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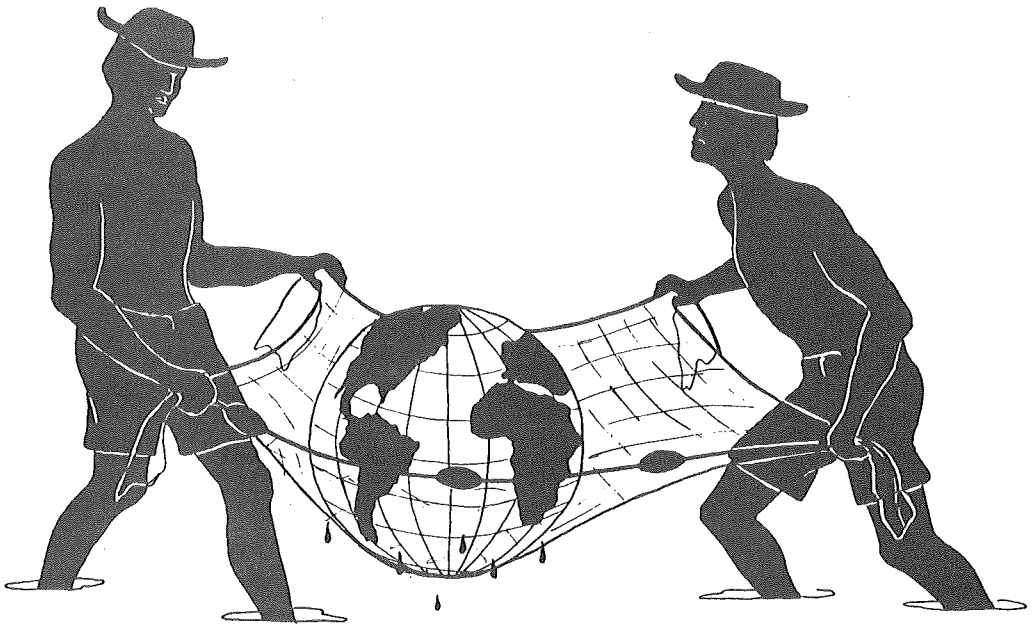
TITLE XII
COLLABORATIVE RESEARCH SUPPORT PROGRAM

POND DYNAMICS/AQUACULTURE

CRSP WORK PLAN: FIRST
EXPERIMENTAL CYCLE

Volume I

Experimental Protocol and
Methods



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INTRODUCTION

The technical goal of the CRSP is to quantitatively describe the physical, chemical and biological principles of pond culture systems. To meet this goal it is essential to distinguish between site specific and more general factors which can be considered principles in an aquaculture system. It will also be important to distinguish between general principles applicable to all production systems and principles which apply only to specific systems.

It will be important in the development of the CRSP to establish points of reference and commonalities to distinguish between general principles and those which are site or system specific. The diverse nature of the CRSP sites will facilitate distinguishing of general principles from specific ones. Such will require standardization, as far as possible, of experimental designs, methods, data collection and analysis.

The program description in the CRSP Grant lists the following objectives for the first year:

1. To compile a quantitative baseline of chemical, physical and biological parameters for each work location.

2. To observe quantitative physical, chemical and biological responses to various levels of inorganic fertilizer application to pond culture systems, and test for significant correlations with and between work locations.

3. To observe and document technical constraints limiting fry availability in each participating host country.

4. To compile a baseline of information on locally available nutrient inputs and local geography, hydrology and water quality in each participating country utilizing available host country resources.

In accordance with the program description, a generalized research plan for the first year of CRSP activities was developed by the CRSP Technical Advisory Committee. This generalized plan was then transformed into specific experimental protocols by the CRSP Research Team composed of U.S. and Host Country Principal Investigator in cooperation with the Technical Advisory Committee and the CRSP Executive Council during a meeting at Davis, California on March 2 and 3, 1983. The following sections outline the standard experimental protocol including design of the experiments, data to be collected, sampling and analytical methods and schedules.

Volume II of this plan, which deals with data collection format and processing will be published separately at a later date.

Considerations in Developing the Work Plan

The sites of the research programs can be considered in several general groupings: brackish to marine tropical locations in Panama and Philippines; warm tropical freshwater locations in Honduras, Jamaica, Panama, Sierra Leone and Thailand with elevations below 700 m and average temperatures above 25°C;

and temperate tropical freshwater locations in Indonesia and Rwanda with elevations above 750 m and average temperatures of 25°C or below. Such groupings offer only very general commonalities and a great deal of variation should be anticipated within these groupings. Continuing effort will be needed to establish common points of reference.

A key element will be the use of a common culture organism. At the freshwater sites the best possibility for a common species is *Tilapia nilotica*. It is currently found in all the participating CRSP countries. It offers great potential as a research organism in that it responds well to a variety of levels of management including inorganic and organic fertilization and several qualities of supplemental feeds. Its reproduction at all the freshwater sites is more feasible than that of other species and will be a major importance in developing fish seed production systems. Its early maturity offers the possibility of using fish of a common genetic line. Honduras, Jamaica and Panama presently have *T. nilotica* of a common genetic history. The Government of Panama has offered to make available *T. nilotica* to interested CRSP sites.

T. nilotica will also be employed in the brackish water experiments in the Philippines project, along with parallel experiments using milkfish, *Chanos chanos*. The use of *T. nilotica* in brackish water experiments in Panama poses institutional problems. Species selection for these experiments can not be made at the present time.

Another point of commonality should be the level and quality of nutrient input. A level of nutrient input should be chosen that would be more than adequate to establish a dynamic pond ecosystem. A system which receives very limited nutrient inputs may not fully illustrate the principles of pond dynamics to be studied by the CRSP, nor would it be at all typical of most aquaculture systems. A source of nutrients common to all CRSP sites is difficult. Feed materials will vary from site to site both in availability and quality. This is also true for animal manures. The most comon nutrient source of comparable quality to all sites may well be inorganic fertilizer.

The first year of the CRSP will be an important one for establishing procedures and refining techniques. One important aspect that should be considered is what degree of variation occurs among ponds for the parameters being measured. It will be essential to establish this variation at the beginning of CRSP activities for the ponds at each site to determine the proper number of replicates for subsequent experiment and the degree of separation required between treatments to give meaningful results. To this end, it is anticipated that in the first year a block of twelve experimental ponds be reserved for use throughout the life of each CRSP project and all receive a common treatment in the initial study.

DESCRIPTION OF EXPERIMENTS

A minimum of 12 earthen ponds to be used for the duration of each country project will be employed in the initial experiments. Although local considerations will dictate the size and configuration of the ponds used, it is noted that the preferred pond size is on the order of 400 to 500 square meters surface area.

During the first year experiments, all ponds will receive the same treatment. This will facilitate determining baseline responses and measuring variances for allocation of replications in future experiments. This baseline will serve as a point of reference (control) for future experiments. Methods of statistical analyses will be described in a later section.

Nutrient Inputs

Superphosphate or triplesuperphosphate will be applied to all fresh-water ponds at a rate of 8 kg/hectare/month of P_2O_5. The fertilizer should be applied biweekly by suspending in a porous bag located in the upper 25 cm of the water column. This fertilization rate should give reasonable fish production at all locations and will serve as a point of reference for future studies. Detailed methods of fertilizer application are outlined in Appendix A.

Pond Preparation

Preparation of ponds for experiments, including the method for satisfying lime requirment 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 between 25 and 50 grams as described in Appendix. 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 experimental cycles will be accomplished during the first year. Each experiment should run for a period of five months (150 days). Each 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 last 90 days of the culture period occur within a single climatic season.

System variables to be Measured

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

I. Physical Environment Measurements

A. Required Measurements for Description and Characterization of Study Sites

1. Light
2. Rainfall
3. Wind Speed and Direction
4. Air Temperature
5. Soil Analysis
6. Morphology
7. Pond Temperature Fluctuation
8. Pond Depth Fluctuation
9. Hydrology

II. Water Analyses

A. Required Analyses During Fish Production Experiments

1. Dissolved Oxygen
2. Temperature
3. pH
4. Alkalinity
5. Total Hardness
6. Water Quality Analysis

B. Recommended Analyses During Fish Production Experiments (not required during year-1)

1. Ammonia
2. Nitrate
3. Total Phosphorus
4. Total Dissolved Phosphorus
5. Total Dissolved Reactive Phosphorus (Ortho-Phosphate)

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

B. Recommended Measurements During Fish Production Experiments (not required during year-1)

1. Light-Dark Bottle Experiments
2. Qualitative Identification of Phytoplankton, Zooplankton and Benthos

SAMPLING PROTOCOL

The various measurements required during the first year of CRSP activities, including sampling methods and frequencies and analytical methods are outlined in this section.

Physical Environment Measurements

The following measurements are required at all locations in order to carefully describe the conditions under which the experiments will be held.

1. Light - It is recommended that a solar monitor and quantum sensor be installed near the study site in order to estimate daily accumulation of quanta that are available for photosynthetic organisms in the ponds. This measurement will also provide information on the daily and seasonal variation in solar radiation at study sites. The LiCor model LI-1776 Solar Monitor and LiCor model LI-190SB Quantum Sensor (Appendix F) or equivalent are recommended.

2. Rainfall - This will be estimated using readings made from three rain gauges placed near the study site. The gauges are readily available from local hardware stores or from Grassroots Co., of Fort Atkinson, Wisconsin. It is suggested that amount of rainfall be read from each gauge at daily intervals and the gauge then emptied.

3. Wind Speed and Direction - These measurements will be made at appropriate intervals to correlate readings with thermal and oxygen stratification of the ponds, i.e. spot readings will be made at the same time as diurnal DO-temp. readings. The Windscope Wind Speed and Direction Indicator available from the Ben Meadows Co., of Atlanta, Georgia (Appendix F) or equivalent is suggested.

4. Air Temperature - Three maximum-minimum thermometers (available from the Taylor Co.) should be placed in the shade near the ponds and read weekly or at appropriate intervals for each site.

5. Soil Analysis - Each site will collect a sample of pond mud from each pond. Analyses will be performed on these samples by the USDA approved Soils Testing Laboratory at Oregon State University. Specific directions for collection preparation and shipment of these samples are outlined in Appendix D.

6. Morphology - Ponds should be mapped including information on areas, depth contours, inlet and outlet locations, pertinent surrounding elevations, wind obstructions and shading features. Basic methods in pond mapping are outlined (Appendix F pgs. 26-31) by Lind (1975).

7. Pond Temperature Fluctuation - In three ponds place 2 maximum-minimum thermometers each at locations of 25 cm below the surface of the water and 25 cm above the bottom of the ponds. Readings from these thermometers should be taken once per week.

8. Pond Depth Fluctuation - A staff gauge should be placed in each pond and read to the nearest 0.5 cm at intervals that seem appropriate to site conditions. Note: Pond depth should be held at 90 cm throughout experiments. Stage height will allow monitoring of this.

9. Hydrology - A water budget will be determined for each pond based upon the information gained from water inputs necessary to maintain the required 90 cm pond depth. Inputs of water to each pond both as rainfall and inlet water should be calculated on the basis of m^3/day. Water losses that could be due to seepage and/or evaporation will equal the amount of water input necessary to maintain 90 cm pond depth throughout the experiment.

Water Analyses

This particular category first describes analyses which are required at all sites for year-1 during the fish production experiments. All special equipment, general laboratory materials and glassware needed for these analyses are listed in Appendix C.

1. Dissolved Oxygen

Method: Yellow Springs Instrument (YSI) Dissolved Oxygen Meter is recommended for measuring DO. The meter should be calibrated once per month with the Winkler method (Appendix F) described by APHA (1981) or with a Hach Digital Titrator for Dissolved Oxygen.

Sampling: Near the center of each pond at 25 cm below the surface, midwater and 25 cm above the bottom.

Frequency: Once per week at all ponds, and diurnal study once per month which involves measuring DO every four hours beginning 30 minutes before sunrise to after sunrise.

2. Temperature

Method: YSI Meter

Sampling: From mid pond at 25 cm below surface, midwater and 25 cm off the bottom of the pond.

Frequency: Once per week and during the once per month diurnal study at four hour intervals, beginning 30 minutes before sunrise until after sunset.

3. pH

Method: Corning or Orion pH meter with an unbreakable Ross combination pH electrode (Appendix F).

Sampling: Measurements will be taken from three pooled 90 cm column samples per pond.

Frequency: Once per week, and as part of the monthly diurnal study at 4 hour intervals. Pooled samples can be taken to the laboratory and pH measured within one hour.

4. Alkalinity

Method: Follow Standard Methods or utilize the Hach Alkalinity Test Kit Model AL-DT with the Digital Titrator (Appendix F).

Sampling: One sample per pond from three pooled 90 cm column samples per pond.

Frequency: One pooled sample per pond will be part of the monthly diurnal. Samples should be taken, kept cool in refrigeration or ice chests and analyzed within 24 hours.

5. Total Hardness

Method: Follow Standard Methods (APHA, 1981) or use Hach Total Calcium Hardness Test Kit Model HAC-DT with Digital Titrator (Appendix F).

Sampling: One sample per pond from three pooled 90 cm column samples per pond.

Frequency: Pooled sample will be part of monthly diurnal from each pond and should be refrigerated and analyzed within 7 days.

6. Water Quality Analysis

Comprehensive water analysis of ponds and supply water at the start and finish of each production study will include measurement of alkalinity, total hardness, pH, ammonia, nitrates, orthophosphate, total phosphorus, chlorides, sulfates, boron, calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc. Conductivity will be estimated from measurements made of major anions and cations. Alkalinity, total hardness, pH, ammonia, nitrates, orthophosphate, and total phosphorus are to be done on-site. Whether these can be done on-site in year-1 will depend on the progress of setting up laboratories in host countries. A water analysis service is available at the Limnology Laboratory at Michigan State University. Additional information regarding this service may be found in Appendix E.

The following water analyses are not required during the fish production experiments of year 1. These are recommended after equipment and time allow.

1. Ammonia

Method: Follow Standard Methods for the Phenate Procedure as outlined in Appendix F. (APHA, 1981).

Sampling: One sample per pond from three pooled 90 cm columns per pond.

Frequency: Once per month as part of monthly diurnal. Samples should be refrigerated and analyzed within 24 hours.

2. Nitrate

Method: Follow Standard Methods for the Phenodisulfonic Procedure in Appendix F (APHA, 197).

Sampling: One sample per pond from three pooled 90 cm column samples per pond.

Frequency: One per month as part of monthly diurnal. Samples should be refrigerated and analyzed within 24 hours.

3. Total Phosphorus

Method: Follow methods in Appendix F with preliminary persulfate digestion (U.S. EPA, 1979).

Sampling: One sample per pond from three pooled 90 cm column samples per pond.

Frequency: Collected once monthly per pond as part of diurnal sampling. Optional: Three additional sampling periods during weeks 1, 10, and 19, samples taken same day before fertilizer application, 12 hours after application and at 1, 2, 3, 6 and 14 days after application. Samples should be refrigerated and analyzed within 24 hours.

4. Total Dissolved Phosphorus

Method: Follow Methods of Appendix F, including the procedure for filtering the sample before analysis.

Sampling: Same as listed for total phosphorus above.

Frequency: Same as listed for total phosphorus above.

5. Ortho-Phosphate (Total Dissolved Reactive Phosphorus - TDRP)

Method: Follow Methods for TDRP and filtering from Appendix F.
Sampling: Same as listed for total phosphorus above.
Frequency: Same as listed for total phosphorus above.

All of the above chemical analyses for water require that the laboratory glassware be very clean. This requires acid washing procedures as outlined in Appendix F. A blank should be run with all standard solutions to help check on the quality control of contaminants in the laboratory.

Fish Production

Fish production will be monitored as follows:

1. Initial Stocking. The initial stock will be weighed as a group and counted and sexed individually. A 10% sample will be individually weighed and measured.

2. During Experiments.

Method: A grab sample from each pond equivalent in number to 10% of the initial stock will be weighed as a group. If substantial size variation is observed or if reproduction is suspected, the sample will be divided into centimeter groups and each group counted and weighed. Any female tilapia observed will be removed and replaced by a male of similar weight. Any other animals collected will be counted, weighed, measured, and discarded. Observations on reproduction and fish health will be recorded.

Frequency: At 30 day intervals throughout each experimental cycle.

2. Termination of experiments. All fish will be removed from the pond. A random sample equivalent to 10% of the initial stocking will be weighed and measured as described above. The total number of fish removed will be determined and the total biomass calculated. Any fish other than tilapia will be counted by species and weighed and measured.

Biological Limnology

The following measurements are required during the fish production experiments of year-1.

1. Secchi Disk Visibility

Method: Standard use of Secchi Disk as outlined in Appendix F (Lind, 1975). Visibility is calculated as the average between 2 depth readings; one depth is that at which the disk disappears when lowered, the second depth is that at which the disk reappears when raised.

Sampling: Two locations per pond.

Frequency: Bi-weekly, between 11:00 am and 2:00 pm on same day as chlorophyll a samples are collected.

2. Chlorophyll a

Method: Follow methods as outlined in Appendix F (Wetzel, 1979).

Sampling: One sample per pond from three pooled 90 cm column samples per pond.

Frequency: Bi-weekly samples with one sampling period coinciding with monthly diurnal.

The following measurements are recommended (not required during fish productions experiments of year-1).

1. Light-Dark Bottles

Method: As outlined in Appendix F by Lind (1975).

Sampling: Three sets of bottles per pond, set at mid-depth in each pond.

Frequency: Monthly.

2. Qualitative Identification of Phytoplankton, Zooplankton, and Benthos

Method & Sampling: Phytoplankton: sample pond with plankton net (with an attached collection bottle) which will be towed through the pond. Several subsamples of this will be observed through compound microscope to make identification of the major groups (green, blue-green, or diatom) and relative abundance of each group (abundant, common, rare).

Zooplankton: sample pond with at least three 90 cm column samples and make microscopic I.D. of invertebrates to at least the level of Order, and make note of relative abundance.

Benthos: sample at least three cores of the pond mud per pond; sieved sample of invertebrates should be identified to Order and noted as to their relative abundance.

Frequency: Whenever possible: recommended once per month or when observation indicates drastic changes in pond biota. Good references for this work include Edmondson (1959) and Needham and Needham (1966).

Brackish Water Projects

This section outlines deviations from the standardized work plan applicable to brackish water sites.

1. Fertilizer Applications

Most of the brackishwater ponds will initially have a benthic algal mat (Lab Lab) type of primary production, rather than phytoplankton based primary production. With Lab Lab ponds, manure (e.g. chicken) is added to the dry pond bottom until the organic content of the upper few centimeters is raised to about 5%.

After manuring the dry pond bottom, water is let into the pond and allowed to seep in overnight. The pond is then refilled the next day with 3 to 5 cm of water, and the manure will start to bubble. A mat will begin to form in 2 to 3 days, at which time the pond depth is carefully increased to 10 cm depth. This leads to the formation of the benthic algal mat in 1

to 2 weeks. When the mat is well formed, fish or shrimp are introduced and the water depth increased to 40 cm for the grow-out phase which takes about 4 months. The dominant primary producers will often change from the Lab Lab at the beginning of grow-out to phytoplankton by harvest time. Additional manure may need to be added during grow-out.

2. Test Species

A. Brackishwater Aquaculture Center, Philippines

We intend to devote 24 ponds to our initial experiments as follows:

Test Species	No. of Ponds	Water Depth (cm)	Primary Production
Milkfish[b/]	12	40	Lab Lab
Tilapia[c/]	6	40	" "
Milkfish	3[a/]	80	Phytoplankton
Tilapia	3[a/]	80	"

a/ artificially circulate one each of the phytoplankton ponds which contain milkfish or tilapia.
b/ stocking rate is about 3,000/ha.
c/ stocking rate is about 2,000/ha.

B. Panama

The test species at Panama was not established during the UC Davis meeting. The preference seemed to be to use *P. vannamei* in 12 grow-out ponds with Lab Lab. *T. nilotica* (females only) were considered, but not confirmed.

3. Monitoring

The monitoring program for water quality and fish growth would be nearly identical to that defined for freshwater sites, except that the brackishwater sites would:

(a) include salinity measurements,
(b) measure Lab Lab standing crop. The measuring techniques were not determined at UCD, but will probably include some method of coring followed by either chlorophyll determination or organic matter determination.

4. Miscellaneous

At the BAC, we intend to install data logging systems for weather parameters and water quality parameters as follow:

Weather: at one to two minute intervals, log solar irradiance, wind speed, wind direction and air temperature.

Water: at five minute intervals, log from the surface and bottom of two ponds each dissolved oxygen, temperature, pH and total gas pressure.

Data will be logged onto magnetic storage chips, which will be taken to the lab where the data will be transferred to the personal computer for computation, and to floppy discs for permanent storage.

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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/1) 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 in the tropics and subtropics.

      2) The lime requirement of each pond's mud must be met.

      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. This analysis should be done at a selected lab in the USA.

   B. Fertilizer for each complete experimental cycle will be purchased in one lot.

      1) A random sample of fertilizer will be collected from each lot for nutrient analysis. A selected lab in the USA will be used by all CRSP participants.

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

C. Each 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 would be good.

1) Prior to introducing the new fertilizer into the bag, mix/crumble any residual in the bag aid in nutrient dispersion.

D. Fertilizer should not be broadcast over the pond surface.

3. Water and its management

A. Staff gauges should be placed in all ponds and water level monitored and recorded (to the nearest 0.5 cm) on a weekly basis.

B. Replacement water for seepage and evaporation will be added on a weekly basis, if needed. Record the water level before and after adding water.

C. Water levels in ponds should be maintained 5 cm below the lip of the drain to prevent overflow.

D. Prevent runoff, into the ponds from adjacent land.

4. Water quality management

A. Observe ponds/fish while measuring D.O. noting any abnormal fish behavior.

B. Aeration and water exchange in cases of low dissolved oxygen concentrations are prohibited. In such an instance, note the D.O. and fish behavior. Remove, enumerate, weigh and measure any mortality.

5. 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.

6. 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.

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 grow 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:

prepare as in Appx A

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 $\underline{2000\ m^2}$

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

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

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

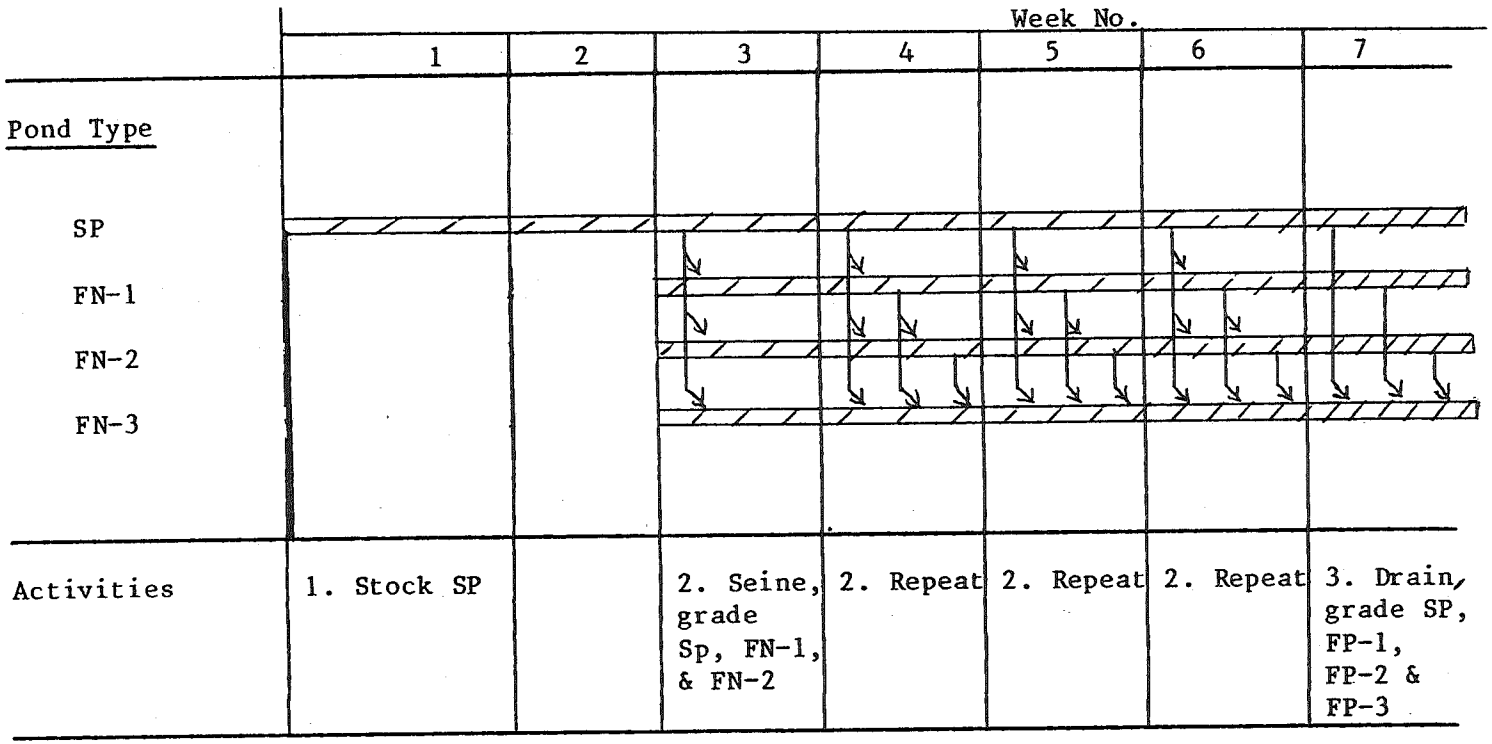
less 50% discarded as females = 4,400/week,

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

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

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

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 TO SUPPLY CRSPS

APPENDIX C

Lists of Suggested Equipment and Chemicals to Supply CRSPs

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' c 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% AI, 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 LiCor Model LI-1776 Quantum Sensor LiCor Model LI-190SB	estimate solar radiation
2.	Rain Gauge	estimates rainfall
3.	Windscope, Ben Meadows Co.	wind speed and direction
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)

APPENDIX D

PROCEDURE FOR POND SOIL SAMPLING AND ANALYSIS

APPENDIX D

PROCEDURE FOR POND SOIL SAMPLING AND ANALYSIS

Pond mud will be analyzed for pH, exchangeable bases (Ca, Mg, K), total exchangeable acidity (generally due to Al), extractable phosphorus, carbonate, zinc, copper, manganese, iron, nitrate-nitrogen, % organic matter and particle size distribution. Analyses will be performed prior to initiating each experimental cycle during the first year. The lime requirement of the pond soil will be determined using the modified Adams-Evans procedure (Boyd, 1979). The above analyses will be done in the USA at a selected, common lab.

Samples should be collected from the top 15 cm of pond mud. Any number of devices can be utilized to collect the sample: a tin can, an Eckman dredge, a core sampler.

Steps:

1. Begin at the shallow end of an empty pond, opposite the drain.

2. Proceed in an 'S' shape toward the deep end of the pond collecting 12 samples.

3. Composite all samples from one pond and mix thoroughly. Wet mud mixes more easily than dry mud.

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

5. Select 200 gms of rock-free dried mud from each pond for shipment to USA. The sample should be packaged in plastic bags, and identified and recorded as to origin.

6. Send all samples, preferably in one box, with the USDA form affixed the outside of the box to the OSU Soil Testing Laboratory as described below.

Six to seven weeks should be allowed for receiving the results of the analysis. It is important that the lime requirement for each pond's mud be met so as to give a base unsaturation of 0.2 or less, or a mud pH of 5.9 or greater. Lime should be present on the station so that, if needed, lime can be applied in a timely fashion, allowing approximately 2 weeks between application of lime and the first fertilizer application. Agricultural limestone, $CaCO_3$ or $CaMg(CO_3)_2$, is preferable for use in fish ponds.

The OSU Soil Testing Laboratory is authorized by the United States Department of Agriculture to receive soil samples from outside the U.S.A.

The following conditions must be met when handling international soil samples:

1. Samples to be shipped in sturdy, leak-proof containers.

2. Samples to be released without treatment at the port of entry.

3. Samples to be used only for soil analysis and only in the laboratory of the permittee at Corvallis, Oregon.

4. All unconsumed soil, containers, and effluent to be autoclaved or incinerated by the permittee at the conclusion of the project.

Each package of samples must bear an authorization sticker (see sample below). These may be obtained at the OSU Soil Testing Laboratory, Agriculture Hall 114, Corvallis, Oregon 97331, or from the CRSP Program Manager.

Be certain to specify the analyses required for each sample. This is best done by completing the OSU order form (sample attached).

The fee schedule for soil analysis is shown below.

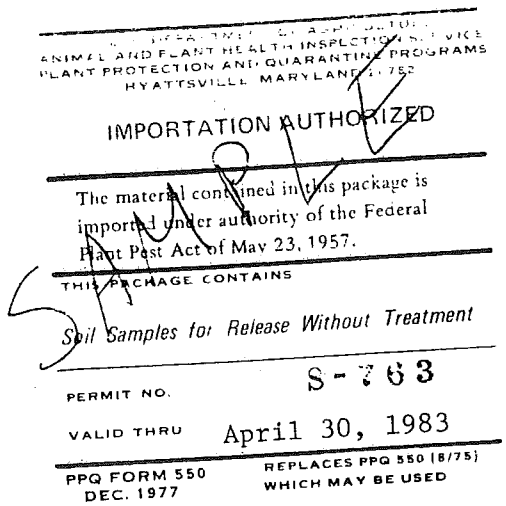
FEE SCHEDULE FOR RESEARCH SOIL ANALYSIS

Sample preparation (grinding, handling, storage) this charge made for every sample	$ .75
pH	1.00
Phosphorus	3.00
Extractable Bases (Ca, Mg, K, or Na)	2.25/each
2 Bases	3.25
3 Bases	4.25
4 Bases	5.25
Organic Matter	5.25
Boron	3.75
Total Nitrogen	8.00
Nitrate Nitrogen (NO_3-N)	4.00
Ammonium Nitrogen (NH_4-N)	4.00
Nitrate Nitrogen & Ammonium Nitrogen	7.00
Cation Exchange Capacity	7.00
Soluble Salts	2.25 conductivity
Heavy Metals (Zn, Mn, or Cu)	5.25/each
2 Heavy Metals	6.75
3 Heavy Metals	8.25
Sulfate-Sulfur (SO_4-S)	8.00
Lime Requirement	1.00
Free $CaCO_3$ or $CaCO_3$ Equivalent	5.50
Exchangeable H	5.50
Exchangeable Na	5.50

Research on a non-rush basis 20% discount

Fe
Al
Cu
Mn

must have comparable elimination



U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE PROGRAMS
HYATTSVILLE, MARYLAND 20782

IMPORTATION AUTHORIZED

The material contained in this package is imported under authority of the Federal Plant Pest Act of May 23, 1957.

THIS PACKAGE CONTAINS

Soil Samples for Release Without Treatment

PERMIT NO. S-763

VALID THRU April 30, 1983

PPQ FORM 550
DEC. 1977

REPLACES PPQ 550 (8/75)
WHICH MAY BE USED

Agricultural Experiment Station
Soil Test Form S103

OREGON STATE UNIVERSITY
Soil Testing Laboratory
Corvallis, Oregon 97331

Cooperative Extension Service
February 1981

SEND RESULTS TO:

Name_____

_____

Address_____

City_____, Oregon_____(Zip)

Phone_____ Date submitted_____

RESEARCH SOIL DATA SHEET

Date sampled_____

Date results desired:

☐ RUSH by_____

☐ NON-RUSH

CHARGE PROJECT NO._____ Experiment Ident._____

Sample disposition:

☐ discard ☐ pick up

Sample from: ☐ East of Cascades

☐ West of Cascades

CHECK IN NARROW COLUMN THE ANALYSIS DESIRED FOR EACH SAMPLE

Sample no.	Sample depth in.	Lab. no.	pH	P (ppm)	Extractable Bases				B (ppm)							
					K (ppm)	Ca meq./100g	Mg meq./100g	Na meq./100g								

APPENDIX E

MSU WATER ANALYSIS SERVICE

APPENDIX E

MSU WATER ANALYSIS SERVICE

The Limnology Laboratory at Michigan State University has offered to do the analyses of chlorides, sulfates, boron, copper, iron, magnesium, manganese, potassium, sodium and zinc at a charge to the individual country budgets of $40.00 per water sample. If you use this service, a payment made out to Michigan State University should be submitted by the USA PI 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 MUS's Customs Broker and their charge of $5.00 per sample to clear customs is included above the total charge of $40.00 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, Dept. 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 USA PIs 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 HC1 are of course not suitable). Since pH probes lose $C1^-$ 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.

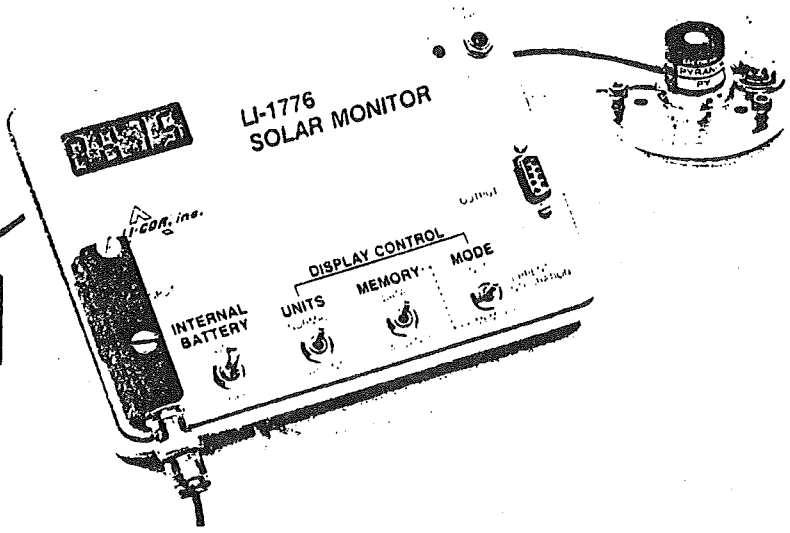
APPENDIX F

MATERIALS AND METHODS

LI-COR

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.

LI-1776 $750.00

* 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 $845.00

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 $945.00

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.

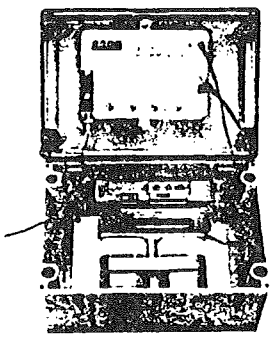
LI-1776/07 $370.00

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

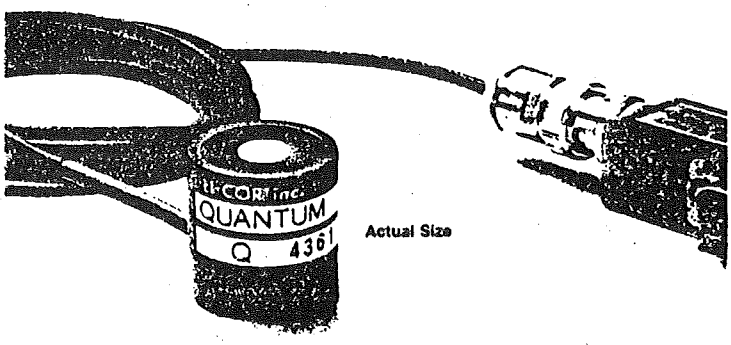
For field operation, this fiberglass case provides a weatherproof enclosure for the LI-1776 and a cassette tape recorder (optional). Included with the 1776-06 is a 12 volt battery pack and external battery leads.

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).

Quantum Sensors

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

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.*



Actual Size

LI-190SB Quantum Sensor $240.00

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}.

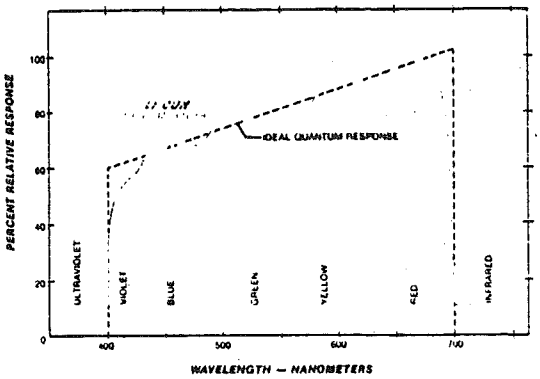


Figure 1. Typical spectral response of LI-COR Quantum Sensors vs. Wavelength and the Ideal Quantum Response (equal response to all photons in the 400-700 nm waveband). An interference filter (for sharp near infrared cutoff at 700 nm), and colored glass filters, tailor the silicon photodiode response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

LI-190SB Specifications

Absolute Calibration: ± 5% traceable to the U.S. National Bureau of Standards (NBS). See page 22.
Relative Error (spectral response): < ± 1% relative error for plant canopies; < ± 5% for standard growth chamber lighting (see Figure 1).
Sensitivity: Typically 8μA per 1000 μE s^{-1} m^{-2}.
Millivolt Output: 5.0 mV per 1000 μE s^{-1} m^{-2} using millivolt adapter (included).
Linearity: Maximum deviation of 1% up to 10,000 μE s^{-1} m^{-2}.

Common Sensor Specifications

(**LI-190SB** Quantum Sensor, **LI-220SB** Near Infrared Sensor, **LI-190SEB** Photosynthetic Irradiance Sensor, **LI-200SB** Pyranometer Sensor, **LI-210SB** Photometric Sensor)

Stability: < ± 2% change over a 1 year period.
Response Time: 10 μs.
Temperature Dependence: ± 0.15% per °C maximum.
Cosine Correction: Cosine corrected up to 80° angle of incidence.
Azimuth: < ± 1% error over 360° at 45° elevation.
Tilt: No error induced from orientation.
Detector: High stability silicon photovoltaic detector (blue enhanced).
Sensor Housing: Weatherproof anodized aluminum case with acrylic diffuser and stainless steel hardware.
Size: 2.38 Dia. x 2.54 cm H (0.94" x 1.0").
Weight: 28 g (1 oz).
Cable Length: 1.5 m (5 ft).
Accessories: 2003S Mounting and Leveling Fixture, 2010S Miniature Shadow Band, 2222SB Extension Cable.

See pages 42 to 46 for explanations of measurement errors.

Indoor/Outdoor Wind Speed and Direction Indicator

Tells wind speed or direction by 2-position knob. Self contained, no outside power source needed. Easy to install and maintain. Outdoor parts corrosion-resistant. Attractive mahogany-finished indoor indicating unit 6½" x 5" Complete with battery, 60 feet of lead-in wire and instructions.

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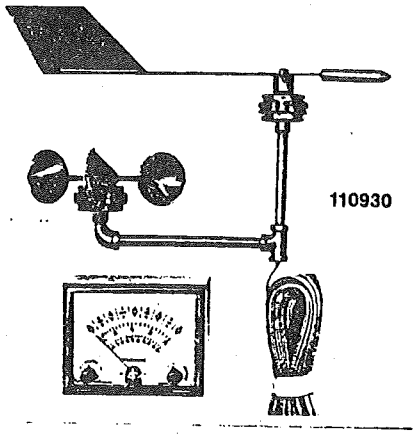

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1 □ Physical limnology

(from Lind, 1975)

MAPPING

Mapping a pond or small lake by plane table method

The mapping of lakes is usually beyond the capability of the limnologist, who must rely on maps made by well-equipped survey teams. Often aerial photographs with known scale will suffice. However, small ponds (generally less than 5 hectares) 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 (crude alidade may be made by setting pins in opposite ends of wooden ruler for "sights")
Compass
Ruler, graduated in millimeters
Map paper
Hard-lead pencils
Round-headed map pins
Steel measuring tape, approximately 30 to 50 m long
2 steel stakes, approximately 0.5 m long
Wooden stakes, approximately 1 m long (25 to 100, depending on size of pond)
Sledgehammer
Stadia rod or other painted pole at least 2 m long

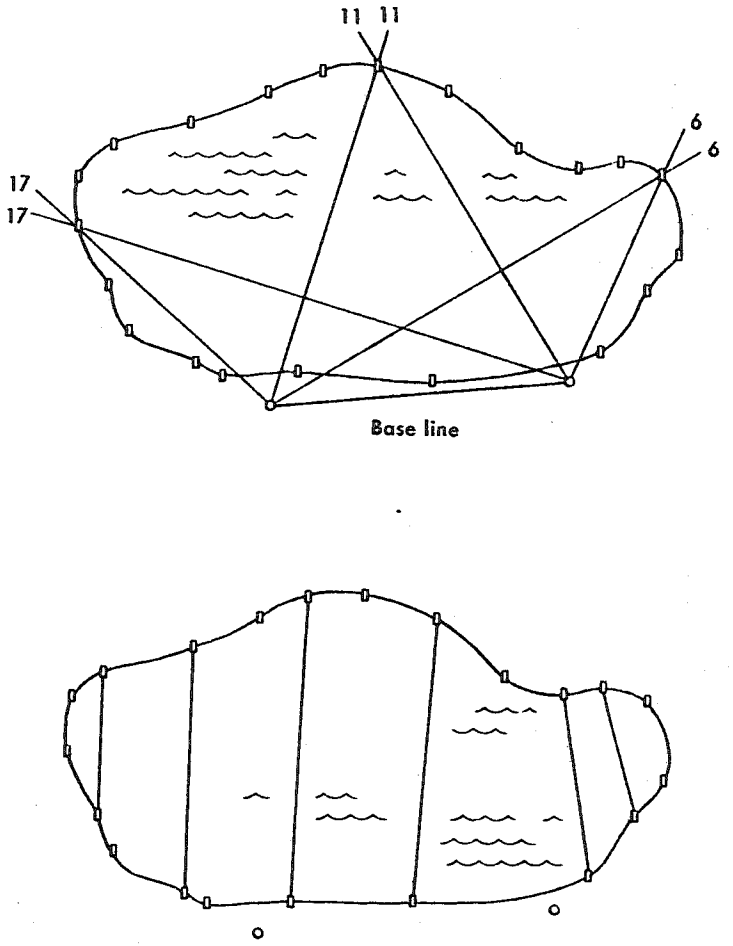

Fig. 1. Plane table map *(top)* showing position of numbered shoreline stakes and the base line. Distances for three shoreline points as determined by triangulation from the ends of the base line are shown. In a small lake or pond, bottom contours may be determined by sounding along a calibrated rope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

Metal-core clothesline rope, of sufficient length to reach across pond, marked in 1 and 5 m increments
Small boat or canoe
Depth sounding line or electronic depth sounder

Procedure

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

2. Drive steel stake near shore for one end of base line. Most of pond should be visible from this point. Repeat for other end of base line at a point at least 30 m 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 20 m. 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 2 to 5 cm 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. 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.*

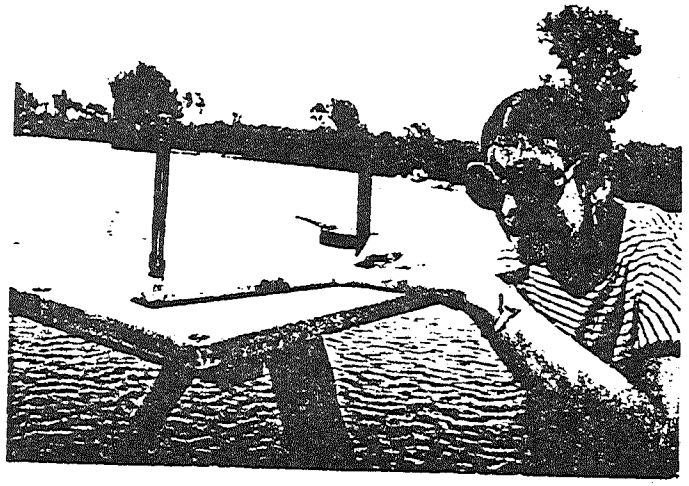

Fig. 2. Student using an open-sight alidade and a plane table to map a small pond. A compass is used to ascertain north orientation of the map. The edge of the alidade at the end nearest the student is always placed against a map pin firmly set in the table. This pin is the map position corresponding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

8. Now in a similar manner, progressively sight alidade around the pond on each wooden stake (flagman holds stadia rod just behind the 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 at which 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 this 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 low 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 ruler; meter stick cut off at 60 to 70 cm is convenient length

Steel-core clothesline rope, of sufficient length to reach across pond's longest dimension, marked in 1 and 5 m increments.

If approximate size of pond is known before going to field, previously prepared table of scale conversions of meters to millimeters will facilitate time spent in 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. Convert this distance to scale in millimeters; 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.

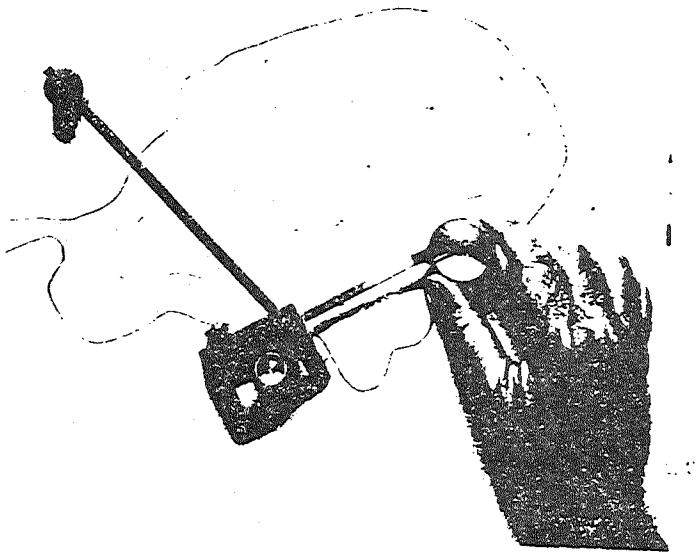

Fig. 3. Compensating polar planimeter used to determine areas. Here the planimeter is tracing the shoreline to determine the total area of a pond drawn by the plane table method.

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 on morphometry).

MORPHOMETRY

Most limnological phenomena, distribution of biota, 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 maps, 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 (Fig. 3) 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. Be sure to observe instructions regarding direction of tracing (usually clockwise) and minimum and maximum allowable angles of the tracing arm.

1. Prepare map by placing it on a 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 three 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 10 cm × 10 cm square is usable) and weigh this piece of paper. Calculate weight per square centimeter.

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

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

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

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 (hypsographic curve). 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 squares that are more than half within, and disregard those which are more than half outside the line. If equal dimensions 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. NOTE: This is the same form of integration described on p. 135.

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 (SLD) 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. A few natural lakes,

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

such as craters and sinkholes, are near unity. Elongate lakes and especially reservoirs impounding water in valleys and side valleys may have SLDs of more than 4. This calculation is of limnological interest because it relates to age—that is, older lakes have had more shoreline erosion, thus are more regular in outline—and to total productivity through more or less littoral areas. This value is calculated as follows:

$$\text{SLD} = \frac{S}{2\sqrt{a\pi}}$$

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. The orientation may be of great significance because it relates to the prevailing seasonal winds of the region.

Maximum depth

The maximum depth (Z_{max}) is the deepest spot in the lake.

Mean depth

The mean depth ($\overline{Z}$) is an important value. It is used in certain other calculations, such as heat budgets, and is generally considered as a reliable morphometric indicator of trophic conditions with $\overline{Z}$ inversely related to production. It is calculated as follows:

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

STREAM FLOW

Physical measurements of flowing waters not only include temperature and light, as in lakes, but also rate of flow and volume of discharge. Stream flow rate is highly variable in different stream portions such as rapids, riffles, and pools. However, it is also variable within one of these types of stream segments. In straight-flowing segments, friction along the stream bottom and sides produces "drag" with maximum velocity occurring in midstream near the surface. In stream bends, the maximum velocity is obtained near the surface at the outside of the curve. Biologists should note that measurement of velocity in such open water portions has little meaning in describing the habitat of most organisms, who usually seek some association with the stream bottom or obstructions to flow. Such considerations are well

(from APHA, 1980)

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.[4] 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

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-

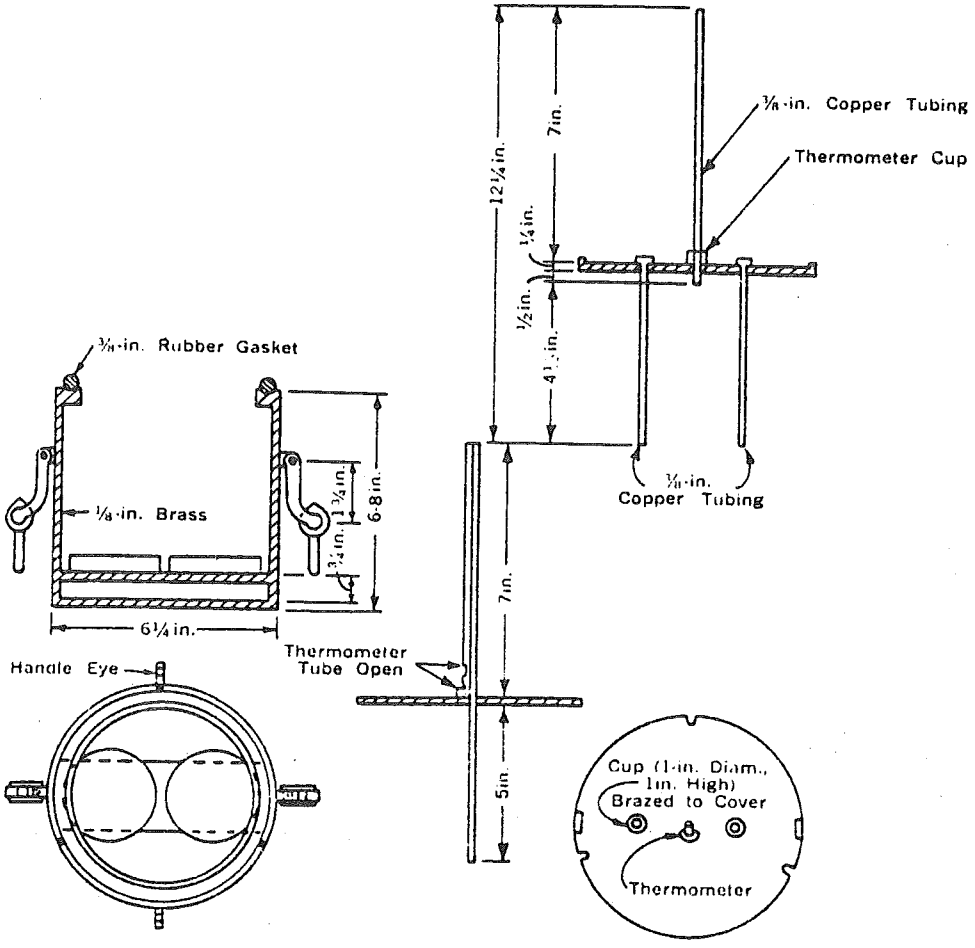

Figure 421:1. DO and BOD sampler assembly.

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-

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.

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.)

TABLE 421:I. SOLUBILITY OF OXYGEN IN WATER EXPOSED TO WATER-SATURATED AIR[a]

Temperature	Chloride Concentration in Water mg/L				
C	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-

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, $AlK(SO_4)_2 \cdot 12H_2O$,

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.

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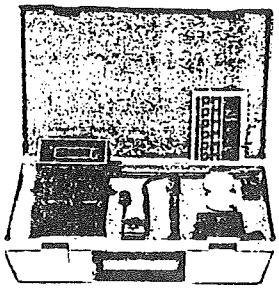

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display	7.5 mm high LED	1 cm high LCD	1 cm high LCD
battery complement	six size AA 1.5 volt batteries	six size AA 1.6 volt batteries	one size D 9 volt battery
battery life	3000 ten second measurements	3000 ten second measurements	3000 ten second measurements
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
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pH range:
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Drift:
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Slope:
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Reference-half-cell filling solution:
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Sizes:
Models 81-55 and 81-56 combination pH: body 120 mm long by 12 mm diameter.

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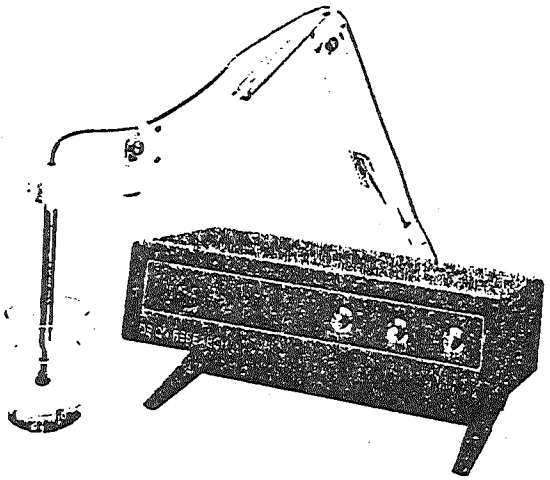

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403 ALKALINITY

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

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,

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

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

c. Standard sulfuric acid or hydrochloric acid, 0.02N: Dilute 200.00 mL 0.1000*N* standard acid to 1,000 mL with distilled or deionized water. Standardize by potentiometric titration of 15.00 mL 0.05*N* 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 0.02*N* 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:

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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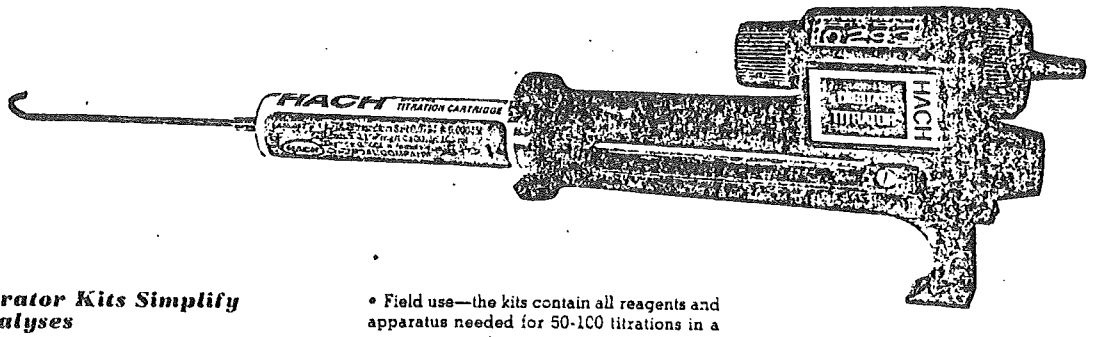

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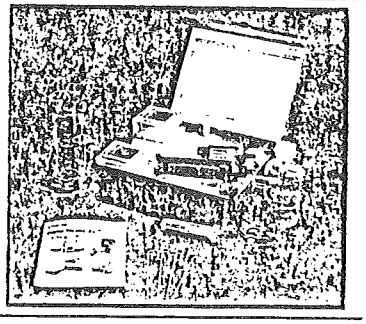
Sample Size: 100 ml, 100 ml and 5 ml, respectively

Order Cat. No. 20635-00.....$108.00



★*Alkalinity Test Kit, High and Low Range,*

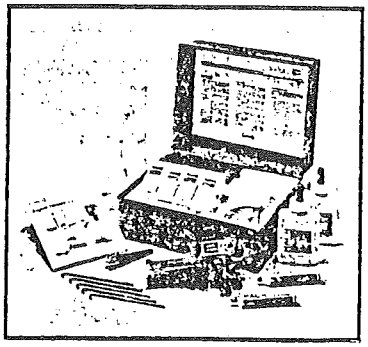
Model AL-DT

Model AL-DT is a higher accuracy version of our Model AL-AP Test Kit. Kit contains the award-winning Digital Titrator and two titration cartridges.

Readout is directly in mg/L. Both phenolphthalein (P) and total (T) alkalinity are determined.

Features:
Ranges: 0-100 and 0-1000 mg/L alkalinity as calcium carbonate
Smallest Increment: 0.1 and 1 mg/L respectively.

Order Cat. No. 20637-00.....$103.00



★*Total and Calcium Hardness Kit, High and Low Range,*

Model HAC-DT

Measure total hardness, calcium and magnesium (by difference) in two ranges with this kit. Tests are conducted using Hach's Digital Titrator and two different EDTA titration cartridges. Analyze for total hardness using ManVer Hardness Indicator; color change is from red to blue. Perform the same titration using CalVer 2 Calcium Indicator to measure calcium content.

Order Cat. No. 20639-00.....$117.00

(from APHA, 1980)

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

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.

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-

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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416 NITROGEN

In waters and wastewaters the forms of nitrogen of greatest interest are, in order of decreasing oxidation state, nitrate, nitrite, ammonia, and organic nitrogen. All these forms of nitrogen, as well as nitrogen gas (N_2), are biochemically interconvertible and are components of the nitrogen cycle. They are of interest for many reasons.

Organic nitrogen is defined functionally as organically bound nitrogen in the trinegative oxidation state. It does not include all organic nitrogen compounds. Analytically, organic nitrogen and ammonia can be determined together and have been referred to as "kjeldahl nitrogen," a term that reflects the technic used in their determination. Organic nitrogen includes such

natural materials as proteins and peptides, nucleic acids and urea, and numerous synthetic organic materials. Typical organic nitrogen concentrations vary from a few hundred micrograms per liter in some lakes to more than 20 mg/L in raw sewage.

Total oxidized nitrogen is the sum of nitrate and nitrite nitrogen. Nitrate generally occurs in trace quantities in surface water but may attain high levels in some groundwater. In excessive amounts, it contributes to the illness known as methemoglobinemia in infants. A limit of 10 mg nitrate as nitrogen/L has been imposed on drinking water to prevent this disorder. Nitrate is found only in small amounts in fresh domestic wastewater but in the effluent of nitrifying biological treatment plants nitrate may be found in concentrations of up to 30 mg nitrate as nitrogen/L. It is an essential nutrient for many photosynthetic autotrophs and in some cases has been identified as the growth-limiting nutrient.

Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and in the reduction of nitrate. Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems, and natural waters. Nitrite can enter a water supply system through its use as a corrosion inhibitor in industrial process water. Nitrite is the actual etiologic agent of methemoglobinemia. Nitrous acid, which is formed from nitrite in acidic solution, can react with secondary amines (RR'NH) to form nitrosamines (RR'N-NO), many of which are known to be potent carcinogens. The toxicologic significance of nitrosation reactions in vivo and in the natural environment is the subject of much current concern and research.

Ammonia naturally is present in surface and wastewaters. Its concentration generally is low in groundwaters because it adsorbs to soil particles and clays and is not readily leached from soils. It is produced largely by the deamination of organic nitrogen-containing compounds and by the hydrolysis of urea. At some water treatment plants ammonia is added to react with chlorine to form a combined chlorine residual.

In the chlorination of wastewater effluents containing ammonia, virtually no free residual chlorine is obtained until the ammonia has been oxidized. Rather, the chlorine reacts with ammonia to form mono- and dichloramines. Ammonia concentrations encountered in water vary from less than 10 μg ammonia nitrogen/L in some natural surface and groundwaters to more than 30 mg/L in some wastewaters.

In this manual, organic nitrogen is referred to as organic N, nitrate nitrogen as NO_3^--N, nitrite nitrogen as NO_2^--N, and ammonia nitrogen as NH_3-N.

417 NITROGEN (AMMONIA)

1. Selection of Method

The two major factors that influence selection of the method to determine ammonia are concentration and presence of interferences. In general, direct manual determination of low concentrations of ammonia is confined to drinking waters, clean surface water, and good-quality nitrified wastewater effluent. In other instances, and where interferences are present and greater precision is necessary, a preliminary distillation step (A) is required. For high ammonia concentrations a distillation and titration technic is preferred. The data presented in 417.4 below and Table 417:I should be helpful in selecting the appropriate method of analysis.

Two manual colorimetric technics—the

nesslerization (B) and phenate (C) methods—and one titration method (D) are presented. An ammonia-selective electrode method (E), which may be used either with or without prior sample distillation, and an automated version of the phenate method (F) also are included. While the stated maximum concentration ranges for the manual methods are not rigorous limits, titration is preferred at concentrations higher than the stated maximum levels for the photometric procedure.

The nessler method is sensitive to 20 μg NH_3-N/L under optimum conditions and may be used for up to 5 mg NH_3-N/L. Turbidity, color, and substances precipitated by hydroxyl ion, such as magnesium and calcium, interfere and may be removed by preliminary distillation or, less satisfactorily, by precipitation with zinc sulfate and alkali.

The manual phenate method has a sensitivity of 10 μg NH_3-N/L and is useful for up to 500 μg NH_3-N/L. Preliminary distillation is required if the alkalinity exceeds 500 mg $CaCO_3$/L or if color or turbidity is present. This step also must precede the phenate technic if the sample has been preserved with acid.

The distillation and titration procedure is used especially for NH_3-N concentrations greater than 5 mg/L.

Distillation into sulfuric acid (H_2SO_4) absorbent is mandatory for the phenate method when interferences are present. Boric acid must be the absorbent following distillation if the distillate is to be nesslerized or titrated.

The ammonia-selective electrode method is applicable over the range from 0.03 to 1,400 mg NH_3-N/L.

2. Interferences

Glycine, urea, glutamic acid, cyanates, and acetamide hydrolyze very slowly in solution on standing but, of these, only urea and cyanates will hydrolyze on distillation at pH of 9.5. Hydrolysis amounts to about 7% at this pH for urea and about 5% for cyanates. Glycine, hydrazine, and some amines will react with nessler reagent to give the characteristic yellow color in the time required for the test. Similarly, volatile alkaline compounds such as hydrazine and amines will influence titrimetric results. Some organic compounds such as ketones, aldehydes, alcohols, and some amines may cause a yellowish or greenish off-color or a turbidity on nesslerization following distillation. Some of these, such as formaldehyde, may be eliminated by boiling off at a low pH before nesslerization. Remove residual chlorine by sample pretreatment.

3. Storage of Samples

Most reliable results are obtained on fresh samples. Destroy residual chlorine immediately after sample collection to prevent its reaction with ammonia. If prompt analysis is impossible, preserve samples with 0.8 mL conc H_2SO_4/L sample and store at 4 C. The pH of the acid-preserved samples should be between 1.5 and 2. Some wastewaters may require more conc H_2SO_4 to achieve this pH. If acid preservation is used, neutralize samples with NaOH or KOH immediately before making the determination.

4. Precision and Accuracy

Six synthetic samples containing ammonia and other constituents dissolved in distilled water were analyzed by five procedures. The first three samples were subjected to direct nesslerization alone, distillation followed by nesslerization, and distillation followed by titration. Samples 4 through 6 were analyzed by direct nesslerization, by distillation followed by nesslerization, by the phenate method alone, and by distillation followed by the phenate method. Results obtained by the participating laboratories are summarized in Table 417:1.

Sample 1 contained the following addi-

TABLE 417.I. PRECISION AND ACCURACY DATA FOR AMMONIA METHODS

Number of Laboratories	Ammonia Nitrogen Concentration µg/L	Relative Standard Deviation					Relative Error				
				Distillation Plus					Distillation Plus		
		Direct Nesslerization %	Direct Manual Phenate Method %	Nessler Method %	Manual Phenate Method %	Titrimetric Method %	Direct Nesslerization %	Direct Manual Phenate Method %	Nessler Method %	Manual Phenate Method %	Titrimetric Method %
20	200	38.1	—	—	—	—	0	—	—	—	—
44	200	—	—	46.3	—	—	—	—	10.0	—	—
21	200	—	—	—	—	69.8	—	—	—	—	20.0
20	800	11.2	—	—	—	—	0	—	—	—	—
42	800	—	—	21.2	—	—	—	—	8.7	—	—
20	800	—	—	—	—	28.6	—	—	—	—	5.0
21	1,500	11.6	—	—	—	—	0.6	—	—	—	—
42	1,500	—	—	18.0	—	—	—	—	4.0	—	—
21	1,500	—	—	—	—	21.6	—	—	—	—	2.6
70	200	—	39.2	—	—	—	—	2.4	—	—	—
3	200	—	—	—	15.1	—	—	—	—	16.7	—
9	200	22.0	—	—	—	—	8.3	—	—	—	—
5	200	—	—	15.7	—	—	—	—	2.0	—	—
66	800	—	15.8	—	—	—	—	1.5	—	—	—
3	800	—	—	—	16.6	—	—	—	—	1.7	—
9	800	16.1	—	—	—	—	0.3	—	—	—	—
6	800	—	—	16.3	—	—	—	—	3.1	—	—
71	1,500	—	26.0	—	—	—	—	10.0	—	—	—
3	1,500	—	—	—	7.3	—	—	—	—	0.4	—
8	1,500	5.3	—	—	—	—	1.2	—	—	—	—
6	1,500	—	—	7.5	—	—	—	—	3.6	—	—

tional constituents: 10 mg chloride/L, 1.0 mg NO_3^--N/L, 1.5 mg organic N/L, 10.0 mg phosphate/L, and 5.0 mg silica/L.

Sample 2 contained the following additional constituents: 200 mg chloride/L, 1.0 mg NO_3^--N/L, 0.8 mg organic N/L, 5.0 mg phosphate/L, and 15.0 mg silica/L.

Sample 3 contained the following additional constituents: 400 mg chloride/L, 1.0 mg NO_3^--N/L, 0.2 mg organic N/L, 0.5 mg phosphate/L, and 30.0 mg silica/L.

Sample 4 contained the following additional constituents: 400 mg chloride/L, 0.05 mg NO_3^--N/L, 0.23 mg organic phosphorus/L added in the form of adenylic acid, 7.00 mg orthophosphate phosphorus/L, and 3.00 mg polyphosphate phosphorus/L added as sodium hexametaphosphate.

Sample 5 contained the following additional constituents: 400 mg chloride/L, 5.00 mg NO_3^--N/L, 0.09 mg organic phosphorus/L added in the form of adenylic acid, 0.6 mg orthophosphate phosphorus/L, and 0.3 mg polyphosphate phosphorus/L added as sodium hexametaphosphate.

Sample 6 contained the following additional constituents: 400 mg chloride/L, 0.4 mg NO_3^--N/L, 0.03 mg organic phosphorus/L added in the form of adenylic acid, 0.1 mg orthophosphate phosphorus/L, and 0.08 mg polyphosphate phosphorus/L added as sodium hexametaphosphate.

For the ammonia-selective electrode in a single laboratory using surface water samples at concentrations of 1.00, 0.77, 0.19, and 0.13 mg NH_3-N/L, standard deviations were +0.038, +0.017, +0.007, and +0.003, respectively. In a single laboratory using surface water samples at concentrations of 0.10 and 0.13 NH_3-N/L, recoveries were 96% and 91%, respectively. The results of an interlaboratory study involving 12 laboratories using the ammonia-selective electrode* on distilled water and effluents are summarized in Table 417:II.

For the automated phenate system, with Automated System I† in a single laboratory using surface water samples at concentrations of 1.41, 0.77, 0.59, and 0.43 mg NH_3-N/L, the standard deviation was +0.005, and at concentrations of 0.16 and 1.44 mg NH_3-N/L, recoveries were 107 and 99%, respectively.

*American Society For Testing and Materials. ASTM Method 1426-79.

†AutoAnalyzer I, Technicon Instrument Corporation, Tarrytown, N.Y. 10591.

TABLE 417:II. PRECISION AND BIAS OF AMMONIA-SELECTIVE ELECTRODE

Level *mg/L*	Matrix	Mean Recovery %	Precision	
			Overall, S_T	Operator, S_o
0.04	Distilled water	200	0.05	0.01
	Effluent water	100	0.03	0.00
0.10	Distilled water	180	0.05	0.01
	Effluent water	470	0.61	0.01
0.80	Distilled water	105	0.11	0.04
	Effluent water	105	0.30	0.06
20	Distilled water	95	2	1
	Effluent water	95	3	2
100	Distilled water	98	5	2
	Effluent water	97	—	—
750	Distilled water	97	78	12
	Effluent water	99	106	10

417 A. Preliminary Distillation Step

1. General Discussion

The sample is buffered at pH 9.5 with a borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds. It is distilled into a solution of boric acid when nesslerization or titration is to be used or into H_2SO_4 when the phenate method is used. The ammonia in the distillate can be determined either colorimetrically by nesslerization or the phenate method or titrimetrically with standard H_2SO_4 and a mixed indicator or a pH meter. The choice between the colorimetric or acidimetric method depends on the concentration of ammonia. Ammonia in the distillate also can be determined by the ammonia-selective electrode method, using 0.04*N* H_2SO_4 to trap the distillate.

2. Apparatus

a. Distillation apparatus: Arrange a borosilicate glass flask of 800 to 2,000-mL capacity attached to a vertical condenser so that the outlet tip may be submerged to at least 2 cm below the surface of the receiving acid solution. Use an all-borosilicate-glass apparatus or one with condensing units constructed of block tin or aluminum tubes.

b. pH meter.

3. Reagents

a. Ammonia-free water: Prepare by ion-exchange or distillation methods:

1) Ion exchange—Prepare ammonia-free water by passing distilled water through an ion-exchange column containing a strongly acidic cation-exchange resin mixed with a strongly basic anion-exchange resin. Select resins that will remove organic compounds that interfere with the ammonia determination. Some anion-exchange resins tend to release ammonia. If this occurs, prepare ammonia-free water with a strongly acidic cation-exchange resin. Regenerate the column according to the manufacturer's instructions. Check ammonia-free water for the possibility of a high blank value.

2) Distillation—Eliminate traces of ammonia in distilled water by adding 0.1 mL conc H_2SO_4 to 1 L distilled water and redistilling. Alternatively, treat distilled water with sufficient bromine or chlorine water to produce a free halogen residual of 2 to 5 mg/L and redistill after standing at least 1 hr. Discard the first 100 mL distillate. Check redistilled water for the possibility of a high blank.

It is very difficult to store ammonia-free water in the laboratory without contamination from ammonia fumes. However, if storage is necessary, store in a tightly stoppered glass container to which is added about 10 g ion-exchange resin (preferably a strongly acidic cation-exchange resin) per liter ammonia-free water. For use, let resin settle and decant ammonia-free water. If a high blank value is produced, replace the resin or prepare fresh ammonia-free water.

Prepare all reagents with ammonia-free distilled water.

b. Borate buffer solution: Add 88 mL 0.1*N* NaOH solution to 500 mL approximately 0.025*M* sodium tetraborate ($Na_2B_4O_7$) solution (9.5 g $Na_2B_4O_7 \cdot 10H_2O$/L) and dilute to 1 L.

c. Sodium hydroxide, 6N: Dissolve 240 g NaOH in ammonia-free distilled water and dilute to 1 L.

d. Dechlorinating agent, N/70: Use 1 mL of any of the following reagents to remove 1 mg/L residual chlorine in 500 mL sample.

1) *Phenylarsine oxide:* Dissolve 1.2 g C_6H_5AsO in 200 mL 0.3*N* NaOH solution, filter if necessary, and dilute to 1 L with ammonia-free water. (CAUTION: *Toxic—take care to avoid ingestion.*)

2) *Sodium arsenite:* Dissolve 1.0 g $NaAsO_2$ in ammonia-free water and dilute to 1 L. (CAUTION: *Toxic—take care to avoid ingestion.*) Prepare fresh weekly.

3) *Sodium sulfite:* Dissolve 0.9 g Na_2SO_3 in ammonia-free water and dilute to 1 L. Prepare fresh daily.

4) *Sodium thiosulfate:* Dissolve 3.5 g $Na_2S_2O_3 \cdot 5H_2O$ in ammonia-free water and dilute to 1 L. Prepare fresh weekly.

e. Neutralization agent: Prepare with ammonia-free water.

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

2) *Sulfuric acid,* H_2SO_4, 1*N*.

f. Absorbent solution, plain boric acid: Dissolve 20 g H_3BO_3 in ammonia-free water and dilute to 1 L.

g. Indicating boric acid solution: See Section 417D.3*a* and *b*.

h. Sulfuric acid, 0.04*N*: Dilute 1.0 mL conc H_2SO_4 to 1 L.

4. Procedure

a. Preparation of equipment: Add 500 mL ammonia-free water and 20 mL borate buffer to a distillation flask and adjust pH to 9.5 with 6*N* NaOH solution. Add a few glass beads or boiling chips and use this mixture to steam out the distillation apparatus until distillate shows no traces of ammonia.

b. Sample preparation: Use 500 mL of dechlorinated sample or a portion diluted to 500 mL with ammonia-free water. When NH_3-N concentration is less than 100 μg/L, use a sample volume of 1,000 mL. Remove residual chlorine by adding, at the time of collection, dechlorinating agent equivalent to the chlorine residual. If necessary, neutralize to approximately pH 7 with dilute acid or base, using a pH meter.

Add 25 mL borate buffer solution and adjust to pH 9.5 with 6*N* NaOH using a pH meter.

c. Distillation: To minimize contamination, leave distillation apparatus assembled after steaming out and until just before starting sample distillation. Disconnect steaming-out flask and immediately transfer sample flask to distillation apparatus. Distill at a rate of 6 to 10 mL/min with the tip of the delivery tube submerged to at least 2 cm below the surface of acid receiving solution. Collect distillate in a 500-mL erlenmeyer flask containing 50 mL plain boric acid solution for nesslerization method. Use 50 mL indicating boric acid solution for titrimetric method. Distill ammonia into 50 mL 0.04*N* H_2SO_4 for the phenate method and for the ammonia-selective electrode method. Collect at least 200 mL distillate. Lower collected distillate free of contact with delivery tube and continue distillation during the last minute or two to cleanse condenser and delivery tube. Dilute to 500 mL with ammonia-free water.

When the phenate method is used for determining NH_3-N, neutralize distillate with 1*N* NaOH solution.

d. Ammonia determination: Determine ammonia by the nesslerization method (Section 417B), the phenate method (Section 417C), the titrimetric method (Section 417D) or the ammonia-selective electrode method (Section 417E).

417 C. Phenate Method

1. General Discussion

a. Principle: An intensely blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by a manganous salt.

b. Interference: Alkalinity over 500 mg as $CaCO_3$/L, acidity over 100 mg as $CaCO_3$/L, color, and turbidity interfere. Remove these interferences by preliminary distillation.

2. Apparatus

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

1) *Spectrophotometer,* for use at 630 nm with a light path of approximately 1 cm.

2) *Filter photometer,* equipped with a red-orange filter having a maximum transmittance near 630 nm and providing a light path of approximately 1 cm.

b. Magnetic stirrer.

3. Reagents

a. Ammonia-free water: Prepare as directed in Section 417A.3*a*.

b. Hypochlorous acid reagent: To 40 mL distilled water add 10 mL 5% NaOCl solution prepared from commercial bleach. Adjust pH to 6.5 to 7.0 with HCl. Prepare this unstable reagent weekly.

c. Manganous sulfate solution, 0.003M: Dissolve 50 mg $MnSO_4 \cdot H_2O$ in 100 mL distilled water.

d. Phenate reagent: Dissolve 2.5 g NaOH and 10 g phenol, C_6H_5OH, in 100 mL ammonia-free water. Because this reagent darkens on standing, prepare weekly. (CAUTION: *Handle phenol with care.*)

e. Stock ammonium solution: Dissolve 381.9 mg anhydrous NH_4Cl, dried at 100 C, in ammonia-free water, and dilute to 1,000 mL; 1.00 mL = 100 μg N = 122 μg NH_3.

f. Standard ammonium solution: Dilute 5.00 mL stock ammonium solution to 1,000 mL with ammonia-free water; 1.00 mL = 0.500 μg N = 0.607 μg NH_3.

4. Procedure

a. Treatment of sample: To a 10.0-mL sample in a 50-mL beaker, add 1 drop (0.05 mL) $MnSO_4$ solution. Place on a magnetic stirrer and add 0.5 mL hypochlorous acid reagent. Immediately add, a drop at a time, 0.6 mL phenate reagent. Add reagent without delay using a bulb pipet or a buret for convenient delivery. Mark pipet for hypochlorous acid at the 0.5-mL level and deliver the phenate reagent from a pipet or a buret that has been calibrated by counting the number of drops previously found to be equivalent to 0.6 mL. Stir vigorously during addition of reagents. Because color intensity is affected by age of reagents, carry a blank and a standard through the procedure with each batch of samples. Measure absorbance using reagent blank to zero the spectrophotometer. Color formation is complete in 10 min and is stable for at least 24 hr. Although the blue color has a maximum absorbance at 630 nm, satisfactory measurements can be made in the 600- to 660-nm region.

b. Preparation of standards: Prepare a calibration curve in the NH_3-N range of 0.1 to 5 μg, treating standards exactly as the sample. Beer's Law governs.

5. Calculation

Calculate ammonia concentration as follows:

$$\text{mg } NH_3\text{-N/L (11.1 mL final volume)} = \frac{A \times B}{C \times S} \times \frac{D}{E}$$

where:

A = absorbance of sample,
B = NH_3-N in standard, μg,
C = absorbance of standard,
S = volume of sample used, mL,
D = volume of total distillate collected, mL, including acid absorbent, neutralizing agent, and ammonia-free water added, and
E = volume of distillate used for color development, mL.

The ratio D/E applies only to distilled samples.

6. Precision and Accuracy

See Section 417A.4 and Table 417:I.

133 A. Phenoldisulfonic Acid Method

1. General Discussion

a. Principle: The yellow color produced by the reaction between nitrate and phenoldisulfonic acid obeys Beer's law up to at least 12 mg/l N at a wavelength of 480 mμ when a light path of 1 cm is used. At a wavelength of 410 mμ, the point of maximum absorption, determinations may be made up to 2 mg/l with the same cell path.

b. Interference: As even small concentrations of chloride result in nitrate losses using this method, it is important that the chloride content be reduced to a minimum, preferably below 10 mg/l. However, the silver sulfate used for this purpose presents problems with some water samples owing to the incomplete precipitation of silver ion, which produces an off color or turbidity when the final color is developed. The preferred alkali for color development in the final stage of the determination is ammonium hydroxide, particularly where chloride removal must be prac-

ticed on the sample. Potassium hydroxide should be used only if ammonia fumes must be reduced to a minimum in the laboratory atmosphere (for example, when trace amounts of ammonia nitrogen are being determined concurrently). A faint tinge of brown is imparted by potassium hydroxide to the final color when a silver compound has been previously applied for chloride precipitation. Nitrite levels in excess of 0.2 mg/l N erratically increase the apparent nitrate concentration. Colored ions and materials physically modifying the color system should be absent.

c. Minimum detectable concentration: In the absence of interference, the phenoldisulfonic acid method is sensitive to 1 μg of nitrate nitrogen, which represents 10 μg/l in a 100-ml sample.

2. Apparatus

COLORIMETRIC EQUIPMENT—One of the following is required:

a. Spectrophotometer, for use at 410 mμ, providing a light path of 1 cm or longer.

b. Filter photometer, providing a light path of 1 cm or longer and equipped with a violet filter having maximum transmittance near 410 mμ.

c. Nessler tubes, matched, 50- or 100-ml.

3. Reagents

Prepare all reagents from chemicals which are white in color and store all solutions in pyrex containers.

a. Standard silver sulfate solution: Dissolve 4.40 g Ag_2SO_4, free from nitrate, in distilled water and dilute to 1.0 liter; 1.00 ml is equivalent to 1.00 mg Cl.

b. Phenoldisulfonic acid reagent: Dissolve 25 g pure white phenol in 150 ml conc H_2SO_4. Add 75 ml fuming H_2SO_4 (15% free SO_3), stir well, and heat for 2 hr on a hot water bath.

c. Ammonium hydroxide, conc: If this cannot be used, prepare 12*N* potassium hydroxide solution by dissolving 673 g KOH in distilled water and diluting to 1 liter.

d. EDTA reagent: Rub 50 g disodium ethylenediamine tetraacetate dihydrate, also called (ethylenedinitrilo)-tetraacetic acid sodium salt, with 20 ml distilled water to form a thoroughly wetted paste. Add 60 ml conc NH_4OH and mix well to dissolve the paste. [The EDTA solution described under Ammonia Nitrogen, Method B, Section 132B.3c(1), can also be used.]

e. Stock nitrate solution: Dissolve 721.8 mg anhydrous potassium nitrate, KNO_3, and dilute to 1,000 ml with distilled water. This solution contains 100 mg/l N.

f. Standard nitrate solution: Evaporate 50.0 ml stock nitrate solution to dryness on a steam or hot water bath; dissolve the residue by rubbing with 2.0 ml phenoldisulfonic acid reagent, and dilute to 500 ml with distilled water; 1.00 ml = 10.0 μg N = 44.3 μg NO_3.

g. Reagents for treatment of unusual interference:

1) ALUMINUM HYDROXIDE SUSPENSION—Dissolve 125 g aluminum potassium or ammonium sulfate, $AlK(SO_4)_2 \cdot 12H_2O$ or $AlNH_4(SO_4)_2 \cdot 12H_2O$ in 1 liter distilled water. Warm to 60 C and add 55 ml conc NH_4OH slowly, with stirring. After permitting the mixture to stand about 1 hr, transfer to a large bottle and wash the precipitate by successive additions (with thorough mixing) and decantations of distilled water, until free from ammonia, chloride, nitrate and nitrite. Finally, after

settling, decant off as much clear liquid as possible, leaving only the concentrated suspension.

2) SULFURIC ACID, 1*N*.

3) POTASSIUM PERMANGANATE, 0.1 *N*—Dissolve 316 mg $KMnO_4$ in distilled water and dilute to 100 ml.

4) DILUTE HYDROGEN PEROXIDE SOLUTION—Dilute 10 ml of 30% hydrogen peroxide (low in nitrate) to 100 ml with distilled water.

5) SODIUM HYDROXIDE, 1*N*.

4. Procedure

a. Color removal: If the sample has a color of more than 10, decolorize by adding 3 ml aluminum hydroxide suspension to 150 ml of sample, stir very thoroughly, allow to stand for a few minutes, then filter, discarding the first portion of the filtrate.

b. Nitrite conversion: To 100 ml of sample add 1 ml of H_2SO_4 and stir. Add dropwise, with stirring, either $KMnO_4$ or H_2O_2 solution. Let the treated sample stand for 15 min to complete the conversion of nitrite to nitrate. (A faint pink color persists for at least 15 min when sufficient $KMnO_4$ is used.) Make the proper deduction at the end of the nitrate determination for the nitrite concentration as determined by the method described in Nitrogen (Nitrite), Section 134 following.

c. Chloride removal: Determine the chioride content of the water (see Section 112) and treat 100 ml of sample with an equivalent amount of standard silver sulfate solution. Remove the precipitated chloride either by centrifugation or by filtration, coagulating the silver chloride by heat if necessary. (Excellent removal of silver chloride can be achieved by allowing the treated sample to stand overnight at laboratory temperature away from strong light. This approach is applicable to samples free of contamination by nitrifying organisms.)

d. Evaporation and color development: Neutralize the clarified sample to approximately pH 7, transfer to a casserole, and evaporate to dryness over a hot water bath. Using a glass rod, rub the residue thoroughly with 2.0 ml phenoldisulfonic acid reagent to insure dissolution of all solids. If need be, heat mildly on the hot water bath a short time to dissolve the entire residue. Dilute with 20 ml distilled water and add, with stirring, about 6 to 7 ml NH_4OH or about 5 to 6 ml KOH until maximum color is developed. Remove any resulting flocculent hydroxides by passing through a filter paper or filtering crucible, or add the EDTA reagent dropwise, with stirring, until the turbidity redissolves. Transfer the filtrate or clear solution to a 50- or 100-ml volumetric flask or nessler tube, dilute to the mark, and mix.

e. Photometric measurement: Make photometric readings in cells with a 1-cm or longer light path at a wavelength of 410 mμ, or with violet filters exhibiting maximum transmittance in the range from 400 to 425 mμ. If available, use a 5-cm light path for measurements in the nitrogen interval from 5 to 50 μg, and a 1-cm light path in a proportionate range. Make readings against a blank prepared from the same volumes of phenoldisulfonic acid reagent and NH_4OH or KOH as used for the samples.

f. Visual comparison: In the case of 50-ml nessler tubes, use the following volumes of standard nitrate solution: 0, 0.1, 0.3, 0.5, 0.7, 1.0, 1.5, 2.0, 3.5, 6.0, 10, 15, 20 and 30 ml. Where it is more convenient to use a total volume

of 100 ml, double the volumes of standard solution. To each of these standards add 2.0 ml phenoldisulfonic acid reagent and the same volume of the same alkali as is used in preparation of the sample. These standards may be kept several weeks without deterioration.

5. Calculation

$$\text{mg/l nitrate N} = \frac{\mu\text{g nitrate N}}{\text{ml sample}}$$

$$\text{mg/l } NO_3 = \text{mg/l nitrate N} \times 4.43$$

6. Precision and Accuracy

Accuracy of the order of ±0.1 mg/l nitrate N can be obtained only by the proper treatment of the chloride and nitrite interference. Many laboratories appear to have difficulty in coping with these interferences.

A synthetic unknown sample containing 1.0 mg/l nitrate N, 10 mg/l chloride, 200 μg/l ammonia N, 1.5 mg/l organic N, 10.0 mg/l phosphate, and 5.0 mg/l silica in distilled water was determined by the phenoldisulfonic acid method, with a relative standard deviation of 74.4% and a relative error of 38.0% in 46 laboratories.

A second synthetic unknown sample containing 1.0 mg/l nitrate N, 200 mg/l chloride, 800 μg/l ammonia N, 800 μg/l organic N, 5.0 mg/l phosphate, and 15.0 mg/l silica in distilled water was determined by the phenoldisulfonic acid method, with a relative standard deviation of 57.9% and a relative error of 31.0% in 46 laboratories.

Phosphorus (from APHA, 1980)

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.

PHOSPHORUS

SAMPLE

Filtered portion (homogenize)

Transfer 50ml by transfer pipet to a labeled 125ml Erlenmeyer flask

Using a pH meter, adjust the sample to pH 7.0± 0.2. Use 5N H_2SO_4 and 1N NaOH

Add 8.0ml of combined reagent and mix thoroughly. After at least 10mins. but not more than 30mins. measure the absorbance at 880.0nm

TOTAL DISSOLVED REACTIVE PHOSPHORUS

Transfer 50ml by transfer pipet to a labeled 125ml Erlenmeyer flask

Add 1ml of 11N H_2SO_4

Add 0.4g of ammonium persulfate

Boil gently on a pre-heated hot plate until a volume of about 10ml is reached (30-40 mins.). DO NOT let the sample go to dryness

Using a pH meter, adjust the sample to pH 7.0± 0.2. Use 5N H_2SO_4 and 1N NaOH

Dilute the sample to 50ml using a volumetric flask. Pour the sample back into the 125ml Erlenmeyer

Add 8.0ml of combined reagent and mix thoroughly. After at least 10mins. but no longer than 30mins. measure the absorbance at 880.0nm

TOTAL DISSOLVED PHOSPHORUS

Non-filtered portion (homogenize)

Transfer 50ml by transfer pipet to a labeled 125ml Erlenmeyer flask

Add 1ml of 11N H_2SO_4

Add 0.4g of ammonium persulfate

Boil gently on a pre-heated hot plate until a volume of about 10ml is reached (30-40 mins.). DO NOT let the sample go to dryness

Using a pH meter, adjust the sample to pH 7.0± 0.2. Use 5N H_2SO_4 and 1N NaOH

Dilute the sample to 50ml using a volumetric flask. Pour the sample back into the 125ml Erlenmeyer

Add 8.0ml of combined reagent and mix thoroughly. After at least 10mins. but no longer than 30mins. measure the absorbance at 880.0nm

TOTAL PHOSPHORUS

REAGENTS FOR PHOSPHORUS

1. 5N H_2SO_4

   Dilute 140ml (measured in a 100ml graduated cylinder) concentrated H_2SO_4 in 800ml of deionized distilled water, let cool, bring up to 1000mL.

2. Antimony potassium tartrate solution

   Weigh 2.7430g $K(SbO)C_4H_4O_6 \cdot 1/2\ H_2O$ in 800ml of deionized distilled water, then dilute to 1000ml. Store at 4°C in a dark, glass-stoppered bottle.

3. Ammonium molybdate solution

   Dissolve 40g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 1000ml of deionized distilled water. Store in plastic bottle at 4°C.

4. Ascorbic acid, 0.1M

   Dissolve 8.80g of ascorbic acid in 500ml of deionized distilled water. Solution stable for 1wk. if stored at 4°C.

5. Combined reagent

   Mix the above reagents in the following proportions for 500ml of mixed reagent in the following order. MIX BETWEEN EACH ADDITION AND ALL REAGENTS MUST HAVE REACHED ROOM TEMPERATURE.
   - 250ml of 5N H_2SO_4 (1.) [Use a 250ml volumetric flask]
   - 25ml of antimony potassium tartrate solution (2.) [Use a 25ml pipet]
   - 75ml of ammonium molybdate solution (3.) [Use a 25ml transfer pipet]
   - 150ml of ascorbic acid solution (4.) [Use a 50ml transfer pipet]

   This must be prepared fresh for each run. From the 500ml of combined reagent 60 samples can be analyzed

6. 11N H_2SO_4

   Add 310ml (use a 500ml graduated cylinder) of concentrated H_2SO_4 to 600ml of deionized distilled water. When cool dilute to 1 liter.

7. Ammonium persulfate

   Need 0.4g amount for each digestion (a heaping glass scoopfull).

8. Stock phosphorus solution

Dissolve 0.2197g of potassium dihydrogen phosphate, KH_2PO_4, which has been dried in an oven at 105°C and stored in a dessicator. Dilute the solution to 1000ml with deionized distilled water; 1.0ml=0.05mg P.

9. Standard phosphorus solution

Dilute 10.0ml of stock phosphorus solution (8.) to 1000ml with deionized distilled water; 1.0ml=0.5ug P.

Using the standard phosphorus solution, prepare the following standards in 200ml volumetric flasks:

ml of std. P soln. (9.)	conc., mg P/l
4.0	0.01
12.0	0.03
20.0	0.05
40.0	0.10
80.0	0.20
120.0	0.30
160.0	0.40
200.0	0.50

10. Sodium hydroxide, 1N

Dissolve 40g NaOH in 600ml of deionized distilled water. Cool and dilute to 1 liter.

MICHIGAN STATE UNIVERSITY

DEPARTMENT OF FISHERIES AND WILDLIFE
NATURAL RESOURCES BUILDING
EAST LANSING · MICHIGAN · 48824
Limnological Research Laboratory

GLASSWARE WASHING PROCEDURES:

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

Page 2 - GLASSWARE WASHING PROCEDURES:

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

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

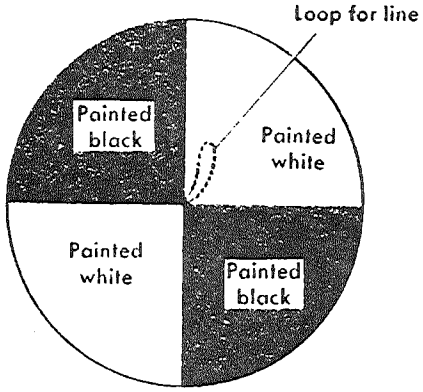

Fig. 7. The Secchi disk may be easily made from a 20 cm diameter metal or weighted wooden disk. Opposite quarters are painted gloss white and gloss black as shown. It is important that the calibrated line be attached so that the disk hangs horizontally in the water. (From Vivian, V. E.: Sourcebook for environmental education, St. Louis, 1973, The C. V. Mosby Co.)

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. 7) 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 little further, 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.

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 AM and 3 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 eyes" 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. The factor should fall between 2 and 5 for most natural waters without unusual turbid layers.

CHLOROPHYLL-a AND PHAEOPIGMENT DETERMINATIONS

For these determinations, the samples should be kept in opaque bottles that are kept cool at 4°C and processed as quickly as possible after sampling. All handling of the samples for the analyses should be done in the dark.

1. Filter approximately 500 ml of the sample through a 47 mm diameter, 0.80 micron GA-4 Gelman Metricel filter. Replace the filter as often as necessary, depending upon the severity of clogging. Record the exact amount of water filtered.
   Note: At this time if it is not possible to go on with the rest of the procedure the filters can be wrapped in aluminum foil and frozen until ready to proceed.

2. Extract the chlorophyll by placing the filter(s) in the bottom of a tissue grinding tube, and add about 10 ml of 90% alkaline aqueous acetone (900 ml of distilled in glass acetone, brought up to 1000 ml with deionized distilled water plus 2-3 drops of NH_3OH).

3. Grind the extract with the tissue grinder for approximately 1½ minutes or until filter is liquified. Pour the sample into a 50 ml volumetric flask to volume with the 90% alkaline acetone.

4. Centrifuge the samples at 3500 rpm for 15 minutes. Make sure the centrifuge is properly balanced. Collect the supernatant, check for clarity. If turbidity is still present, continue centrifuging.

5. All absorbance readings are to be done using a spectrophotometer. The slit band width shoudl be set at around 2.0 nm or low as possible and a 10 cm pathlength cell is to be used. The machine should be zereoed using a 90% alkaline acetone blank.

   A. Read and record the absorbance of the supernatant at 750 nm and 665 nm.

   B. Acidfy the sample by pipetting 2-3 drops of dilute HCl (dilute 50 ml of concentrated HCl to 100 ml with deionized distilled water) into the 10 cm cell, shake vigorously and let set for 5 minutes. Then read and record the absorbance at 750 nm and at 665 nm.

6. Calculate corrected chlorophyll-a according to the following equation:

$$\text{Chlorophyll-a (in mg/m}^3\text{)} = \frac{26.7\ (665_o - 665_a) \times v}{V \times 1}$$

Where: 665_o = [(absorbance at 665 nm before acidification) - (absorbance at 750 nm)]

665_a = [(absorbance at 665 nm after acidification) - (absorbance at 750 nm after acidification)]

v = volume of acetone used for extraction, in ml

V = volume of water filtered, in liters

l = pathlength, in cm

7. Calculate the phaeopigments according to the following equation:

$$\text{Phaeopigments (in mg/m}^3\text{)} = \frac{26.7[1.7(665_a) - 665_o] \times v}{V \times l}$$

Where: 665_o = [(absorbance at 665 nm before acidification) - (absorbance at 750 nm)]

665_a = [(absorbance at 665 nm after acidification) - (absorbance at 750 nm after acidification)]

v = volume of acetone used for extraction, in ml

V = volume of water filtered, in liters

l = cell pathlength, in cm

Oxygen method

The light- and dark-bottle method (Gaarder and Gran, 1927) of measuring the synthetic rate of phytoplankton is suitable for eutrophic waters. Precise analysis of small changes in oxygen concentration may be difficult in oligotrophic waters. This method compares the oxygen changes that occur in plankton communities contained in clear bottles with those occurring in dark bottles during a 24-hour period. In the light bottles, oxygen is evolved during photosynthesis and consumed by plant and animal respiration. In the dark bottle only respiration occurs.

Apparatus

3 glass-stoppered 125 to 300 ml reagent or BOD bottles for each measurement, one painted black (or wrapped with black plastic tape) and wrapped in aluminum foil (provide a square of aluminum foil for final capping)
Rope or other means to suspend pairs of bottles in lake at depth of collection
Various means of attaching paired bottles to rope (snaps and rings, clamps, or wire baskets)
Water collection and analysis apparatus as described in section on dissolved oxygen

Reagents

As for dissolved oxygen.

Procedure

1. Select stations that will adequately represent the major morphological characteristics of the lake and depths that will adequately represent conditions of the water column. These depths may be at regular intervals or at percentages of surface illumination (100%, 75%, 50%, 25%, 1%).

2. Take a sample from each depth, using the best oxygen techniques. Use two light bottles and one dark bottle for each depth. It is preferable to use a large enough sampler (3 liters) to fill all three bottles.

3. Return one light and one dark bottle to their original depth for incubation. Be certain that glass stoppers are firmly in place and that foil cap completely seals area between cap and shoulder of the dark bottle. It is important to avoid even pinhole light leaks. Record the time.

4. Fix remaining bottle from each depth with Winkler oxygen reagents, and determine initial (I) oxygen concentration of the water sample by titration.

5. After 24 hours recover other bottles, and determine oxygen concentration.

Calculations

$$\text{Plankton community respiration (as mg oxygen consumed per hour)} = \frac{I - D}{24}$$

$$\text{Net plankton photosynthesis (as mg oxygen per hour)} = \frac{L - I}{24}$$

Gross plankton photosynthesis (as mg oxygen per hour) =

Net photosynthesis + Community respiration

where

I, D, and L = Oxygen concentrations as mg/L for initial bottle, dark bottle, and light bottle, respectively

These data may be converted to grams of oxygen per cubic meter by multiplying numerator and denominator by 1,000. To estimate the photosynthetic activity per square meter of lake surface, either (1) multiply cubic meter value by the depth, in meters, that it represents, and sum each of these; or (2) plot actual cubic meter values on linear graph paper against depth (m), and then integrate area beneath curve by planimeter or counting of squares.