A Manual for the Measurement of Chlorophyll *a* in Netphytoplankton and Nannophytoplankton

by

C. A. Evans and J. E. O'Reilly

Northeast Fisheries Center
Sandy Hook Laboratory
Division of Environmental Assessment
Highlands, New Jersey 07732

Ocean Pulse Technical Manual No. 3
Report No. SHL 80-17

TABLE OF CONTENTS

1.s Introduction and Brief Overview of Methods

Chlorophyll a concentration is considered an index of phytoplankton biomass. This manual describes standardized methods for making shipboard measurements of chlorophyll a concentrations during the Northeast Fisheries Center's Ocean Pulse and MARMAP surveys of northeastern U. S. coastal and continental shelf water between Cape Hatteras and Nova Scotia. Our survey area and sampling locations are depicted in section 26.10.

Our fluorometric method for determining chlorophyll a is based on the method and suggestions provided in Strickland and Parsons (1972), Holm-Hansen et al. (1965, 1978), UNESCO (1966), Yentsch and Menzel (1963), and Turner Designs (1976). Shortly after collection of seawater, filtration of phytoplankton, grinding and extraction of pigments in 90% acetone and measurement of fluorescence are performed at sea.

In our baseline surveys we are particularly interested in determining the relative biomass of netphytoplankton (>20 µm) and nannophytoplankton (<20 µm). Consequently, all seawater samples are size-fractionated by serial filtration through 20 µm mesh and 0.7 µm mesh filters to yield concentrations of chlorophyll a in netplankton and nannoplankton. "The Ocean's Food Web, A Changing Paradigm" (Pomeroy, 1974) provides a useful description of the structure and function of marine planktonic ecosystems, and emphasizes the importance of the smaller nannoplankton as a major component of the phytoplankton community in many marine environments. Our own studies of phytoplankton in the Raritan-Hudson estuary (O'Reilly et al., 1976), New York Bight (Thomas et al., 1978), and Georges Bank (Thomas et al., 1979) as well as other studies (Malone, 1976; Malone et al., 1979; Durbin et al., 1975; McCarthy et al., 1974) have demonstrated the quantitative importance of nannoplankton assemblages.

We use a fluorometric method rather than a spectrophotometric method for measuring chlorophyll a because the fluorometric method is about 20 times more sensitive (Holm-Hansen et al., 1965) and consequently smaller volumes of seawater are required for an accurate determination. Concentrations approaching 0.01 µgChla/l may be measured on the fluorometer when the plankton in one liter of seawater are filtered and extracted. This is important since in most multidisciplinary field surveys such as ours where subsamples are taken from a single water sampling bottle (i.e. dissolved oxygen, chlorophyll, ^{14}C-primary productivity, nutrients, pH, alkalinity, phytoplankton species, salinity, etc.) the volumes of seawater for each analysis must be minimal.

Additionally, the fluorometric method, because of its enhanced sensitivity, enables us to size-fractionate the phytoplankton community and obtain estimates of chlorophyll a concentration in netphytoplankton and nannophytoplankton throughout an annual cycle, even during periods when either netplankton or nannoplankton are sparse. Other advantages of fluorometric methods are summarized by Turner Designs (1976).

Samples for chlorophyll a analyses are usually collected from the surface, 5, 10, 15, 20, 25, 30, 35, 50, and 75 meters. Deeper depths are sampled depending on the vertical distribution of the phytoplankton and objectives of the sampling program. At stations where primary productivity is measured, additional depths corresponding to 69, 46, 25, 10, 3, and 1% of surface irradiance, are sampled for chlorophyll so that phytoplankton production can be directly related to phytoplankton biomass (O'Reilly and Thomas, 1979). Additionally, a "bottom-trip" Niskin water sampling bottle (rigged to close when a tripping device contacts the seabed) is used routinely during our surveys to better define phytoplankton biomass near the seabed. In shelf and coastal water less than 50 m deep we have frequently observed large concentrations of phytoplankton in water collected within a meter of the seabed.

Chlorophyll a is measured fluorometrically using a Turner Designs fluorometer.* The fluorometer is calibrated using a pure chlorophyll a extract obtained from Sigma Chemical Company. The fluorometer is also checked against a pure chlorophyll a calibration standard obtained from the U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Quality Assurance Branch, Cincinnati, Ohio 45256 USA.

At sea, the fluorescence of chlorophyll a in plankton extracts is read on the fluorometer, two drops of 5% HCl are added to the extract, and the fluoroescence is reread. The "before acid" and "after acid" fluorescence readings and relevant field information concerning sampling location, time, depth, volume of seawater filtered, etc. are coded at sea on a standard chlorophyll computer form.

The data are keypunched and processed through a FORTRAN program to generate concentrations of chlorophyll a (mg/m^3), phaeophytin a (mg/m^3) and the sample acidification ratio (Fo/Fa). The chlorophyll program also sums netphytoplankton and nannophytoplankton chlorophyll a concentrations to generate "total" chlorophyll a for each sampling depth, and generates a weighted average of chlorophyll a concentrations for the water column by arithmetically integrating chlorophyll concentrations over depth and then dividing the integral by the deepest sampling depth (generally 75 m).

*Use of trade names throughout this paper does not imply endorsement by NOAA/National Marine Fisheries Service.o

2.a Equipment Setup and Precautions

To assure the highest quality of chlorophyll analyses, consideration should be given to the location of chlorophyll related activities on shipboard and the compatibility of these analyses with other analyses performed in the same shipboard laboratory.

2.1 Work requiring strong acids should not be done in areas where chlorophyll analyses are being performed. If possible, cleaning of all chlorophyll equipment should be done in an acid-free area (a sink where no acid is dispensed, poured, etc.). Because nutrient analysis, ^{14}C, and dissolved oxygen analysis all require the use of acid at some stage in processing, these analyses should be performed outside of chlorophyll working area, if possible, in another laboratory.

2.2 Light can breakdown chlorophyll. Because of this, samples must be filtered and analyzed under subdued light. Analysis should be conducted away from windows, portholes, etc. If analysis must be conducted near these light sources, it is necessary to opaque them.

2.3 There should not be a strong flow of air going through the laboratory while filtering as this may result in unwanted particles coming in contact with the filters and/or the loss of a filter while transferring filter from filter holder to grinding vessels. Portholes and hatches should be closed during filtering and analysis.

2.4 Precautions should also be taken to minimize the amount of acetone vapor in the shipboard laboratory. During grinding, the box containing the electric drill is vented to the outside through a small exhaust fan connected to the grinding box with 75 mm diameter flexible plastic duct.

3. Presail Equipment Testing

Fluorometers are calibrated before and after each survey (sect. 18, 19).

3.1 Before sailing, the fluorometer is checked to insure that it is functioning properly and readings of standards are consistent with laboratory calibration (sec. 21). A pure chlorophyll a and a secondary coproporphyrin standard are read on the fluorometer before sailing and once each day at sea.

3.2 Remove chlorophyll and coproporphyrin standards from freezer and allow to warm up to ambient room temperature in their opaque containers. If the samples are cold when placed in the fluorometer, condensation may occur and readings will be in error. Also, the temperature at which standards (and samples) are read should be consistent since fluoroescence varies with temperature.

3.3 Under subdued light, blank the fluorometer with 90% acetone, and read the fluorescence of chlorophyll a and coproporphyrin standards. Record observations on the field log for recording daily readings of primary (chlorophyll a) and secondary (coproporphyrin) standards (26.2).

3.4 If the reading obtained with the chlorophyll a standard is lower by more than a few percent of the expected fluorescence, the fluorometer calibration may no longer be valid or the chlorophyll standard may have degraded.

If the coproporphyrin readings agree with the expected (laboratory calibration) reading the chlorophyll a standard has probably degraded.

The fluorescense of the second (backup) chlorophyll a standard is read. If this agrees with the expected reading, the first standard probably degraded.

3.5 To test if the chlorophyll a standard has degraded, 2 drops of 5% HCl are added to the standard in question. The acidification ratio of the standard is dteremined (the ratio of fluorescence units before acidification/fluorescence units of the standard after acidification (F_o/F_a)). This ratio is compraed to the laboratory determined τ. If the ratio of the standard is significantly lower than τ the standard probably degraded, if it is equal to τ the instrument probably is not holding calibration and the backup fluorometer should be used after it has been tested(sect. 3.1).

4. Prestation Preparation

4.1 Be sure all glassware is clean and dry. Fluorometer cuvettes must be clear and unscratched.

4.2 Prepare 90% acetone solution (100 ml distilled water Q.S. to 1000 ml with spectroquality acetone).

4.3 Place a 47 mm diameter Whatman GF/F filter in a 47 mm diameter Gelman filter holder. This is used to generate filtrate used to wash plankton off inner walls of filtration funnels. prefiltere seawa er

Place 25 mm diameter 20 um Nitex, and 25 mm diameter GF/F glass fiber filter in the upper and lower filtration funnels, respectively.

Forceps should always be used when handling glass fiber (GF/F) and nylon (Nitex) filters. Do not use fingers! When not in use, the forcep tips are covered with a short length of plastic tubing.

4.4 Check 300 um le diameter inline filter in the drawing tube to insure that it is clean, free of detritus and zooplankton, and properly centered in the filter holder.

4.5 Check that the vacuum setting is less than 55 mm Hg (~30 inches of water). Adjust vacuum so that the water level in the manometer registers 30 inches. (A vacuum pressure higher than 55 mm Hg can lyse phytoplankton cells causing underestimates of chlorophyll a.)

4.6 Empty the water trap located between the vacuum pump and the filtration manifold.

5.e Seawater Collectione

5.1 Samples are usually collected from surface, 5, 10, 15, 20, 25, 30, 35, 50, and 75 meters using opaque PVC Niskin bottles. At stations where primary productivity is measured, usually at sunrise and noon, additional depths are sampled corresponding to 100% (surface), 69%, 46%, 25%, 10%, 3%, and 1% light penetration (determined with a quantum-response photometer). At least 6-7 depths are sampled throughout the euphotic layer. When sampling, care should be taken to insure that the sample is free from ship discharges.

5.2 Bottles are placed on the hydrowire so that standard depths of 1, 5, 10, 15, 20, 25, 30, 35, 50, and 75 m are sampled when the wire is perpendicular to the surface plane of the water with a wire angle of 0°.

5.3 The Niskin sampling bottles are hung on the hydrowire and "soaked" for 5 minutes at the sampling depth before the messenger is released and the bottles closed. This helps to insure that the Niskin bottles are thoroughlye flushed and equilibrated with sample water.

5.4 Record the following information on the chlorophyll coding form for each station (see section26.1).

Year, month, day, and time (EST) when the messenger is released and water bottles closed (columns 1 through 10).

Also, record the consecutive station number in columns 11, 12, 13 and the predetermined station number in space provided on the left side of the coding form.

←The wire angle at the time the messenger is released is recorded.
←--Using this angle appropriate depth corrections are made (Because wire angles less than 10° do not affect the sampling depth significantly, depth corrections are made for wire angles of 10° or greater only.)

To determine the actual sampling depth when the wire angle is 10° or greater use Table 1 (26.9). Find the standard sampling depth at the top of the table and the wire angle in the extreme left of the table. The intersection of the corresponding column and row in the table will approximate the true sampling depth.

In cases where standard depths are not sampled, i.e. "primary productivity" stations, the actual sampling depth is calculated using the following formula:

Actual depth = (cosine wire angle) x (anticipated depth (m))

where:

Angle (<°)	Cosine
10	0.98
15	0.97
20	0.94
25	0.91
30	0.87
35	0.82
40	0.77
45	0.71

The wire angle, bottom depth, Secchi disappearance depth are recorded in the spaces provided on the left side of the coding form.

6.d Sampling Chlorophyll and Removal of Zooplankton Greater than 300 microns

As soon as the first (surface) Niskin bottle is in the rack begin drawing samples. Do not delay as settling of plankton will result.

Dissolved oxygen samples should be taken from the water bottle before any other samples are drawn. The following subsamples, ^{14}C-productivity, chlorophyll, nutrients and phytoplankton species are taken immediately following oxygen sampling, with ^{14}C-productivity sampling having the "right of way". Samples for salinity determination are taken last. Reversinge thermometers are read after salinity samples have been taken.

All sampling should be performed as soon as possible.e There will be a certain amount of "crowding" at the water bottle racke if sampling is efficient and proper.

Water for chlorophyll analyses is immediately transferred from the Niskin into an opaque 1-liter polyethylene bottle that has been rinsed twice with sample water. During the transfer, the water passes from the Niskin petcok through silicon tubing (with a 300 micron nylon mesh filter held in line by a 1" plastic Gelman filter holder) into the bottom of the opaque bottle. The purpose of this filter is to remove larger zooplankton, which if present, could interfere with chlorophyll a measurements.

When filling the opaque plastic 1-liter chlorophyll bottles, leave an air space so that the contents can be mixed easily before filtration.

7.e Prefiltered Seawatere

Before beginning sample filtration, between 600-800 ml of prefiltered seawatere is prepared by filtering surface seawater through a Whatman GF/F glass fibere filter (47 mm diameter). The filtrate is transferred to a plastic squeeze bottlee and used to rinse filtration funnels.e

8.e Filtratione

A subsample from the opaque chlorophyll sampling bottle is size-fractionated through an upper stage 20 μm mesh filter (Nitex, 25 mm diameter), and a lower stage ~0.7 μm mesh filter (Whatman GF/F, 25 mm diameter) to collect netphytoplanktone and nannophytoplankton, respectively. During filtration, a manometer is used to regulate vacuum pressure at <55 mm Hg (2.2 inches Hg). Higher vacuum pressuree may lyse phytoplankton cells.e

8.1 Seawater samples for chlorophyll a analyses are filtered immediately after water from all sampling depths is collected .

8.2 The filtration rack can simultaneously filter 10 samples. Filter the samples in order of increasing sampling depth, beginning with the surface sample.

8.3 Mix the sample by gently inverting the sample bottle 5 times. The purpose of this is to resuspend particulates that may have settled to the bottom of the container.

8.4 Rinse the graduated cylinder twice with about 50 mls of sample. Measure the aliquots to be filtered (in our coastal/shelf surveys generally between 0.200 and 0.900 l).

8.5 Record the volume of seawater filtered (liters) in columns 24, 25, 26, 27, 28 of the chlorophyll data coding form.

8.6 Pour the sample into the upper stage netplankton filter funnel. The seawater will quickly pass through the 20 µm mesh filter (via gravity) into the lower stage nannoplankton filter funnel.

8.7 Immediately rinse down the inside of the netplankton filter funnel with about 30 ml of prefiltered seawater. The rinse water will collect in the lower stage funnel. This step is necessary to remove and collect netplankton and nannoplankton adhering to filter funnel. It also insures that the filter funnel and filter base are "clean" before the filter funnel is used for the next sample.

8.8 As soon as the seawater rinse passes through the netplankton filter and into the nannoplankton filter funnel the netplankton filter is removed from the filter funnel according to the instructions given in section 10, "Removing Netplankton Filters.".

8.9 When the weak vacuum has drawn almost all the seawater through the lower stage nannoplankton filter (2-5 mls remain above the filter) rinse the nannoplankton filter funnel walls with about 15 ml of prefiltered seawater. Repeat this again, when only one or two ml of seawater rinse is covering the nannoplankton filter. As soon as the rinse seawater passes through the nannoplankton filter, turn off the air valve connecting each individual funnel stem to the vacuum line so that remaining nannoplankton samples will continue to filter.

9. Volume of Seawater Filtered

The volume of seawater filtered for chlorophyll analyses will vary with the concentration of phytoplankton. Usually 200 to 900 ml of seawater is filtered during our coastal/shelf surveys. The seawater volume is chosen so that the fluorescence readings of acetone extract is approximately in the mid-range region of the fluorometer (1, 31.6; or 100,1 ranges) and yields around 100-900 fluorescence units (see sec. 19).

Fluorescence readings of dilute chlorophyll extracts requiring the lowest ranges of the fluorometer do not fall within the above fluorescence range. In such cases larger volumes of seawater are filtered. If the fluorescence of both netplankton and nannoplankton chlorophyll a extracts is too low (20 fluorescence units is the lowest we accept) a larger volume of seawater is serially filtered through both netplankton and nannoplankton filters.

If the nannoplankton size-fraction is very abundant and the netplankton size-fraction of the phytoplankton is very scarce, additional volumes of seawater are filtered only through the 20 um netplankton filter to obtain fluorescence readings within the desired ranges of the fluorometer.

10.e Removing Netplankton Filters

10.e Remove the netplankton filter funnel from the filter base support.e

10.2 Using clean forceps, place a clean unused 25 mm diameter glass fiber GF/F filter disc on top of the netplankton-nylon filter. This facilitates the transfer of netplankton to the grinding vessel and insures that the glass fibers from the GF/F filter come in good contact with the netplankton on the Nitex filter during grinding.

10.3 Using clean forceps, remove the two filters together, and transfer the filters to a grinding vessel. The intact pair of filters is placed near the bottom of the grinding vessel with the glass fiber GF/F facing the center of the grinding vessel, and the nylon filter against the inner wall of the grinding vessel.

10.4. Rinse the forceps with 90% acetone, quantitatively collecting the rinse in the grinding vessel.

10.5. Add approximately 3-4 ml of 90% acetone to the grinding vessel, insuring that the filter is completely submerged in acetone.

10.6. Temporarily store the vessel in a dark test tube box which contains a frozen plastic ice pack at the bottom of the test tube box (this insures that samples are chilled before grinding to minimize heating of the extract).

11. Removing Nannoplankton Filters where there are no plankton

11.1. Using clean forceps grab the perimeter of the filter and fold the filter in half so that the nannoplankton are "inside" the filter semi-circle.

11.2. Using forceps, carefully transfer the filter to the grinding vessel. Place the nannoplankton filter near the bottom of the vessel.

11.3. Rinse the forceps with 90% acetone, quantitatively collecting the rinsee in the grinding vessel.

11.4. Add approximately 3-4 ml of 90% acetone to the grinding vessel, insuring that the filter is completely submerged in acetone.

11.5. Temporarily store the vessel in a dark test tube box which contains a frozen plastic ice pack at the bottom of the test tube box (this insures that the samples are chilled before grinding to minimize heating of the

12.eGrinding and Extracting Chlorophyll a

After all netplankton and nannoplankton samples from a station aree filtered, transferred to the grinding vessels, and covered with 3-4 ml 90%e acetone and chilled, the extraction of chlorophyll a is accelerated bye grinding samples in the grinding vessel with a Teflon tip grinding rode driven by an electric hand drill which is firmly anchored to a ring stand.

12.1 The samples are ground and extracted, in the order which they were filtered, at maximum drill speed (500 rpm) until the glass fiber filter and plankton are pulverized and the entire extract is homogeneous. This takes one minute. Grinding the extract for periods greater than one minute may result in excessive heating of the extract, and pigment degradation.

Generally after the first 20 seconds of grinding the glass fiber filter is fragmented. The remaining 40 seconds of grinding insures thorough contact among phytoplankton, glass fibers, the Teflon rod and the wall of the grinding vessel.

12.2 For a rapid efficient chlorophyll extraction, the grinding rod should bee moved up and down as it revolves against the side of the grinding vessel where the filter is placed. When grinding the netplankton sample, it is necessary to keep the glass fiber filter between the Nitex filter and the grinding rod to insure that the cells on the Nitex filter are broken.

12.3 At the completion of grinding, lower grinding vessel, keeping the rod directly over the vessel and rinse rod with 90% acetone, quantitatively collecting the rinse in the grinding vessel.

12.4 Using 90% acetone in a plastic squeeze bottle, bring the level of extract to 10 mls using the prescribed mark on the grinding vessel (grinding vessels purchased from A. H. Thomas Company, Philadelphia, Pennsylvania, USA, Type A. A 10 ml mark is scribed on the vessel by us in our laboratory).

12.5 The grinding vessels are sealed with a soft rubber stopper. (Stoppers used should be tested to determine if they leach and interfere with fluorescence readings.) Alternatively, the extract can be quantitatively transferred to a plastic or glass graduated 10-15 ml centrifuge tube with screw cap and sealed.

We prefer to conduct all steps in preparation of sample in one vessel and therefore grind, extract, and centrifuge directly in the grinding vessels. This saves time, eliminates a transfer step, and minimizes the amount of glassware needed at sea.)

12.6 After stoppering thoroughlye mix the extracts by completely inverting each grinding vessel 10 times and return the vessel to the dark test tube box containing a frozen plastic ice pack.

12.7 Allow 5 minutes to elapse before the vessels are again completely inverted 10 times to accelerate the extraction of pigments.

12.8 Again, after a second 5 minute extraction period, completely invert each grinding vessel 10 times.

12.9 Immediately centrifuge samples in grinding vessels at 4000 rpm for 2 minutes. Samples should be clarified after 2 minutes, if not centrifuge another 2 minutes.

13.a Measuring the Fluorescence of Chlorophyll Extracts

13.1a Following centrifugation and clarification of pigment acetone extracts, approximately 5-6 ml of the extract is carefully decanted into a fluorometer cuvette (13 mm x 100 mm) and the fluorescence is measured using a Turner Designs Fluorometer equipped with the 10-045 Blue lamp, red sensitive photomultiplier tube, a Corning filters 10-050, 10-051, and 10-052 for excitation, emission, and reference, respectively.

13.2 As suggested in the Turner Designs manual, we have changed the labelling of the sensitivity ranges of the fluorometer from the factory-printed ranges to the new ranges indicated in the tables below, so that the product of range 1 times range 2 times upper-scale needle reading is always directly proportional to chlorophyll a concentration.

Range 1

Original Factory Label	New Label
1	100
100	1

Range 2

Original Factory Label	New Label
31.6	1
10	3.16
3.16	10
Min sens(?)	31.6

13.3 Before reading the fluorescence of chlorophyll extracts, 90% acetone blank measurements (fluorescence of 90% acetone) are made on each of the eight possible range combinations (1 x 1, 1 x 3.16, 1 x 10, 1 x 31.6, 100 x 1, 100 x 3.16, 100 x 10, 100 x 31.6) and recorded in the appropriate "before acid" boxes on the chlorophyll data coding form. Two drops of 5% HCl are added to the cuvette and the fluorescence is recorded for the eight range-combinations in the appropriate "after acid"

13.4 When reading the fluorescence of chlorophyll extracts, only the upper-scale (0-10) is used. ~~and~~ The ~~two~~ sensitivity ranges (1 and 2) of the fluorometer are adjusted to generate a needle reading between 3 and 10 units on the upper-scale for the unacidified sample.

13.5 The fluorescence of the sample is read and recorded in column 36 through 40 of the chlorophyll coding form (26.1). Two drops of 5% HCl are added to the sample using a small eyedropper (1 drop = ~0.05 ml). The acidified sample is mixed and as soon as the needle stabilizes (15 to 30 seconds after acidification) the fluorescence is reread and recorded in columns 48 through 52 of the chlorophyll coding form. The fluorescence of the sample after acidification should be measured using the same fluorometer range combination which was used for the unacidified sample. This insures that the Fo/Fa (fluorescence before/fluorescence after acid) determined for each sample will be precise.

14.e Cleaning Glasswaree

Immediately after use, the grinding vessels are placed in a plastic tub containing soapy water ("Liqui-Nox", manufactured by Alconox, Inc., New York,e NY 10003, USA) until they can be thoroughly cleaned. The vessels are scrubbed and rinsed 5 times with tap water, and air dried on a polyethelene pegboard drying rack. Immediately after use, fluorometer cuvettes containing acidified acetone pigment extracts are placed in a second plastic tub containing Liqui-Nox solution. The cuvettes are thoroughly rinsed 5 times with tap water and dried on a plastic drying rack. If a film develops on the cuvettes they ~~should be~~ are rinsed with 90% acetone.

15.e Pre aration of Primare chloro h ll a and Secondar tandards

15.e Chlorophyll a standarde

Approximately 1 mg of pure chlorophyll a (Sigma Chemical Company) is dissolved in 1-liter of 90% spectroquality acetone. This stock solution is stored in the freezer in a glass, stoppered 1-liter volumetric flask which is opaqued by covering it with aluminum foil. This standard is used to calibrate flurometers. The chlorophyll a stock solution is also used to fill several stoppered flurometer cuvettes which are taken to sea to insure that the fluorometers are maintaining calibration (sect. 3.1).

15.2 Coproporphyrin standard

Coproporphyrin can be purchased from the Sigma Chemical Company, P. O. Box 14508, St. Louis, MO 63718. One mg is dissolved in 10 mle 1.5N HCl in a 100 ml volumetric. Once dissolved, it is diluted to 100 mle with more 1.5N HCle and stored in glass stoppered amber bottle in a refrigerator (Turner Designs). This stock solution is used to fill several fluorometer cuvettes which are taken to sea as the "secondary standardse". The cuvettes are sealed with a stopper. The secondary standard is refrigerated and kept in darkness during the cruise.e

16. Recording Information on the Chlorophyll Coding Forme

A standard chlorophyll data format has been developed to enter data into a computer so that calculations of chlorophyll a concentration may be made easily for a large number of field samples, and so that calculated data may be computer-archived with related information such as sampling date, location, depth, etc. (sect. 26.1).

16.1 The following table explains the locations on the coding form where data are coded. An 80 column format is used in recording essential data which is keypunched. Additional ancillary information is recorded on the chlorophyll coding sheet but is not keypunched.

INFORMATION	COLUMNS USED ON CODING FORM
Year	1,2
Month	3,4
Day	5,6
Time (hours, eastern standard time when messenger is released	7,8,9,10
Consecutive station number	11,12,13
Actual sampling depth (m)	14,15,16
Size fraction (NETplankton, NANnoplankton or PPP if water is not fractionated	17,18,19
Sampler type (N = Niskin bottle; B = surface bucket sample; P = submersible pump, etc.)	20
Tube number (number on grinding vessel)	21,22,23
Volume of seawater filtered (liters)	24,25,26,27,28
Final volume of acetone extract (ml, usually 10.0 ml)	29,30,31,32
Fluorescence Before Acidification of Extract	
Range 1 on Turner Designs Fluorometer (1 or 100)	33,34,35
Range 2 on Turner Designs Fluorometer (1 or 3.16 or 10 or 31.6)e	36,37,38,39,40

INFORMATION	COLUMNS USED ON CODING FORM
Upper-scale reading on Turner Designs Fluorometer	41,42,43,44e
Fluorescence After Acidification of Extract	
Range 1 on Turner Designs Fluorometer (1 or 100)	45,46,47
Range 2 on Turner Designs Fluorometer (1 or 3.16 or 10 or 31.6)	48,49,50,51,52
Upper-scale reading on Turner Designs Fluorometer	53,54,55,56
Blank reading (fluorescence of 90% acetone using the same range 1 and range 2 used for reading fluorescence ofe chlorophyll extract)	57,58,59,60
Columns not used	61,62,63,64
F, fluorometer calibration factor (µg chlorophyll a/10 ml acetone/ fluorescence unit) using pure chlorophyll a	65,66,67,68,69,70,71,72
τ, calibration factor (ratio of fluorescence before acid/fluorescence after acid) using pure chlorophyll a	73,74,75,76
Columns not used	77,78,79,80

should be on the same line

should be left blank

17.e Recording Ancillary Information on the Chlorophyll Coding Form

17.1 The research ship, cruise number, predetermined station number, latitude,e longitude, local time, and Greenwich mean time are recorded on the left side of the coding form. The predetermined station number is the number assigned to the intended sampling location before the station is actually occupied and given a consecutive station number.

17.2 The hydrocast wire angle is measured with an inclinometer at the time the messenger is released. The cosine of the wire angle multiplied by the anticipated sampling depths yields the locations of actual "corrected" sampling depths. Between columns 13 and 14 of the coding form, there are three columns (this data is not keypunched) for recording the anticipated sampling depths (m). Following the hydrocaste the anticipated sampling depths are corrected (using cosine formula) and the corrected sampling depths (to nearest whole meter) are coded and keypunched in columns 14, 15, 16 of the coding form (Sect. 5.4).

17.3 The bottom trip sampler (a Niskin bottle rigged to close when a trip weight contacts the seabed) is frequently used in shelf surveys at stations shallower than 75 meters.

17.4. The bottom depth determined using the ship's fathometer and determined from the winch meter wheel are recorded.

17.5. The disappearance-depth of a 30 cm diameter white Secchi disc is recorded to the nearest tenth of a meter.

17.6. The names of technicians and individuals responsible for seawater collection and measurement of chlorophyll concentrations are recorded for each station sampled.

17.7. In the upper right hand corner of the data coding form, columns are provided for recording the fluorescence of the blank (90% acetone) before

and after two drops of 5% HCl are added to the blank. The fluorescence of the blank is read using the eight possible combinations of range 1 and range 2 (Section 13). The blank fluorescence is measured at each station, before the fluorescence of chlorophyll extracts is measured. With 90% acetone in the fluorometer cuvette, zero the instrument on the most commonly used range combination, then proceed to record fluorescence of the blank using the seven remaining range combinations. The blank fluorescence readings from range 1 and range 2 are paired with the chlorophyll extract fluorescence readings made on the same range 1 and same range 2. For example, if a chlorophyll a extract gave a mid-scale needle reading using range 1 (=1) and range 2 (=31.6) then the readings of blank fluorescence before acid made on range 1(=1) and range 2 (=31.6) are transcribed to columns 57, 58, 59, and 60 of the coding form. Readings of fluorescence of the 90% acetone blank after the addition of 5% HCl are made and recorded but are not keypunched and are not paired up with the fluorescence readings of chlorophyll extracts since the "after acid" blank is usually identical to the "before acid" blank. It is good practice, however, to make the "after acid" blank fluorescence readings to insure that the acid added to chlorophyll extracts is not systematically contaminating these extracts and altering the sample acidification ratio (Fo/Fa).

18.e Calibration of Fluorometere

The Turner Designs Fluorometer is calibrated spectrophotometrically using pure chlorophyll a purchased from Sigma Chemical Company, P. O. Box 14508, St. Louis, MO 63178, USA. Fluorometers are calibrated immediately before and after a survey. Additionally, several aliquots of pure chlorophyll a are taken to sea in stoppered fluorometer cuvettes, stored in a freezer and in the dark. At sea, each day (preferably at night), the fluorescence of pure chlorophyll a is recorded to determine if significant drift in the fluorometer calibration has occurred (26.2)(3 e).

Also, as a check on our laboratory calibration procedure, the fluorescence of a pure chlorophyll a standard solution available (no charge) from U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Quality Assurance Branch, Cincinnati, OH 45268, USA is measured to determine the accuracy of our calibration factor.

Two calibration factors (F and τ) are required to equate fluorescence readings with chlorophyll a concentrations, corrected for the presence of phaeophytin a. F is the ratio of chlorophyll a to one fluorescent unit. One fluorescent unit, using the Turner Designs Fluorometer, is equivalent to a needle reading on upper fluorometer scale of 1.0 when using a setting of 1 on range 1 and 1 on range 2, that is, the most sensitive combination of ranges for the fluorometer. F is in units of µg Chl a/10 ml acetone/fluorescence unit. VARY among fluorometer. Factors Calibration factors for five different fluorometers ranged between 1.0×10^{-3}e to 8.0×10^{-4} µg Chl a/10 ml acetone/fluorescence unit.e

τ is the ratio of fluorescence before acidification of a pure chlorophyll a solution to the fluorescence of the solution after acidification with 2 drops of 5% HCl. Generally, τ ranges between 2.2 and 2.4, and is unitless sincee it is a ratio of two fluorescence readings.e

19. Calibration Procedure

During preparation of standard chlorophyll solutions and during the entire calibration procedure, work is performed under subdued light with window shades drawn to prevent the breakdown of chlorophyll by strong illumination.

A 50 ml aliquot of the pure chlorophyll a stock solution (Sigma Chemical Company) is equilibrated to ambient temperature in a stoppered volumetric flask opaqued with aluminum foil. While this aliquot is warming to ambient temperature, the procedure given in the "Fluorometer Calibration Worksheet #1e' is followed (26.3).

20.e Determining the Chlorophyll a Concentration of the Stock Solution one Spectrophotometer

In our work we use a dual beam Perkin Elmer #550 UV-visible spectrophotometer.e

A spectrophotometer cuvette with a 5 cm light path is used.

20.2 The wavelength alignment and accuracy is checked using a deuterium lamp which has a sharp emission line (656.1 nm) near the region of maximum absorbance for chlorophyll. Using a 10% neutral density filter absorbance is adjusted to read 1.000A and percent transmittance to read 10.0%T.

20.3 Both Reference and Sample spectrophotometer cuvette are filled with 90% acetone to determine the cuvette to cuvette "blanke.

20.4 At a wavelength setting of 663 nm, the spectrophotometer is adjusted to read 100.0% transmittance in the "T" mode and 0.000 absorbance in the "A" mode. ~~Record~~ the absorbances of the cuvette blank are recorded on the worksheet.

20.5 An aliquot of pure chlorophyll a solution is placed in the "sample" spectrophotometer cuvette and absorbances are read at several wavelengths. The absorbances at 663 and 750 nm are used in the calculation of chlorophyll a concentration. The absorbances at other wavelengths as well as a ~~the~~ scan of the sample between 400 and 800 nm wavelengths are used to determine the presence of other chlorophyll pigments or breakdown products from the "pure" chlorophyll a stock solution.

20.6 The wavelength yielding the highest absorbance is recorded to provide an additional check on the wavelength calibration and purity of the stock solution of chlorophyll a.

The spectrophotometer acid ratio (usually about 1.77) is measured to determine the purity of the aliquot used for spectrophotometric measurement of Ca.

20.7 Calculation of Ca, the concentration of chlorophyll a (ug Chla/10 ml acetone) is based on a specific absorption coefficient of 89.31 liter/g Cm for chlorophyll a (SCOR/UNESCO, 1966).

21.e Determining Calibration Factors F and τ

Following the spectrophotometric determination of Ca (the concentration of chlorophyll a in 10 ml of stock solution) the fluorescence of the pure chlorophyll a stock solution and the fluorescence of several dilutions of the stock solution is recorded on the "Fluorometer Calibration Worksheet #2". The fluorescence of several dilutions of the chlorophyll a standard is measuredе to evaluate the linearity of each fluorometer (fluorescence units versus chlorophyll a concentration). Generally, fluorescence readings using the highest (100, 31.6) and the lowest (1, 1) fluorometer range combinations fall outside the linear range of our fluorometers.

21.1 One ml from the chlorophyll stock solution is used for each dilution. Each dilution is made up individually and not serially to prevent carrying through dilution errors.

21.2 The calibration factors F and τ (26.4) are computed for each dilution. Generally, the calibration F and τ measured using the fluorometer range combination (Range 1 = 1, Range 2 = 31.6) is used in the formula to calculate chlorophyll a concentration since most fluorometric readings of field samples fall in this range of the fluorometer.

22.e Calculation of Chlorophyll a, Phaeophytin a, and the Sample Acidification Ratioe

The concentration of chlorophyll a in seawater is calculated using thee following formula:

$$\text{Chlorophyll a ug/L, or mg/m}^3 = e$$

$$\left(F, \frac{\text{ug Chla}}{\text{10 ml acetone}}{\text{fluorescence unit}}\right) \cdot \left(\frac{T}{T-1}\right) \left(\text{Fluorescence units before acidification} - \text{Fluorescence units after acidification}\right)e$$

$$\cdot e\left(\frac{\text{Acetone extract, ml}}{\text{Seawater filtered, liters}}\right)e$$

The concentration of phaeophytin a in seawater is calculated using the following formula:

$$\text{Phaeophytin a ug/L, or mg/m}^3 = \left(F, \frac{\text{ug Chlao}}{\text{10 ml acetone}}{\text{fluorescence unit}}\right)\left(\frac{T}{T-1}\right)\left((T)\cdot(\text{Fluorescence units after acidification}) - (\text{Fluorescence units before acidification})\right) \cdot \left(\frac{\text{Acetone extract, ml}}{\text{Seawater filtered, liters}}\right)$$

The sample acidification ratio "Fo/Fa" is calculated according to the following formula:

$$\frac{\text{Sample fluorescence before acidification}}{\text{Sample fluorescence after acidification}}$$

Fo/Fa ranges between values of 1 (no chla) and T (pure chla).

23.0 Computer Program for Calculating Chlorophyll Concentrations

The information on the chlorophyll coding sheet is keypunched and used as input to a FORTRAN program which calculates concentrations of chlorophyll a, phaeophytin a, and the sample acidification ratio (Fo/Fa). The program also sums the measured values for netplankton and nannoplankton size fractions for each sampling depth to yield "total" chlorophyll concentrations.

Additionally, a subroutine of the chlorophyll program calculates the weighted mean concentration of chlorophyll a in the water column for each station.

The chlorophyll computer program generates output at several stages of the data reduction.

Stage 1 (26.5) consists of a listing of keypunched fluorometric measurements by date, time, station number, depth, and size fraction.

At Stage 2 (26.6) the listing contains input data from stage 1 as well as calculated concentrations of chlorophyll a, phaeophytin a, and sample acidification ratio (Fo/Fa) by depth and size fraction. The sum of chlorophyll a concentrations measured for each size fraction is also caluclated and listed.

In Stage 3, the program reformats the data so that all measurements from one sampling depth are listed in one row of output according to date, station number and sampling depth. Additionally, the percent nannoplankton, the percent of total chlorophyll a passing 20 um mesh, is calculated and listed to provide an indication of community size composition for each sampling depth.

In Stage 4 of the program (26.8), concentrations of chlorophyll a, and phaeophytin a are arithmetically integrated over the depth of the water column (to 75 m or bottom whichever is less). The resulting integral is divided by the deepest sampling depth in the integral to yield average water column concentrations of chlorophyll a and phaeophytin a in netphytoplankton and nannophytoplankton. The "total phytoplankton" chlorophyll a and phaeophytin a water column average concentrations listed are calculated by the addition of average water column values for netphytoplankton and nannophytoplankton. The "percent nannoplankton" data listed in the output from Stage 4 are calculated by dividing the average water column nannophytoplankton concentrations by average water column total phytoplankton concentrations.

24.0 Additivity of Size-Fractions

In our fractionation filtration method a 20 micron mesh nylon filter is used to separate the netphytoplankton (> 20 um) from nannophytoplankton (< 20 um) which pass the 20 um mesh and are retained on the glass fiber GF/F filter. In our studies we are interested in the size composition of the phytoplankton community as well as the "total biomass" of the phytoplankton. Consequently, it is important that pigment extraction efficiency is high for both netplankton and nannoplankton so that the sum of netplankton plus nannoplankton chlorophyll a concentrations agrees with "total chlorophyll a" measurements conventionally made on a non-fractionated "whole water" sample filtered through a glass fiber GF/F filter.

We have generally found good agreement between total chlorophyll a concentrations derived by summing netplankton and nannoplankton concentrations and concentrations of total chlorophyll a (unfractionated) measured on subsamples of seawater from the same sampling depths used for size-fractionation

Nevertheless, if size-fractionation is employed, investigators should evaluate the additivity of size-fractions for the particular phytoplankton communities and areas being studied since some species may be relatively more refractory to pigment extraction and since pigment extraction efficiency is dependent upon thorough and standardized grinding and extraction technique.

The coefficient of variability (standard error x 100/average) for fractionated chlorophyll a analyses of 10 replicate samples of seawater from a Niskin bottle is 6-7% at concentrations of 1 ug Chla/l.

25.o List of Equipment Used to Measure Chlorophyll

Equipment	Description	Distributor
Niskin bottle	#1010-5 5-L, PVC, with reversing thermometer frame attachedo	1
Messengers	Go Devil messengers Model 4000	1
Acetone	MallincKrodt (2438) Acetone SpectrAR	local scientific equipment distributor
Nitex/netplankton filters	nylon, monofilament screen cloth, nitex, 1" dia. discs (custom order #HC-3-20)	3
Glass fiber filters	Whatman GF/F, pore size ∿ 0.7 micrometre 25 mm diameter	local distributor
	Whatman GF/F, pore size ∿ 0.7 micrometre, 47 mm diameter	local distributor
Vacuum pump	Ships with (60 Hz) Millipore vacuum pressure pump 115 V 60 Hz #60 000 00	5
	Foreign ships with (50 Hz) #7055-10 vacuum pressure pump 220 V 50 Hz	4
Coproporphyrin	1 mg type I crystalline coproporphyrin tetramethyl ester	6
Chlorophyll a	chlorophyll a from Spinach 99% pure	6
Drills for grinding	Rockwell 3/8" adjustable variable speed drill (500 rpm) with lock in drill speed	local hardware store
Grinding vessel	#3431-E45 grinding vessel Size A (Thomas)	7
Tissue grinder	#3431-E15 Serrated Pestle, Teflon Tissue Grinder Size A (Thomas)	7
Filter holder for 300um filter	Delrin filter holder, inline type, 25 mm, pkg. 6	local distributor
Nylon 300 um filter disc	Nylon, monofiliment screen cut to 25 mm diameter	3

Equipment	Description	Distributor
Detergent	Liqui-Nox cleaning compound	local distributor
Fluorometer vessels	13 x 100 mm fluorometer tubes	8
Net and nan filter funnels	Gelman #4203 200 ml filter funnel	local distributor
Plastic volumetric flasks	Nalgene volumetric flasks (1000 ml, 100 ml)	
Forceps	flat edge and tip Millipore stainless MF forceps #xx6200 06	5
Centrifuge	IEC Model HN-S11 angled centrifuge head (IEC 816) (8 x 50 ml) Shield 50 ml long (IEC 305) set of 8 (cushions incl.)	local distributor
Fluorometer	10-005R field fluorometero 10-030 13 100 mm cuvette holdero 10-031-13 13 x 100 mm duvetteso 10-040 chlorophyll accessory kito 10-066 fuseo 10-065 fuseo 10-045 lamp, blueo	2
Stoppers	For grinding vessels, #8754-010	
1oliter chlorophyll sampling bottleo	Polyethylene bottles (Nalgene #2004) (1706-N26)	7
Plastic squeeze bottles	Polyethylene bottle for 90% acetone and for prefiltered seawater (Nalgene #2410) (1758-U55)	7
Dropping bottle	Glass, contains 5% HCl (1760-D33)	7
Test tube rack	Polypropylene, for fluorometer cuvettes (S9259-1)	8
Test tube rack	Polypropylene, for grinding vessels (S9259-2)	8
Graduated Cylinders	Plastic, 250 ml, 1000 ml	local distribution

Distributors

1 - General Oceanics
5535 Northwest 7th Avenue
Miami, Florida 33127
305-754-6658e

2 - Turner Designs
3132 Alexis Drive
Palo Alto, California 94304

3 - Tetko, Inc.
420 Saw Mill River Road
Elmsford, New York 10523
914-592-5010e

4 - Cole Parmer
7425 North Oak Park Avenue
Chicago, Illinois 60648
312-647-0272e

5 - Millipore Corporation
Bedford, Massachusetts 01730

6 - Sigma Chemical Company
P.e O. Box 14508e
St. Louis, Missouri 63178e
314-771-5750e

7 - Arthur H. Thomas
Vine Street at Third
P.e O. Box 779e
Philadelphia, Pennsylvania 19105e

8 - Scientific Products
100 Raritan Center Parkway
Edison, New Jersey 08817
201-494-4000e

(75°W)

YEAR	MONTH	DAY	Messenger Time	Consecutive Station Number	Anticipated Sampling Depth (m)	Actual Corrected Sampling Depths (m)	Size Fraction	Sampler Type	Tube Number	Volume of Seawater Filtered (liters)	Final Volume of Acetone Extract (ml)	Fluorescence Before Acidification: Range 1 (1 or 100)	Fluorescence Before Acidification: Range 2 (1 or 3.16; 10 or 31.6)	Fluorescence Before Acidification: Upper Scale Reading	Fluorescence After Acidification: Range 1 (1 or 100)	Fluorescence After Acidification: Range 2 (1 or 3.16; 10 or 31.6)	Fluorescence After Acidification: Upper Scale Reading	90% Acetone Blank
							NET											
							NAN											
							NET											
							NAN											
							NET											
							NAN											
							NET											
							NAN											
							NET											
							NAN											
							NET											
							NAN											
							NET											
							NAN											
							NET											
							NAN											
							NET											
							NAN											
							NET											
							NAN											

Ship ________

Cruise # ________

Predetermined Station Number ______

Latitude __° __.__'

Longitude __° __.__'

Time (messenger)
Local _____
GMT _____

Hydrocast Wire Angle ____°

Bottom Trip Sampler Used
Yes ___ No ___

Bottom Depth by Fathometer ____m

Bottom Depth by Meter Wheel ____m

Secchi depth _._m

Technicians sampling and measuring chlorophyll at this station

____

Fluorometer Serial No.

____

Calibration Factors

F	τ
.	.

90% Acetone Blank

Range 1	Range 2	Before Acid	After Acid
1	1	.	.
1	3.16	.	.
1	10	.	.
1	31.6	.	.
100	1	.	.
100	3.16	.	.
100	10	.	.
100	31.6	.	.

REMARKS

Additional Sampling
pp nuts. p TSS

Field log for recording daily readings of primary (chlorophyll a) and secondary (coproporphyrin) calibration standards.

Fluorometer Serial No. _____	Date	Leg	Fluorescence of Standards						Initials	Remarks
			Primary-chlorophyll a			Secondary-coproporphyrin				
			Range 1	Range 2	Scale	Range 1	Range 2	Scale		
Laboratory reading-1 day prior to equipment departure										
Shipboard reading prior to sailing (instrument check)										
Daily readings taken at night										

Date _____ Technician _____ Chlorophyll source _____

Spectrophotometer used _____ Cuvette path length _____ cm

1)e Check wave length alignment using Deuterium line ate 656.1 nme

2)e Using 10% neutral density filter check that absorbancee = 1.000A and transmittancee= 10.0%T.e

3)e Fill both reference and sample spectrophotometere cuvettes with 90% acetone to determine cuvettee "blank" (sample cuvette is nearest to operator when using P.E. 550).e

4)e At 663 nm, adjust spectrophotometer to read 100.0%e transmittance in the "T" mode and 0.000 absorbancee in the "A" mode.e

5)e With both cuvettes containing 90% acetone, determinee the absorbance (+ or -) of the sample cuvette ate the wave lengths below.e

Wave Length(nm)	Absorbance
480.0	_____
630.0	_____
645.0	_____
663.0	_____ =a
665.0	_____
750.0	_____ =b

6)e Discard 90% acetone from "sample" spectrophotometer cuvette and allow cuvette to dry.e

7)e Rinse "sample" cuvette twice with aliquots taken frome the pure chlorophyll a solution. Fill cuvette withe chlorophyll a solution and read absorbances at the wave lengths below.

Wave Length(nm)	Absorbance
480.0	_____
630.0	_____
645.0	_____
663.0	_____ =c
665.0	_____
750.0	_____ =d

8)e Adjust wave length dial on spectrophotometer until you obtain the highest absorbance, and record the wavee length ___.__ nme

9)e Scan the extract from 400 to 800 nm (by slowly turninge wave length dial) to determine the presence of othere chlorophylls or breakdown products in the stock chlorophyll solution.e

10) Add 5% HCl (2 drops per 10 ml aliquot) to spectrophotometer cuvette containing pure chlorophyll a and to thee 90% acetone in the "reference" cuvette and record absorbances at the following wave lengths:e

Wave Length(nm)	Absorbance
663.0	_____ =e
750.0	_____ =f

$$\frac{(c-a)-(d-b)}{(e-a)-(f-b)} = e \_\_\_\_\_, \text{ spec. acid ratioe}$$

If the chlorophyll a aliquot has not degraded, then this ratio is usually ∿ 1.77

11)e Calculate chlorophyll a concentration, Ca as follows:

$$Ca = \frac{c-a - d-b}{\text{cuvette path length cme}} \left(\frac{10{,}000}{89.31\ell/gcm}\right) = \_\_\_ \text{ ug Chla/10 ml acetone}$$

Fluorometer Calibration Worksheet #2

Date _____ Technician _____

Ca _____ ug chlorophyll a/10 ml acetone (from Worksheet #1)

Fluorometer Serial Number _____

Dilution Factor (df)	Range 1		Range 2		Upper Scale Needle Reading		Fluorescence Units Before Acid	Range 1		Range 2		Upper Scale Needle Reading		Fluorescence Units After Acid	Calibration Factors F		τ
1(no dilution)		x		x		=			x		x		=				
1/10	___	x	____	x	____	=	____	___	x	____	x	____	=	____	.	____	____
1/25	___	x	____	x	____	=	____	___	x	____	x	____	=	____	.	____	____
1/50	___	x	____	x	____	=	____	___	x	____	x	____	=	____	.	____	____
1/100		x		x		=			x		x		=				
1/250		x		x		=			x		x		=				
1/500		x		x		=			x		x						
1/1000e		x		x		=			x		x		=				

$$F \frac{\text{ug chlorophyll a/10 ml acetone}}{\text{fluorescence unit}} = \frac{\text{Ca x df}}{\text{fluorescence units before acidification}}$$

$$\tau = \frac{\text{fluorescence units before acidification}}{\text{fluorescence units after acidification}}$$

Comments on using above worksheet: Range 1 - enter either 1 or 100 (using Turner Designs Fluorometer)
Range 2 - enter either 1 or 3.16 or 10 or 31.6
Upper scale needle reading - read only upper scale on Turner Designs fluorometer

26.5 STAGE 1. LISTING OF KEYPUNCHED CHLOROPHYLL DATA.

```
DATE MAR 09/25/80        CHLOROPHYLL PUNCHED DATA

         1         2         3         4         5         6         7         8
12345678901234567890123456789012345678901234567890123456789012345678901234567890
790123083U  1  1NETN  10.30010.0100 1.005.58100 1.002.7000,0     .006709 2.35          1
790123083U  1  1NANN  20.10010.0   131.805.20   131.803.1000,0                          2
790123083U  1  3NETN  30.30010.0100 1.005.18100 1.002.5000,0                          3
790123083U  1  3NANN  40.10010.0   131.805.70   131.802.1900,0                          4
790123083U  1  4NETN  50.30010.0100 1.004.80100 1.002.3000,0                          5
790123083U  1  4NANN  60.10010.0   131.804.15   131.802.5000,0                          6
790123083U  1  7NETN  70.30010.0100 1.004.38100 1.002.5906,0                          7
790123083U  1  7NANN  80.10010.0   131.805.70   131.803.3900,0                          8
790123083U  1  9NETN  90.30010.0100 1.005.10100 1.002.5800,0                          9
790123083U  1  9NANN 100.10010.0   131.803.85   131.802.3000,0                         10
790123083U  1 15NETN 110.30010.0100 1.004.40100 1.002.1000,0                         11
790123083U  1 15NANN 120.10010.0   131.803.20   131.801.8500,0                         12
790123083U  1 25NETN 130.30010.0100 1.000.00100 1.002.9900,0                         13
790123083U  1 25NANN 140.10010.0   131.805.90   131.803.8000,0                         14
790123083U  1 30NETN 150.30010.0100 3.102.45100 3.101.2000,0                         15
790123083U  1 30NANN 160.10010.0   131.807.20   131.804.4200,0                         16
```

26.6 STAGE 2. PROGRAM FOR CALCULATING CONCENTRATIONS OF CHLOROPHYLL A, PHAEOPHYTIN A, AND FO/FA.

```
 CHLORO.CNTL
//MBCHLOR JOB  (0000,MBLS),'EVANS',CLASS=L                                  00000100
//STEP1  EXEC  FORTGCL                                                      00000200
//FORT.SYSIN  DD  *                                                         00000300
C  CHPGM1       GREGORY B. HOWARD       MAY 15, 1977                       00000400
C  THIS PROGRAM READS IN CHLOROPHYLL DATA CALCULATES CHLOROPHYLL A,         00000500
C  PHAEO-PIGMENTS AND FO/FA. A NEW OUTPUT RECORD IS THEN PRODUCED CONTAI00000600
C  ING THESE CALCULATED VALUES. A SUM CARD IS ALSO ADDED FOR EACH SET OF00000700
C  NET, NAN AND PPP CARDS AND CONTAINS  A SUM FOR CHLOROPHYLL A AND A SU00000800
C  FOR PHAEO-PIGMENTS THIS CARD WILL FOLLOW THE NAN CARD IN EACH SET.    00000900
C     ** CRITICAL NOTE:  THERE MUST BE NO, ABSOLUTELY NO TUBE NUMBER ON  00001000
C                        CARDS THAT HAVE NO DATA AND ARE THERE JUST TO   00001100
C                        KEEP THE CORRECT SEQ - NET, NAN, PPP.           00001200
C                                                                        00001300
      REAL MGCHLA,MGPHAC,NET/'NET'/,NAN/'NAN'/,PPP/'PPP'/                00001400
      INTEGER T,W,OTHER(5),TREAT,TUBE,BLANK/'    '/                      00001500
      DIMENSION OUT(3)                                                   00001600
      DATA ID/'  '/                                                      00001630
      TRB=0.                                                             00001700
      TRA=0.                                                             00001800
      TFOFA=0.                                                           00001850
      SUMPHA=0.                                                          00001900
      SUMCHL=0.                                                          00002000
C  READ IN NET CARD DO PROCESSING THEN COME BACK AND GET NAN CARD.       00002100
   10 READ(5,1000,END=99)OTHER,FRAC,TREAT,TUBE,SWV,AV,T,U,V,W,X,Y,AB,    00002200
     (EFF,AU,DEPTH                                                       00002300
      IF(EFF.EQ.0.0)GO TO 12                                             00002400
      F=EFF                                                              00002450
   12 IF(AU.EQ.0.0)GO TO 15                                              00002500
      TAU=AU                                                             00002600
C  COMPUTE TAU/TAU-1 AND LET A = THIS VALUE.                             00002700
      A=TAU/(TAU-1)                                                      00002800
C  IF X OR U IS EQUAL TO 3.20 CHANGE IT TO 3.16                          00002900
   15 IF (U.EQ.3.20)U=3.16                                               00003000
      IF (X.EQ.3.20) X=3.16                                              00003100
C  INITIALIZE CALCULATED VALUES SO THAT BLANK DATA CARDS WON'T GET       00003200
C  PREVIOUS VALUES.                                                      00003300
      RB=0.                                                              00003400
      TT=T                                                               00003450
      WW=W                                                               00003460
      RA=0.                                                              00003500
      MGCHLA=0.                                                          00003600
      FOFA=0.                                                            00003700
      MGPHAC=0.                                                          00003800
      RB=T*U*(V-AB)                                                      00004000
      RA=W*X*(Y-AB)                                                      00004100
      IF(RB.LE.0.0.OR.RA.LE.0.0)GO TO 30                                 00004150
      MGCHLA = F*A*(RB-RA)*(AV*.10)*(1/SWV)                              00004200
      MGPHAC = F*A*(TAU*RA-RB)*(AV*.10)*(1/SWV)                          00004300
      FOFA = RB/RA                                                       00004400
C  WRITE OUTPUT RECORD WITH YOUR NEW CALCULATED VALUES ON DISK.          00004500
   20 WRITE (8,1002) OTHER,FRAC,TREAT,TUBE,SWV,AV,T,U,V,W,X,Y,AB,MGCHLA,00004900
     X  MGPHAC,FOFA,ID,F,TAU                                             00004950
      GO TO 40                                                           00004960
   30 WRITE (8,1002) OTHER,FRAC,TREAT,ID,F,TAU                           00005100
   40 IF (FRAC.EQ.PPP) GO TO 10                                          00005500
      IF(SWV.LE.0.0)GO TO 45                                             00005550
```

```
                                                                        00005700
      IF(MGCHLA.LE.0.0)MGCHLA=0.0,                                      00005750
      IF(MGPHAC.LE.0.0)MGPHAC=0.0                                       00005760
      SUMCHL  = SUMCHL  + MGCHLA,                                       00005800
      SUMPHA  = SUMPHA  + MGPHAC                                        00005900
      TRB=TRB+(RB/SWV)                                                  00006000
      TRA=TRA+(RA/SWV)                                                  00006100
C  VALUES AND SUMS FOR CHLOROPHYLL AND PHAEO SHOULD HAVE BEEN CALCULATED00006200
C  SO AT THIS POINT WE WILL WRITE OUT THE SUM CARD. ALSO WE MUST INITIAL00006300,
C  THE SUMING VARIABLES FOR THE NEXT GROUP.                             00006400
   40 IF(FRAC.EQ.NAN)GO TO 30                                           00006500
      OUT(1)=MGCHLA                                                     00006600
      OUT(2)=MGPHAC,                                                    00006700
      OUT(3)=FOFA,                                                      00006800
      GO TO 10,                                                         00006900
   50 IF(OUT(3).GT.0.0.AND.FOFA.GT.0.0)GO TO 60                         00006910
      IF(OUT(3).EQ.0.0.AND.FOFA.EQ.0.0)GO TO 80                         00006920
      IF(OUT(3).EQ.0.0)GO TO 70                                         00006930
      WRITE(9,1010)OTHER,OUT,                                           00006940,
      GO TO 80,                                                         00006950,
   70 WRITE(9,1020)OTHER,MGCHLA,MGPHAC,FOFA                             00006960
   80 WRITE(8,1030)OTHER,TREAT                                          00006980
      GO TO 90                                                          00006990
   60 TFOFA=TRB/TRA                                                     00007000
      WRITE(8,1004)OTHER,TREAT,      SUMCHL,SUMPHA,TFOFA,ID,F,TAU       00007100
      WRITE(9,1009)OTHER,OUT,MGCHLA,MGPHAC,FOFA,SUMCHL,SUMPHA,TFOFA     00007200
   90 TFOFA=0.0                                                         00007210
      TRB=0.                                                            00007300
      TRA=0.                                                            00007400
      SUMCHL = 0.0                                                      00007500
      SUMPHA = 0.0                                                      00007600
      GO TO 10                                                          00007700
   99 END FILE 8                                                        00007800
      STOP                                                              00007900
 1000 FORMAT (3A2,A4,A3,A3,A3,A1,A3,F5.3,F4.1,I3,F5.2,F4.2,I3,F5.2,F4.2,00008100
     1F4.1,4X,F7.6,F5.2,A4)                                             00008200
 1002 FORMAT (3A2,A4,A3,A3,A3,A1,A3,F5.3,F4.1,I3,F5.2,F4.2,I3,F5.2,     00008500
     1F4.2,F4.1,F6.2,F6.2,F7.4,A2,F9.7,F6.2)                            00008600
 1004 FORMAT(   3A2,A4,2A3,'SUM',A1,   T61,2F6.2,F7.4,A2,F9.7,          00008800
     XF6.2)                                                             00008900
 1008 FORMAT (3A2,A4,3A3,A1,T80,A2,F9.7,F6.2)                           00009200
 1009 FORMAT(3A2,A4,2A3,3(2F6.2,F6.4))                                  00009300
 1010 FORMAT(3A2,A4,2A3,2F6.2,F6.4)                                     00009310,
 1020 FORMAT(3A2,A4,2A3,18X,2F6.2,F6.4)                                 00009320,
 1030 FORMAT(3A2,A4,2A3,'SUM',A1)                                       00009339,
      END                                                               00009400,
/*                                                                      00009410,
//LKED.SYSLMOD DD DSN=NE.CAS.LOADLIB,DISP=MOD                           00009420,
//LKED.SYSIN DD *                                                       00009430,
  NAME MBCHLORO(R)                                                      00009440,
/*,                                                                     00009450,
//                                                                      00009460,
READY
```

STAGE 2. LISTING OF CALCULATED CONCENTRATIONS OF CHLOROPHYLL A, PHAEOPHYTIN A, AND Fo/Fa BY DEPTH AND SIZE-FRACTION.

```
                                                        CHL A   PHAEO   FO/FA
79012308 30  1 1NETN  10.30010.0100 1.005,50100 1.002,70 0.0  1.15,  0.35 2.0370  0.0007090  2.35
79012308 30  1 1NANN  20.10010.0  131.805.20  131.803.10 0.0  0.82   0.81 1.8778  0.0007090  2.35
79012308 30  1 1SLMN                                         1.97   1.16 1.8494  0.0007090  2.35
79012308 30  1 3NETN  30.30010.0100 1.005,18100 1.002,50 0.0  1.10   0.29 2.0720  0.0007090  2.35
79012308 30  1 3NANN  40.10010.0  131.803,70  131.802,19 0.0  0.59   0.56 1.8495  0.0007090  2.35
79012308 30  1 3SLMN                                         1.89   0.85 1.8985  0.0007090  2.35
79012308 30  1 5NETN  50.30010.0100 1.004,80100 1.002,30 0.0  1.03   0.25 2.0870  0.0007090  2.35
79012308 30  1 5NANN  60.10010.0  131.804,15  131.802,50 0.0  0.84   0.87 1.8400  0.0007090  2.35
79012308 30  1 5SLMN                                         1.87   0.92 1.8703  0.0007090  2.35
79012308 30  1 7NETN  70.30010.0100 1.004,38100 1.002,59 0.0  0.74   0.70 1.8911, 0.0007090  2.35
79012308 30  1 7NANN  80.10010.0  131.805.70  131.803,39 0.0  0.90   0.88 1.8814, 0.0007090  2.35
79012308 30  1 7SLMN                                         1.84   1.59, 1.8857  0.0007090  2.35
79012308 30  1 9NETN  90.30010.0100 1.005,10100 1.002,30 0.0  1.07   0.32 2.0400  0.0007090  2.35
79012308 30  1 9NANN 100.10010.0  131.805.85  131.802,30 0.0  0.80   0.81 1.8739  0.0007090  2.35
79012308 30  1 9SLMN                                         1.87   0.93, 1.8845  0.0007090  2.35,
79012308 30  1 15NETN 110.30010.0100 1.004,40100 1.002,10 0.0  0.95   0.22, 2.0952  0.0007090  2.35
79012308 30  1 15NANN 120.10010.0  131.803.20  131.801,85 0.0  0.53   0.45 1.7297  0.0007090  2.35
79012308 30  1 15SLMN                                        1.47   0.67 1.9289  0.0007090  2.35
79012308 30  1 25NETN 130.30010.0100 1.000,30100 1.002,90 0.0  1.24   0.42 2.0067  0.0007090  2.35
79012308 30  1 25NANN 140.10010.0  131.805.90  131.803,80 0.0  0.90   1.00, 1.8369  0.0007090  2.35
79012308 30  1 25SLMN                                        2.14   1.42 1.9108  0.0007090  2.35
79012308 30  1 50NETN 150.30010.0100 3.102,45100 3.101,20 0.0  1.05   0.48, 2.0417  0.0007090  2.35
79012308 30  1 50NANN 160.10010.0  131.807.20  131.804,42 0.0  1.00   1.26, 1.8290  0.0007090  2.35
79012308 30  1 50SLMN                                        2.71   1.72, 1.8290  0.0007090  2.35
```

26.7 STAGE 3. PROGRAM FOR REARRANGING CHLOROPHYLL A DATA BY SAMPLING DEPTH.

```
FORTRAN IV G LEVEL 21          MAIN          DATE = 80270          09/23/37          PAGE 0001

0001              DIMENSION INPU(4)
0002              REAL * 8 VALUE(9)
0003            5 WRITE(6,300)
0004              LINES=3
0005           10 READ(5,100,END=99)INPC,STA,DEPTH,VALUE,VAL4,VAL7
0006              PERMAN=0.0
0007              IF(VAL7.GT.0.0)PERMAN=(VAL4/VAL7)*100.
0008              WRITE(6,200)INPC,STA,DEPTH,VALUE,PERMAN
0009              LINES=LINES+1
0010              IF(LINES-40)10,10,5
0011           99 STOP
0012          100 FORMAT(3I2,A4,A3,F3.0,3(2A6,A4,2X),I3S,F6.0,I53,F6.0)
0013          200 FORMAT(1X,2(I2,'/'),I2,3X,A4,4X,A3,5X,F4.0,3(3X,A6,1X,A4,3X,A4),
                 ?11X,F6.2)
0014          300 FORMAT(1H1,///139,'NET PHYTOPLANKTON',T81,'NANNOPHYTOPLANKTON',
                 ?T84,'TOTAL PHYTOPLANKTON',T108,'% NANNOPLANKTON',//1X,
                 ?' DATE      TIME  CONSEC. SAMPLE',
                 ?3(5X,' MG/M3 ',10A),//1X,'YR MO DY    EST  STATION  DEPTH',
                 ?3(4X,'CHL A  PHAEO  FC/FA'))
                  END
```

STAGE 3. LISTING OF CHLOROPHYLL DATA BY SAMPLING DEPTH.

DATE	TIME	CONSEC.	SAMPLE	NET PHYTOPLANKTON MG/M3			NANNOPHYTOPLANKTON MG/M3			TOTAL PHYTOPLANKTON MG/M3			% NANNOPLANKTON
YR MO DY	EST	STATION	DEPTH	CHL A	PHAEO	FC/FA	CHL A	PHAEO	FC/FA	CHL A	PHAEO	FC/FA	
79/ 1/23	0830	1	1.	1.15	0.35	2.03	0.82	0.81	1.67	1.97	1.16	1.88	41.62
79/ 1/23	0830	1	3.	1.19	0.29	2.07	0.59	0.58	1.68	1.69	0.85	1.89	34.91
79/ 1/23	0830	1	4.	1.03	0.25	2.06	0.64	0.67	1.66	1.67	0.92	1.87	38.32
79/ 1/23	0830	1	7.	0.74	0.70	1.89	0.90	0.88	1.68	1.64	1.59	1.68	54.88
79/ 1/23	0830	1	9.	1.07	0.32	2.08	0.60	0.81	1.67	1.67	0.93	1.86	35.93
79/ 1/23	0830		15.	0.99	0.22	2.09	0.53	0.45	1.72	1.47	0.67	1.92	36.05
79/ 1/23	0830		25.	1.24	0.42	2.00	0.90	1.00	1.83	2.14	1.42	1.81	42.08
79/ 1/23	0830		30.	1.03	0.48	2.04	1.08	1.24	1.82	2.71	1.72	1.82	39.85

26.8 STAGE 4. PROGRAM FOR INTEGRATING AND AVERAGING ( ) CONCENTRATIONS OVER SAMPLING DEPTH BY STATION.

```
FORTRAN IV G LEVEL 21          MAIN          DATE = 80270          09/24/25          PAGE 0001

0001              REAL LDEPTH
0002              INTEGER STA,PSTA,BLNK,CHCK(9),FINFG(4)
0003              DIMENSION INPU(4),VALUE(9),DIF(9),CDEP(9),OVAL(9),CALC(9),
                 XTCALC(9),AVEN(9)
0004              DATA PSTA/'  '/,PDEPTH/0.0/,BLNK/'  '/,LINES/60/,TCALC/9*0.0/
0005              LOGICAL FIRST,FINISH
0006              FIRST=.TRUE.
0007              FINISH=.FALSE.
0008              WRITE(6,300)
0009              LINES=3
0010              READ(5,500)LDEPTH
0011           10 READ(5,100,END=70)INPC,STA,DEPTH,VALUE,CHCK
0012              IF(FIRST)GO TO 50
0013              IF(STA.NE.PSTA.OR.DEPTH.LT.PDEPTH)GO TO 40
0014              PDEPTH=DEPTH
0015              IF(DEPTH.GT.LDEPTH)GO TO 10
0016           20 DO 30 I=1,9
0017              CALC(I)=0.0
0018              IF(CHCK(I).EQ.BLNK)GO TO 30
0019              DIF(I)=DEPTH-CDEP(I)
0020              AVEN(I)=(VALUE(I)+OVAL(I))/2.
```

```
0021              IF(DDEP(I).EQ.0.0)AVEN(I)=VALUE(I)
0022              CALC(I)=DIF(I)*AVE=(I)
0023              TCALC(I)=TCALC(I)+CALC(I)
0024              DDEP(I)=DEPTH
0025              DVAL(I)=VALUE(I)
0026           30 CONTINUE
0027              GO TO 10
                C  40 CONTINUE
0028              DO 42 I=1,9
0029                 IF(TCALC(I).EQ.0.0)GO TO 42
0030                 TCALC(I)=TCALC(I)/DDEP(I)
0031              42 CONTINUE
0032                 TCALC(7)=TCALC(1)+TCALC(4)
0033                 TCALC(8)=TCALC(2)+TCALC(5)
0034                 PERCENT=(TCALC(8)*100)/TCALC(7)
0035              45 WRITE(6,200)DDEP(1),(TCALC(I),I=1,3),DDEP(4),(TCALC(J),J=4,6),
                   7DDEP(7),(TCALC(K),K=7,9),PERCENT
                C     WRITE(7,600)PINFO,PSTA,DDEP(1),(TCALC(I),I=1,3),DDEP(4),
                C    X(TCALC(J),J=4,6),DDEP(7),(TCALC(K),K=7,9)
0036                 LINES=LINES+1
0037                 IF(LINES.LT.60)GO TO 50
0038                 WRITE(6,300)
0039                 LINES=3
0040              50 IF(FINISH)GO TO 99
0041                 DO 60 I=1,9
0042                 DDEP(I)=0.0
0043                 DVAL(I)=0.0
0044              60 TCALC(I)=0.0
0045                 WRITE(6,400)INFO,STA
0046                 DO 65 I=1,4
0047              65 PINFO(I)=(INFO(I)
0048                 PDEPTH=DEPTH
0049                 PSTA=STA
0050                 FIRST=.FALSE.
0051                 GO TO 20
0052              70 FINISH=.TRUE.
0053                 GO TO 40
0054              99 STOP
0055             100 FORMAT(3I2,4A,A3,F3.0,3(2F6.2,F6.4),T17,9(2X,A4))
0056             400 FORMAT(1H0,20A,3(2X,F4.0,2F6.2,1X,F7.2,3X),2X,F6.2)
0057             300 FORMAT(1H1,T35,'METHYPHYTOPLANKTON',T66,'NANNOPHYTOPLANKTON',
                   XT90,'TOTAL PHYTOPLANKTON',T114,'% NANNOPLANKTON',/1X,
                   X' DATE      TIME CONSEC.',3(3X,'  INT.     MG/M3 ',10A),/1X,
                   X'YR MO DY     EST STATION',3(4X,'DEPTH CHL A PHAEO   FC/PA'))
0058             400 FORMAT(1X,2(I2,'/'),I2,3X,A4,3X,A3)
0059             500 FORMAT(F3.0)
0060             600 FORMAT(3I2,4A,A3,3(F4.0,2F6.2,F6.2))
0061                 END
```

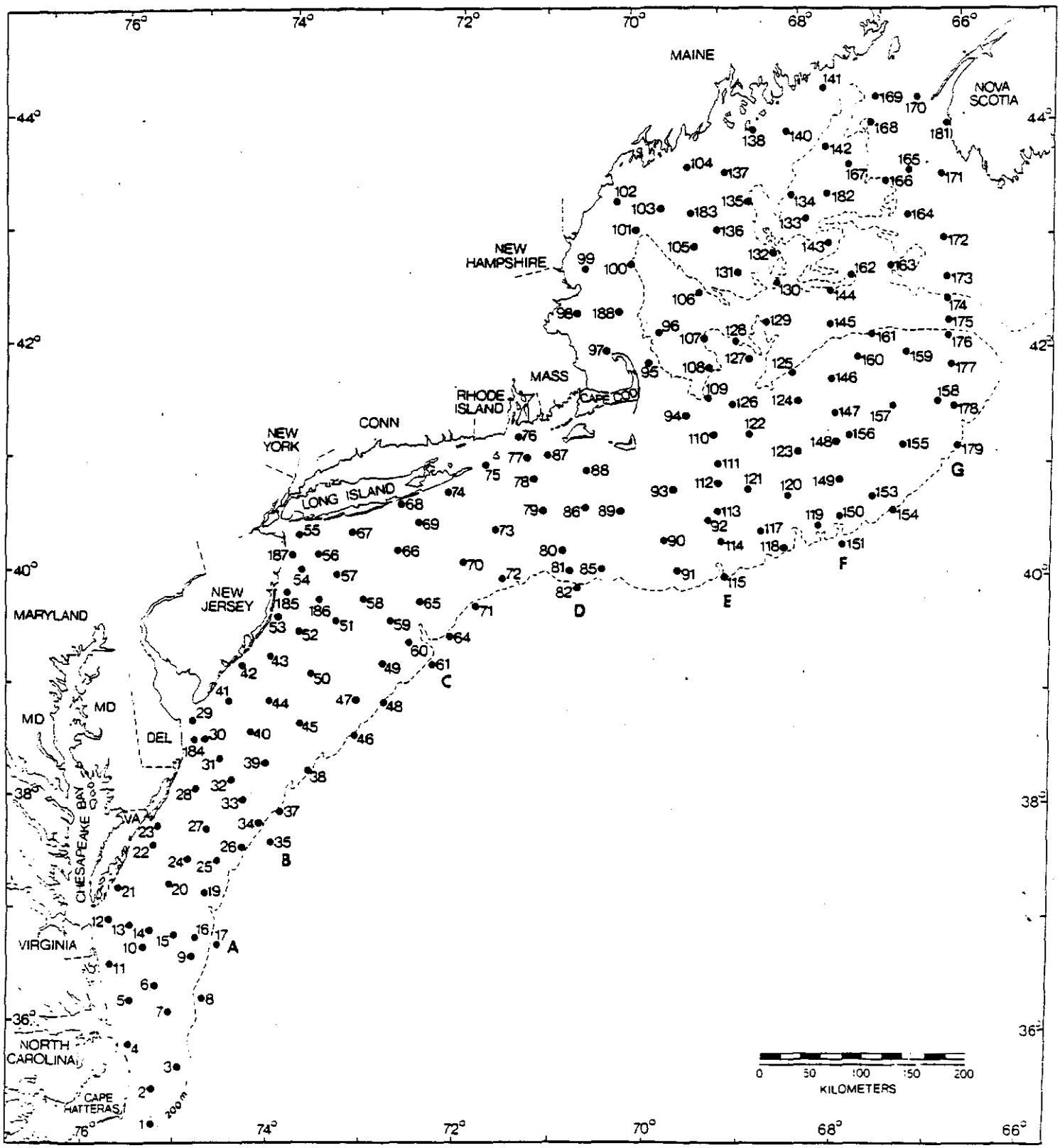
STAGE 4. LISTING OF WEIGHTED AVERAGE WATER COLUMN CONCENTRATIONS OF CHLOROPHYLL A AND PHAEOPHYTIN A BY STATION.

DATE	TIME CONSEC.	METHYPHYTOPLANKTON INT. MG/M3	NANNOPHYTOPLANKTON INT. MG/M3	TOTAL PHYTOPLANKTON INT. MG/M3	% NANNO
YR MO DY	EST STATION	DEPTH CHL A PHAEO	DEPTH CHL A PHAEO	DEPTH CHL A PHAEO	
79/ 1/23	0830 1	30. 1.10 0.36	30. 0.74 0.75	30. 1.88 1.11	40.37

Table I. Corrections for standard depth s when wire angle is 10° or greater

Wire Angle Degrees	Stardard Depths (meters)																		
0	1	5	10	15	20	25	30	35	50	75	100	125	150	175	200	250	300	400	500
10	1	5	10	15	201	25	30	34	49	74	98	123	148	172	197	246	295	394	492
15	1	5	10	14	19	24	29	34	48	72	97	121	145	169	193	241	290	386	483
20	1	51	9	14	19	23	28	33	47	70	94	1171	141	164	188	235	282	376	470
25	1	5	9	14	18	23	27	32	45	68	91	113	136	159	181	227	272	363	453
30	1	41	9	13	17	22	26	30	43	65	87	108	130	152	173	217	260	346	433
35	1	41	8	12	16	20	25	29	41	61	82	102	123	143	164	205	246	328	410
40	1	41	8	11	15	19	23	27	38	57	77	96	115	134	153	192	230	306	383
45	1	41	7	11	14	18	21	25	35	53	71	88	106	124	141	177	212	283	354

SURVEY AREA BETWEEN CAPE HATTERAS AND NOVA SCOTIA.



27. Acknowledgements

We thank T. Malone for assistance with size fractionation, and P. Falkowski for assistance with calibration procedures using purified chlorophyll a. We also appreciate the comments on the manual provided by D. Busch, A. Draxler, J. Pearce, K. Sherman, J. Thomas, J. Duggan, S. Fromm, K. Workman, and R. Fitzgerald.

28.a References

Durbin, E. G., R. W. Krawiec, and T. J. Smayda. 1975. Seasonal studies on the relative importance of different size fractions of phytoplankton in Narragansett Bay (USA). Mar. Biol. 32: 271-287.

Holm-Hansen, O., and B. Riemann. 1978. Chlorophyll a determination: Improvements in methodology. Oikos. 30, pp. 438-447.

Holm-Hansen, O., C. J. Lorenzen, R. W. Holmes, J. D. Strickland. 1965. Fluorometric determination of chlorophyll. J. Cons. perm. int. Explor. Mer. 30, no. 1, pp. 3-15.

Malone, T. C. 1976. Phytoplankton productivity in the apex of the New York Bight: Environmental regulation of productivity/chlorophyll a. In Limnol. Oceanogr. Spec. Symp. Vol. 2, Middle Atlantic Continental Shelf and the New York Bight.

Malone, T. C., and M. B. Chervin. 1979. The production and fate of phytoplankton size fractions in the plume of the Hudson River, New York Bight. Limnol. Oceanogr. 24(4): 686-696.

McCarthy, J. J., W. R. Taylor, and M. E. Loftus. 1974. Significance of nannoplankton in the Chesapeake Bay estuary and problems associated with the measurement of nannoplankton productivity. Mar. Biol. 24: 7-16.a

O'Reilly, J. E. and J. P. Thomas. 1979. A manual for the measurement of total daily primary productivity on MARMAP and OCEAN PULSE cruises using 14C simulated in situ sunlight incubation. NOAA, Northeast Fisheries Center, Ocean Pulse Technical Manual No. 1, Report No. SHL 79-06.a

O'Reilly, J. E., J. P. Thomas, and C. Evans. 1976. Annual primary production (nannoplankton, netplankton, dissolved organic matter) in the Lower New York Bay. Paper # 19, in W. H. McKeon and G. J. Lauer [eds.]. Fourth Symposium on Hudson River Ecology. Hudson River Environmental Society, Inc., New York.

Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. Bio. Sci. 24(9): 499-504.

Ricker, W. E. 1973. Linear regressions in fishery research. J. Fish. Bd. Canada 30: 409-434.

Strickland, J. D. H., and T. R. Parsons. 1972. A practical handbook of seawater analysis. Fish. Res. Bd. Canada 167, 310 p.

Thomas, J. P., J. E. O'Reilly, C. N. Robertson, and W. C. Phoel. 1978. Primary productivity and respiration over Georges Bank during March and July, 1977. ICES Paper C.M. 1978/L:37. Biological Oceanography Committee.

Thomas, J. P., J. E. O'Reilly, A. F. J. Draxler, J. A. Babinchak, C. N. Robertson, W. C. Phoel, R. I. Waldhauer, C. A. Evans, A. Matte, M.n S. Cohn, M. F. Nitkowski, and S. Dudley. 1979. Biological processes: Productivity and respiration. Chapter 10. In Oxygen depleation and associated benthic mortalities in New York Bight, 1976. R. L. Swanson and C. J. Sindermann (eds.). NOAA Professionaln Paper 11. U. S. Gov. Printing Office, Washington, D. C.n

Turner Designs. 1977. Fluorometric facts. Chlorophyll and phaeophytin. Turner Designs 2247A Old Middlefield Way, Mountain View, CA 95043 USA.

UNESCO. 1966. Determination of photosynthetic pigments in sea-water. United Nations Educational, Scientific, and Cultural Organization. Place de Fontenoy, Paris-7e. pp. 1-69.

Yentsch, C. S., and D. W. Menzel. 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep Sea Research, vol. 10, pp. 221-231.

COASTAL OCEANOGRAPHY AND CLIMATOLOGY NEWS

Recent Events in United States Coastal Waters

Volume 2 Number 3 Spring 1980

The purpose of Coastal Oceanography and Climatology News (*COCN*) is to provide timely dissemination of information concerning environmental events and research activities in U.S. coastal waters. We will publish brief articles describing recent events and unusual phenomena. Also, announcements of cruises, meetings, and investigations will be posted. Since the emphasis is on timely reporting of early results, observations older than six months will not be accepted unless they are used as a basis for comparison with more recent observations. The newsletter is not a substitute for publication in professional journals or presses. *COCN* is not copyrighted, and any reference to material printed ind the newsletter must be approved by the author.

Editors:
Merton Ingham, *National Marine Fisheries Service*
H.dPerry Jeffries, *University of Rhode Island*

Technical Editor:
Nancy Ingham

Published by:
Center for Ocean Management Studies
University of Rhode Island, Kingston, RI 02881

John Knauss, *Chairman, Executive Board*
Virginia K. Tippie, *Executive Director*

In This Issue

Environmental Events

Monitoring of *Ceratium tripos* Continues Between Nova Scotia and Cape Hatteras

The abundance and distribution of *Ceratium tripos*, a dinoflagellate which contributed to extensive and persistent anoxia off the coast of New Jersey in 1976, was monitored during surveys of coastal-shelf water between Nova Scotia and Cape Hatteras during the fall and winter of 1979-80. Quantitative collections of netphytoplankton were taken twice daily, coinciding with measurements of primary productivity. The samples were collected by making double oblique tows with a 53 μm mesh net from the surface to within a few meters of the bottom at shelf stations and to a maximum depth of 200 m at stations off the shelf.

Figures 1 and 2 indicate the abundance of *C. tripos* on the shelf in November-December 1979 and February-April 1980. Densities are expressed as cells per liter and cells x $10^5 \cdot m^{-2}$, which is an estimate of the *C. tripos* population contained beneath a square meter of sea surface to the depth of the tow.

During the November-December 1979 survey highest densities were found off the coast of Massachusetts (517-636 $\cdot 1^{-1}$), where *C. tripos* represented a relatively high percentage of the total cell count (41-47%). Lowest densities occurred at a station in the northern portion of the Gulf of Maine ($0.6 \times 10^5 \cdot m^{-2}$ or $0.7 \cdot 1^{-1}$). Population densities $\cdot m^{-2}$ were similar on and off the shelf near the Hudson Canyon and south of Cape Cod near the 200 m isobath (Fig. 1). During the survey from February 27 to April 5, 1980, relatively high densities of *C. tripos* were found in the New York Bightd (257 cells$\cdot 1^{-1}$), on Georges Bank south of Cape Cod (435 cells$\cdot 1^{-1}$), and southeast of Cape Cod (378 cells$\cdot 1^{-1}$). *Ceratium* population densities were relatively lower on the shelf south of the New York Bight and at the 200 m isobath southeast of Cape Cod.

Ceratium tripos was the dominant member of the netphytoplankton at four of the fifteen stations in the November-December 1979 survey. At the other stations diatoms (*Thalassionema nitzschioides*, *Coscinodiscus* sp., *Rhizosolenia* sp., and *Nitzschia seriata*) were predominant. Similarly, *C. tripos* was dominant at three of the thirty-one stations reported for the February-April 1980 survey. Again, the most numerous netphytoplankton at the other stations were diatoms (*Guinardia* sp., *Coscinodiscus* sp., *T. nitzschioides*, *Thalassiosira* sp., *Leptocylindrus* sp., and *N. seriata*). *Ceratium fusus* and

Ceratium macroceros, which were present in most of the samples examined, were less abundant than *C. tripos*.

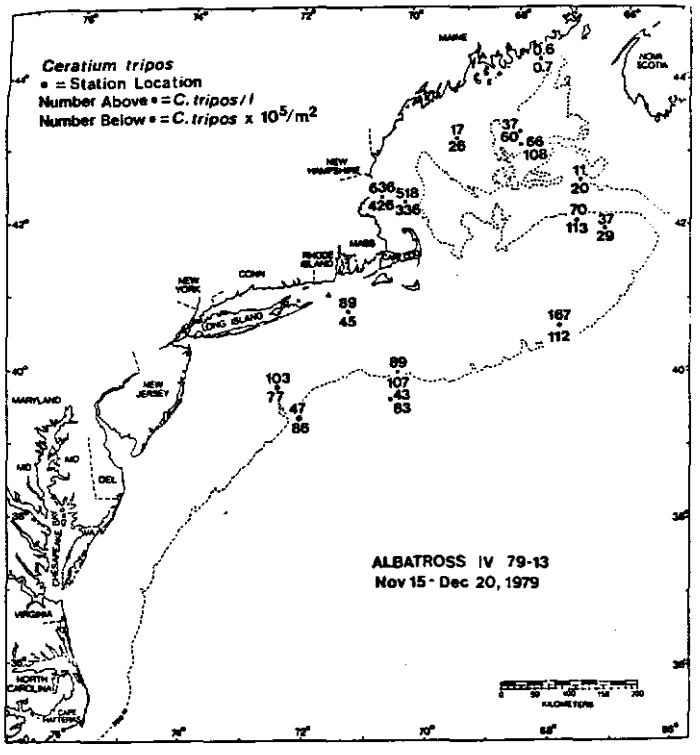


Figure 1. Abundance of *Ceratium tripos* during the November-December 1979 survey.

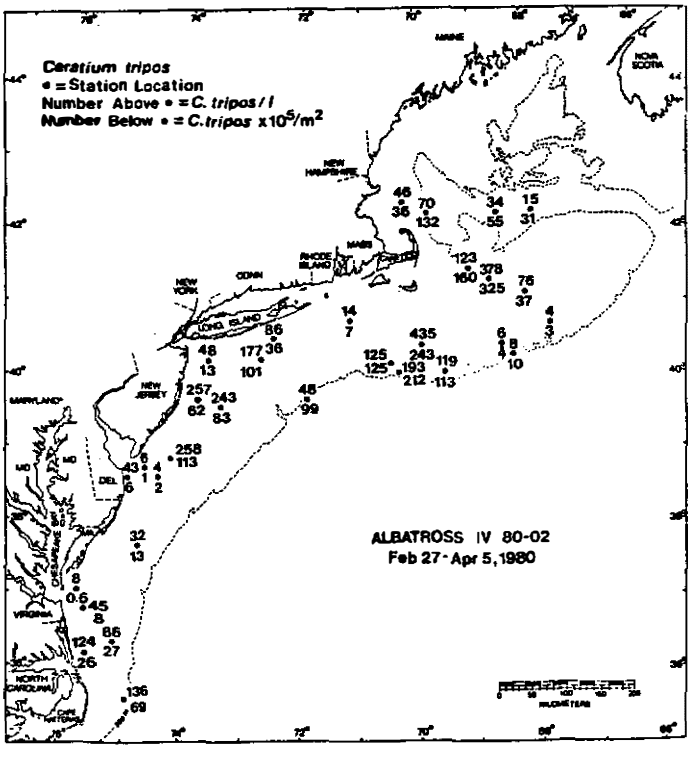


Figure 2. Abundance of *Ceratium tripos* during the February-April 1980 survey.

Prior to the episode of severe anoxia in the New York Bight in 1976, *C. tripos* was distributed relatively homogeneously throughout the water column in February and March 1976. Mean water column densities of this dinoflagellate, which were 10 cells $\cdot m1^{-1}$ in the outer Bight near the end of March 1976 (Malone 1978), were probably half this value in early March. By May 1976 *C. tripos* was concentrated (100-1000 cells $\cdot m1^{-1}$) in a thin stratum 1-3 m thick at the base of the pycnocline (Malone 1978). The *C. tripos* population in early. March 1980 in the mid-shelf area of the New York Bight. was approximately 5% the density of this organism for the. same period of 1976..

During the February 25-April 5, 1980 survey of New York Bight, Georges Bank, and Gulf of Maine, one-liter discrete samples from seven depths in the water column (corresponding to 100, 69, 46, 25, 10, 3, and 1% of surface light intensities) were filtered through 2.54 cm diameter 20μm mesh filters to reveal the depth profile of *C. tripos* abundance. Samples from the New York Bight in early March showed a relatively homogeneous vertical distribution of *C. tripos* and chlorophyll in the euphotic layer.

Several environmental factors acted synergistically to produce the anoxia off the New Jersey coast in 1976 (Malone 1978 and Swanson and Sindermann in press). We show one element of the synergism: the early spring abundance of *Ceratium tripos* in 1976 and 1980. Monitoring of *Ceratium* and other possible indicators of potential anoxia, including phytoplankton primary productivity, chlorophyll *a*, nutrient, and oxygen concentrations, will continue through the spring and summer.

References

Malone, T.C. 1978. The 1976 *Ceratium tripos* bloom in the New York Bight: Causes and consequences. NOAA Tech. Rep., NMFS Circ. 410, 14 p.

Swanson, R.L., and C.J. Sindermann [eds.]. In press. Oxygen depletion and associated benthic mortalities in the New York Bight, 1976. NOAA Professional Paper, 1979.

S. Esser.
New Jersey Marine Science Consortium
Sandy Hook, NJ 07732
201-872-1300

J. O'Reilly.
National Marine Fisheries Service
Sandy Hook Laboratory
Highlands, NJ 07732
201-872-0200

D. Busch.
National Marine Fisheries Service
Narragansett Laboratory
Narragansett, RI 02882
401-789-9326

Winter Coastal Climate Review

The winter of 1979-80, in contrast to the previous three winters, was not characterized by the development of extreme temperature anomalies. Mean temperatures were near normal. (+2°F) along much of the Gulf and Atlantic coasts. Departures exceeded 4°F along the southern California coast (see