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A Handbook for Algal Monitoring in Drinking Water Reservoirs by Flow-through Fluorimetry

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A HANDBOOK FOR ALGAL MONITORING IN DRINKING WATER RESERVOIRS BY FLOW-THROUGH FLUORIMETRY

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CONTENTS

I. Introduction

Nuisance algal growth continues to pose problems for the water treatment industry. Algal growth may produce taste and odor in treated water, and incur unacceptably short filter run times in filtration systems. A link may exist between algae in raw water and the amount of contaminants, such as halogenated hydrocarbons, in finished water.

Whether you are a water treatment specialist concerned with the costs of filtering and treating drinking water, or a lake manager concerned with the efficiency of the management strategy being applied to a lake, there is a need for basic information on the quantity and distribution of algae in lakes and reservoirs.

Algal monitoring by flow-through fluorometry has been used for algal monitoring in oceanography and limnology for many years, and has the potential for being a powerful tool in lake and reservoir management. However, like many new technologies, the proper use of fluorometry requires a thorough understanding of its strengths and weaknesses. This manual provides step-by-step instruction in the use of this technique.

This handbook is meant to complement other references by explaining in general terms the steps in chlorophyll monitoring by in vivo fluorescence (IVF). References are cited for more specific instructions on standard laboratory methods of chlorophyll analysis.

II. The Method

A. Synopsis: The amount of algae (phytoplankton) suspended in a water body is measured by the amount of fluorescence produced by the green photosynthetic pigment (chlorophyll) in the algal cells as they pass through a fluorometer. The technique is called flow-through in vivo chlorophyll fluorometry. Flow-through refers to pumping water directly from the lake or reservoir through the fluorometer. In vivo (within the living organism) means measurement of algal fluorescence without killing or disrupting the algal cells. Fluorometry is measurement of the

fluorescence of a substance. Fluorescence is a property of certain materials which absorb one form of energy and radiate another. This property permits highly specific, sensitive, and precise measurement. Subsequent references to in vivo fluorescence will be abbreviated as IVF.

B. Algae and pigments: The algae are a diverse group of plants related by their primitive level of structural development. Algae are divided into groups according to their pigments. All algae contain the green pigment chlorophyll A, and may contain several ancillary pigments such as chlorophyll B, C and several forms of the yellow pigments carotene and xanthophyll. The bluegreen algae (actually bacteria) also contain red and blue phycobilins. Photosynthesis, the process by which plants produce energy, depends upon these pigments. Pigments function in photosynthesis by capturing the energy of sunlight and converting that radiant energy into chemical energy used by the algal cell.

The subgroups of algae which commonly cause problems in lake and reservoirs are the green algae and the bluegreen algae, although other groups (diatoms, chrysophytes, dinoflagellates, and euglenoids) also may form troublesome 'blooms'.

C. Chlorophyll and fluorescence: Fluorescence is the emission of light energy by a substance. Energy is absorbed in one form by a fluorescent substance and emitted in altered form, such as the conversion of electrical energy to light in fluorescent light bulbs. Chlorophyll A fluorescence is the absorbance of blue light (436 nm) by the chlorophyll A molecule and the emission of red light (685 nm). The wavelength change represents a drop in the energy content of light roughly equivalent to the amount of energy captured and diverted to photosynthesis.

D. Fluorometry: A fluorometer is a device which measures fluorescence. A sample substance receives light in a selected range of wavelengths, called the excitation light. The range is created by placing special, colored glass filters over a light source. Light emitted by the sample upon excitation (fluorescence) enters an optical system situated at right angles to the source (to avoid receiving excitation light directly). A

second filter assembly (secondary filter) is used to determine the wavelength range which will enter the optical system to be measured (Turner Designs Fluorimetric Facts).

E. Vertical and horizontal profiles: The differences in algal concentration at different places in a reservoir (horizontal profiles) are measured by slowly towing a weighted intake hose along transects across the reservoir. Differences in algal concentration at different depths (vertical profile) are measured by slowly lowering a weighted intake hose from the surface to near the bottom. Vertical profiles disclose the location of horizontal layers of algae in stratified water bodies.

III. Equipment

A. General: The system of instruments used to obtain a vertical profile of chlorophyll fluorescence consists of a fluorometer, a pump, a chart recorder (or digitizer), an energy supply (a portable generator or storage battery), and a length of plastic tubing equal to the depth of the reservoir. The pump pushes water through the fluorometer as the tubing is slowly lowered through the water column or towed along a transect, and the chart recorder (or digitizer) records the fluorescence.

B. Fluorometer: There is presently only one fluorometer manufactured which is suitable for field conditions. The Turner Designs Model 10-005R Field Fluorometer is specifically designed for field use and will even withstand accidental emersion. It is possible to modify a laboratory instrument for field use, however, this is not recommended. Laboratory models are not designed to be used under field conditions, and many electrical components in laboratory models will not withstand conditions commonly encountered in the field, such as high humidity and vibration. Gas bubbles will cause the fluorometer to respond, but can be easily eliminated by placing the pump before (upstream of) the fluorometer, optimizing the pump rate and pumping water through the fluorometer from bottom to top (in the bottom port and out the top).

C. Pump: I have used peristaltic pumps exclusively in my work, and I recommend them. There is no contact between the pump and the water. The pump should have a variable speed control, and have a range from about one half to two liters (ca. quarts) per minute. Submersible pumps have been used by other researchers (Fee, 1976) and may be equally suitable (beware of electrical shock hazard).

D. Tubing: Flexible opaque plastic tubing is available from most hardware stores or scientific supply houses. The tubing must be flexible, but have enough strength to resist collapsing when drawing water from deeper water bodies. Tygon or a similar product will perform well up to depths of 20 meters. The vinyl tubing available at hardware stores is usually too flexible, and will collapse easily. A narrow diameter tubing (0.5 in. O.D.) is advantageous because of a shorter clearing time.

E. Chart recorder: There are many field chart recorders available. I recommend a rechargeable battery-powered recorder such as the Linear Model 142. Clamping bar recorders are not as suitable as pen recorders for this application because of the requirement of high resolution records.

There are advantages to obtaining a multiple pen recorder. The tubing used for fluorometry can easily be attached to the lowering cable of a field dissolved oxygen meter such as the Y.S.I. Model 58. Temperature and fluorescence, or dissolved oxygen and fluorescence may then be recorded simultaneously on the same set of axes. This will facilitate relating chlorophyll A fluorescence peaks to ambient temperature and dissolved oxygen conditions.

F. Digitizer: A digitizer records data as numerical values at short time intervals rather than as ink lines on a chart. Data output from the fluorometer in this form may be input directly to a data logging device of portable computer for analysis, thereby eliminating time consuming and error producing intermediate steps. Although I have not used a digitizer,

and therefore cannot recommend a particular model, this is an ideal application for one.

G. Power Supply: I recommend a small gasoline powered electrical generator. I use the Kawasaki Model 550A. It is powerful enough to run the fluorometer and the pump, and is quiet. Storage batteries may be used. The fluorometer draws about 0.3 amps at 105 to 130 volts and the pump draws about 1.5 amps. If a chart recorder is employed which is not battery powered, the power requirements of the recorder should also be considered when judging the size of a generator or battery.

IV. Sampling Routines

A. Field

1. Vertical Profiles: Vertical chlorophyll fluorescence profiles are used to determine the vertical distribution and relative abundance of algae in lakes and reservoirs. The depth of algal layers can be determined rapidly by flow-through in vivo fluorometry.

Vertical profiles are usually performed on water bodies which are thermally stratified. When a lake or reservoir is stratified, the water column is divided into distinct layers: a warm, mixed, surface layer (the epilimnion), a narrow layer where temperature changes rapidly with depth (the metalimnion), and a cold bottom layer (the hypolimnion).

Most stratified lakes and reservoirs contain horizontal layers of algae. Layers form due to temperature, density, and light differences in the water column. The reason why layers form where they do is complex and not completely understood, but thermal stratification is usually required for thin layer formation.

For treatment plants which can vary the depth of their intake, vertical IVF profiles can be used to find and avoid layers containing algae.

To perform a vertical profile, a length of opaque flexible plastic tubing marked in depth units and equal to the depth of the water body is required. Opaque tubing is recommended because exposure of water to sunlight as the water enters the fluorometer cell may influence algal fluorescence. As an alternative to using opaque tubing, only the tubing near the fluorometer may be wrapped with electrical tape, and the remaining tubing kept under the water surface during measurement. Electrical tape is also useful for marking depths on the tubing. The tubing is connected to the pump, and water is pumped (pushed) through the fluorometer so that a positive pressure is maintained in the fluorometer cell. The pump should not be placed after the fluorometer because water will then be pulled through the cell resulting in a negative pressure in the cell and possible bubble formation. If bubbles enter the system, the fluorometer will respond to the passage of bubbles which distorts the fluorescence profile. The lower port on the fluorometer should be used as the inflow port and the top port as the outflow port. This arrangement prevents bubbles from being held in the cell by the water flow.

Before recording fluorescence profiles, distilled water must be circulated through the system to provide a zero baseline.
Following the measurement of profiles, distilled water is again circulated through the system to rinse the tubing and prevent algal growth on the tubing walls or in the fluorometer cell.

One end of the tubing is connected to the fluorometer and the other end is slowly lowered through the water while the pump is drawing water through the fluorometer. The lowered end should be weighted to hang vertically in the water. A lag will occur between the time water from any depth enters the tubing and when that water passes through the fluorometer. The lag time may be easily measured by holding the end of the tubing under the water surface until the entire system is filled with water, then raising the end out of the water for a few seconds to allow a bubble to enter the tubing. The chart should be marked adjacent to the pen just as the tubing is lifted to allow the bubble to enter. The fluorometer will respond when the bubble passes through the cell by

making a small peak on the chart, and the distance between the original mark on the chart and the peak is the response time. As the tubing is lowered through the water, a mark is placed on the chart as each depth interval passes below the surface. The depths recorded on the chart will all be displaced along the profile by a distance equal to the lag time.

The fluorescence profile recorded on the chart is a recording of the changes in fluorescence encountered with increasing depth. When the tubing passes through a region of high algal concentration, the fluorometer will respond to the increased chlorophyll fluorescence by forming a peak on the chart. The depth of the peaks may be calculated by adding the chart distance produced by the lag time to the depths marked on the chart. The total amount of chlorophyll in the water column may be calculated by integrating the area under the profile curve. Traditional methods of chlorophyll sampling involve removing water samples at discrete depth intervals. Thin layers of algae located between the sampling depths can be missed entirely. The fluorescence profile represents a continuous measurement of chlorophyll fluorescence with depth.

Two duplicate vertical profiles should be recorded at each location to be ensure that peaks encountered are not artifacts of sampling, such as bubbles (Figure 1). The shape of the fluorescence profiles will depend upon the rate at which the tubing is lowered, the chart speed of the recorder, and the pump rate. The peak definition depends upon the sensitivity setting on the fluorometer. With a little practice, lowering rates may be easily duplicated. The chart speed and pump rate should not be changed between duplicate profiles.

Samples for chlorophyll extraction, to calibrate the profiles, may be taken directly from the outflow of the fluorometer as the tubing is being held stationary at a fixed depth. Duplicate samples for extracted chlorophyll should also be taken.

Data may be presented either by tracing the profile onto appropriate axes, or by transcribing the fluorescences at small depth intervals, then

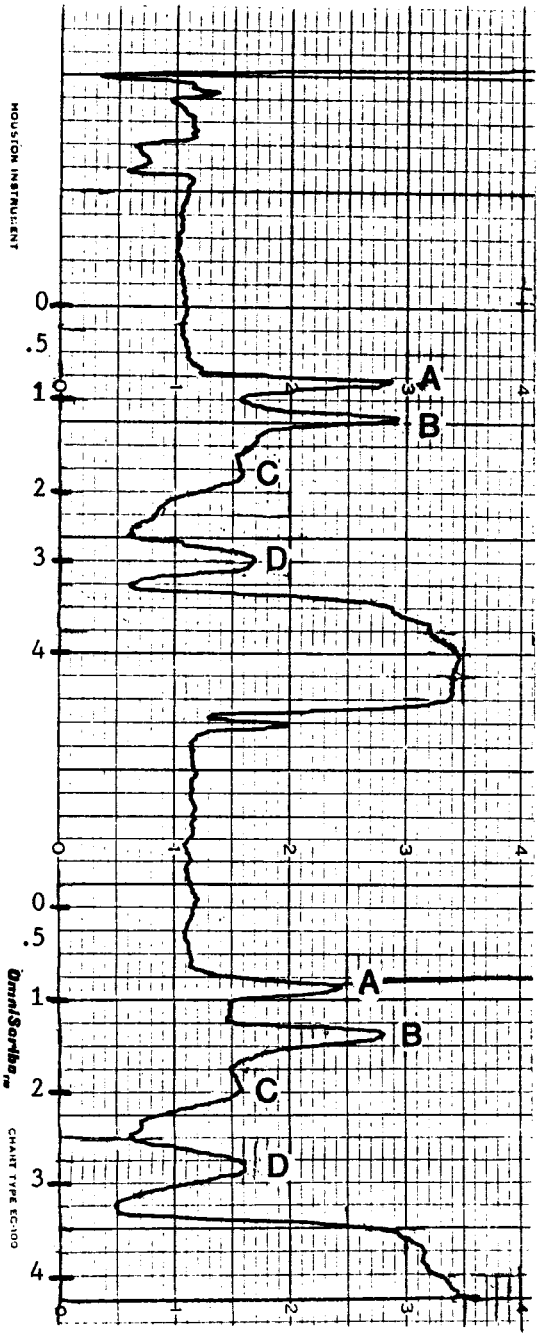

Figure 1. Example of fluorescence profiles recorded in Dunham Pond, CT on June 4, 1982. Four distinct fluorescence peaks were located and duplicated on successive profiles (A-D). Lag time was approximately one vertical square.

finding the mean of the two profiles, and plotting the mean and variance as a function of depth.

2. Horizontal Transects: Horizontal monitoring may be useful in large lakes and reservoirs to discover localized "hot spots" of algal abundance. It may also be useful in lakes with irregular or complex basins (lakes with more than one deep basin).

The sampling equipment needed is identical to that used for vertical profiles. For horizontal transects, a weighted tubing is held at a constant depth as the boat moves slowly along a transect. It may be useful to delineate the transect with a string with knots at regular intervals. As each knot is passed, the distance along the string is recorded on the chart next to the recorded IVF value. The data may then be presented as a graph of chlorophyll fluorescence as a function of distance from a fixed point, or between two points.

B. Laboratory

1. Extracted Chlorophyll Analysis: The IVF technique requires calibration by extracted chlorophyll analysis. The number of calibration samples depends entirely upon the degree of precision the user desires in predicting chlorophyll from IVF values. If the user is interested only in locating major concentrations of algae, a minimum number of extractions is necessary. For example, samples should be collected from the water masses yielding a range of IVF values. The IVF values and the chlorophyll concentrations would then be plotted (i.e. Figure 2.). Estimates of intermediate chlorophyll concentrations may then be obtained by interpolating values between points. Precision and accuracy depend on the strength of the relationship between IVF and extracted chlorophyll a.

2. Chlorophyll Extraction Procedure: The standard method for chlorophyll analysis involves three steps: filtration, extraction, and measurement.

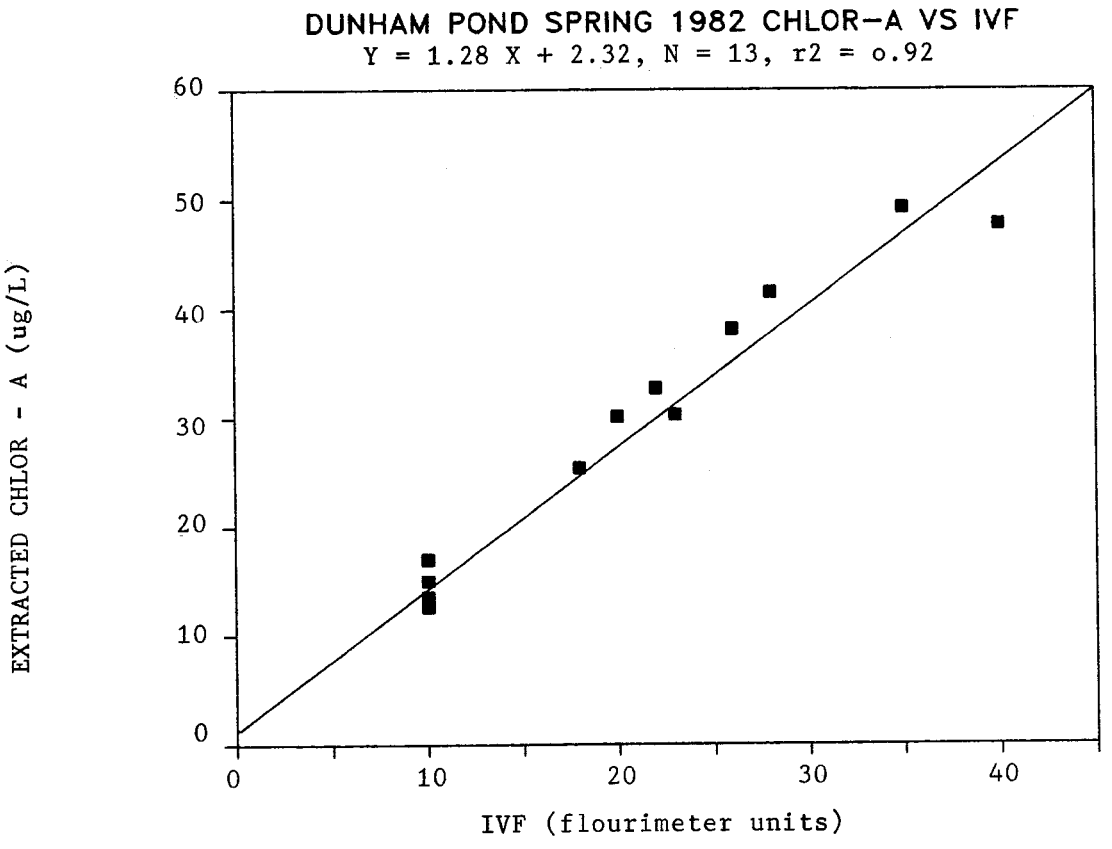

Figure 2. Relationship between extracted chlorophyll-a and in vivo fluorescence in Dunham Pond, Connecticut, April-May 1982 (N=13, r^2=0.96).

Filtration: a known volume of sample is filtered through a glass microfiber filter. The filter is then frozen for future analysis (for up to three months) or extracted immediately.

Extraction: the filter is ground with a small mortar and pestle or in a tissue grinder in a small amount (a few ml) of 90% acetone (prepare 90% acetone by adding reagent grade acetone to 100 ml of distilled, deionized water to make 1 Liter in a volumetric flask, then add two drops of reagent grade ammonium hydroxide to produce an alkaline solution). Care should be taken to ensure that the filter is not touched with the fingers or come in contact with any acidic material. Chlorophyll is readily degraded by acids. Grinding should continue until the filter is completely homogeneous. After grinding, the sample is refiltered through another glass fiber filter with more washings of 90% acetone to remove the ground filter and residual particulate material.

Measurement: the filtrate obtained from the second filtration should be colored but free of all turbidity, and will contain most of the chlorophyll in solution. The extracted chlorophyll is light sensitive, and the filtrate should be kept under subdued light. Measurement consists of determining the absorbance at selected wavelengths in a spectrophotometer. The wavelengths correspond to the absorbance bands of chlorophyll a = 665 nm, chlorophyll b = 645 nm, and chlorophyll c = 630 nm. In addition, absorbance at 750 nm is measured as a background estimate because chlorophyll will not absorb at that wavelength.

The resulting absorbances are then converted to chlorophyll concentration through formulae available in several references (Standard Methods current edition, Strickland and Parsons 1968, Vollenweider 1974). The formulae presented below are found in A Practical Handbook for Seawater Analysis by Strickland and Parsons.

FORMULA FOR CALCULATING CHLOROPHYLL A FROM ABSORBANCES:

$$\text{CHLOROPHYLL A (ug/L)} = C = 11.6 \times E_{665} - 1.31 \times E_{645} - 0.14 \times E_{630}$$

where: E_{665} = absorbance at 665 nm.

E645 = absorbance at 645 nm.

E630 = absorbance at 630 nm.

This formula is based on an extraction using a total of 10 ml of 90% acetone and a spectrophotometric cell with a 10 cm light path. More acetone may be used, but a correction factor must be applied to the absorbances to account for the greater dilution before applying the coefficients. For example, if 15 ml of acetone are used, absorbances must be multiplied by 15/10 = 1.5 before substitution into the above formula. Cells or cuvettes with shorter path lengths may be used, but again, a correction factor must be applied to the absorbances to account for the decrease due to a shorter path length through the liquid. For example, if a 5 cm cell is used, the absorbances must be multiplied by 10/5 = 2.0 before substitution in the above formula.

It is also possible to estimate the proportions of active (live) chlorophyll to degraded (dead) chlorophyll (phaeophytin) by performing an additional step. After all samples have been measured, the samples are acidifed and the absorbances remeasured at 750 nm and 665 nm.
Acidification converts all chlorophyll in the sample to phaeophytin. Again, a formula is used to convert the changes in absorbance due to acidification to the concentrations of active and degraded chlorophyll.

Estimation of phaeophytin is important because the proportion of phaeophytin increases with depth and may constitute a significant proportion of total chlorophyll in some lakes.

FORMULA FOR CALCULATING ACTIVE CHLOROPHYLL AND PHAEOPHYTIN:

$$\text{ACTIVE CHLOROPHYLL A (ug/L)} = \frac{26.7\ (665_o - 665_a) \times v}{V \times 1}$$

$$\text{PHAEOPIGMENTS (ug/L)} = \frac{26.7\ (1.7\ [665_a] - 665_o) \times v}{V \times 1}$$

where: 665_o = net unacidified absorbance at 665 nm ($E_{665}-E_{750}$)
665_a = net acidifed absorbance at 665 nm ($E_{665}-E_{750}$)
v = volume of acetone used for extraction in mL (milliliters)
V = volume of sample filtered in L (Liters)
1 = pathlength of the cell of cuvette used

Alternative methods of standardizing IVF results with chlorophyll involve use of the fluorometer to measure the fluorescence of extracted chlorophyll or fluorescent standards. These methods are faster and more sensitive than spectrophotometric determination, but require calibration by spectrophotometry or by standards prepared from either native chlorophyll a (available from chemical supply houses) or by chemicals which possess fluorescence characteristics very similar to chlorophyll a. Detailed instructions for calibration with pure chlorophyll or chlorophyll substitutes is included in literature accompanying the fluorometer when purchased.

V. Correcting IVF for interferences

A. General guidelines: When ambient chlorophyll levels are low (less than 10 ug/L of ppb), other factors may strongly influence the reliability of the IVF technique to accurately predict chlorophyll concentration and, thus, algal distribution. The large difference in temperature encountered in stratified lakes affects IVF. Also, at low chlorophyll levels, background fluorescence due to dissolved substances such as humic acid may comprise a significant proportion of the fluorescence response. Neither temperature nor background fluorescence appears to greatly influence IVF values if the chlorophyll concentration is greater than 10 ug/L.

Many of the problems encountered with the IVF method involve the reliability of IVF to precisely and accurately estimate extracted chlorophyll. Scientists involved in basic research usually require a high level of both precision (repeatability) and accuracy (nearness to the 'true' value). An acceptable precision is usually between 5-10%, while acceptable accuracy is usually determined statistically and also is in the range of 5%.

However, for drinking water suppliers and lake managers, whose main interest may be qualitative (i.e. where are major algal accumulations on any given day?) rather than quantitative, most of the problems with the method would not apply. The true value of the method depends upon the nature of the information needed.

B. Corrections for temperature effects: IVF values may be corrected for temperature effects by measuring the difference in IVF response of a sample as the temperature of the sample is cooled or warmed through a range similar to that found in the study lake (i.e. 4 - 30 C.). Measure the decrease in IVF values measured at warm temperatures and the increase in IVF values measured at cold temperatures. Calculate the temperature effects relative to the IVF of the sample at 25 C. and correct IVF values measured in the lake at warmer and cooler temperatures by the same

amount. This procedure will eliminate temperature effects from your values.

C. Correction for background fluorescence: If you suspect high background fluorescence, measure IVF on several samples yielding a range of values. Filter the samples through glass fiber filters or membrane filters as though you were performing a chlorophyll analysis. Remeasure the IVF values of the filtered samples and calculate the proportion of IVF due to particulate material (filterable) by subtracting the IVF value of the filtered sample from the IVF of the unfiltered sample and dividing by the total IVF of the unfiltered sample. The proportion of IVF due to background fluorescence is the IVF value of the filtered sample divided by the IVF of the unfiltered sample.

VI. Examples

A. Dunham Pond: Dunham Pond is a small softwater lake near the campus of the University of Connecticut in rural eastern Connecticut. Dunham Pond is shallow (max. depth = 4.5 m), but humic-stained. Most sunlight is absorbed in the surface 2 m, and the lake is thermally stratified for six or seven months each year. The water beneath the thermocline at 2.5 m is anoxic through most of the stratified period.

A strong correlation exists between extracted chlorophyll a and IVF in Dunham Pond (Figure 2). The relationship was used to convert IVF to chlorophyll concentration.

Two zones of phytoplankton growth were discovered using IVF monitoring. One was located in the surface water just above the thermocline developing soon after thermal stratification in early summer. Another layer formed near the sediments, at a depth of 3.5 m, soon after the onset of anoxia and remained at that depth throughout the stratified period.

The upper layer was composed of flagellated green algae, while the deeper layer was dominated by bluegreen pigmented bacteria.

Figure 3 is a plot of the distribution of IVF, converted to chlorophyll a concentration using the relationship in Figure 2, over time and depth in Dunham Pond.

The upper chlorophyll layer is representative of layers found in most temperate stratified lakes. Although the ultimate cause of layer formation has not been clearly established, it is thought that the lack of turbulence in the thermocline region contributes to the accumulation of algae.

The lower layer in Dunham Pond is believed to be a result of the growth of chlorophyll-pigmented bacteria specially adapted to a low light and low oxygen environment. These bacteria make use of the increased concentration of nutrients and other chemical substances found near the sediment.

B. New Haven Reservoirs: IVF was used to study the distribution and abundance of phytoplankton in reservoirs of the South-central Connecticut Regional Water Authority. The distribution of IVF in four low-productivity reservoirs is shown in Figure 4.

It was found that IVF profiles were not strongly related to the extracted chlorophyll values in these reservoirs because the extracted chlorophyll concentrations were very low, and with low chlorophyll, fluorescence from dissolved humic substances interferred with accurate IVF measurements. In the fall, when the extracted chlorophyll a concentrations increased above approximately 10 ug/L, IVF again provided a good estimate of extracted chlorophyll a in these reservoirs.

However, even with the decreased sensitivity, the IVF profiles still pinpointed the depth of the major algal accumulations near the thermocline, and permitted improved management decisions. During routine winter IVF sampling, an algal layer was located at the same depth as the filtration plant intake. After adjustment to avoid the algal layer,

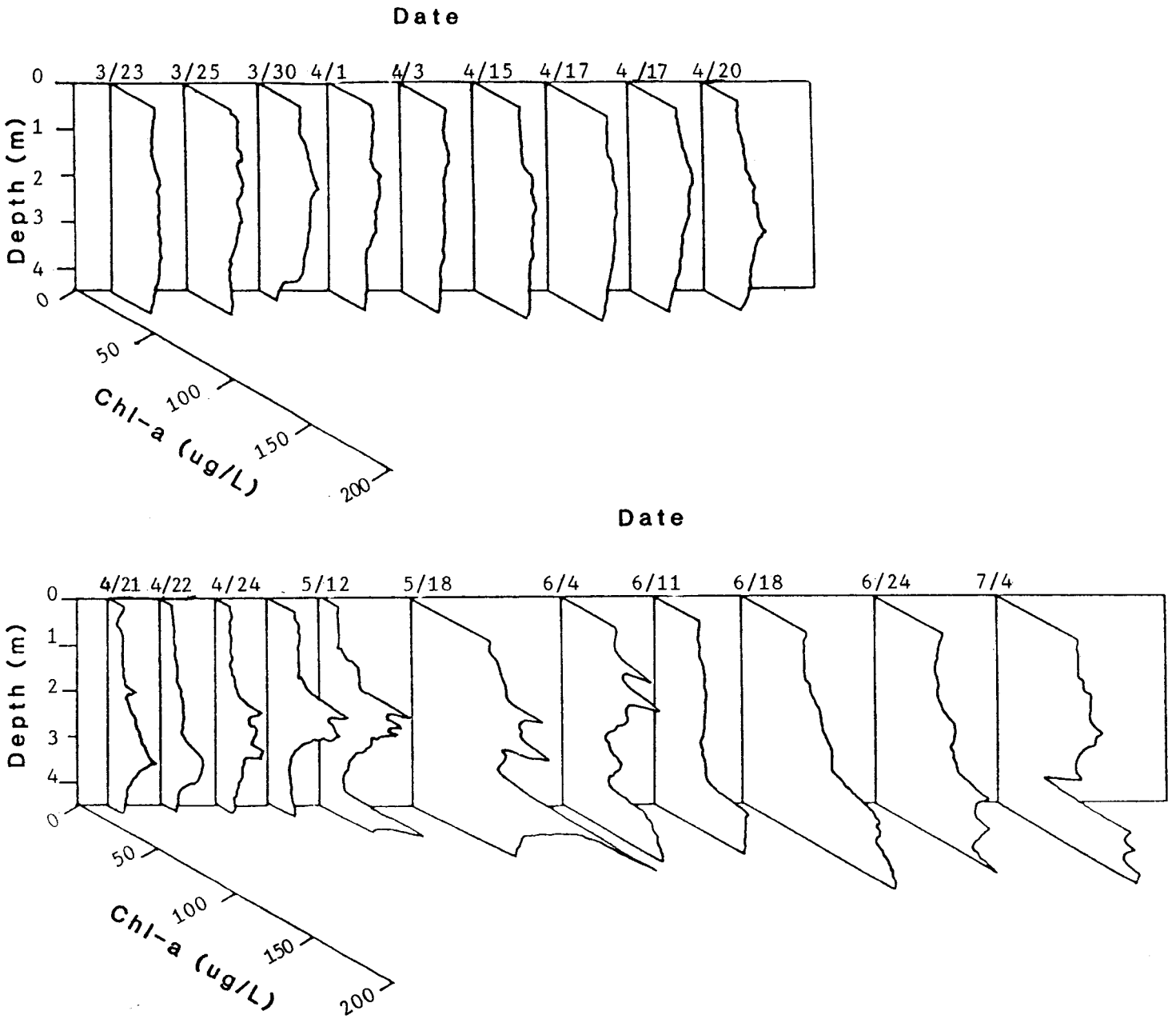

Figure 3. In vivo fluorescence profiles from Dunham Pond, CT, for spring and summer 1982. Fluorescence values were converted to chlorophyll-a concentrations using the relationship shown in Figure 2.

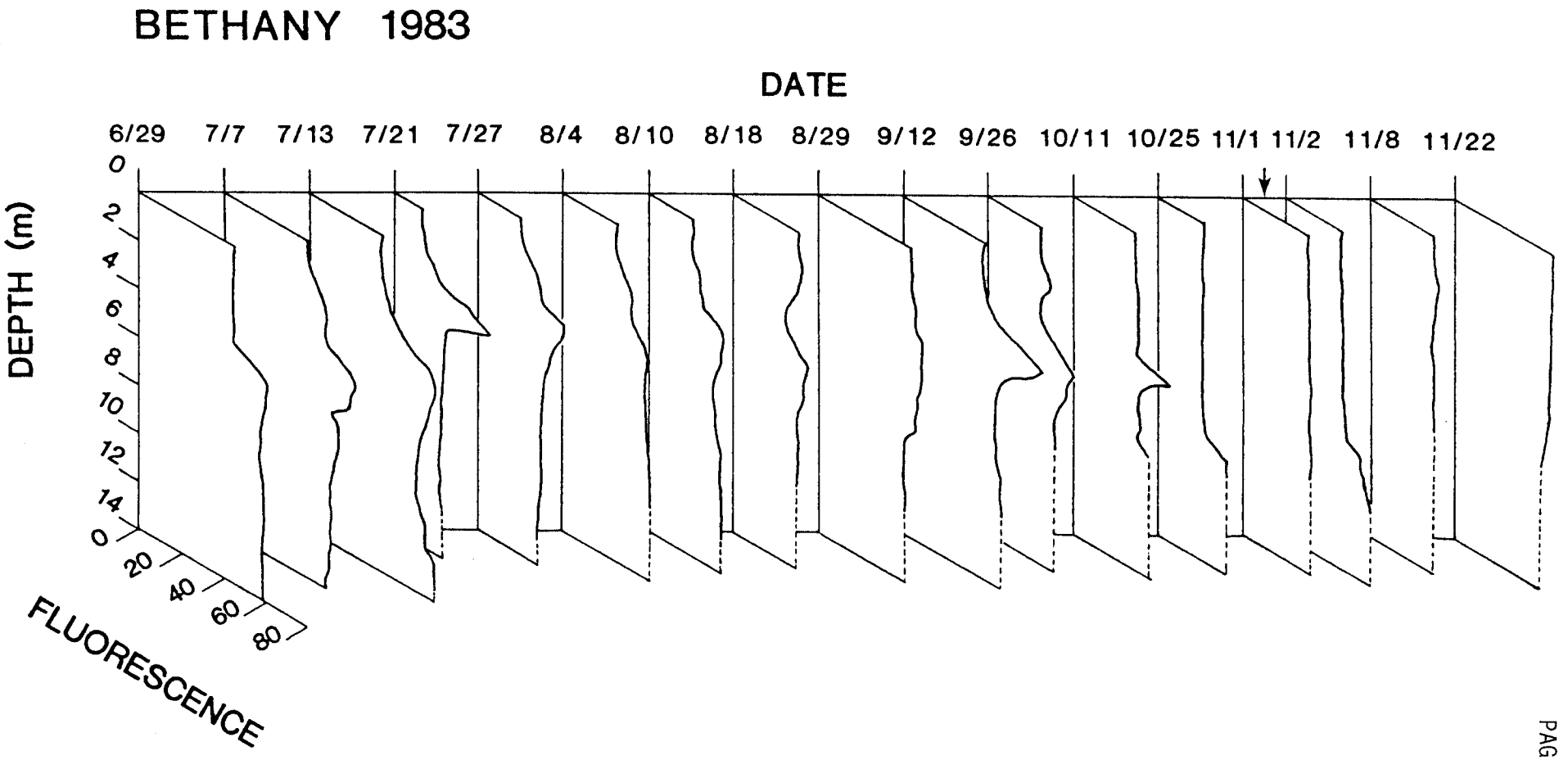

Figure 4A. IVF profiles from four low productivity reservoirs in Connecticut (A. Bethany, B. Chamberlain, C. Glen, D. Watrous).

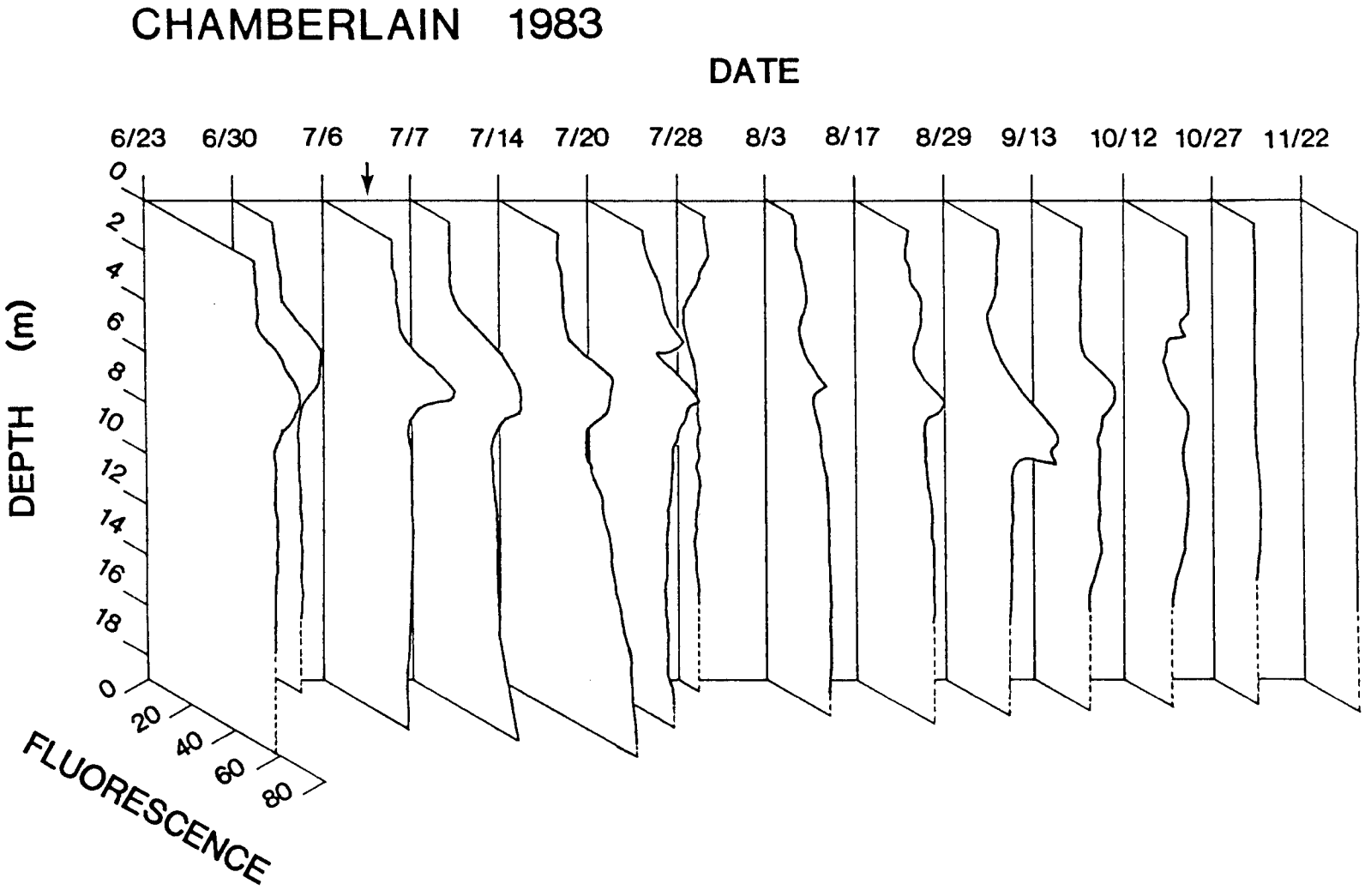

Figure 4B. IVF profiles from four low productivity reservoirs in Connecticut (A. Bethany, B. Chamberlain, C. Glen, D. Watrous).

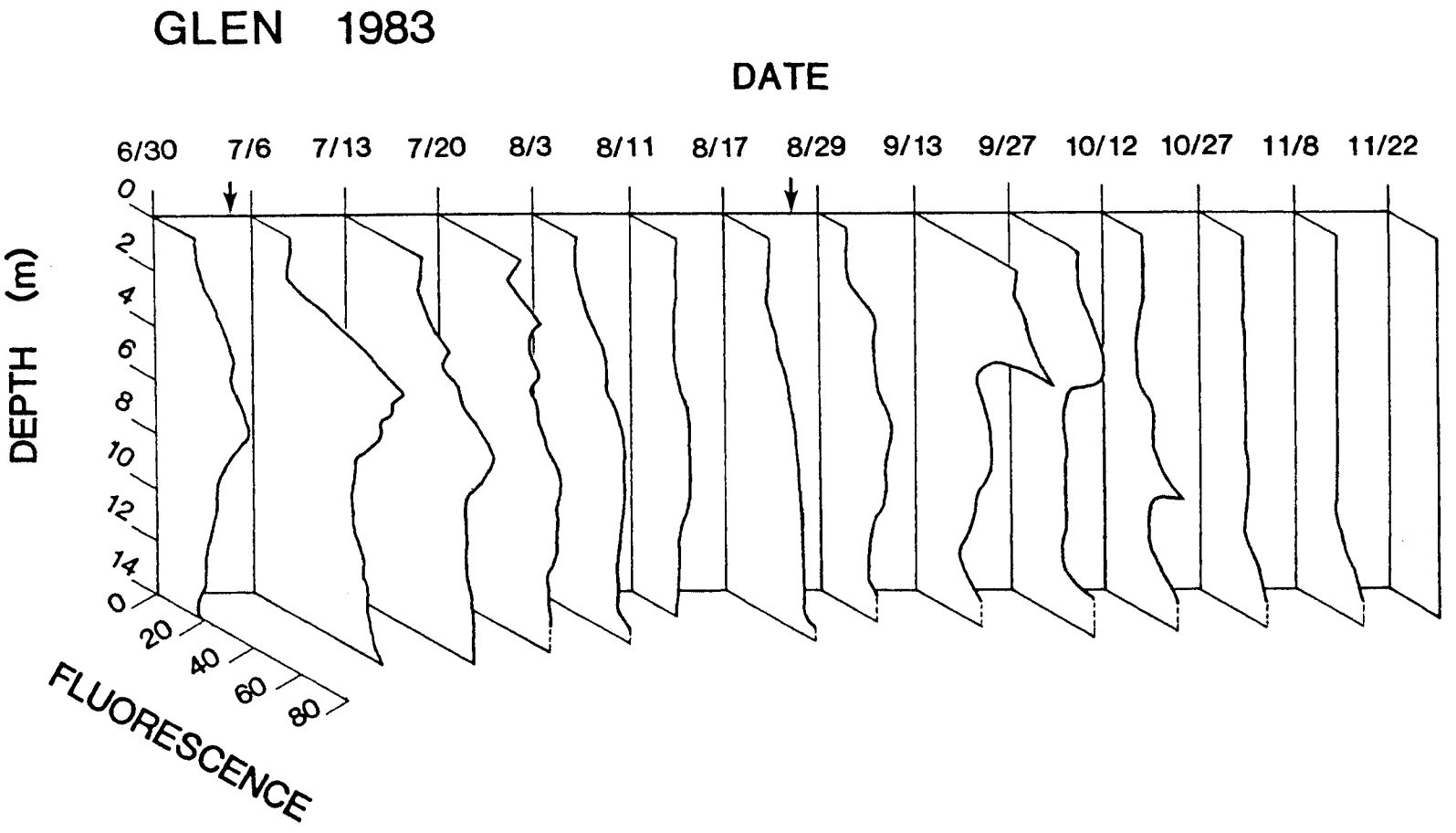

Figure 4C. IVF profiles from four low productivity reservoirs in Connecticut (A. Bethany, B. Chamberlain, C. Glen, D. Watrous).

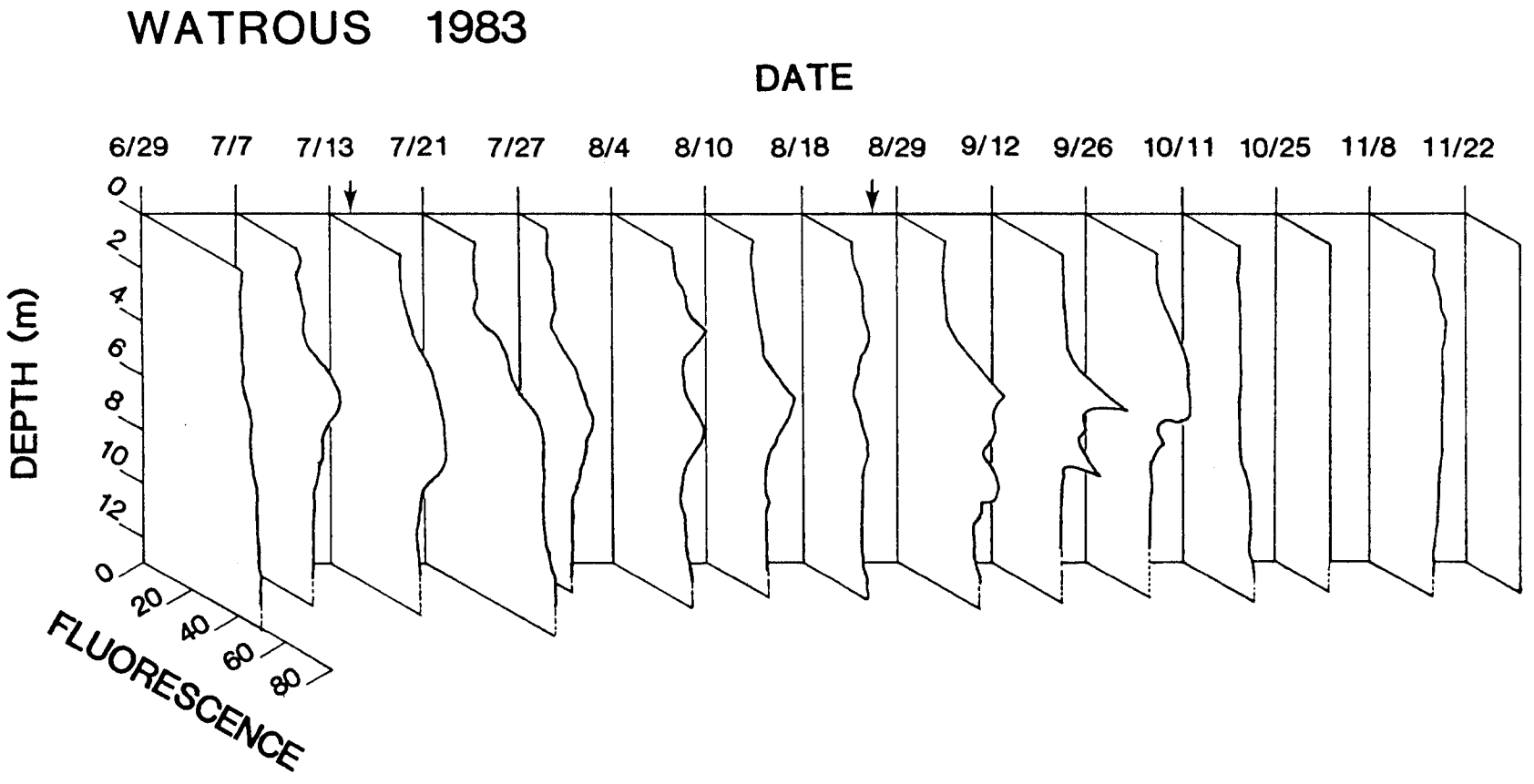

Figure 4D. IVF profiles from four low productivity reservoirs in Connecticut (A. Bethany, B. Chamberlain, C. Glen, D. Watrous).

filter run times increased dramatically, and the amount of treatment chemicals required decreased, resulting in a savings to the company.

Lake Saltonstall, another SCCRWA reservoir, is more fertile and has a much higher algal productivity. It was sampled by fluorometry on August 1, 1983. The distribution of IVF and extracted chlorophyll is shown in Figure 5. A strong relationship existed between IVF and extracted chlorophyll a in Lake Saltonstall on this date (Figure 6). Lake Saltonstall is typical of the type of water body in which IVF can provide quick and accurate estimates of algal chlorophyll; a lake or reservoir in which the eutrophication process has resulted in increased algal growth and is producing serious problems for the water suppliers.

C. Lake Annie: Lake Annie is a subtropical soft-water sinkhole lake in south-central Florida. IVF and extracted chlorophyll profiles were measured weekly for over a year. A strong relationship exists between IVF and extracted chlorophyll a (Figure 7). IVF and extracted chlorophyll a values from eight depths (1,5,9,11,13,15,and 17 m) measured weekly between February through October 1984 are plotted in Figure 7. The regression of extracted chlorophyll and IVF yields a coefficient of determination of 0.76, meaning that 76% of the variation in extracted chlorophyll may be accounted for by the variation in IVF. Given that the data set includes variation due to both seasonal and depth differences in fluorescence, this relationship is remarkably strong.

Only three data points of 187 can be considered outliers, and they are due to IVF measurements within a dense, deep phytoplankton layer which existed in Lake Annie through most of the period of stratification. The reason for the low relative IVF values is not clear. However, although the depth of the layer could be measured with great precision, the thinness of the phytoplankton layer made extremely accurate and reproducible measurements of IVF values within the layer difficult. The layer thickness was approximately 20 cm, and the passage of the sampling tube was sufficient to disrupt the layer.

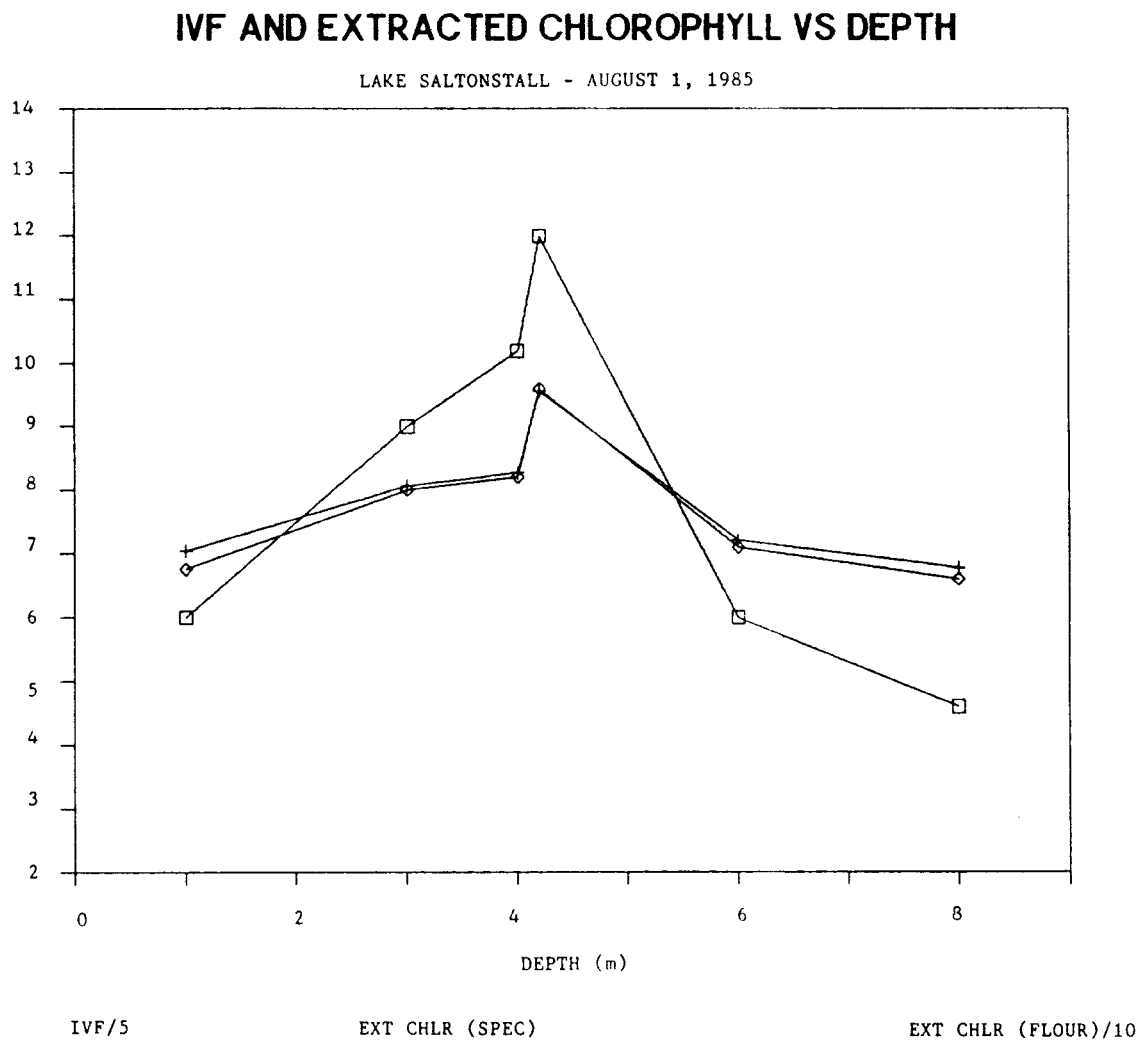

Figure 5. The relationship between <u>in vivo</u> fluorescence and two measurements of extracted chlorophyll-a in Lake Saltonstall at several depths in the water column (IVF is divided by 5 and extracted chlorophyll fluorescence is divided by 10 for scale, extracted chlorophyll measured by spectrophotometer is given in ug/L).

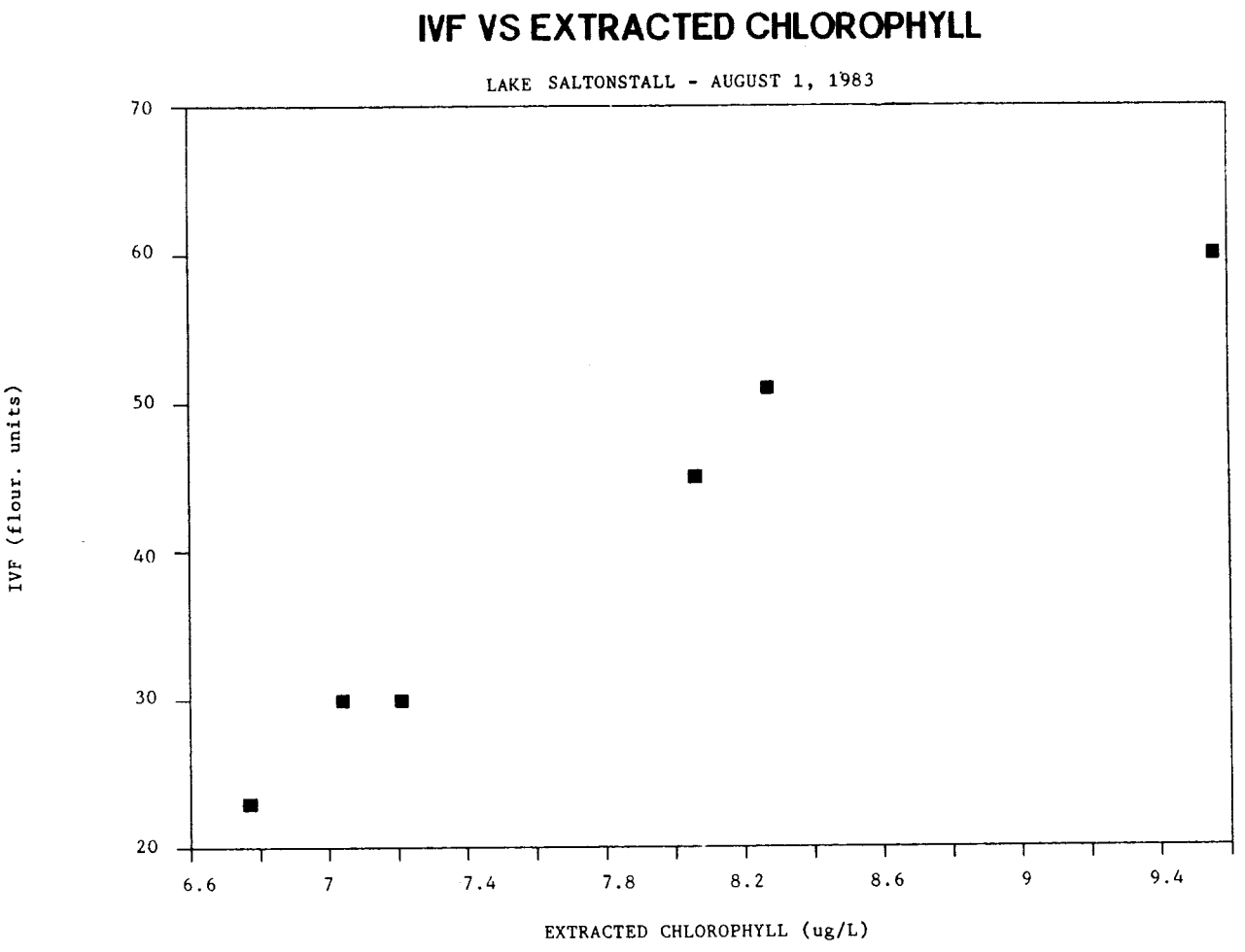

Figure 6. The relationship between <u>in vivo</u> fluorescence and extracted chlorophyll-a in Lake Saltonstall on August 1, 1983 (samples taken from depths indicated in Figure 5.) (slope=13.5, r^2=0.95).

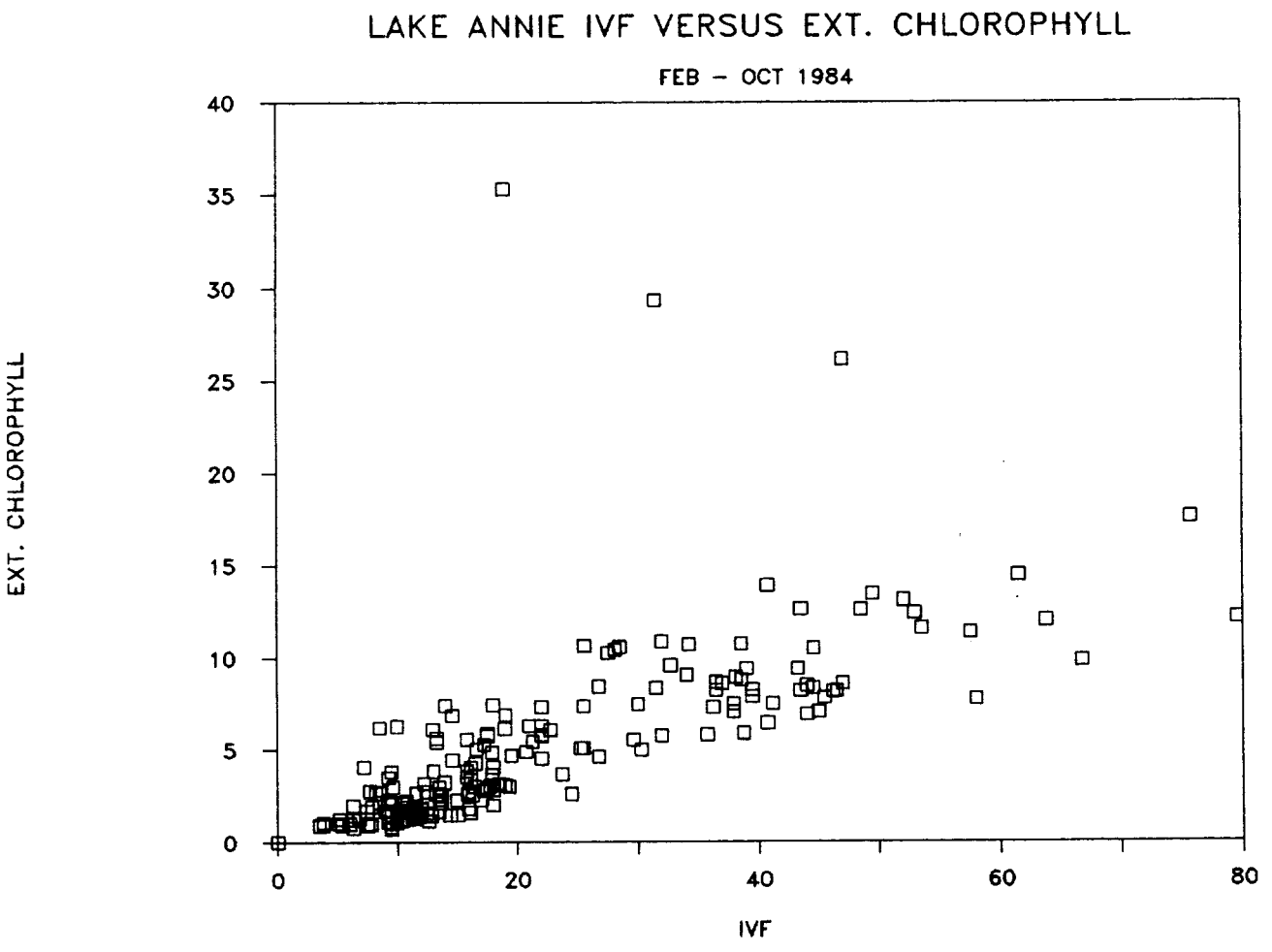

Figure 7. Relationship between in vivo fluorescence and extracted chlorophyll-a in Lake Annie, a sinkhole lake in south-central Florida. Of N=187, three outliers represent measurements from a dense deep phytoplankton layer.

The distribution and abundance of extracted chlorophyll a and IVF showed the same overall trend. Figures 8 and 9 show both the 3-dimensional surface and the isopleth diagram views of extracted chlorophyll and IVF distributions with depth and time.

The presence of the phytoplankton layer at approximately 13 meters is the most prominent characteristics of both the extracted chlorophyll and the IVF distribution. As noted above, the IVF values tend to inflate estimates of extracted chlorophyll in deeper waters due to enhanced fluorescence at cooler temperatures. This effect can be seen in the elevated IVF values at greater depths in Figure 9. In July, the depth of the phytoplankton layer decreased from 13 meters to about 12 meters, and this movement can be seen in both figure 8 and 9 (see especially the isopleths, 8b and 9b). The IVF method proved invaluable for tracking the depth of the deep algal layer. Tracking of such a thin layer would be much more difficult with conventional but less sensitive and more disruptive sampling techniques.

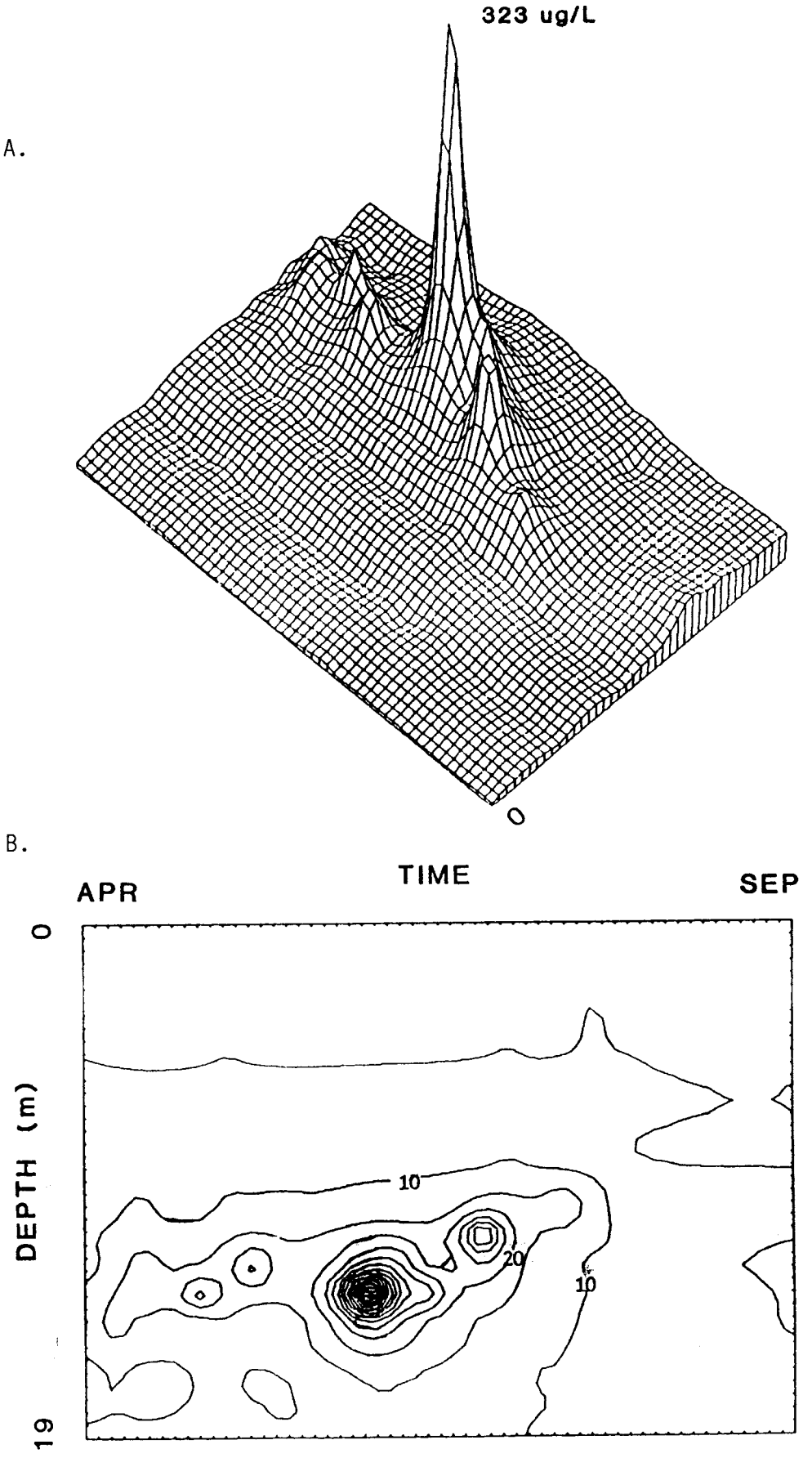

Figure 8. Three dimensional surface plot (A.) and isopleth plot (B.) of the distribution of extracted chlorophyll-a over time and depth in Lake Annie.

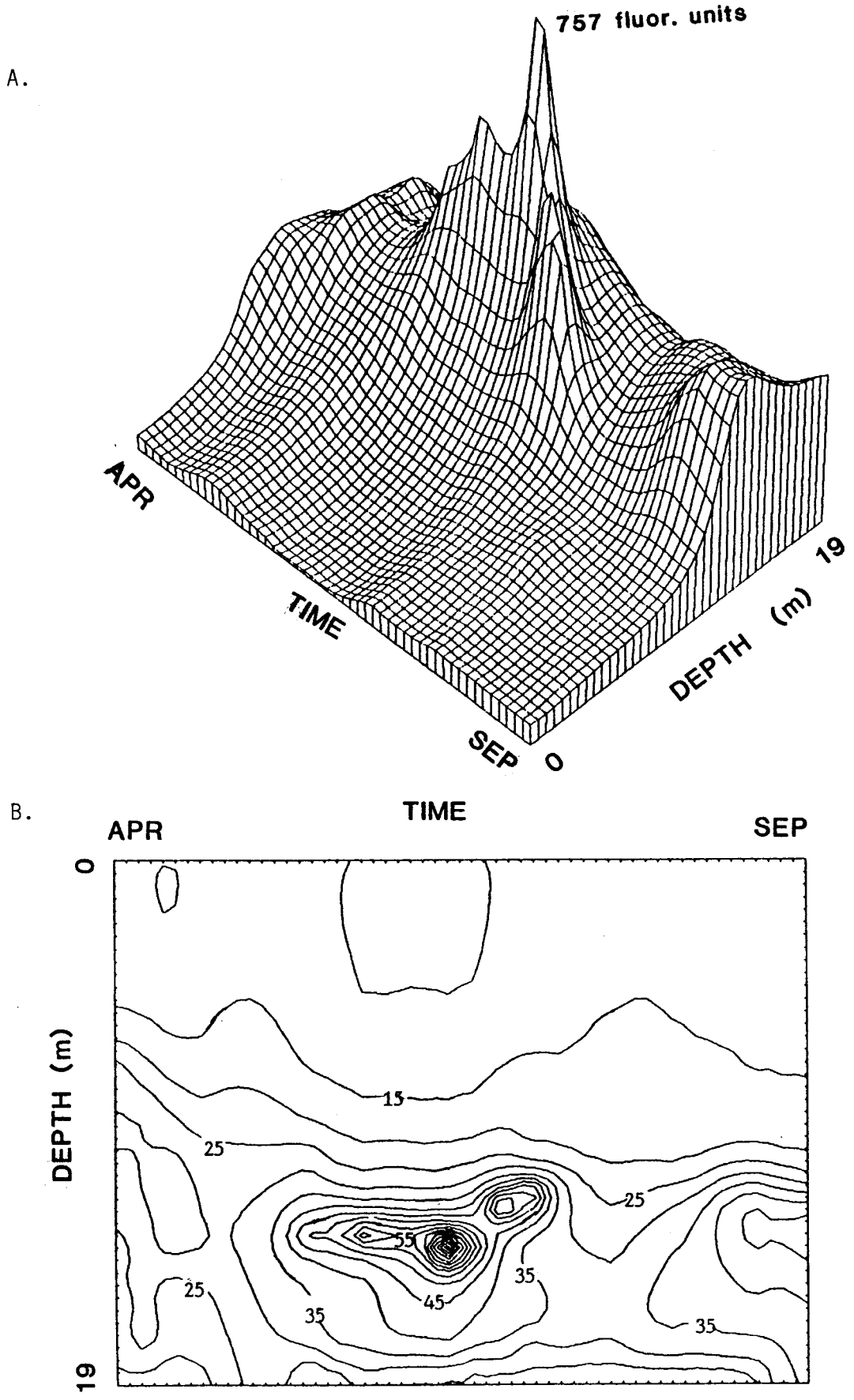

Figure 9. Three dimensional surface plot (A.) and isopleth plot (B.) of the distribution of <u>in vivo</u> fluorescence over time and depth in Lake Annie.

D. References

The following list of references should include any additional information needed to employ the IVF method for most applications.

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