing (overflow for approximately 10 sec), and prevent turbulence and formation of bubbles while filling. Record sample temperature to nearest degree Celsius or more precisely.

4. Preservation of Samples

Determine DO immediately on all samples containing an appreciable oxygen or iodine demand. Samples with no iodine demand may be stored for a few hours without change after addition of manganous sulfate ($MnSO_4$) solution, alkali-iodide solution, and H_2SO_4, followed by shaking in the usual way. Protect stored samples from strong sunlight and titrate as soon as possible.

For samples with an iodine demand, preserve for 4 to 8 hr by adding 0.7 mL conc H_2SO_4 and 1 mL sodium azide solution (2 g NaN_3/100 mL distilled water) to the BOD bottle. This will arrest biological activity and maintain DO if the bottle is stored at the temperature of collection or water-sealed and kept at 10 to 20 C. As soon as possible, complete the procedure, using 2 mL $MnSO_4$ solution, 3 mL alkali-iodide solution, and 2 mL conc H_2SO_4.

421 B. Azide Modification

1. General Discussion

Use the azide modification for most sewage, effluent, and stream samples, especially if samples contain more than 50 μg NO_2^--N/L and not more than 1 mg ferrous iron/L. Other reducing or oxidizing materials should be absent. If 1 mL KF so-

DISSOLVED OXYGEN (continued)

lution is added before the sample is acidified and there is no delay in titration, the method is applicable in the presence of 100 to 200 mg ferric iron/L.

2. Reagents

a. Manganous sulfate solution: Dissolve 480 g $MnSO_4 \cdot 4H_2O$, 400 g $MnSO_4 \cdot 2H_2O$, or 364 g $MnSO_4 \cdot H_2O$ in distilled water, filter, and dilute to 1 L. The $MnSO_4$ solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.

b. Alkali-iodide-azide reagent: Dissolve 10 g NaN_3 in 500 mL distilled water. Add 480 g sodium hydroxide (NaOH) and 750 g sodium iodide (NaI), and stir until dissolved. There will be a white turbidity due to sodium carbonate (Na_2CO_3), but this will do no harm. CAUTION—*Do not acidify this solution because toxic hydrazoic acid fumes may be produced.*

c. Sulfuric acid, H_2SO_4, conc; One milliliter is equivalent to about 3 mL alkali-iodide-azide reagent.

d. Starch: Use either an aqueous solution or soluble starch powder mixtures.

To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 100 mL hot distilled water.

e. Standard sodium thiosulfate titrant: Dissolve 6.205 g $Na_2S_2O_3 \cdot 5H_2O$ in distilled water. Add 1.5 mL 6*N* NaOH or 0.4 g solid NaOH and dilute to 1,000 mL. Standardize with bi-iodate solution.

f. Standard potassium bi-iodate solution, 0.0250N: Dissolve 812.4 mg $KH(IO_3)_2$ in distilled water and dilute to 1,000 mL.

Standardization: Dissolve approximately 2 g KI, free from iodate, in an erlenmeyer flask with 100 to 150 mL distilled water. Add 1 mL 6*N* H_2SO_4 or a few drops of conc H_2SO_4 and 20.00 mL standard bi-iodate solution. Dilute to 200 mL and titrate liberated iodine with thiosulfate titrant, adding starch toward end of titration, when a pale straw color is reached. When the solutions are of equal strength, 20.00 mL 0.0250*N* $Na_2S_2O_3$ should be required. If not, adjust the $Na_2S_2O_3$ solution to 0.0250*N*.

g. Potassium fluoride solution: Dissolve 40 g $KF \cdot 2H_2O$ in distilled water and dilute to 100 mL.

3. Procedure

a. To the sample collected in a 250- to 300-mL bottle, add 1 mL $MnSO_4$ solution, followed by 1 mL alkali-iodide-azide reagent. If pipets are dipped into sample, rinse them before returning them to reagent bottles. Alternatively, hold pipet tips just above liquid surface when adding reagents. Stopper carefully to exclude air bubbles and mix by inverting bottle a few times. When precipitate has settled sufficiently (to approximately half the bottle volume) to leave clear supernate above the manganese hydroxide floc, add 1.0 mL conc H_2SO_4. Restopper and mix by inverting several times until dissolution is complete. Titrate a volume corresponding to 200 mL original sample after correction for sample loss by displacement with reagents. Thus, for a total of 2 mL (1 mL each) of $MnSO_4$ and alkali-iodide-azide reagents in a 300-mL bottle, titrate 200 × 300/(300-2) = 201 mL.

b. Titrate with 0.0250*N* $Na_2S_2O_3$ solution to a pale straw color. Add a few drops of starch solution and continue titration to first disappearance of blue color. If end point is overrun, back-titrate with 0.0250*N* bi-iodate solution added dropwise, or by adding a measured volume of treated sample. Correct for amount of bi-iodate solution or sample. Disregard subsequent recolorations due to the catalytic effect of nitrite or to traces of ferric salts that have not been complexed with fluoride.

4. Calculation

a. For titration of 200 mL sample, 1 mL 0.0250*N* $Na_2S_2O_3$ = 1 mg DO/L.

DISSOLVED OXYGEN (continued)

TABLE 421:I. SOLUBILITY OF OXYGEN IN WATER EXPOSED TO WATER-SATURATED AIR*

Temperature C	Chloride Concentration in Water mg/L				
	0	5,000	10,000	15,000	20,000
0	14.60	13.72	12.90	12.13	11.41
1	14.19	13.35	12.56	11.81	11.11
2	13.81	12.99	12.23	11.51	10.83
3	13.44	12.65	11.91	11.22	10.56
4	13.09	12.33	11.61	10.94	10.30
5	12.75	12.02	11.32	10.67	10.05
6	12.43	11.72	11.05	10.41	9.82
7	12.12	11.43	10.78	10.17	9.59
8	11.83	11.16	10.53	9.93	9.37
9	11.55	10.90	10.29	9.71	9.16
10	11.27	10.65	10.05	9.49	8.96
11	11.01	10.40	9.83	9.28	8.77
12	10.76	10.17	9.61	9.08	8.58
13	10.52	9.95	9.41	8.89	8.41
14	10.29	9.73	9.21	8.71	8.24
15	10.07	9.53	9.01	8.53	8.07
16	9.85	9.33	8.83	8.36	7.91
17	9.65	9.14	8.65	8.19	7.78
18	9.45	8.95	8.48	8.03	7.61
19	9.26	8.77	8.32	7.88	7.47
20	9.07	8.60	8.16	7.73	7.33
21	8.90	8.44	8.00	7.59	7.20
22	8.72	8.28	7.85	7.45	7.07
23	8.56	8.12	7.71	7.32	6.95
24	8.40	7.97	7.57	7.19	6.83
25	8.24	7.83	7.44	7.06	6.71
26	8.09	7.69	7.31	6.94	6.60
27	7.95	7.55	7.18	6.83	6.49
28	7.81	7.42	7.06	6.71	6.38
29	7.67	7.30	6.94	6.60	6.28
30	7.54	7.17	6.83	6.49	6.18
31	7.41	7.05	6.71	6.39	6.08
32	7.28	6.94	6.61	6.29	5.99
33	7.16	6.82	6.50	6.19	5.90
34	7.05	6.71	6.40	6.10	5.81
35	6.93	6.61	6.30	6.01	5.72
36	6.82	6.51	6.20	5.92	5.64

* At a total pressure of 101.3 kPa. Under any other barometric pressure, P, obtain the solubility, S (mg/L) from the corresponding value in the table by the equation:

$$S' = S \frac{P-p}{760-p}$$

in which S is the solubility at 101.3 kPa and p is the pressure (mm) of saturated water vapor at the water temperature. For elevations less than 1,000 m and temperatures below 25 C, ignore p. The equation then becomes:

$$S' = S \frac{P}{760} = S \frac{P'}{29.92}$$

Dry air is assumed to contain 20.90% oxygen. (Calculations made by Whipple and Whipple, 1911. *J. Amer. Chem. Soc.* 33:362.)

DISSOLVED OXYGEN (continued)

TABLE 421:I. SOLUBILITY OF OXYGEN IN WATER EXPOSED TO WATER-SATURATED AIR*

Temperature C	Chloride Concentration in Water mg/L				
	0	5,000	10,000	15,000	20,000
37	6.71	6.40	6.11	5.83	5.56
38	6.61	6.31	6.02	5.74	5.48
39	6.51	6.21	5.93	5.66	5.40
40	6.41	6.12	5.84	5.58	5.33
41	6.31	6.03	5.76	5.50	5.25
42	6.22	5.94	5.68	5.42	5.18
43	6.13	5.85	5.60	5.35	5.11
44	6.04	5.77	5.52	5.27	5.04
45	5.95	5.69	5.44	5.20	4.98
46	5.86	5.61	5.37	5.13	4.91
47	5.78	5.53	5.29	5.06	4.85
48	5.70	5.45	5.22	5.00	4.78
49	5.62	5.38	5.15	4.93	4.72
50	5.54	5.31	5.08	4.87	4.66

b. To obtain results in milliliters oxygen gas per liter, corrected to 0 C and 101.3 kPa, multiply mg DO/L by 0.70.

c. To express results as percent saturation at 101.3 kPa, use the solubility data in Table 421:I. Equations for correcting solubilities to barometric pressures other than mean sea level are given below the table.

5. Precision and Accuracy

DO can be determined with a precision, expressed as a standard deviation, of about 20 μg/L in distilled water and about 60 μg/L in wastewater and secondary effluents. In the presence of appreciable interference, even with proper modifications, the standard deviation may be as high as 100 μg/L. Still greater errors may occur in testing waters having organic suspended solids or heavy pollution. Avoid errors due to carelessness in collecting samples, prolonging the completion of test, or selecting an unsuitable modification.

421 C. Permanganate Modification

1. General Discussion

Use the permanganate modification only on samples containing ferrous iron. Interference from high concentrations of ferric iron (up to several hundred milligrams per liter), as in acid mine water, may be overcome by the addition of 1 mL potassium fluoride (KF) and azide, provided that the final titration is made immediately after acidification.

This procedure is ineffective for oxidation of sulfite, thiosulfate, polythionate, or the organic matter in wastewater. The error with samples containing 0.25% by volume of digester waste from the manufacture of sulfite pulp may amount to 7 to 8 mg DO/L. With such samples, use the al-

DISSOLVED OXYGEN (continued)

kali-hypochlorite modification.[10] At best, however, the latter procedure gives low results, the deviation amounting to 1 mg/L for samples containing 0.25% digester wastes.

2. Reagents

All the reagents required for Method B, and in addition:

a. Potassium permanganate solution: Dissolve 6.3 g $KMnO_4$ in distilled water and dilute to 1 L.

b. Potassium oxalate solution: Dissolve 2 g $K_2C_2O_4 \cdot H_2O$ in 100 mL distilled water; 1 mL will reduce about 1.1 mL permanganate solution.

3. Procedure

a. To a sample collected in a 250- to 300-mL bottle add, below the surface, 0.70 mL conc H_2SO_4, 1 mL $KMnO_4$ solution, and 1 mL KF solution. Stopper and mix by inversion. Never add more than 0.7 mL conc H_2SO_4 as the first step of pretreatment. Add acid with a 1-mL pipet graduated to 0.1 mL. Add sufficient $KMnO_4$ solution to obtain a violet tinge that persists for 5 min. If the permanganate color is destroyed in a shorter time, add additional $KMnO_4$ solution, but avoid large excesses.

b. Remove permanganate color completely by adding 0.5 to 1.0 mL $K_2C_2O_4$ solution. Mix well and let stand in the dark to facilitate the reaction. Excess oxalate causes low results; add only an amount of $K_2C_2O_4$ that completely decolorizes the $KMnO_4$ without having an excess of more than 0.5 mL. Complete decolorization in 2 to 10 min. If it is impossible to decolorize the sample without adding a large excess of oxalate, the DO result will be inaccurate.

c. From this point the procedure closely parallels that in Section 421B.3. Add 1 mL $MnSO_4$ solution and 3 mL alkali-iodide-azide reagent. Stopper, mix, and let precipitate settle a short time; acidify with 2 mL conc H_2SO_4. When 0.7 mL acid, 1 mL $KMnO_4$ solution, 1 mL $K_2C_2O_4$ solution, 1 mL $MnSO_4$ solution, and 3 mL alkali-iodide-azide (or a total of 6.7 mL reagents) are used in a 300-mL bottle, take $200 \times 300/(300 - 6.7) = 205$ mL for titration.

This correction is slightly in error because the $KMnO_4$ solution is nearly saturated with DO and 1 mL would add about 0.008 mg oxygen to the DO bottle. However, because precision of the method (standard deviation, 0.06 mL thiosulfate titration, or 0.012 mg DO) is 50% greater than this error, a correction is unnecessary. When substantially more $KMnO_4$ solution is used routinely, use a solution several times more concentrated so that 1 mL will satisfy the permanganate demand.

421 D. Alum Flocculation Modification

1. General Discussion

Samples high in suspended solids may consume appreciable quantities of iodine in acid solution. The interference due to solids may be removed by alum flocculation.

2. Reagents

All the reagents required for the azide modification (Section 421B.2) and in addition:

a. Alum solution: Dissolve 10 g aluminum potassium sulfate, $AlK(SO_4)_2 \cdot 12H_2O$,

DISSOLVED OXYGEN (continued)

in distilled water and dilute to 100 mL.

b. Ammonium hydroxide, NH_4OH, conc.

3. Procedure

Collect sample in a glass-stoppered bottle of 500 to 1,000 mL capacity, using the same precautions as for regular DO samples. Add 10 mL alum solution and 1 to 2 mL conc NH_4OH. Stopper and invert gently for about 1 min. Let sample settle for about 10 min and siphon clear supernate into a 250- to 300-mL DO bottle until it overflows. Avoid sample aeration and keep siphon submerged at all times. Continue sample treatment as in Section 421B.3 or an appropriate modification.

421 E. Copper Sulfate-Sulfamic Acid Flocculation Modification

1. General Discussion

This modification is used for biological flocs such as activated sludge mixtures, which have high oxygen utilization rates.

2. Reagents

All the reagents required for the azide modification (Section 421B.2) and, in addition:

Copper sulfate-sulfamic acid inhibitor solution: Dissolve 32 g technical-grade NH_2SO_2OH without heat in 475 mL distilled water. Dissolve 50 g $CuSO_4 \cdot 5H_2O$ in 500 mL distilled water. Mix the two solutions and add 25 mL conc acetic acid.

3. Procedure

Add 10 mL $CuSO_4$-NH_2SO_2OH inhibitor to a 1-L glass-stoppered bottle. Insert bottle in a special sampler designed so that bottle fills from a tube near bottom and overflows only 25 to 50% of bottle capacity. Collect sample, stopper, and mix by inverting. Let suspended solids settle and siphon relatively clear supernatant liquor into a 250- to 300-mL DO bottle. Continue sample treatment as rapidly as possible by the azide (Section 421B.3) or other appropriate modification.

421 F. Membrane Electrode Method

1. General Discussion

Various modifications of the iodometric method have been developed to eliminate or minimize effects of interferences; nevertheless, the method still is inapplicable to a variety of industrial and domestic wastewaters.[11] Moreover, the iodometric method is not suited for field testing and cannot be adapted easily for continuous monitoring or for DO determinations in situ.

Polarographic methods using the dropping mercury electrode or the rotating platinum electrode have not been reliable always for the DO analysis in domestic and industrial wastewaters because impurities in the test solution can cause electrode poisoning or other interferences.[12,13] With membrane-covered electrode systems these problems are minimized, because the sensing element is protected by an oxygen-permeable plastic membrane that serves as a diffusion barrier against

DISSOLVED OXYGEN (continued)

impurities.[14-16] Under steady-state conditions the current is directly proportional to the DO concentration.*

Membrane electrodes of the polarographic[14] as well as the galvanic[15] type have been used for DO measurements in lakes and reservoirs,[17] for stream survey and control of industrial effluents,[18,19] for continuous monitoring of DO in activated sludge units,[20] and for estuarine and oceanographic studies.[21] Being completely submersible, membrane electrodes are suited for analysis in situ. Their portability and ease of operation and maintenance make them particularly convenient for field applications. In laboratory investigations, membrane electrodes have been used for continuous DO analysis in bacterial cultures, including the BOD test.[15,22]

Membrane electrodes provide an excellent method for DO analysis in polluted waters, highly colored waters, and strong waste effluents. They are recommended for use especially under conditions that are unfavorable for use of the iodometric method, or when that test and its modifications are subject to serious errors caused by interferences.

a. Principle: Oxygen-sensitive membrane electrodes of the polarographic or galvanic type are composed of two solid metal electrodes in contact with supporting electrolyte separated from the test solution by a selective membrane. The basic difference between the galvanic and the polarographic systems is that in the former the electrode reaction is spontaneous (similar to that in a fuel cell), while in the latter an external source of applied voltage is needed to polarize the indicator electrode. Polyethylene and fluorocarbon membranes are used commonly because they are permeable to molecular oxygen and are relatively rugged.

Membrane electrodes are commercially available in some variety. In all these instruments the "diffusion current" is linearly proportional to the concentration of molecular oxygen. The current can be converted easily to concentration units (e.g., milligrams per liter) by a number of calibration procedures.

Membrane electrodes exhibit a relatively high temperature coefficient largely due to changes in the membrane permeability.[16] The effect of temperature on the electrode sensitivity, ϕ (microamperes per milligram per liter), can be expressed by the following simplified relationship:[16]

$$\log \phi = 0.43\, mt + b$$

where:

t = temperature, degrees C,
m = constant that depends on the membrane material, and
b = constant that largely depends on membrane thickness.

If values of ϕ and m are determined for one temperature (ϕ_0 and t_0), it is possible to calculate the sensitivity at any desired temperature (ϕ and t) as follows:

$$\log \phi = \log \phi_0 + 0.43\, m\, (t - t_0)$$

Nomographic charts for temperature correction can be constructed easily[2] and are available from some manufacturers. An example is shown in Figure 421:2, in which, for simplicity, sensitivity is plotted versus temperature in degrees Celsius on semilogarithmic coordinates. Check one or two points frequently to confirm original calibration. If calibration changes, the new calibration should be parallel to the original, provided that the same membrane material is used.

Temperature compensation also can be made automatically by using thermistors in the electrode circuit.[14] However, thermistors may not compensate fully over a wide temperature range. For certain applications where high accuracy is required,

*Fundamentally the current is directly proportional to the activity of molecular oxygen.[2]

DISSOLVED OXYGEN (continued)

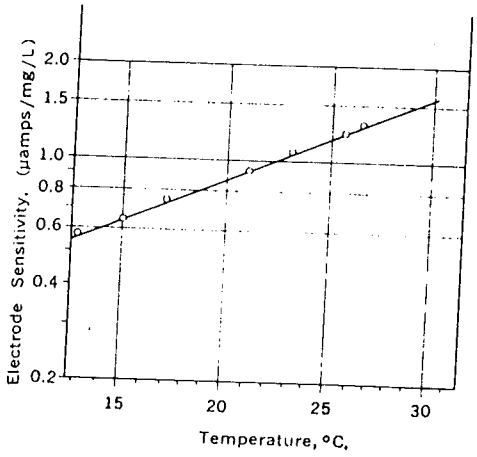


Figure 421:2. Effect of temperature on electrode sensitivity.

use calibrated nomographic charts to correct for temperature effect.

To use the DO membrane electrode in estuarine waters or in wastewaters with varying ionic strength, correct for effect of salting-out on electrode sensitivity.[2,16] This effect is particularly significant for large changes in salt content. Electrode sensitivity varies with salt concentration according to the following relationship:

$$\log \phi_S = 0.43\, m_S C_S + \log \phi_0$$

where:

ϕ_S, ϕ_0 = sensitivities in salt solution and distilled water, respectively,
C_S = salt concentration (preferably ionic strength), and
m_S = constant (salting-out coefficient).

If ϕ_0 and m_S are determined, it is possible to calculate sensitivity for any value of C_S. Conductivity measurements can be used to approximate salt concentration (C_S). This is particularly applicable to estuarine waters. Figure 421:3 shows calibration curves for sensitivity of varying salt solutions at different temperatures.

b. Interference: Plastic films used with membrane electrode systems are permeable to a variety of gases besides oxygen, although none is depolarized easily at the indicator electrode. Prolonged use of membrane electrodes in waters containing such gases as hydrogen sulfide (H_2S) tends to lower cell sensitivity. Eliminate this interference by frequently changing and calibrating the membrane electrode.

c. Sampling: Because membrane electrodes offer the advantage of analysis in situ they eliminate errors caused by sample handling and storage. If sampling is required, use the same precautions suggested for the iodometric method.

2. Apparatus

Oxygen-sensitive membrane electrode, polarographic or galvanic, with appropriate meter.

3. Procedure

a. Calibration: Follow manufacturer's calibration procedure exactly to obtain guaranteed precision and accuracy. Generally, calibrate membrane electrodes by reading against air or a sample of known DO concentration (determined by iodometric method) as well as in a sample with

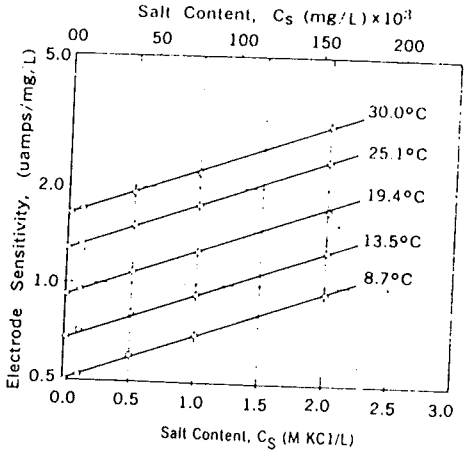


Figure 421:3. The salting-out effect at different temperatures.

DISSOLVED OXYGEN (continued)

zero DO. (Add excess sodium sulfite, Na_2SO_3, and a trace of cobalt chloride, $CoCl_2$, to bring DO to zero.) Preferably calibrate with samples of water under test. Avoid an iodometric calibration where interfering substances are suspected. The following illustrate the recommended procedures:

1) Fresh water—For unpolluted samples where interfering substances are absent, calibrate in the test solution or distilled water, whichever is more convenient.

2) Salt water—Calibrate directly with samples of seawater or waters having a constant salt concentration in excess of 1,000 mg/L.

3) Fresh water containing pollutants or interfering substances—Calibrate with distilled water because erroneous results occur with the sample.

4) Salt water containing pollutants or interfering substances—Calibrate with a sample of clean water containing the same salt content as the sample. Add a concentrated potassium chloride (KCl) solution (see Conductivity, Section 205 and Table 205:I) to distilled water to produce the same specific conductance as that in the sample. For polluted ocean waters, calibrate with a sample of unpolluted seawater.

5) Estuary water containing varying quantities of salt—Calibrate with a sample of uncontaminated seawater or distilled or tap water. Determine sample chloride or salt concentration and revise calibration to account for change of oxygen solubility in the estuary water.[2]

b. Sample measurement: Follow all precautions recommended by manufacturer to insure acceptable results. Take care in changing membrane to avoid contamination of sensing element and also trapping of minute air bubbles under the membrane, which can lead to lowered response and high residual current. Provide sufficient sample flow across membrane surface to overcome erratic response (see Figure 421:4 for a typical example of the effect of stirring).

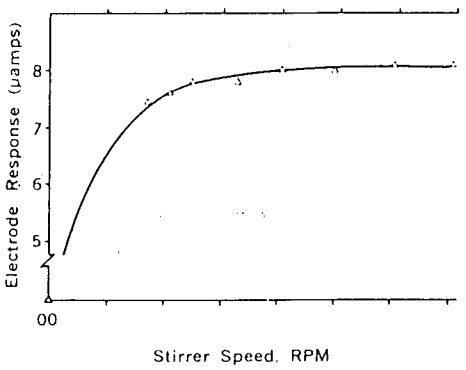


Figure 421:4. Typical trend of effect of stirring on electrode response.

c. Validation of temperature effect: Check frequently one or two points to verify temperature correction data.

4. Precision and Accuracy

With most commercially available membrane electrode systems an accuracy of ±0.1 mg DO/L and a precision of ±0.05 mg DO/L can be obtained.

421 G. References

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DISSOLVED OXYGEN (continued)

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ANALYTICAL METHOD

ALKALINITY

REFERENCE

American Public Health Association, 1980. Standard methods for the examination of water and waste water, 15th ed. APHA, Washington, D.C. pp. 253-257.

403 ALKALINITY

Alkalinity of a water is its quantitative capacity to react with a strong acid to a designated pH. The measured value may vary significantly with the end-point pH used. Alkalinity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known.

Alkalinity is significant in many uses and treatments of natural and wastewaters. Because the alkalinity of many surface waters is primarily a function of carbonate, bicarbonate, and hydroxide content, it is taken as an indication of the concentration of these constitutents. The measured values may include contributions from borates, phosphates, or silicates if these are present. Alkalinity in excess of alkaline earth metal concentrations is significant in determining the suitability of a water for irrigation. Alkalinity measurements are used in the interpretation and control of water and wastewater treatment processes. Raw domestic wastewater has an alkalinity less than or only slightly greater than that of the water supply. Properly operating anaerobic digesters typically have supernatant alkalinities in the range of 2,000 to 4,000 mg calcium carbonate ($CaCO_3$)/L.[1]

1. General Discussion

a. Principle: Hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard acid. Alkalinity thus depends on the end-point pH used. For methods of determining inflection points from titration curves and the rationale for titrating to fixed pH end points, see Section 402.1*a*.

For samples of low alkalinity (less than 20 mg $CaCO_3$/L) use an extrapolation technic based on the near proportionality of concentration of hydrogen ions to excess of titrant beyond the equivalence point. The amount of standard acid required to reduce pH exactly 0.30 pH unit is measured carefully. Because this change in pH corresponds to an exact doubling of the hydrogen ion concentration, a

ALKALINITY (continued)

simple extrapolation can be made to the equivalence point.[2,3]

b. End points: When alkalinity is due entirely to hydroxide, carbonate, or bicarbonate content, the pH at the equivalence point of the titration is determined by the concentration of carbon dioxide (CO_2) at that stage. CO_2 concentration depends, in turn, on the total carbonate species originally present and any losses that may have occurred during titration. The following pH values are suggested as the equivalence points for the corresponding alkalinity concentrations as milligrams $CaCO_3$ per liter:

	End point pH	
	Total	Phenolphthalein
Alkalinity, mg $CaCO_3$/L:		
30	5.1	8.3
150	4.8	8.3
500	4.5	8.3
Silicates, phosphates known or suspected	4.5	8.3
Routine or automated analyses	4.5	8.3
Industrial waste or complex system	3.7	8.3

c. Interferences: Soaps, oily matter, suspended solids, or precipitates may coat the glass electrode and cause a sluggish response. Allow additional time between titrant additions to let electrode come to equilibrium. Do not filter, dilute, concentrate, or alter sample.

d. Selection of method: Determine sample alkalinity from volume of standard acid required to titrate a portion to a designated pH taken from ¶ 1*b*. Titrate at room temperature with a properly calibrated pH meter or electrically operated titrator, or use color indicators.

Report alkalinity less than 20 mg $CaCO_3$/L only if it has been determined by the low-alkalinity method of ¶ 4*d*.

Construct a titration curve for standardization of reagents.

Color indicators may be used for routine and control titrations in the absence of interfering color and turbidity and for preliminary titrations to select sample size and strength of titrant (see below).

e. Sample size: See Section 402.1*e* for selection of size sample to be titrated and normality of titrant, substituting 0.02 *N* or 0.1*N* sulfuric (H_2SO_4) or hydrochloric (HCl) acid for the standard alkali of that method. For the low-alkalinity method, titrate a 200-mL sample with 0.02*N* H_2SO_4 from a 10-mL buret.

f. Sampling and storage: See Section 402.1*f*.

2. Apparatus

See Section 402.2.

3. Reagents

a. Sodium carbonate solution, approximately 0.05*N*: Dry 3 to 5 g primary standard Na_2CO_3 at 250 C for 4 hr and cool in a desiccator. Weigh 2.5 ± 0.2 g (to the nearest mg), transfer to a 1-L volumetric flask, fill flask to the mark with distilled water, and dissolve and mix reagent. Do not keep longer than 1 wk.

b. Standard sulfuric acid or hydrochloric acid, 0.1 N: Dilute 3.0 mL conc H_2SO_4 or 8.3 mL conc HCl to 1 L with distilled or deionized water. Standardize against 40.00 mL 0.05 *N* Na_2CO_3 solution, with about 60 mL water, in a beaker by titrating potentiometrically to pH of about 5. Lift out electrodes, rinse into the same beaker, and boil gently for 3 to 5 min under a watch glass cover. Cool to room temperature, rinse cover glass into beaker, and finish titrating to the pH inflection point. Calculate normality:

$$\text{Normality, } N = \frac{A \times B}{53.00 \times C}$$

where:

A = g Na_2CO_3 weighed into 1 L flask,

ALKALINITY (continued)

B = mL Na_2CO_3 solution taken for titration, and
C = mL acid used.

Use measured normality in calculations or adjust to 0.1000N; 1 mL 0.1000 N solution = 5.00 mg $CaCO_3$.

c. Standard sulfuric acid or hydrochloric acid, 0.02N: Dilute 200.00 mL 0.1000N standard acid to 1,000 mL with distilled or deionized water. Standardize by potentiometric titration of 15.00 mL 0.05N Na_2CO_3 according to the procedure of ¶ 3*b*; 1 mL = 1.00 mg $CaCO_3$.

d. Mixed bromcresol green-methyl red indicator solution: Use either the aqueous or the alcoholic solution:

1) Dissolve 100 mg bromcresol green sodium salt and 20 mg methyl red sodium salt in 100 mL distilled water.

2) Dissolve 100 mg bromcresol green and 20 mg methyl red in 100 mL 95% ethyl alcohol or isopropyl alcohol.

e. Methyl orange solution.

f. Phenolphthalein solution, alcoholic.

g. Sodium thiosulfate, 0.1N: See Section 402.3*b*.

4. Procedure

a. Color change: See Section 402.4*a*. The color response of the mixed bromcresol green-methyl red indicator is approximately as follows: above pH 5.2, greenish blue; pH 5.0, light blue with lavender gray; pH 4.8, light pink-gray with bluish cast; and pH 4.6, light pink. Check color changes against reading of a pH meter under the conditions of the titration. Because colors are difficult to distinguish, the method is subject to relatively large operator error.

b. Potentiometric titration curve: Follow the procedure for determining acidity (Section 402.4*b*), substituting the appropriate normality of standard acid solution for standard NaOH, and continue titration to pH 3.7 or lower. Do not filter, dilute, concentrate, or alter the sample.

c. Potentiometric titration to preselected pH: Determine the appropriate end-point pH according to ¶ 1*b*. Prepare sample and titration assembly (Section 402.4*b*). Titrate to the end-point pH without recording intermediate pH values and without undue delay. As the end point is approached make smaller additions of acid and be sure that pH equilibrium is reached before adding more titrant.

d. Potentiometric titration of low alkalinity: For alkalinities less than 20 mg/L titrate 100 to 200 mL according to the procedure of ¶ 4*c*, above, using a 10-mL microburet and 0.02N standard acid solution. Stop the titration at a pH in the range 4.3 to 4.7 and record volume and exact pH. Carefully add additional titrant to reduce the pH exactly 0.30 pH unit and again record volume.

5. Calculations

a. Potentiometric titration to end-point pH:

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times N \times 50{,}000}{\text{mL sample}}$$

where:

A = mL standard acid used and
N = normality of standard acid

or

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times t \times 1{,}000}{\text{mL sample}}$$

where:

t = titer of standard acid, mg $CaCO_3$/mL.

Report pH of end point used as follows: "The alkalinity to pH _____ = _____ mg $CaCO_3$/L" and indicate clearly if this pH corresponds to an inflection point of the titration curve.

b. Potentiometric titration of low alkalinity:

ALKALINITY (continued)

Total alkalinity, mg $CaCO_3$/L

$$= \frac{(2B - C) \times N \times 50{,}000}{\text{mL sample}}$$

where:

B = mL titrant to first recorded pH,
C = total mL titrant to reach pH 0.3 unit lower, and
N = normality of acid.

c. Calculation of alkalinity relationships: The results obtained from the phenolphthalein and total alkalinity determinations offer a means for stoichiometric classification of the three principal forms of alkalinity present in many waters. The classification ascribes the entire alkalinity to bicarbonate, carbonate, and hydroxide, and assumes the absence of other (weak) inorganic or organic acids, such as silicic, phosphoric, and boric acids. It further presupposes the incompatibility of hydroxide and bicarbonate alkalinities. Because the calculations are made on a stoichiometric basis, ion concentrations in the strictest sense are not represented in the results, which may differ significantly from actual concentrations especially at pH >10. According to this scheme:

1) Carbonate (CO_3^{2-}) alkalinity is present when phenolphthalein alkalinity is not zero but is less than total alkalinity.

2) Hydroxide (OH^-) alkalinity is present if phenolphthalein alkalinity is more than half the total alkalinity.

3) Bicarbonate (HCO_3^-) ions are present if phenolphthalein alkalinity is less than half the total alkalinity. These relationships may be calculated by the following scheme, where P is phenolphthalein alkalinity and T is total alkalinity (¶ 1*b*):

Select the smaller value of P or (T-P). Then, carbonate alkalinity equals twice the smaller value. When the smaller value is P, the balance (T-2P) is bicarbonate. When the smaller value is (T-P), the balance (2P-T) is hydroxide. All results are expressed as $CaCO_3$. The mathematical conversion of the results is shown in Table 403:I.

TABLE 403.I. ALKALINITY RELATIONSHIPS*

Result of Titration	Hydroxide Alkalinity as $CaCO_3$	Carbonate Alkalinity as $CaCO_3$	Bicarbonate Concentration as $CaCO_3$
P = 0	0	0	T
P < ½T	0	2P	T − 2P
P = ½T	0	2P	0
P > ½T	2P − T	2(T − P)	0
P = T	T	0	0

*Key: P—phenolphthalein alkalinity; T—total alkalinity.

Alkalinity relationships also may be computed nomographically (see Carbon Dioxide, Section 406). Accurately measure pH, calculate OH^- concentration as milligrams $CaCO_3$ per liter, and calculate concentrations of CO_3^{2-} and HCO_3^- as milligrams $CaCO_3$ per liter from the OH^- concentration, and the phenolphthalein and total alkalinities by the following equations:

$$CO_3^{2-} = 2P - 2[OH^-]$$

$$HCO_3^- = T - 2P + [OH^-]$$

Similarly, if difficulty is experienced with the phenolphthalein end point, or if a check on the phenolphthalein titration is desired, calculate phenolphthalein alkalinity as $CaCO_3$ from the results of the nomographic determinations of carbonate and hydroxide ion concentrations:

$$P = 1/2\,[CO_3^{2-}] + [OH^-]$$

6. Precision and Accuracy

No general statement can be made about precision because of the great variation in sample characteristics. The precision of the titration is likely to be much greater than the uncertainties involved in

sampling and sample handling before the analysis.

In the range of 10 to 500 mg/L, when the alkalinity is due entirely to carbonates or bicarbonates, a standard deviation of 1 mg $CaCO_3$/L can be achieved. Forty analysts in 17 laboratories analyzed synthetic samples containing increments of bicarbonate equivalent to 120 mg $CaCO_3$/L. The titration procedure of ¶ 4*b* was used, with an end point pH of 4.5. The standard deviation was 5 mg/L and the average bias (lower than the true value) was 9 mg/L.[4]

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ANALYTICAL METHOD

HARDNESS

REFERENCE

American Public Health Association, 1980. Standard methods for the examination of water and waste water, 15th ed. APHA, Washington, D.C. pp. 194-199.

314 HARDNESS

Originally, water hardness was understood to be a measure of the capacity of water to precipitate soap. Soap is precipitated chiefly by the calcium and magnesium ions present. Other polyvalent cations also may precipitate soap, but they often are in complex forms, frequently with organic constituents, and their role in water hardness may be minimal and difficult to define. In conformity with current practice, total hardness is defined as the sum of the calcium and magnesium concentrations, both expressed as calcium carbonate, in milligrams per liter.

When hardness numerically is greater than the sum of carbonate and bicarbonate alkalinity, that amount of hardness equivalent to the total alkalinity is called "car-

HARDNESS (continued)

bonate hardness''; the amount of hardness in excess of this is called ''noncarbonate hardness.'' When the hardness numerically is equal to or less than the sum of carbonate and bicarbonate alkalinity, all hardness is carbonate hardness and noncarbonate hardness is absent. The hardness may range from zero to hundreds of milligrams per liter in terms of calcium carbonate, depending on the source and treatment to which the water has been subjected.

1. Selection of Method

Two methods are presented for the determination of hardness. Method A, hardness by calculation, is applicable to all waters and yields the higher accuracy. If a mineral analysis is performed, hardness by calculation can be reported. Method B, the EDTA titration method, measures the calcium and magnesium ions and may be applied with appropriate modification to any kind of water. The procedure described affords a means of rapid analysis.

2. Reporting Results

When reporting hardness, state the method used, for example, ''hardness (calc.)'' or ''hardness (EDTA)''.

314 A. Hardness by Calculation

1. Discussion

The preferred method for determining hardness is to compute it from the results of separate determinations of calcium and magnesium.

2. Calculation

$$\text{Hardness, mg equivalent } CaCO_3/L = 2.497\,[\text{Ca, mg/L}] + 4.118\,[\text{Mg, mg/L}]$$

314 B. EDTA Titrimetric Method

1. General Discussion

a. Principle: Ethylenediaminetetraacetic acid and its sodium salts (abbreviated EDTA) form a chelated soluble complex when added to a solution of certain metal cations. If a small amount of a dye such as Eriochrome Black T or Calmagite is added to an aqueous solution containing calcium and magnesium ions at a pH of 10.0 ± 0.1, the solution becomes wine red. If EDTA is added as a titrant, the calcium and magnesium will be complexed, and when all of the magnesium and calcium has been complexed the solution turns from wine red to blue, marking the end point of the titration. Magnesium ion must be present to yield a satisfactory end point. To insure this, a small amount of complexometrically neutral magnesium salt of EDTA is added to the buffer; this automatically introduces sufficient magnesium and obviates the need for a blank correction.

The sharpness of the end point increases with increasing pH. However, the pH cannot be increased indefinitely because of the danger of precipitating calcium carbonate, $CaCO_3$, or magnesium hydroxide, $Mg(OH)_2$, and because the dye changes color at high pH values. The specified pH of 10.0 ± 0.1 is a satisfactory compromise. A limit of 5 min is set for the duration of

the titration to minimize the tendency toward $CaCO_3$ precipitation.

b. Interference: Some metal ions interfere by causing fading or indistinct end points or by stoichiometric consumption of EDTA. Reduce this interference by adding certain inhibitors before titration. Adding MgCDTA [see 2*b*3)], which is *not* an inhibitor, permits titrating all polyvalent cations listed in Table 314:I but yields erroneously high hardness values in proportion to the concentration of such cations. Because it is nontoxic it is the complexing agent of choice if the interferences are known to be so low that no significant addition to the hardness will result from their titration. When the indicated cations are present in significant concentrations, use the inhibitors listed in Table 314:I. The figures in Table 314:I are intended as a rough guide only and are based on using a 25-mL sample diluted to 50 mL.

TABLE 314:I. MAXIMUM CONCENTRATIONS OF INTERFERENCES PERMISSIBLE WITH VARIOUS INHIBITORS*

Interfering Substance	Max. Interference Concentration *mg/L*	
	Inhibitor I	Inhibitor II
Aluminum	20	20
Barium	†	†
Cadmium	†	20
Cobalt	over 20	0.3
Copper	over 30	20
Iron	over 30	5
Lead	†	20
Manganese (Mn^{2+})	†	1
Nickel	over 20	0.3
Strontium	†	†
Zinc	†	200
Polyphosphate		10

*Based on 25-mL sample diluted to 50 mL.
†Titrates as hardness.

Suspended or colloidal organic matter also may interfere with the end point. Eliminate this interference by evaporating the sample to dryness on a steam bath and heating in a muffle furnace at 550 C until the organic matter is completely oxidized. Dissolve the residue in 20 mL 1*N* hydrochloric acid (HCl), neutralize to pH 7 with 1*N* sodium hydroxide (NaOH), and make up to 50 mL with distilled water; cool to room temperature and continue according to the general procedure.

c. Titration precautions: Conduct titrations at or near normal room temperature. The color change becomes impractically slow as the sample approaches freezing temperature. Indicator decomposition becomes a problem in hot water.

The specified pH may produce an environment conducive to $CaCO_3$ precipitation. Although the titrant slowly redissolves such precipitates, a drifting end point often yields low results. Completion of the titration within 5 min minimizes the tendency for $CaCO_3$ to precipitate. The following three methods also reduce precipitation loss:

1) Dilute the sample with distilled water to reduce the $CaCO_3$ concentration. This simple expedient has been incorporated in the procedure. If precipitation occurs at this dilution of 1 + 1 use modification 2) or 3). Using too small a sample contributes a systematic error due to the buret-reading error.

2) If the approximate hardness is known or is determined by a preliminary titration, add 90% or more of titrant to sample *before* adjusting the pH with buffer.

3) Acidify the sample and stir for 2 min to expel CO_2 *before* pH adjustment. Determine alkalinity to indicate the amount of acid to be added.

HARDNESS (continued)

2. Reagents

a. Buffer solution:

1) Dissolve 16.9 g ammonium chloride (NH_4Cl) in 143 mL conc ammonium hydroxide (NH_4OH). Add 1.25 g magnesium salt of EDTA (available commercially) and dilute to 250 mL with distilled water.

2) If the magnesium salt of EDTA is unavailable, dissolve 1.179 g disodium salt of ethylenediaminetetraacetic acid dihydrate (analytical reagent grade) and 780 mg magnesium sulfate ($MgSO_4 \cdot 7H_2O$) or 644 mg magnesium chloride ($MgCl_2 \cdot 6H_2O$) in 50 mL distilled water. Add this solution to 16.9 g NH_4Cl and 143 mL conc NH_4OH with mixing and dilute to 250 mL with distilled water. To attain the highest accuracy, adjust to exact equivalence through appropriate addition of a small amount of EDTA or $MgSO_4$ or $MgCl_2$.

Store Solution 1) or 2) in a plastic or resistant-glass container for no longer than 1 month. Stopper tightly to prevent loss of ammonia (NH_3) or pickup of carbon dioxide (CO_2). Dispense buffer solution by means of a bulb-operated pipet. Discard buffer when 1 or 2 mL added to the sample fails to produce a pH of 10.0 ± 0.1 at the titration end point.

3) Satisfactory alternate "odorless buffers" also are available commercially. They contain the magnesium salt of EDTA and have the advantage of being relatively odorless and more stable than the NH_4Cl-NH_4OH buffer. They usually do not provide as good an endpoint as NH_4Cl-NH_4OH because of slower reactions and they may be unsuitable when this method is automated. Prepare one of these buffers by mixing 55 mL conc HCl with 400 mL distilled water and then, slowly and with stirring, adding 300 mL 2-aminoethanol (free of aluminum and heavier metals). Add 5.0 g magnesium salt of EDTA and dilute to 1 L with distilled water.

b. Complexing agents: For most waters no complexing agent is needed. Occasionally water containing interfering ions requires the addition of an appropriate complexing agent to give a clear, sharp change in color at the end point. The following are satisfactory:

1) *Inhibitor I:* Adjust acid samples to pH 6 or higher with buffer or 0.1*N* NaOH. Add 250 mg sodium cyanide (NaCN) in powder form to the sample. Add sufficient buffer to adjust to pH 10.0 ± 0.1 (CAUTION: *NaCN is extremely poisonous. Take extra precautions in its use.* Flush solutions containing this inhibitor down the drain with large quantities of water after insuring that no acid is present to liberate volatile poisonous hydrogen cyanide.)

2) *Inhibitor II:* Dissolve 5.0 g sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) or 3.7 g $Na_2S \cdot 5H_2O$ in 100 mL distilled water. Exclude air with a tightly fitting rubber stopper. This inhibitor deteriorates through air oxidation. It produces a sulfide precipitate that obscures the end point when appreciable concentrations of heavy metals are present. Use 1 mL in ¶ 3*b* below.

3) *MgCDTA:* Magnesium salt of 1, 2-cyclohexanediaminetetraacetic acid. Add 250 mg per 100 mL sample and dissolve completely before adding buffer solution. Use this complexing agent to avoid using toxic or odorous inhibitors when interfering substances are present in concentrations that affect the end point but will not contribute significantly to the hardness value.

Commercial preparations incorporating a buffer and a complexing agent are available. Such mixtures must maintain pH 10.0 ± 0.1 during the titration and give a clear, sharp end point when the sample is titrated.

c. Indicators: Many types of indicator solutions have been advocated and may be used if the analyst demonstrates that they yield accurate values. The prime difficulty with indicator solutions is deterioration with aging, giving indistinct end points. For example, alkaline solutions of Erio-

HARDNESS (continued)

chrome Black T are sensitive to oxidants and aqueous or alcoholic solutions are unstable. In general, use the least amount of indicator providing a sharp end point. It is the analyst's responsibility to determine individually the optimal indicator concentration.

1) *Eriochrome Black T:* Sodium salt of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid; No. 203 in the Color Index. Dissolve 0.5 g dye in 100 g 2,2',2''-nitrilotriethanol (also called triethanolamine) or 2-methoxymethanol (also called ethylene glycol monomethyl ether). Add 2 drops per 50 mL solution to be titrated. Adjust volume if necessary.

2) *Calmagite:* 1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid. This is stable in aqueous solution and produces the same color change as Eriochrome Black T, with a sharper end point. Dissolve 0.10 g Calmagite in 100 mL distilled water. Use 1 mL per 50 mL solution to be titrated. Adjust volume if necessary.

3) Indicators 1 and 2 can be used in dry powder form if care is taken to avoid excess indicator. Prepared dry mixtures of these indicators and an inert salt are available commercially.

If the end point color change of these indicators is not clear and sharp, it usually means that an appropriate complexing agent is required. If NaCN inhibitor does not sharpen the end point, the indicator probably is at fault.

d. Standard EDTA titrant, 0.01M: Weigh 3.723 g analytical reagent-grade disodium ethylenediaminetetraacetate dihydrate, also called (ethylenedinitrilo)-tetraacetic acid disodium salt (EDTA), dissolve in distilled water, and dilute to 1,000 mL. Standardize against standard-calcium solution (¶ 2*e*) as described in ¶ 3*b* below.

Because the titrant extracts hardness-producing cations from soft-glass containers, store in polyethylene (preferable) or borosilicate glass bottles. Compensate for gradual deterioration by periodic restandardization and by using a suitable correction factor.

e. Standard calcium solution: Weigh 1.000 g anhydrous $CaCO_3$ powder (primary standard or special reagent low in heavy metals, alkalis, and magnesium) into a 500-mL erlenmeyer flask. Place a funnel in the flask neck and add, a little at a time, 1 + 1 HCl until all $CaCO_3$ has dissolved. Add 200 mL distilled water and boil for a few minutes to expel CO_2. Cool, add a few drops of methyl red indicator, and adjust to the intermediate orange color by adding 3*N* NH_4OH or 1 + 1 HCl, as required. Transfer quantitatively and dilute to 1,000 mL with distilled water; 1 mL = 1.00 mg $CaCO_3$.

f. Sodium hydroxide, NaOH, 0.1 *N*.

3. Procedure

a. Pretreatment of polluted water and wastewater samples: Follow the procedure described in Section 302E or F.

b. Titration of sample: Select a sample volume that requires less than 15 mL EDTA titrant and complete titration within 5 min, measured from the time of buffer addition.

Dilute 25.0 mL sample to about 50 mL with distilled water in a porcelain casserole or other suitable vessel. Add 1 to 2 mL buffer solution. Usually 1 mL will be sufficient to give a pH of 10.0 to 10.1. The absence of a sharp end-point color change in the titration usually means that an inhibitor must be added at this point in the procedure (¶ 2*b* et seq.) or that the indicator has deteriorated.

Add 1 to 2 drops indicator solution or an appropriate amount of dry-powder indicator formulation [¶ 2*c*3)]. Add standard EDTA titrant slowly, with continuous stirring, until the last reddish tinge disappears from the solution. Add the last few drops at 3- to 5-sec intervals. At the end point the solution normally is blue. Daylight or a daylight fluorescent lamp is highly recom-

mended because ordinary incandescent lights tend to produce a reddish tinge in the blue at the end point.

If sufficient sample is available and interference is absent, improve accuracy by increasing sample size, as described in ¶ 3*c* below.

c. Low-hardness sample: For ion-exchanger effluent or other softened water and for natural waters of low hardness (less than 5 mg/L), take a larger sample, 100 to 1,000 mL, for titration and add proportionately larger amounts of buffer, inhibitor, and indicator. Add standard EDTA titrant slowly from a microburet and run a blank, using redistilled, distilled, or deionized water of the same volume as the sample, to which identical amounts of buffer, inhibitor, and indicator have been added. Subtract volume of EDTA used for blank from volume of EDTA used for sample.

4. Calculation

$$\text{Hardness (EDTA) as mg } CaCO_3/L = \frac{A \times B \times 1{,}000}{\text{mL sample}}$$

where:

A = mL titration for sample and
B = mg $CaCO_3$ equivalent to 1.00 mL EDTA titrant.

5. Precision and Accuracy

A synthetic sample containing 610 mg/L total hardness as $CaCO_3$ contributed by 108 mg Ca/L and 82 mg Mg/L, and the following supplementary substances: 3.1 mg K/L, 19.9 mg Na/L, 241 mg Cl/L, 0.25 mg NO_2^--N/L, 1.1 mg NO_3^--N/L, 259 mg sulfate/L, and 42.5 mg total alkalinity/L (contributed by $NaHCO_3$) in distilled water was analyzed in 56 laboratories by the EDTA titrimetric method with a relative standard deviation of 2.9% and a relative error of 0.8%.

314 C. Bibliography

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ANALYTICAL METHOD

TOTAL KJELDAHL NITROGEN, AMMONIA

REFERENCE

Michigan State University Limnological Research Laboratory, 1984. Unpublished memo on total Kjeldahl nitrogen determination. East Lansing, MI. 5 pp.

This method estimates the total organic nitrogen plus the ammonia nitrogen in natural water samples. The classical Kjeldahl digestion converts the organic nitrogen to ammonium nitrogen. The sample is digested with sulfuric acid containing potassium sulfate to raise the digestion temperature and mercuric oxide, a required catalyst. The ammonia is estimated colorimetrically after steam distillation by Nesslerization. The analytical procedure described below is an application of methods for natural waters described by Golterman *et al.* (1978) and U.S. Environmental Protection Agency (1979) to the semi-micro Kjeldahl digestion and distillation apparatus of Knotes Scientific Glassware (Vineland, NJ). Note that this same glassware can be used to determine ammonia nitrogen separately if the digestion step is omitted. In addition, the Kjeldahl method for nitrogen and its compounds in biological materials and the apparatus provided by Kontes permits nitrogen determinations to be made on a wide range of sample matrices, i.e., soils, soil extracts, feeds, fertilizers, plant and animal tissues, rainwater, fresh and sea waters.

EQUIPMENT

Digestion apparatus, Kjeldahl, rotary, Kontes (Cat. No. K-551000-0000) (Kontes Scientific Glassware, Spruce Street, P.O. Box 729, Vineland, NJ 08360)
Transformer, variable voltage, 750 Watt, input: to match voltage at project site; output: 115 V, 50/60 Hz.

GLASSWARE

Distillation apparatus, Kjeldahl, Kontes (Cat. No. K-551100-0000) (1 or 2 units)
Flasks, erlenmeyer, 50 ml, with ground glass stoppers
Flask, Kjeldahl digestion/distillation, 100 ml, Kontes (Cat. No. K-551600-0100)
Fume hood, Kjeldahl digestion, Kontes (Cat. No. K-551001-0030), with 30 mm dia. holes to accept K-551600 30 and 100 ml flasks. (Accessory permits Kjeldahl digestion outside of a laboratory fume hoods, requires proximity to a faucet for water aspirator)

SUPPLIES

Granules, micro, for smooth boiling, 10 mesh, plain, Hengar (Cat. No. 136-CC) (American Scientific Products Cat. No. N3916-2)
Grease, silicone, high vacuum, Dow Corning

TOTAL KJELDAHL NITROGEN, AMMONIA (Continued)

Aspirator, water (Nalgene Cat. No. 6140, pump, vacuum) (required for use of K-551001-0030 fume hood)
Protector, Hot-Hand, silicone rubber (Fisher Scientific Cat. No. 11-394-300)
Glasses, safety
Ring stands, 3-prong clamps, clamp holders (for distillation glassware)
Tubing, laboratory, Tygon, 5/8 in. ID, 1/16 in. wall and 1/4 in., 1/16 in. wall

GLASSWARE PREPARATION

1. The glassware is soaked overnight in a solution of Liqui-Nox detergent and is rinsed with tap water.
2. The glassware is soaked in 0.1 N HCl until ready for use. Glassware to be used is rinsed with distilled water with a final rinse with deionized distilled water for low-level Kjeldahl-N or ammonia-N determinations. The glassware is best protected from pick-up of ammonia from the laboratory atmosphere by storage in the 0.1 N HCl acid bath.

REAGENTS

Mercuric sulfate solution:

Dissolve 8 g of mercuric oxide, red, HgO, in 50 ml of 1:4 sulfuric acid (10 ml concentrated sulfuric acid : 40 ml deionized distilled water) and dilute to 100 ml with deionized distilled water.

Sulfuric acid-mercuric sulfate-potassium sulfate solution:

Dissolve 133.5 g of potassium sulfate, K_2SO_4, in 650 ml of deionized distilled water and 200 ml of concentrated sulfuric acid. Add 25 ml of mercuric sulfate solution and dilute to 1 liter.

Sodium hydroxide-sodium thiosulfate solution:

Dissolve 500 g of sodium hydroxide, NaOH, and 16 g of anhydrous sodium thiosulfate, $Na_2S_2O_3$, in deionized distilled water and dilute to 1 liter.

Boric acid solution:

Dissolve 20 g of boric acid, H_3BO_3 in deionized distilled water and dilute to 1 liter.

Nessler Reagent:

Dissolve 100 g of mercuric iodide, HgI_2, (Fisher M-166 or equivalent) and 70 g potassium iodide, KI, in a small volume of deionized distilled water. Add this mixture slowly with stirring, to a cooled solution of 160 g of sodium hydroxide in 500 ml of deionized distilled water. Dilute this mixture to 1 liter. Allow to stand for 24-48 hours and decant off the clear solution for use. This

TOTAL KJELDAHL NITROGEN, AMMONIA (Continued)

solution is stable for at least 1 year if stored in a pyrex bottle out of direct sunlight.

Nitrogen stock solution, 1000 microgram ml^{-1} NH_3-N:

Dissolve 3.819 g of ammonium chloride, NH_4Cl, in deionized distilled water and dilute to 1000 ml. Preserve with 1 ml of chloroform.

Nitrogen standard solution, 20 microgram ml^{-1} NH_3-N:

Dilute 20.0 ml of nitrogen stock solution with deionized distilled water to 1000 ml.

Nitrogen working standard solutions:

Using 200 ml volumetric flasks, dilute the following volumes of the 20 microgram ml^{-1} NH_3-N standard solution to volume with deionized distilled water. Prepare fresh for each set of samples.

ml of nitrogen standard solution diluted to 200 ml	mg NH_3-N / 50 ml
0.0	0.00 (Blank)
2.0	0.01
10.0	0.05
20.0	0.10
50.0	0.25
100.0	0.50

DIGESTION PROCEDURE

1. Transfer 50.0 ml of a well homogenized sample or an aliquot diluted to 50.0 ml into a 100 ml Kjeldahl flask.
2. Add 10 ml of sulfuric acid-mercuric sulfate-potassium sulfate solution and 3-5 Hengar granules. The Hengar granules are soaked in 0.1 N HCl overnight, rinsed with deionized distilled water, dried and stored closed to the atmosphere.
3. Place the prepared samples on the digestion apparatus in a laboratory fume hood or outfit the digestion apparatus with the accessory glass fume hood (Kontes). This glass fume hood is attached to a water aspirator.
4. The heat controls on the digestion apparatus are set near the "high" to boil off the water (about 1.5-2.0 hours).

TOTAL KJELDAHL NITROGEN, AMMONIA (Continued)

5. When white, cloudy fumes of SO_3 are given off, increase the heat control setting to "high" and begin timing the digestion.
6. Allow the digestion to proceed for 40 minutes or until the digestion is complete. During this time, rotate and gently swirl the flasks at intervals to dislodge any material adhering to the walls of the flask and to bring it into contact with the acid. The digestion is complete when the digest becomes clear, colorless or pale yellow and there are no particles of charred material remaining.
7. After the flasks have cooled, add about 30 ml of deionized distilled water to each flask to dissolve the cake of material. It may be necessary to warm the flask gently to complete the dissolution.
8. Digest the standards and, at least, 2 blanks by the same procedure.

DISTILLATION PROCEDURE

1. Assemble the distillation apparatus according to the manufacturer's instructions.
2. Fill the steam generator flask with deionized distilled water. Turn on the heating element and the condenser cooling water. Adjust the velocity at which the steam in generated by changing the output voltage of the variable voltage transformer.
3. The distillation apparatus should be pre-steamed before use. Attach a clean Kjeldahl flask with about 30 ml of deionized distilled water and 10 ml of sodium hydroxide-sodium thiosulfate solution. Adjust the vent plug on the steam generator flask and the three-way steam control stopcock (stopcock B) on the distillation head to allow steam to enter the flask. The entrance tube stopcock (stopcock A) should be closed. After 10 minutes, begin collecting the effluent and testing for ammonia by the addition of the Nessler Reagent. Continue steaming the apparatus until the effluent is ammonia-free. Repeat this procedure if the apparatus is idle for more than 4 hours.
4. Adjust the vent plug on the steam generator flask to the open position to allow the generated steam to exit through the vent tube. Close both stopcocks (A and B) on the distillation head.
5. Add 5 ml of boric acid solution into a 50 ml erlenmeyer flask and set the ground glass stopper aside. Place the flask with the tip of the extension tube of the condenser below the level of the boric acid solution. The erlenmeyer flask is considered in the "up" position when the condenser tip is submerged in the boric acid solution.
6. The distillation can be carried out directly from the Kjeldahl digestion flask. Attach a digestion flask containing a sample to the distillation head. Dispense 10 ml of sodium hydroxide-sodium thiosulfate solution into the reservoir above the entrance tube stopcock (stopcock A). Open the entrance tube stopcock and allow the sodium hydroxide-sodium thiosulfate solution to slowly enter the digestion flask without mixing. Close the stopcock immediately. Do NOT allow steam to enter the Kjeldahl flask before or during the addition of the sodium hydroxide-sodium thiosulfate solution, otherwise a violent bumping may occur when that alkaline solution is added.

TOTAL KJELDAHL NITROGEN, AMMONIA (Continued)

7. Adjust the three-way steam control stopcock (stopcock B) to permit steam to enter the Kjeldahl flask thru the steam entrance tube. Adjust the vent control plug on the steam generator to the closed position to allow the generated steam to go to the distillation head.
8. Distill about 35 ml of distillate at a rate of 6-10 ml min^{-1} with the erlenmeyer flask in the up position. Lower the erlenmeyer flask to the "down" position so that the tip of the extension tube of the condenser is above the level of the boric acid. This will permit the distillate to wash the tip of the tube. Collect about 10 ml of additional distillate. It may be necessary to adjust the rate of distillation or the volumes collected in the "up" and "down" position to insure maximum recovery of ammonia with a minimum distillation time.
9. Dilute the collected distillate to 50.0 ml and replace the ground-glass stopper on the flask. Set the flasks aside until all samples, standards and blanks have been distilled.

NESSLERIZATION

1. To a 50.0 ml sample or an aliquot diluted to 50.0 ml, add 1 ml of Nessler Reagent and mix.
2. After 20 minutes read the absorbance at 425 nm against a deionized distilled water reference. Subtract the mean absorbance of the blanks from the absorbances of the samples and standards. From the values obtained prepare a standard curve of absorbance vs. mg NH_3-N.
3. Calculate the TKN in the original sample as follows:

$$\text{TKN, mg } l^{-1} = \frac{A\ (1000)\ B}{C\ (D)}$$

where:
A = mg NH_3-N read from standard curve.
B = ml of total distillate collected including the boric acid.
C = ml of distillate taken for Nesslerization.
D = ml of original sample taken for digestion.

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U.S. Environmental Protection Agency, 1979. Methods for Chemical Analysis of Water and Wastes. Office of Research and Development, EPA-600/4-79-020, Cincinnati, OH. pp. 351.1-351.3.

ANALYTICAL METHOD

NITRATE

REFERENCE

Michigan State University Limnological Research Laboratory, 1984. Unpublished memo on nitrate-nitrite nitrogen determination. East Lansing, MI. 5 pp.

Nitrate is quantitatively reduced to nitrite by a cadmium-copper couple in an alkaline buffered solution (pH 8.5). This nitrite and any nitrite originally present in the sample is reacted with sulphanilamide in a strongly acidic medium to form a diazonium compound which reacts quantitatively with N-(1-napthyl)ethyl-enediamine dihydrochloride to form a strongly colored azo compound. The procedure described below is an application of the methods of the American Public Health Association (1980), Strickland and Parsons (1972) and Wood *et al.* (1967). If separate, rather than combined nitrate-nitrite values are required, nitrite standards can be prepared and nitrite can be determined without the reduction step. Nitrate is obtained by subtraction.

Sensitivity: 0.01 mg NO_3-N l^{-1}
Working range: 0.01 to 1.0 mg NO_3-N l^{-1}

GLASSWARE:

Flasks, erlenmeyer, 125 ml, pyrex (sufficient number for blanks, standards and samples)
Cylinders, graduated, 50 ml
Pipets, transfer, 2, 5, 10, 20, 25 and 75 ml
Reduction columns (custom construction by glassblowing shop or from Fisher Scientific, Cat. No. 13-942) (see: American Public Health Association (1980) Standard Methods, 15th ed., p. 371, for construction details)
Ring stand and buret holders

GLASSWARE PREPARATION:

1. Glassware is soaked overnight in a solution of Liqui-Nox detergent and is rinsed with tap water.
2. The glassware is soaked in 1 N hydrochloric acid for a minimum of 15 minutes and is rinsed with deionized distilled water.
3. After the glassware has dried in an inverted position, it is capped with aluminum foil to protect the interior from dust.
4. Chromic acid cleaning solution may be used to clean this glassware initially or as necessary.

NOTE: Do not use nitric acid to clean this glassware.

NITRATE (continued)

REAGENTS:

Cadmium metal:

Cadmium course powder, for filling reductors, EM Reagents (Cat. No. 2001) (particle size: 0.3-1.5 mm) (available from: VWR Scientific, Cat. No. EM-2001-2, 250 g/btl.)

Color Reagent:

Dissolve 10 g of sulfanilamide (Baker V153, Fisher O-4525 or equivalent) and 1 g of N-(1-naphthyl)ethylenediamine dihydrochloride, reagent ACS grade (Kodak 4835 or equivalent) in a mixture of 100 ml concentrated phosphoric acid and 800 ml deionized distilled water and dilute to 1 liter. Store in a low actinic glass bottle (dark brown or deep red bottle). Renew once a month or immediately if reagent develops a dark brown coloration.

Ammonium Chloride-EDTA Solution:

Dissolve 13 g of ammonium chloride and 1.7 g of disodium ethylenediamine tetracetate (EDTA) in 900 ml of deionized distilled water. Adjust the pH to 8.5 with concentrated ammonium hydroxide and dilute to 1 liter.

Dilute Ammonium Chloride-EDTA Solution:

Dilute 300 ml of ammonium chloride-EDTA solution to 500 ml with deionized distilled water.

Copper Sulfate Solution, 2%:

Dissolve 20 g of cupric sulfate penta-hydrate ($CuSO_4 \cdot 5H_2O$) in 500 ml of deionized distilled water and dilute to 1 liter.

Hydrochloric Acid, 6 N:

Carefully dilute 50 ml of concentrated hydrochloric acid to 100 ml with deionized distilled water.

Nitrate Stock Standard Solution, 1000 microgram ml^{-1} NO_3-N:

Dissolve 7.218 g of potassium nitrate, KNO_3, (dried for 1 hour at 110°C) in deionized distilled water and dilute to 1000 ml. Preserve with 2 ml of chloroform per liter. This solution is stable for at least 6 months.

Nitrate Intermediate Standard Solution, 10 microgram ml^{-1} NO_3-N:

Dilute 10.0 ml of nitrate stock standard solution (1000 microgram ml^{-1}) to 1000 ml with deionized distilled water. Prepare fresh for each new set of samples.

NITRATE (continued)

Nitrite Stock Standard Solution, 1000 microgram ml^{-1} NO_2-N:

Dissolve 4.926 g of sodium nitrite, $NaNO_2$, in deionized distilled water and dilute to 1000 ml. Preserve with 2 ml chloroform per liter. This solution is stable for 2 months.

Nitrite Intermediate Standard Solution, 10 microgram ml^{-1} NO_2-N:

Dilute 10.0 ml of nitrite stock solution (1000 microgram ml^{-1}) to 1000 ml with deionized distilled water. Prepare fresh for each new set of samples.

REDUCTION COLUMN PREPARATION:

1. Prepare cadmium for all columns in one batch. Prepare enough cadmium to fill all columns to be used to a depth of 20 cm. Four reduction columns are a convenient number for use.
2. Wash the cadmium with 100-150 ml of 6 N hydrochloric acid and rinse well with distilled water until the wash is no longer acid (pH greater than 5). Decant as much of the final liquid as possible.
3. Stir the cadmium in 100-150 ml of the 2% cupric sulfate solution until all the blue color has disappeared from the solution and brown, semi-colloidal copper particles begin to appear in the supernatant liquid. After the addition of the cupric sulfate solution, the cadmium particles should never be exposed to the air. Wash the cadmium with distilled water until the brown copper particles are removed.
4. Insert a small plug made of fine copper turnings into the bottom of each reduction column.
5. Fill the column with dilute ammonium chloride solution and pour in sufficient cadmium to produce a column 20 cm in length. Add the cadmium a little at a time, pausing to tap the column after each addition to make sure that the cadmium is well settled. Wash the column thoroughly with the dilute ammonium chloride solution. During this washing procedure, check the flow rate of the packed column. The flow rate should be such that 100 ml of solution takes between 8 and 12 minutes to pass through the column. If the 100 ml of solution takes less than 8 minutes, slow the column by restricting the outlet by adding a short piece of tygon tubing to the outlet and using a clamp-screw compressor (nylon, Bel-Art Products, Cat. No. F18225, doz.). If the flow takes more than 12 minutes, loosen the plug at the bottom of the column. Add a plug of copper turnings to the top of the column to prevent cadmium particles from being washed into the reservoir when solutions are added to the column. When the columns are not in use, the cadmium must be completely covered with dilute ammonium chloride solution.

NITRATE (continued)

PREPARATION OF WORKING STANDARDS

Nitrate and/or Nitrite Standard Solutions:

Using 200 ml volumetric flasks, dilute the following volumes of the 10 microgram ml^{-1} intermediate standard solution. Prepare fresh for each new set of samples.

ml of 10 microgram ml^{-1} intermediate standard solution diluted to 200 ml	mg NO_3-NO_2-N l^{-1} or mg NO_2-N l^{-1}
0.0	0.00 (Blank)
2.0	0.10
5.0	0.25
10.0	0.50
15.0	0.75
20.0	1.00

PROCEDURE

1. Activate the columns by passing thru each column 100 ml of solution composed of 25 ml of 1.0 mg NO_3-NO_2-N standard and 75 ml of ammonium chloride-EDTA solution.
2. Filter the samples through a glass fiber filter or 0.45 micrometer membrane filter to remove suspended matter. This avoids potential clogging of the top of the columns.
3. To a 25.0 ml sample, add 75 ml of the ammonium chloride-EDTA solution and mix. The pH of this prepared sample should be 8.5 after the addition of the ammonium chloride-EDTA solution (Note 1).
4. Pour about 5 ml of the sample into the reduction column and allow it to pass through the column. This insures that the liquid at the top of the column has the same composition as the sample avoiding possible dilution. Add the balance of the sample to the column.
5. Discard the first 35 ml of column effluent.
6. Collect the next 50 ml in a graduated cylinder and return that volume to the original sample flask.
7. Collect the remaining 10-15 ml of sample (until flow from the column ceases) in the graduated cyclinder and save temporarily. If the concentration of the sample exceeds 1.0 mg NO_3-NO_2-N l^{-1}, this remaining volume of reduced sample can be used to make an appropriate dilution. Using a second graduated cylinder for this column, begin passing a new sample through the column (step 4). This will save time while waiting for the color development of the previous sample.

NITRATE (continued)

8. Within 15 minutes after passage of the sample through the column, add 2.0 ml of color reagent to 50.0 ml of sample. Allow 10 minutes for complete color development. Measure the absorbance at 540 nm against deionized distilled water. The color is stable for at least 2 hrs.
9. If the absorbance of a sample appears to exceed that of the highest standard (1.00 mg NO_3-NO_2-N l^{-1}), make a dilution with deionized distilled water of a portion of the reduced sample saved. Add color reagent and read the absorbance after the color development period. Record the dilution used.
10. Carry out the reduction of the nitrate standards and a blank exactly as described for the samples. A complete set of standards and a blank should be run thru each column in use.
11. At least one nitrite standard should be compared to a reduced nitrate standard at the same concentration (preferably the 1.0 mg NO_3-N l^{-1} standard) to verify the efficiency of the reduction columns. The efficiency should be greater than 95%. If not, repack the column according to the reduction column preparation procedure given above. That same nitrite standard may also be passed through the column to check on possible reduction past the NO_2 oxidation state. This is especially important if the nitrite concentration exceeds 30% of the nitrate-nitrite concentration.
12. Subtract the mean absorbance of all blanks run from the absorbances of the samples and standards. In addition, subtract any absorbance at 540 nm due to humic and fulvic substances from the samples absorbances (Note 2). From the values obtained, prepare a standard curve of absorbance vs. mg NO_3-NO_2-N l^{-1}. If nitrite is not determined separately, report the results as mg NO_3-NO_2-N l^{-1}.

NOTES:

1. If samples are acidified for transport or storage, they should be brought to a pH of 7-8 with concentrated ammonium hydroxide (Keep this reagent away from ammonia or kjeldahl nitrogen glassware and apparatus). The strong acid added for preservation will exceed the base buffering capacity of the ammonium chloride-EDTA solution. The sample solution pH must be 8.5 or greater for reduction to take place in the column. Samples preserved with acid cannot be later analyzed for nitrite since the acid treatment alters the proportions of nitrate and nitrite in the sample. However, a nitrate-nitrite-nitrogen determination can be made.
2. If the samples are visibly brownish in their natural color due to humic or fulvic acids, the absorbance of each sample must be measured separately and subtracted from the absorbance of that sample after color development.

REFERENCES:

American Public Health Association, 1980. Standard methods for the examination of water and wastewater, 15th ed. APHA, Washington, D.C. pp. 350-388.

NITRATE (continued)

Strickland, J.D.H. and T. R. Parsons, 1972. A practical handbook of seawater analysis. Bull. 167 (2nd edition). Fish. Res. Bd. Canada, Ottawa, 310 p.

Wood, E. E., F.A.J. Armstrong and F. A. Richards, 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. Mar. Biol. Assoc. U.K. 47:23-31.

*a. Reduction column:** Purchase or construct the column (Figure 418:1) from a 100-mL volumetric pipet by removing the top portion. The column also can be constructed from two pieces of tubing joined end to end: join a 10-cm length of 3-cm-ID tubing to a 25-cm length of 3.5-mm-ID tubing.

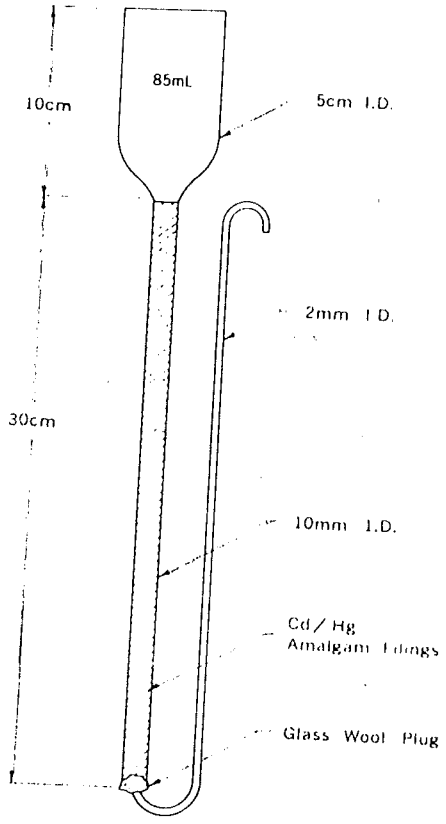

Figure 418:1: Reduction column.

ANALYTICAL METHOD

CHLOROPHYLL a, b, and c

REFERENCE

American Public Health Association, 1980. Standard methods for the examination of water and waste water, 15th ed. APHA, Washington, D.C. pp. 950-954.

1002 G. Chlorophyll

The characteristic algal pigments are chlorophylls, xanthophylls, and carotenes. The three chlorophylls commonly found in planktonic algae are chlorophylls *a*, *b*, and *c*. Chlorophyll *a* constitutes approximately 1 to 2% of the dry weight of organic material in all planktonic algae and is the preferred indicator for algal biomass estimates. Chlorophyll content of cells varies with species or taxonomic groups and is affected by age, growth rate, light, and nutrient conditions.[31]

Two methods for determining chlorophyll *a* in phytoplankton are available, the spectrophotometric[9,32,33] and fluorometric.[9,31,35,36] The latter is more sensitive, requires less sample, and has been adapted for in vivo measurements.[37] A specific method for chlorophyll *c*, more sensitive than the trichromatic method described below, especially for samples of low pigment content, is available[38] but is not included here.

Pheophytin *a*, a common degradation product of chlorophyll *a*, can interfere with the determination of chlorophyll *a* because it absorbs light and fluoresces in the same region of the spectrum as chlorophyll *a* and, if present, may cause errors in chlorophyll *a* values.[39,40] When measuring chlorophyll *a* measure also the concentration of pheophytin *a*. The ratio of chlorophyll *a* to pheophytin *a* serves as a good indicator of physiological condition of phytoplankton. Another useful water quality indicator is the ratio of biomass to chlorophyll *a* (Autotrophic Index). In unpolluted waters the plankton population is composed largely of autotrophic (food-producing), chlorophyllous algae. As waters become organically enriched, the proportion of heterotrophic (consuming), nonchlorophyllous organisms, such as the filamentous bacteria and stalked protozoa, increases. The Autotrophic Index (AI) is a means of relating changes in plankton species composition to changes in water quality.[41] Calculate as:

$$AI = \frac{\text{Biomass (ash-free wt of organic matter), mg/m}^3}{\text{Chlorophyll } a\text{, mg/m}^3}$$

Normal AI values range from 50 to 200. Larger AI values (above 200) indicate poor water quality.

1. Spectrophotometric Determination of Chlorophyll *a*, *b*, and *c* (Trichromatic Method)

The pigments are extracted from the plankton concentrate with aqueous acetone and the optical density (absorbance)

CHLOROPHYLL a, b, AND c (Continued)

of the extract is determined with a spectrophotometer. When immediate pigment extraction is not possible (as described below), the samples may be stored frozen for as long as 30 days if kept in the dark. The ease with which the chlorophylls are removed from the cells varies considerably with different algae. To achieve complete extraction of the pigments, it is necessary usually to disrupt the cells mechanically with a tissue grinder.

a. Equipment and reagents:

1) *Spectrophotometer,* with a narrow band (0.5 to 2.0 nm) because the chlorophyll absorption peak is relatively narrow. At a spectral band width of 20 nm the chlorophyll *a* concentration may be underestimated by as much as 40%.

2) *Cuvettes* with 1 cm, 4 cm, and 10 cm path length.

3) *Clinical centrifuge.*

4) *Tissue grinder.** Successfully macerating glass fiber filters in tissue grinders with grinding tube and pestle of conical design may be difficult. Preferably use grinding tubes and pestles with rounded bottoms.

5) *Centrifuge tubes,* 15 mL, graduated, screw-cap.

6) *Filtration equipment,* filters, membrane (0.45 μm porosity, 47-mm diam) or glass fiber (GF/C or GF/A, 4.5-cm diam); vacuum pump.

7) *Magnesium carbonate suspension:* Add 1.0 g finely powdered $MgCO_3$ to 100 mL distilled water.

8) *Aqueous acetone solution:* Mix 90 parts acetone (reagent grade BP 56 C) with 10 parts water (v/v).

b. Procedure:

1) Concentrate the sample by centrifuging or filtering (membrane or glass fiber filter). Add 0.2 mL $MgCO_3$ suspension before centrifuging or during the final phase of filtering. Store concentrated samples frozen in a desiccator in the dark if extraction is delayed. Use glassware and cuvettes that are clean and acid-free.

2) Place sample in a tissue grinder, cover with 2 to 3 mL 90% aqueous acetone solution, and macerate. Use TFE/glass grinder for a glass-fiber filter and glass/glass grinder for a membrane filter.

3) Transfer sample to a screw-cap centrifuge tube, rinse grinder with a few milliliters 90% aqueous acetone, and add the rinse to the extraction slurry. Adjust total volume to a constant level, 5 to 10 mL with 90% aqueous acetone. Use solvent sparingly and avoid excessive dilution of pigments. Steep samples overnight at 4 C in the dark.

4) Clarify extract by centrifuging in closed tubes for 20 min at 500 *g*. Decant the clarified extract into a clean, calibrated, 15-mL, screw-cap centrifuge tube and measure the total volume of extract.

5) Transfer extract to a 1-cm cuvette and measure optical density (OD) at 750, 663, 645, and 630 nm. Choose a cell path length or dilution to provide an OD663 greater than 0.2 and less than 1.0.

c. Calculations: Use the optical density readings at 663, 645, and 630 nm for the determination of chlorophyll *a*, *b*, and *c*, respectively. The OD reading at 750 nm serves as a correction for turbidity. Subtract this reading from each of the pigment OD values of the other wavelengths before using them in the equations below. Because the OD of the extract at 750 nm is very sensitive to changes in the acetone-to-water proportions, adhere rigidly to the 90 parts acetone: 10 parts water (v/v) formula for pigment extraction.

To avoid using the 750-nm reading, clear the pigment solution by centrifuging for 20 min at 1,000 *g* and use a light path limited to 1 cm. However, when the possibility of resuspending sediment exists, make the 750-nm reading. This is commonly a problem when using glass fiber filters

*Kontes Glass Company, Vineland, N.J. 08360: Glass/glass grinder, Model No. 885500; Glass/teflon grinder, Model No. 886000; or equivalent.

CHLOROPHYLL a, b, AND c (Continued)

and a centrifuge with a slant head. To reduce this difficulty use a swing-out centrifuge head and additional amounts of $MgCO_3$ added immediately before centrifuging.

1) Calculate the concentrations of chlorophyll *a*, *b*, and *c* in the extract by inserting the corrected optical densities in following equations:

a) Chl *a*, mg/L = 11.64 (OD663) − 2.16 (OD645) + 0.10 (OD630)

b) Chl *b*, mg/L = 20.97 (OD645) − 3.94 (OD663) − 3.66 (OD630)

c) Chl *c*, mg/L = 54.22 (OD630) − 14.81 (OD645) − 5.53 (OD663)

where:

OD663, OD645, and OD630 = corrected optical densities (with a 1 cm light path) at the respective wavelengths.

2) After determining the concentration of pigment in the extract, calculate the amount of pigment per unit volume as follows:

$$\text{Chlorophyll } a,\ \text{mg/m}^3 = \frac{\text{Chl } a \times \text{extract volume, L}}{\text{Volume of sample, m}^3}$$

where:

Chl *a* = chlorophyll concentration in the extract determined by Equation a) above.

2. Fluorometric Method for Chlorophyll *a*

The fluorometric method for chlorophyll *a* is more sensitive than the spectrophotometric method, requires a smaller sample, and does not require the wavelength resolution needed for the spectrophotometric method. Optimum sensitivity for in vitro chlorophyll *a* measurements is obtained at an excitation wavelength of 430 nm and an emission wavelength of 663 nm. A method for continuous measurement of chlorophyll *a* in vivo is available,[17] but is reported to be less efficient than the in vitro method given here, yielding about one-tenth as much fluorescence per unit weight as the same amount in solution. Pheophytin *a* also can be determined fluorometrically.[9]

a. Equipment and reagents:

1) *Fluorometer*, equipped with a high-intensity F4T.5 blue lamp, photomultiplier tube R-136 (red sensitive), sliding window orifices 1×, 3×, 10×, and 30×, and filters for light emission (CS-2-64) and excitation (CS-5-60), and a high-sensitivity door.†

2) Other equipment and reagents as specified for the Spectrophotometric Determination of Chlorophyll, above.

b. Procedure:

1) Calibrate fluorometer with a chlorophyll solution of known concentration as follows:

a) Prepare chlorophyll extract and analyze spectrophotometrically.

b) Prepare serial dilutions of the extract to provide concentrations of approximately 2, 6, 20, and 60 μg chlorophyll *a*/L.

c) Make readings for each solution at each sensitivity setting (sliding window orifice): 1×, 3×, 10×, and 30×.

d) Using the values obtained above, derive calibration factors to convert fluorometric readings in each sensitivity level to concentrations chlorophyll *a*, as follows:

$$F_s = \frac{C_a}{R_s}$$

where:

F_s = calibration factor for sensitivity setting S,

R_s = reading of the fluorometer for sensitivity setting S, and,

C_a = concentration of chlorophyll *a* determined spectrophotometrically, μg/L.

2) Measure sample fluorescence at sensitivity settings that will provide a mid-

†Model 111, Turner Assoc., 2524 Pulgas Ave., Palo Alto, Calif., or equivalent.

CHLOROPHYLL a, b, AND c (Continued)

scale reading. Convert fluorescence readings to concentrations of chlorophyll *a* by multiplying the readings by the appropriate calibration factor.

3) Avoid using the 1× window because of quenching effects.

3. Spectrophotometric Determination of Chlorophyll *a* in the Presence of Pheophytin *a*

Chlorophyll *a* may be overestimated by including pheopigments that absorb near the same wavelength as chlorophyll *a*. Chlorophyll *a*, acidified with dilute acid, degrades to pheophytin *a*, which has maximum absorption at wavelengths of 410 and 665 (667) nm. Additional acidification with more concentrated acid results in further degradation to pheophorbide-like compounds.[31] Addition of acid to chlorophyll *a* results in loss of the magnesium atom, converting it to pheophytin *a*. When a solution of pure chlorophyll *a* is converted to pheophytin *a* by acidification, the absorption peak is reduced to approximately 60% of its original value and shifts from 663 nm to 665 nm. This results in a before-to-after acidification absorption-peak-ratio (OD663/OD665) of 1.70 and is used in correcting the apparent chlorophyll *a* concentration for pheophytin *a*.

Samples with an OD663 before/OD665 after acidification ratio ($663_b/665_a$) of 1.70 are considered to contain little if any pheophytin *a* and to be in excellent physiological condition. Solutions of pure pheophytin show no reduction in OD665 upon acidification and have a $663_b/665_a$ ratio of 1.0. Thus, mixtures of chlorophyll *a* and pheophytin *a* have absorption peak ratios ranging between 1.0 and 1.7. These ratios are based on the use of 90% acetone as solvent. Using 100% acetone as solvent results in a chlorophyll *a* before-to-after acidification ratio of about 2.0.[31,32]

a. Equipment and reagents:

1) See Section 1002G.1*a*.

2) *Hydrochloric acid*, HCl, 1*N*.

b. Procedure:

1) Extract the pigment with 90% acetone (v/v), clarify by centrifuging (see Section 1002G.1*b*), and read OD at 750 nm and 663 nm.

2) Acidify extract in a 1-cm cuvette with 2 drops 1*N* HCl. If a larger cell is used add a proportionately larger volume of acid. Gently agitate the acidified extract and read OD at 750 nm and at 665 nm not sooner than 1 min or later than 2 min after acidification. Treat all samples identically.

3) Subtract the 750-nm OD value from the readings before (OD663 nm) and after acidification (OD665 nm).

c. Calculations: Using the corrected values calculate chlorophyll *a* (*C*) and pheophytin *a* (*P*) per cubic meter as follows:

$$1)\ C,\ \text{mg/m}^3 = \frac{26.73\,(663_b - 665_a) \times V_1}{V_2 \times L}$$

$$2)\ P,\ \text{mg/m}^3 = \frac{26.73\,[1.7\,(665_a) - 663_b] \times V_1}{V_2 \times L}$$

where:

V_1 = volume of extract, L,
V_2 = volume of sample, m³,
L = light path length or width of cuvette, cm, and
663_b,
665_a = optical densities of 90% acetone extract before and after acidification, respectively.

The value 26.73 is the absorbance correction and equals $A \times K$
where:

A = absorbance coefficient for chlorophyll *a* at 663 nm = 11.0, and
K = ratio expressing correction for acidification,

$$= \frac{\left(\frac{663_b}{665_a}\right)_{\text{pure chlorophyll } a}}{\left(\frac{663_b}{665_a}\right)_{\text{pure chlorophyll } a} - \left(\frac{663_b}{665_a}\right)_{\text{pure pheophytin } a}}$$

$$= \frac{1.7}{1.7 - 1.0} = 2.43$$

CHLOROPHYLL a, b, AND c (Continued)

4. Fluorometric Determination of Chlorophyll *a* in the Presence of Pheophytin *a*

To determine fluorometrically the concentration of pheophytin *a* requires the measurement of the fluorescence of acetone extracts before and after acidification. Acidification of acetone extracts of chlorophyll *a* and the resultant conversion of chlorophyll *a* to pheophytin *a* causes a reduction in fluorescence, which can be used to determine the concentration of pheophytin *a* in the extract.

a. Equipment and reagents:

1) See Section 1002G.2*a*.

2) *Hydrochloric acid*, HCl, 1*N*.

3) *Pure chlorophyll a*‡ (or a plankton chlorophyll extract with before-and-after acidification ratio of 1.70).

b. Procedure: Calibrate fluorometer as in Section 1002G.2*b*. Determine extract fluorescence at each sensitivity setting before and after acidification. Calculate calibration factors (F_s) and before-and-after acidification fluorescence ratio by dividing the fluorescence reading obtained before acidification by the reading obtained after acidification. Avoid readings on the 1 × scale and those outside the range of 20 to 80 fluorometric units.

c. Calculations: Determine the "corrected" chlorophyll *a* and pheophytin *a* in extracts of plankton samples, using the following equations:[9,316]

$$\text{Chlorophyll } a, \text{ mg/m}^3 = F_s \frac{r}{r-1}(R_b - R_a)$$

$$\text{Pheophytin } a, \text{ mg/m}^3 = F_s \frac{r}{r-1}(rR_a - R_b)$$

where:

F_s = conversion factor for sensitivity setting "S" (see 1002G.2*b*),

R_b = fluorescence of extract before acidification,

R_a = fluorescence of extract after acidification, and

$r = R_b/R_a$, as determined with pure chlorophyll *a* for the instrument. Redetermine *r* if filters or light source are changed.

‡Purified chlorophyll *a*, Sigma Chemical Company, St. Louis, Mo., or equivalent.

ANALYTICAL METHOD

PHOSPHORUS (TOTAL, DISSOLVED ORTHOPHOSPHATE)

REFERENCE

American Public Health Association, 1980. Standard methods for the examination of water and waste water, 15th ed. APHA, Washington, D.C. pp. 409-426.

For phosphorus forms, the methods presented in the previous workplan represented an adaptation of "Method 365.2 (Colorimetric, Ascorbic Acid, Single Reagent)" published by the U.S. Environmental Protection Agency (1979). These methods are consistent in principle with the procedures reproduced below that were outlined by the American Public Health Association (1980). To facilitate cross referencing, relevant sections from both sources are as follows:

Parameter	Procedure	American Public Health Association (1980)	U.S. Environmental Protection Agency (1979)
Total Phosphorus	Persulfate digestion	424 C (III)	8.1
	Ascorbic acid/ colorimetric method	424 F	8.3.2
Dissolved Orthophosphate (Filterable Reactive Phosphorus)	Preliminary filtration	424 A	4.2
	Ascorbic acid/ colorimetric method	424 F	8.3

424 PHOSPHORUS

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in solution, in particles or detritus, or in the bodies of aquatic organisms.

These forms of phophate arise from a variety of sources. Small amounts of certain condensed phosphates are added to some water supplies during treatment. Larger quantities of the same compounds may be added when the water is used for laundering or other cleaning, because these materials are major constituents of many commercial cleaning preparations. Phosphates are used extensively in the treatment of boiler waters. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm runoff and to a lesser extent with melting snow. Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues and also may be formed from orthophosphates in biological treatment processes or by receiving water biota.

Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. In instances where phosphate is a growth-limiting nutrient, the discharge of raw or treated wastewater, agricultural drainage, or certain industrial wastes to that water may stimulate the growth of photosynthetic aquatic micro- and macroorganisms in nuisance quantities.

Phosphates also occur in bottom sediments and in biological sludges, both as precipitated inorganic forms and incorporated into organic compounds.

1. Definition of Terms

Phosphorus analyses embody two general procedural steps: *(a)* conversion of the phosphorus form of interest to dissolved orthophosphate, and *(b)* colorimetric determination of dissolved orthophosphate. The separation of phosphorus into its various forms is defined analytically but the analytical differentiations have been selected so that they may be used for interpretive purposes.

Filtration through a 0.45-μm membrane filter separates "filtrable" from "nonfiltrable" forms of phosphorus. No claim is made that filtration through 0.45-μm filters is a true separation of suspended and dissolved forms of phosphorus; it is merely a convenient and replicable analytical technic designed to make a gross separation. This is reflected in the use of the term "filtrable" (rather than dissolved) to describe the phosphorus forms determined in the filtrate that passes the 0.45-μm filter.

Membrane filtration is selected over depth filtration because of the greater likelihood of obtaining a consistent separation of particle sizes. Prefiltration through a glass fiber filter may be used to increase the filtration rate.

PHOSPHORUS (TOTAL, DISSOLVED ORTHOPHOSPHATE) (Continued)

Phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample are termed "reactive phosphorus." While reactive phosphorus is largely a measure of orthophosphate, a small fraction of any condensed phosphate present usually is hydrolyzed unavoidably in the procedure. Reactive phosphorus occurs in both filtrable and nonfiltrable forms.

Acid hydrolysis at boiling-water temperature converts filtrable and particulate condensed phosphates to filtrable orthophosphate. The hydrolysis unavoidably releases some phosphate from organic compounds, but this may be reduced to a minimum by judicious selection of acid strength and hydrolysis time and temperature. The term "acid-hydrolyzable phosphorus" is preferred over "condensed phosphate" for this fraction.

The phosphate fractions that are converted to orthophosphate only by oxidative destruction of the organic matter present are considered "organic" or "organically bound" phosphorus. The severity of the oxidation required for this conversion depends on the form—and to some extent on the amount—of the organic phosphorus present. Like reactive phosphorus and acid-hydrolyzable phosphorus, organic phosphorus occurs both in the filtrable and nonfiltrable fractions. With minor variations, the filtrable and nonfiltrable fractions of a sample correspond to dissolved and particulate phosphates, respectively.

The total phosphorus as well as the filtrable and nonfiltrable phosphorus fractions each may be divided analytically into the three chemical types that have been described; reactive, acid-hydrolyzable, and organic phosphorus. Figure 424:1 shows the steps for analysis of individual phosphorus fractions. As indicated, determinations usually are conducted only on the unfiltered and filtered samples. Nonfiltrable fractions generally are determined by difference.

2. Selection of Method

a. Digestion methods: Because phosphorus may occur in combination with organic matter, a digestion method to determine total phosphorus must be able to oxidize organic matter effectively to release phosphorus as orthophosphate. Three digestion methods are given. The perchloric acid method, the most drastic and time-consuming method, is recommended only for particularly difficult samples such as sediments. The nitric acid-sulfuric acid method is recommended for most samples. By far the simplest method is the persulfate oxidation technic. It is recommended that this method be checked against one or more of the more drastic digestion technics and be adopted if identical recoveries are obtained.

b. Colorimetric methods: Three methods of orthophosphate determination are described. Selection depends largely on the concentration range of orthophosphate. The vanadomolybdic acid method (D) is most useful for routine analyses in the range of 1 to 20 mg P/L. The stannous chloride method (E) or the ascorbic acid method (F) is more suited for the range of 0.01 to 6 mg P/L. An extraction step is recommended for the lower levels of this range and when interferences must be overcome. An automated version of the ascorbic acid method also is presented.

3. Precision and Accuracy

To aid in method selection, Table 424:I presents the results of various combinations of digestion, hydrolysis, and colorimetric technics for three synthetic samples of the following compositions:

Sample 1: 100 μg orthophosphate phosphorus (PO_4–P)/L, 80 μg condensed phosphate phosphorus/L (sodium hexametaphosphate), 30 μg organic phosphorus/L (adenylic acid), 1.5 mg NH_3-N/L, 0.5 mg NO_3-N/L, and 400 mg chloride/L.

Sample 2: 600 μg PO_4-P/L, 300 μg con-

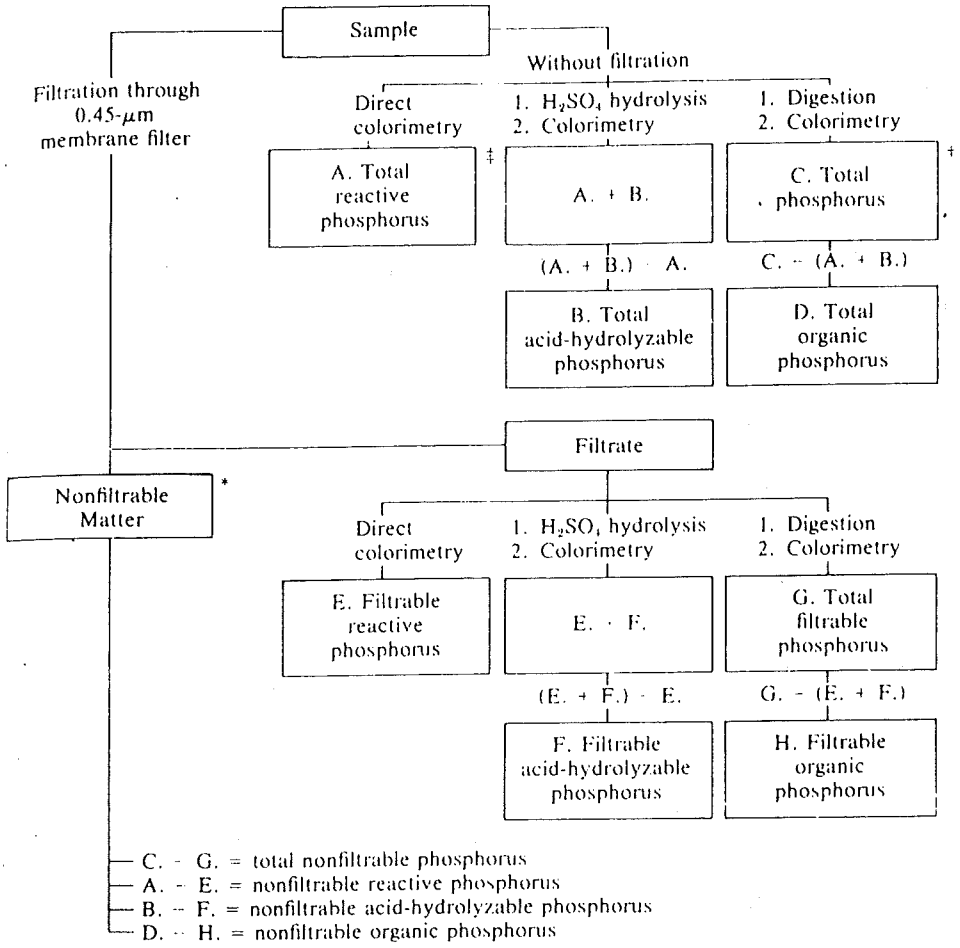

Figure 424:1. Steps for analysis of phosphate fractions.

*Direct determination of phosphorus on the membrane filter containing nonfiltrable matter will be required where greater precision than that obtained by difference is desired. Digest filter with HNO_3 and follow by perchloric acid. Then perform colorimetry.

†Total phosphorus measurements on highly saline samples may be difficult because of precipitation of large quantities of salt as a result of digestion technics that drastically reduce sample volume. For total phosphorus analyses on such samples, directly determine total filtrable phosphorus and total nonfiltrable phosphorus and add the results.

‡When determining total filtrable or total nonfiltrable reactive phosphorus, anomalous results may be obtained on samples containing large amounts of suspended sediments. Very often results depend largely on the degree of agitation and mixing to which samples are subjected during analysis because of a time-dependent desorption of orthophosphate from the suspended particles.

densed phosphate phosphorus/L (sodium hexametaphosphate), 90 μg organic phosphorus/L (adenylic acid), 0.8 mg NH_3-N/L, 5.0 mg NO_3-N/L, and 400 mg chloride/L.

Sample 3: 7.00 mg PO_4-P/L, 3.00 mg condensed phosphate phosphorus/L (sodium hexametaphosphate), 0.230 mg organic phosphorus/L (adenylic acid), 0.20 mg NH_3-N/L, 0.05 mg NO_3-N/L, and 400 mg chloride/L.

4. Sampling and Storage

If phosphorus forms are to be differentiated, filter sample immediately after collection. Preserve by freezing at or below −10 C. Add 40 mg $HgCl_2$/L to the samples, especially when they are to be stored for long periods. Do not add either acid or $CHCl_3$ as a preservative when phosphorus forms are to be determined. If total phosphorus alone is to be determined, add 1 mL conc HCl/L or freeze without any additions.

Do not store samples containing low concentrations of phosphorus in plastic bottles unless kept in a frozen state because phosphates may be adsorbed onto the walls of plastic bottles.

Rinse all glass containers with hot dilute HCl, then rinse several times in distilled water. Never use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.

424 A. Preliminary Filtration Step

Filter samples for determination of filtrable reactive phosphorus, filtrable acid-hydrolyzable phosphorus, and total filtrable phosphorus through 0.45-μm membrane filters. A glass fiber filter may be used to prefilter hard-to-filter samples.

Wash membrane filters by soaking in distilled water before use because they may contribute significant amounts of phosphorus to samples containing low concentrations of phosphate. Use one of two washing technics: *(a)* soak 50 filters in 2 L distilled water for 24 hr; *(b)* soak 50 filters in 2 L distilled water for 1 hr, change distilled water, and soak filters an additional 3 hr. Membrane filters also may be washed by running several 100-mL portions of distilled water through them. This procedure requires more frequent determination of blank values to ensure consistency in washing and to evaluate different lots of filters.

424 B. Preliminary Acid Hydrolysis Step for Acid-Hydrolyzable Phosphorus

1. Discussion

The acid-hydrolyzable phosphorus content of the sample is defined operationally as the difference between reactive phosphorus as measured in the untreated sample and phosphate found after mild acid hydrolysis. Generally, it includes condensed phosphates such as pyro-, tripoly-, and higher-molecular-weight species such as hexametaphosphate. In addition, some natural waters contain organic phos-

phate compounds that are hydrolyzed to orthophosphate under the test conditions. Polyphosphates generally do not respond to reactive phosphorus tests but can be hydrolyzed to orthophosphate by boiling with acid.

After hydrolysis, determine reactive phosphorus by a colorimetric method (D, E, or F). Interferences, precision, accuracy, and sensitivity will depend on the colorimetric method used.

2. Apparatus

Autoclave or pressure cooker, capable of operating at 98 to 137 kPa.

3. Reagents

a. Phenolphthalein indicator aqueous solution.

b. Strong acid solution: Slowly add 300 mL conc H_2SO_4 to about 600 mL distilled water. When cool, add 4.0 mL conc HNO_3 and dilute to 1 L.

c. Sodium hydroxide, NaOH, 6*N*.

4. Procedure

To 100-mL sample or a portion diluted to 100 mL, add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add strong acid solution dropwise, to just discharge the color. Then add 1 mL more.

Boil gently for at least 90 min, adding distilled water to keep the volume between 25 and 50 mL. Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa. Cool, neutralize to a faint pink color with NaOH solution, and restore to the original 100-mL volume with distilled water.

Prepare a calibration curve by carrying a series of standards containing orthophosphate (see colorimetric method D, E, or F) through the hydrolysis step. Do not use orthophosphate standards without hydrolysis, because the salts added in hydrolysis cause an increase in the color intensity in some methods.

Determine reactive phosphorus content of treated portions, using Method D, E, or F. This gives the sum of polyphosphate and orthophosphate in the sample. To calculate its content of acid-hydrolyzable phosphorus, determine reactive phosphorus in a sample portion that has not been hydrolyzed, using the same colorimetric method as for treated sample, and subtract.

424 C. Preliminary Digestion Steps for Total Phosphorus

Total phosphorus includes all orthophosphates and condensed phosphates, both dissolved and particulate, organic and inorganic. To release phosphorus from combination with organic matter, digest and oxidize. The rigor of digestion required depends on the type of sample. The three digestion technics presented, in order of decreasing rigor, are perchloric acid digestion, sulfuric acid-nitric acid digestion, and persulfate digestion. Compare phosphorus recovery by each digestion technic for the specific type of sample being tested; if the less tedious persulfate method gives good phosphorus recovery, use this method.

After digestion, determine liberated orthophosphate by Method D, E, or F. The colorimetric method used, rather than the digestion procedure, governs in matters of interference and minimum detectable concentration.

orimetric method used). Add 1 mL conc H_2SO_4 and 5 mL conc HNO_3.

Digest to a volume of 1 mL and then continue until solution becomes colorless to remove HNO_3.

Cool and add approximately 20 mL distilled water, 0.05 mL (1 drop) phenolphthalein indicator, and as much 1*N* NaOH solution as required to produce a faint pink tinge. Transfer neutralized solution, filtering if necessary to remove particulate material or turbidity, into a 100-mL volumetric flask. Add filter washings to flask and adjust sample volume to 100 mL with distilled water.

Determine phosphorus by Method D, E, or F, for which a separate calibration curve has been constructed by carrying standards through the acid digestion procedure.

III—PERSULFATE DIGESTION METHOD

1. Apparatus

a. Hot plate: A 30- × 50-cm heating surface is adequate.

b. Autoclave: An autoclave or pressure cooker capable of developing 98 to 137 kPa may be used in place of a hot plate.

c. Glass scoop, to hold required amounts of persulfate crystals.

2. Reagents

a. Phenolphthalein indicator aqueous solution.

b. Sulfuric acid solution: Carefully add 300 mL conc H_2SO_4 to approximately 600 mL distilled water and dilute to 1 L with distilled water.

c. Ammonium persulfate, $(NH_4)_2S_2O_8$, solid, or potassium persulfate, $K_2S_2O_8$, solid.

d. Sodium hydroxide, NaOH, 1*N*.

3. Procedure

Use 50 mL or a suitable portion of thoroughly mixed sample. Add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add H_2SO_4 solution dropwise to just discharge the color. Then add 1 mL H_2SO_4 solution and either 0.4 g solid $(NH_4)_2S_2O_8$ or 0.5 g solid $K_2S_2O_8$.

Boil gently on a preheated hot plate for 30 to 40 min or until a final volume of 10 mL is reached. Cool, dilute to 30 mL with distilled water, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH. Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa. Cool, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH. Make up to 100 mL with distilled water. In some samples a precipitate may form at this stage, but do not filter. For any subsequent subdividing of the sample, shake well. The precipitate (which is possibly a calcium phosphate) redissolves under the acid conditions of the colorimetric reactive phosphorus test. Determine phosphorus by Method D, E, or F, for which a separate calibration curve has been constructed by carrying standards through the persulfate digestion procedure.

424 D. Vanadomolybdophosphoric Acid Colorimetric Method

1. General Discussion

a. Principle: In a dilute orthophosphate solution, ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the

Transfer to a 50-mL volumetric flask, add 15 to 16 mL alcoholic H_2SO_4 solution, swirl, add 0.50 mL (10 drops) dilute stannous chloride reagent II, swirl, and dilute to the mark with alcoholic H_2SO_4. Mix thoroughly. After 10 min, but before 30 min, read against the blank at 625 nm. Prepare blank by carrying 40 mL distilled water through the same procedure used for the sample. Read phosphate concentration from a calibration curve prepared by taking known phosphate standards through the same procedure used for samples.

5. Calculation

Calculate as follows:

a. Direct procedure:

$$\text{mg P/L} = \frac{\text{mg P (in approximately 104.5 mL final volume)} \times 1{,}000}{\text{mL sample}}$$

b. Extraction procedure:

$$\text{mg P/L} = \frac{\text{mg P (in 50 mL final volume)} \times 1{,}000}{\text{mL sample}}$$

6. Precision and Accuracy

See Table 424:I.

424 F. Ascorbic Acid Method

1. General Discussion

a. Principle: Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid—phosphomolybdic acid—that is reduced to intensely colored molybdenum blue by ascorbic acid.

b. Interference: Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1 mg arsenic/L interfere with the phosphate determination. Hexavalent chromium and nitrite interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. Sulfide (Na_2S) and silicate do not interfere at concentrations of 1.0 and 10 mg/L.

c. Minimum detectable concentration: Approximately 10 μg P/L. P ranges are as follows:

Approximate P Range *mg/L*	Light Path *cm*
0.30-2.0	0.5
0.15-1.30	1.0
0.01-0.25	5.0

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* with infrared phototube for use at 880 nm, providing a light path of 2.5 cm or longer.

2) *Filter photometer,* equipped with a red color filter and a light path of 0.5 cm or longer.

b. Acid-washed glassware: See Method D, ¶ 2*b* above.

3. Reagents

a. Sulfuric acid, H_2SO_4, 5N: Dilute 70 mL conc H_2SO_4 to 500 mL with distilled water.

b. Potassium antimonyl tartrate solution: Dissolve 1.3715 g $K(SbO)C_4H_4O \cdot \frac{1}{2}H_2O$ in 400 mL distilled water in a 500-mL volumetric flask and dilute to volume. Store in a glass-stoppered bottle.

c. Ammonium molybdate solution: Dissolve 20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 mL distilled water. Store in a glass-stoppered bottle.

d. Ascorbic acid, 0.01M: Dissolve 1.76 g ascorbic acid in 100 mL distilled water. The solution is stable for about 1 week at 4 C.

PHOSPHORUS (TOTAL, DISSOLVED ORTHOPHOSPHATE) (Continued)

e. Combined reagent: Mix the above reagents in the following proportions for 100 mL of the combined reagent: 50 mL 5*N* H_2SO_4, 5 mL potassium antimonyl tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. *Mix after addition of each reagent.* Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 hr.

f. Stock phosphate solution: See Method D, ¶ 3*e*.

g. Standard phosphate solution: Dilute 50.0 mL stock phosphate solution to 1,000 mL with distilled water; 1.00 mL = 2.50 μg P.

4. Procedure

a. Treatment of sample: Pipet 50.0 mL sample into a clean, dry test tube or 125-mL erlenmeyer flask. Add 0.05 mL (1 drop) phenolphthalein indicator. If a red color develops add 5*N* H_2SO_4 solution dropwise to just discharge the color. Add 8.0 mL combined reagent and mix thoroughly. After at least 10 min but no more than 30 min, measure absorbance of each sample at 880 nm, using reagent blank as the reference solution.

b. Correction for turbidity or interfering color: Natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a blank by adding all reagents except ascorbic acid and antimonyl potassium tartrate to the sample. Subtract blank absorbance from absorbance of each sample.

c. Preparation of calibration curve: Prepare individual calibration curves from a series of six standards within the phosphate ranges indicated in Section 424F.1*c*. Use a distilled water blank with the combined reagent to make photometric readings for the calibration curve. Plot absorbance vs. phosphate concentration to give a straight line passing through the origin. Test at least one phosphate standard with each set of samples.

5. Calculation

$$\text{mg P/L} = \frac{\text{mg P (in approximately 58 mL final volume)} \times 1{,}000}{\text{mL sample}}$$

6. Precision and Accuracy

The precision and accuracy values given in Table 424:I are for a single-solution procedure given in the 13th edition. Procedure 424F differs in reagent-to-sample ratios, no addition of solvent, and acidity conditions. It is superior in precision and accuracy to the previous technic in the analysis of both distilled water and river water at the 228 μg P/L level (Table 424:II).

TABLE 424:II. COMPARISON OF PRECISION AND ACCURACY OF ASCORBIC ACID METHODS

Ascorbic Acid Method	Phosphorus Concentration, Filtrable Orthophosphate μg/L	No. of Laboratories	Relative Standard Deviation %		Relative Error %	
			Distilled Water	River Water	Distilled Water	River Water
13th Edition (Edwards, Molof, and Schneeman)	228	8	3.87	2.17	4.01	2.08
Current method (Murphy and Riley)	228	8	3.03	1.75	2.38	1.39

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ANALYTICAL METHOD

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1002 I. Metabolic Rate Measurements

The physiological condition of the aquatic community and the spectrum of biological interactions must be considered. Earlier, numbers, species composition, and biomass were the prime considerations. Recognition of the limitations of this approach, however, led to the measurement of rates of metabolic processes such as photosynthesis (productivity), nitrogen fixation, respiration, and electron transport. These provide a better understanding of the complex nature of the aquatic ecosystem. An indication of photosynthetic efficiency can be determined by the productivity index (mg C fixed/unit chlorophyll *a*).[49]

1. Nitrogen Fixation

The ability of an organism to fix nitrogen is a great competitive advantage and plays a major role in population dynamics. Two reliable methods for estimating nitrogen fixation rates in the laboratory are the ^{15}N isotope tracer method[50,51] and the acetylene reduction method.[52] Because the rate of nitrogen fixation varies greatly with different organisms and with the concentration of combined nitrogen, nitrogen fixation rates cannot be used to estimate biomass of nitrogen-fixing organisms. However, the acetylene reduction method is useful in measuring nitrogen budgets and in algal assay work.[53]

2. Productivity, Oxygen Method

Productivity is defined as the rate at which inorganic carbon is converted to an organic form. Cholorphyll-bearing plants (phytoplankton, periphyton, macrophytes) serve as primary producers in the aquatic food chain. Photosynthesis results in the formation of a wide range of organic compounds, release of oxygen, and depletion of carbon dioxide (CO_2) in the surrounding waters. Primary productivity[54] can be determined by measuring the changes in oxygen and CO_2 concentrations.[55] In poorly buffered waters, pH can be a sensitive property for detecting variations in the system. As CO_2 is removed during photosynthesis, the pH rises. This shift can be used to estimate both photosynthesis and respiration.[56] The sea and many fresh waters are too highly buffered to make this useful, but it has been applied successfully to productivity studies in some lake waters.

Two methods of measuring the rate of carbon uptake and net photosynthesis in situ are: (*a*) the oxygen method[57] and (*b*) the carbon 14 method.[58] In both methods, clear (light) and darkened (dark) bottles are filled with water samples and suspended at regular depth intervals for an incubation period of several hours or samples are incubated under controlled conditions in environmental growth chambers

PRIMARY PRODUCTIVITY (Continued)

in the laboratory.

The basic reactions in algal photosynthesis involve uptake of inorganic carbon and release of oxygen, summarized by the relationship:

$$CO_2 + H_2O \rightarrow (CH_2O)_x + O_2$$

The chief advantages of the oxygen method are that it provides estimates of gross and net productivity and respiration and that analyses can be performed with inexpensive laboratory equipment and common reagents. The DO concentration is determined at the beginning and end of the incubation period. Productivity is calculated on the assumption that one atom of carbon is assimilated for each molecule of oxygen released.

a. Equipment:

1) *BOD bottles*, numbered, 300-mL, clear borosilicate glass, with ground-glass stopper and flared mouth, for sample incubation. Acid-clean the bottles, rinse thoroughly with distilled water, and just before use, rinse with the water being tested. Do not use phosphorus-containing detergents.

If suitable opaque bottles are not available, make clear BOD bottles opaque by painting them black and wrapping with black waterproof tape. As a further precaution, wrap the entire bottle in aluminum foil or place in a light-excluding container during incubation.

2) *Supporting line or rack* that does not shade the suspended bottles.

3) *Nonmetallic opaque acrylic Van Dorn sampler* or equivalent, of 3- to 5-L capacity.

4) *Equipment and reagents for dissolved oxygen determinations* (see Section 421).

5) *Pyrheliometer.*

6) *Submarine photometer.*

b. Procedure:

1) Obtain a profile of the input of solar radiation for the photoperiod with a pyrheliometer.

2) Determine depth of euphotic zone (the region that receives 1% or more of surface illumination) with a submarine photometer. Select depth intervals for bottle placement. The photosynthesis-depth curve will be closely approximated by placing samples at intervals equal to one-tenth the depth of the euphotic zone. Estimate productivity in relatively shallow water with fewer depth intervals.

3) Introduce samples taken from each preselected depth into duplicate clear, darkened, and initial-analysis bottles. Insert delivery tube of sampler to bottom of sample bottle and fill so that three volumes of water are allowed to overflow. Remove tube slowly and close bottle. Use water from the same grab sample to fill a "set" (one light, one dark, and one initial bottle).

4) Immediately treat (fix) samples taken for the chemical determination of initial dissolved oxygen (see Dissolved Oxygen, Section 421) with manganous sulfate ($MnSO_4$), alkaline iodide, and sulfuric acid (H_2SO_4) or check with an oxygen probe. Analyses may be delayed several hours if necessary, if samples are fixed or iced and stored in the dark.

5) Suspend duplicate paired clear and darkened bottles at the depth from which the samples were taken and incubate for at least 2 hr, but never longer than it takes for oxygen-gas bubbles to form in the clear bottles or DO to be depleted in the dark bottles.

6) At the end of the exposure period, immediately determine DO as described above.

c. Calculations: The increase in oxygen concentration in the light bottle during incubation is a measure of net production which, because of the concurrent use of oxygen in respiration, is somewhat less than the total (or gross) production. The loss of oxygen in the dark bottle is used as an estimate of respiration. Thus:

Note: Instead of using a pyrheliometer, researchers should take solar monitor readings at the start and end of a 10:00 AM - 2:00 PM incubation period and for the entire photoperiod. If a submarine photometer is unavailable, suspend bottles at mid-depth (45 cm).

PRIMARY PRODUCTIVITY (Continued)

Net photosynthesis = light bottle DO − initial DO
Respiration = initial DO − dark bottle DO
Gross photosynthesis = light bottle DO − dark bottle DO

Average results from duplicates.

1) Calculate the gross or net production for each incubation depth and plot:

mg carbon fixed/m^3
= mg oxygen released/L × 12/32 × 1,000

Use the factor 12/32 to convert oxygen to carbon; 1 mole of O_2 (32 g) is released for each mole of carbon (12 g) fixed.

2) Productivity is defined as the rate of production and generally is reported in grams carbon fixed per square meter per day. Determine the productivity of a vertical column of water 1 m square by plotting productivity for each exposure depth and graphically integrating the area under the curve.

3) Using the solar radiation profile and photosynthesis rate during incubation adjust the data to represent phytoplankton productivity for the entire photoperiod. Because photosynthetic rates vary widely during the daily cycle,[59,60] do not attempt to convert data to other test circumstances.

APPENDIX G

LITERATURE CITED

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