EVALUATION OF THE CHLOROPHYLL/FLUORESCENCE SENSOR OF THE YSI MULTIPROBE: COMPARISON TO AN ACETONE EXTRACTION PROCEDURE

Patricia Lambert, B.S., M.S.

Thesis Prepared for the Degree of

MASTER OF SCIENCE

UNIVERSITY OF NORTH TEXAS

May 2001

APPROVED:

William T. Waller, Major Professor
Robert Doyle, Committee Member
Miguel Acevedo, Committee Member
Earl Zimmerman, Chair of the Department of Biological Sciences
C. Neal Tate, Dean of the Robert B. Toulouse School of Graduate Studies

Lambert, Patricia, Evaluation of the Chlorophyll/Fluorescence Sensor of the YSI Multiprobe: Comparison to an Acetone Extraction Procedure. Master of Science (Environmental Science), May 2001, 61 pages, 7 tables, 21 figures, 16 references.

The purpose of this study was to examine the suitability of the YSI model 6600 Environmental Monitoring System (multiprobe) for long term deployment at a site in Lewisville Lake, Texas. Specifically, agreement between a laboratory extraction procedure and the multiprobe chlorophyll/fluorescence readings was examined. Preliminary studies involved determining the best method for disrupting algal cells prior to analysis and examining the precision and linearity of the acetone extraction procedure. Cell disruption by mortar and pestle grinding was preferable to bath sonication. Comparison of the chlorophyll/fluorescence readings from the multiprobe and the extraction procedure indicated that they were significantly correlated but temperature dependent.

TABLE OF CONTENTS

Page

LIST OF TABLES iii

LIST OF ILLUSTRATIONS iv

Chapter

1. INTRODUCTION 1

2. ABOUT ECOPLEX/EMPACT 7
3. MATERIALS AND METHODS 9
   Laboratory Analysis of Chlorophyll 9
   Fluorescence Measurement with YSI 6025 Chlorophyll Sensor 12

4. RESULTS OF PRELIMINARY STUDIES 15
   Mortar and Pestle Grinding vs. Sonication 15
   Short Term Correlation Study 16
   Precision and Linearity 17
   Laboratory Spectrophotometric Analysis vs. YSI Chlorophyll Readings 18

5. RESULTS OF FURTHER STUDIES 21
   Calibration with a Phytoplankton Solution 21
   Calibration with Acridine Orange 22
   Comparison of the Turner Fluorometer with the YSI Multiprobe and the Acetone Extraction Procedure 23

6. DISCUSSION 24
   Sources of Error 24
   Conclusions 26

7. TABLES AND FIGURES 29

REFERENCE LIST 59

LIST OF TABLES

Table Page

1. Mortar and pestle grinding vs. bath sonication 30

2. Cook's D influence statistic on all data from Sept. 9, 1999 to Dec. 12, 1999. Dependent variable: multiprobe chlorophyll..... 36

3. Cook's D influence statistic after deletion of first high outlier. Dependent variable: multiprobe chlorophyll..... 38

4. Cook's D influence statistic after deleting second high outlier. Dependent variable: multiprobe chlorophyll..... 41

5. Data for Turner fluorometer, YSI multiprobe and acetone extraction comparison study 49

6. Comparison of Turner fluorometer raw fluorescence, YSI fluorescence and extracted chlorophyll using correlation and linear regression with no intercept..... 50

7. Data from 9/9/99 to 8/22/00..... 55

LIST OF ILLUSTRATIONS

Figure Page

1. Comparisons of bath sonication to mortar and pestle for cell disruption..... 31
2. YSI chlorophyll vs. extracted chlorophyll on Lake Lewisville. 32
3. Typical dilution study..... 33
4. Comparison of standard solution of Chlorophyll *a* with spectrophometric readings.34
5. Extracted chlorophyll vs. YSI chlorophyll 9-9-99 to 12-29-99 35
6. Cook's D influence statistic for data from 9-9-99 to 12-29-99..... 37
7. Cook's D for 9-9-99 to 12-29-99, first high outlier deleted..... 39
8. Extracted chlorophyll vs. YSI chlorophyll, calibration by zeroing fluorescence 40
9. Cook's D influence statistic after deleting second high outlier. 42
10. Predicted values of YSI chlorophyll with upper and lower 95% confidence limits . 43
11. Plot of residuals vs extracted chlorophyll 44
12. Multiprobe chlorophyll vs. extracted chlorophyll 2-8-00 to 5-8-00 45
13. Percent full-scale fluorescence vs. extracted chlorophyll 2-8-00 to 5-8-00 46
14. Multiprobe chlorophyll vs. extracted chlorophyll data from 5-9-00 to 8-22-00..... 47
15. Percent full-scale fluorescence vs. extracted chlorophyll 5-9-00 to 8-22-00..... 48
16. Percent full-scale fluorescence vs. Turner fluorometer raw fluorescence 51
17. Date vs. temperature for all data 52
18. Date vs. ratio of fluorescence to extracted chlorophyll for all data 53
19. Temperature vs. ratio for all data 54

20. Extracted chlorophyll and % FS fluorescence 11-27-00..... 57

21. Dilution study 11-27-00 58

CHAPTER 1

INTRODUCTION

All green plants contain chlorophyll *a*, which constitutes approximately 1 to 2% of the dry weight of planktonic algae. The concentration of photosynthetic pigments such as chlorophyll *a* has been used extensively to estimate phytoplankton biomass (Standard Methods 1992, Axler and Owen 1994, Carpenter et al. 1998, Schloss 2000). The concentration of chlorophyll *a* has also been shown to relate to primary productivity and can be used to assess the physiological health of algae by determining the relative amount of pheophytin *a* (Axler and Owen 1994, Carpenter et al. 1998). A growing problem in recent years has been the procurement of suitable water supplies for our increasing population. In the past, a large portion of the country has relied on ground water supplies, but the water tables have been constantly lowering. More and more cities are turning to lakes, streams or reservoirs for their water supplies (Palmer 1959). This change from ground water to surface sources has created new problems for the procurement and treatment of water. Ground water, in contrast to surface water, is essentially free of organisms causing problems for water supply systems. Surface waters contain organisms capable of producing unpleasant odor and taste, clogging filters and pipes, infestations in finished waters, and toxicity (Palmer 1959). Foul-tasting extracellular metabolites from algae species present in lake water could require the installation of activated carbon filtration systems. It is also possible for the breakdown products of algae, mixed with chlorine, to produce carcinogenic trihalomethanes (Schloss

2000). Fertilizing materials such as sewage and organic waste increase the productivity of surface waters and their crops of algae and other plankton organisms, many of which produce problems when they become abundant (Palmer 1959). Pesticides from agricultural, commercial or residential areas can kill the algae-eating zooplankton, allowing algae populations to flourish (Schloss 2000). It is apparent that monitoring algal growth in lakes can be important to those involved in supplying drinking water to communities as well as to concerned consumers. Since reservoirs in our area are generally multi-use and heavily utilized for recreation, lake managers might need to predict the occurrence of algal blooms. Blue-green algae, or cyanobacteria, can create potent toxins that may be harmful to lake users. Also, algal blooms may deplete oxygen through shading and as a result of their decomposition resulting in suffocation of fish and benthic organisms (Schloss 2000).

A key characteristic of chlorophyll is that it fluoresces. When irradiated with light of a particular wavelength, it emits light of a higher wavelength (or lower energy). This characteristic of fluorescence is the basis for all commercial fluorometers capable of measuring chlorophyll *in situ.* Most chlorophyll measurement systems use a light with a peak wavelength of approximately 470 nm. On irradiation with this blue light, chlorophyll in whole cells emits light in the 650-700 nm region of the spectrum. To quantify fluorescence the system detector is a photodiode that is screened by an optical filter that restricts the detected light. The filter prevents the 470 nm exciting light from being detected when it is backscattered off particles in the water (YSI Operations Manual 1999). Although fluorescence intensity should be directly proportional to the

concentration of chlorophyll in the sample, other pigments and chlorophyll degradation products affect these measurements. Many factors may also affect the fluorescence intensity of chlorophyll such as temperature, species, diurnal cycles and nutrients (YSI Operations Manual 1999). Although all phytoplankton produce chlorophyll *a*, concentrations of chlorophyll can vary widely by species, age, temperature, depth, nutrient levels and other factors (Schloss 2000). Due to these considerations direct fluorescence *in vivo* should be compared to an accepted laboratory method such as extractive analysis using a fluorometer or spectrophotometer. These laboratory methods provide fairly controlled conditions of analysis. All *in situ* sensors operate under whole-cell, heterogeneous conditions where essentially everything that fluoresces above 630 nm when irradiated with 470 nm light will be measured. Hopefully, most of this fluorescence is due to suspended plant and algal matter and the fluorescence is due to chlorophyll. However, it is impossible to exclude interferences from other fluorescent species. Also, while most laboratory methods can differentiate between chlorophyll *a*, *b*, and *c* and pheophytin *a*, *in situ* fluorometers cannot differentiate between the different forms of chlorophyll (YSI Operations Manual 1999).

There are numerous methods in the literature for the determination of chlorophyll *a* in phytoplankton. The most common methods in use are the fluorometric, spectrophotometric and high-performance liquid chromatographic (HPLC) techniques. Early field fluorometers enabled researchers to take chlorophyll measurements directly in the water body from a boat or platform. These measurements were made difficult by the need to carry fairly heavy equipment and to pump the sample water up to the instrument

(Schloss 2000). Newer, compact data sondes with built in chlorophyll sensors and data uploading capabilities have simplified fluorometric measurements.

Although fluorometric methods have been used extensively for the quantitative analysis of chlorophyll, errors can be introduced when chlorophylls *b* and *c* and pheopigments are present. Studies have found that both over and under estimations occur with fluorometric methods with errors ranging from –68 to +53% in marine environments using HPLC as the standard method (Trees et al. 1985). The U.S. Environmental Protection agency Method 445.0 for the *in vitro* determination of chlorophyll *a* cautions that fluorescence detection may have uncorrectable interferences depending on the type of algae present. It is recommended that in cases where taxonomic classification is unavailable, a spectrophotometric or HPLC method should be used. The spectrophotometric method was chosen as the primary chlorophyll analysis method used in this study due to the availability of instrumentation and the fact that the absorption coefficient of chlorophyll *a* at 664 nm in 90% acetone has been well established. The method used is that found in Standard Methods for the Examination of Water and Wastewater (APHA 1992).

Methods of detection and quantification of chlorophyll are well established, however, there is some disagreement as to the best method of extracting the pigments from algae. Several procedures are found in the literature: measurements without disruption of the cell wall (Golterman et al. 1978); or cell wall disruption by sonication or tissue grinding (Simon and Helliwell 1998). Simon and Helliwell found that mechanical disruption was necessary to optimize pigment recovery from algal cells. The methods they examined in order of extraction efficiency were probe sonication, bath sonication,

mortar and pestle and tissue grinding when methanol was the solvent. The order of efficiency when acetone was the solvent was mortar and pestle grinding, tissue grinding, bath sonication and probe sonication. Using acetone as the solvent yielded essentially the same results as methanol when mortar and pestle were used for cell disruption (Simon and Helliwell 1998). In another study the effects of different solvents and methods of cell disruption were evaluated on laboratory cultured algae. Sartory and Grobbelaar (1984) found that 90% acetone without homogenization was the poorest extractor of algal pigments. They also concluded that homogenization did not improve pigment extraction with methanol or 95% ethanol as solvents, but did improve chlorophyll *a* extraction with 90% acetone. This same study also looked at the effect of sonication but only looked at this procedure for 95% ethanol as solvent. They found that 10 to 15 minutes sonication did not improve extraction efficiency. Marker et al., (1980) suggests that samples should be kept cool and dark and should be processed without delay. They state that freezing of samples after filtration is acceptable but the practice of adding MgCO3 to the filter before freezing should be avoided. Aggregates may be formed, retaining chlorophyll during subsequent extractions. In addition, Marker et al. (1980) found that the most commonly used extraction solvent was 90% acetone, but that extraction efficiency with this solvent may be poor. Alcohol (methanol or ethanol) was superior for many green and blue-green algae but for diatoms slightly higher extraction efficiency was achieved with acetone. Methanol was not recommended due to a suspicion of formation of altered chlorophyll products and the fact that it is more hazardous than ethanol.

For this study, the efficiencies of bath sonication to grinding with mortar and pestle were compared specifically for a site located in Lake Lewisville, Texas. It is

obvious from the literature that varying levels of extraction efficiency may be obtained due to factors such as species composition, solvent, and length of extraction time. Since bath sonication is much less time consuming and technique oriented, it would be preferable to grinding if the values obtained were found to be not significantly different with a high degree of power.

The purpose of this study was to evaluate the efficacy of the YSI 6600 Environmental Monitoring System for water quality measurements and data collection. The goal was to determine if there are significant differences between laboratory analysis of chlorophyll and multiprobe chlorophyll readings and whether or not the two can be reconciled if there are differences. The null hypothesis (Ho) is: There is no significant difference between chlorophyll values obtained by the laboratory extraction procedure and those obtained by readings from the YSI multiprobe. The primary focus is the measurement of chlorophyll *in situ* along with other water quality parameters such as temperature, turbidity, pH, dissolved oxygen and specific conductance. The instrument was also used for the *in vitro* measurement of these parameters. In this respect, serial dilutions of water samples with high chlorophyll content were analyzed to determine the linearity of the laboratory analysis and the YSI Multiprobe.

CHAPTER 2

ABOUT ECOPLEX/EMPACT

EMPACT (Environmental Monitoring for Public Access and Community Tracking) is a program funded by the U.S. Environmental Protection Agency with the goal of providing up-to-date environmental information to Americans in 86 of the largest metropolitan areas in the country by the year 2001. The city of Denton, Texas along with the University of North Texas were 1998 grant recipients and are now in the process of implementing these goals for the Denton-Dallas/Ft.Worth area. The local program is known as ECOPLEX (Environmental Conditions On-Line DFW Metroplex). Parameters now being measured at a platform located in Lake Lewisville are water quality (pH, temperature, dissolved oxygen, specific conductance), chlorophyll, and clam gape behavior (a biomonitor of pollution in the lake). Lewisville Lake was completed in 1955 and serves to fulfill a variety of needs for the surrounding communities. The lake provides a capacity of 325,700 acre-feet for floodwater storage and 436,000 acre feet of water is stored in the lake for municipal and industrial purposes. Recreational activities such as fishing, boating, and water skiing are also provided by the lake (ECOPLEX 2000). The ECOPLEX web site (www.ecoplex.unt.edu) displays environmental information in real time and offers links to environmental news as well as Metroplex news. In the future weather data (temperature, relative humidity, wind speed and

direction, solar radiation, rainfall and barometric pressure) from a weather station located on shore near the platform will also be displayed in real-time. Also available on-line are ozone concentrations, ultraviolet light levels, and air clarity monitored at the Environmental Education, Science and Technology building on the University of North Texas campus.

CHAPTER 3

MATERIALS AND METHODS

1. Laboratory analysis of chlorophyll

The basic method for chlorophyll analysis was that found in Standard Methods for the Examination of Water and Wastewater (18th edition, 1992) for spectrophotometric determination (APHA 1992).

a. Sampling – Samples were taken from the Lake Lewisville ECOPLEX platform using a Kemmerer sampler at a depth of 1 m. Samples were taken in triplicate and collected in plastic screw cap containers. Approximately 2 liters of water were collected for each sample. The sampler was placed in the water separately for each sample.

b. Filtration – Water samples were taken immediately to the lab and 500 ml of the sample was filtered using vacuum filtration with Gelman glass microfiber filters type A/E (47 mm). The volume of sample filtered varied with the concentration of particulates in the water and the actual volume filtered was recorded for each sample. All samples were filtered on the same day as collection except for samples taken on 9/29/99 which were held in the refrigerator at 4°C overnight.

c. Cell disruption -

1) Grinding with mortar and pestle – Filters were placed in the mortar and 2 ml of 90% acetone, 10% saturated magnesium carbonate were added and the filters were macerated for approximately 1 minute. A 1% magnesium carbonate suspension was prepared and used to make the saturated magnesium carbonate solution used in preparation of the 90% acetone solution. Another 2 ml of 90% acetone were added and the sample was transferred to a 50 ml conical bottom screw cap tube and the mortar and pestle were rinsed with another 6 ml solvent and this was transferred to the same tube. Samples were adjusted to 10 ml using the volume marks on the screw cap tubes.

2) Bath sonication – Filters were placed in 50 ml tubes and 10 ml 90% acetone buffered with saturated magnesium carbonate were added to the tube. Tubes were placed in the bath sonicator (VWR Scientific, Model P250T) in a water-ice mixture and sonicated for 10 minutes.

d. Extraction – Sample tubes were placed in a walk-in refrigerator at a temperature of 4°C for 24 hr. plus or minus 6 hr. in the dark.

Note: Although Standard Methods specifies that samples should be steeped for at least 2 hours, studies presented in EPA Method 445.0 indicate that precision was better for samples extracted for 24 hours.

e. Clarification – Sample tubes were centrifuged at full speed (approximately 3000 rpm) for 20 minutes in an IEC Model HN table top centrifuge. The clear

extract was decanted into clean 50 ml tubes and analyzed spectrophotometrically.

f. Spectrophotometric analysis – Samples were analyzed on a Beckman DU 64 Spectrophotometer with a wavelength accuracy of +/- 2 nm. Three milliliters of extract were transferred to a Quartz cuvette with a 1 cm light path and readings were taken at 750 nm and 664 nm, then extracts were acidified by adding 0.1 ml of 0.1 N HCl to the 3 ml of extract in the cuvette. This was mixed well and allowed to stand for 90 seconds to allow chlorophyll a to be converted to pheophytin a. At this time the absorbance was taken at 665 nm and again at 750 nm. If the solution is pure chlorophyll a, the ratio OD664/OD665 is 1.70. If the ratio is near this number, then the specimen contains little or no pheophytin a. Mixtures of chlorophyll a and pheophytin a should have absorption peak ratios between 1.0 and 1.7 depending on the condition of the sample. 664b/665a ratios were calculated for each sample. The OD750 reading is a correction for turbidity. The OD750b readings were subtracted from the OD664b readings and the OD750a readings were subtracted from the OD665a readings. For accurate analysis, volumes of samples and/or extraction solvent were varied to keep the OD664 readings between 0.1 and 1.0.

g. Calculation of chlorophyll a concentration –

The first reading at 750 nm was subtracted from the reading at 664 nm and the second reading at 750 nm was subtracted from the 665 nm reading to give 664b and 665a for the following formula.

$$\text{Chlorophyll a (mg/m3)} = \frac{26.7\,(664b-665a)\,V1}{V2\ (L)}$$

V1 = volume of extract in liters

V2 = volume of sample in m3

L = width of cuvette in cm

664b/665a = optical density of 90% acetone extract before and after acidification.

26.7 = absorbance correction

2. Fluorescence measurement with YSI 6025 chlorophyll sensor

a. Deployment of multiprobes – The YSI multiprobes were deployed at the Lake Lewisville ECOPLEX water quality monitoring platform. Multiprobe A was positioned at a depth of 1 meter and multiprobe B was positioned at approximately 1 ft. from the bottom. All chlorophyll comparison data were taken from the multiprobe at 1 meter.

b. Calibration of multiprobes – Multiprobes were initially calibrated while still attached to the platform. However, due to the difficulty of properly cleaning the multiprobes and performing routine maintenance and troubleshooting, they were detached and brought into the lab for these procedures after preliminary studies were complete. There are three methods of chlorophyll sensor calibration described in the Operations Manual.

1) Calibration by zeroing fluorescence – This option is used to zero the sensor in a medium that is chlorophyll-free such as de-ionized water. This

procedure will zero your fluorescence sensor and use the default sensitivity for calculation of chlorophyll concentration in ug/L.

2) Two-point calibration with a phytoplankton suspension of known concentration – In this case the zero standard must be de-ionized water and the second standard is a water sample from the area of interest which has been previously analyzed for chlorophyll a by the acetone extraction procedure. During calibration the software will prompt you to enter the concentration of the second standard. At this point the sonde should be immersed in the water sample and the concentration derived from the previous analysis entered.

3) Two-point calibration with acridine orange solution – This calibration procedure requires first preparing a stock solution of the fluorescent dye, acridine orange (100 mg/L). This solution is stable if stored in a dark bottle at 4°C. A working standard is prepared from this stock solution immediately before calibration and should be discarded after calibration is complete. A good estimate of sensor stability can be determined by comparing fluorescence readings from the previous calibration to present fluorescence readings. This solution is also easier to prepare and is more stable than the phytoplankton solution. Due to the above considerations this calibration procedure is the method that I would recommend.

Note: More complete and detailed instructions for sensor calibration are available in the YSI Operations Manual (1999).

c. Data acquisition – Data were uploaded from the multiprobes to a laptop computer and then copied to disk during the preliminary phase. Starting in January 2000 data were available on-line at the ECOPLEX website.

CHAPTER 4

RESULTS OF PRELIMINARY STUDIES

Comparison of Mortar and Pestle Grinding and Bath Sonication

A total of 22 observations were taken with 3 replicates for each procedure for each observation (Table 1). Three of these observations were from water samples taken from the Eagle Point golf course duck pond and one was from the ODELA (Out-Door Environmental Learning Area) pond. Of the remaining 18, four were from the Lake Lewisville shoreline and 14 were from the platform itself. The means of each group of three were used in the calculations. The data for mortar and pestle grinding versus sonication were subjected to the matched-pairs t test. The differences between the two methods were found to be normally distributed by the Shapiro-Wilk normality test ($p = 0.0821$). A difference of 4.31 ug/L was found between the two means and this was found to be statistically significant ($t = 4.18$, $p = 0.0004$). In order to determine the smallest difference (δ) detectable by the matched-pairs t test with $1-\beta$ power, at the α level of significance, using a sample size n, the formula found in Zar (1999) was used:

$$\delta = (s^2/n)^{1/2} (t_{\alpha,\nu} + t_{\beta(1),\nu})$$

Where $\beta = 0.10$, $1-\beta = 0.90$, $\nu = n-1$ and $\alpha = 0.05$

The minimum detectable difference was found to be 3.51 ug/L and the power of the test to detect a difference of this amount was 0.90 since the power of the test is 1-β (Zar 1999). In this case we would be able to detect this difference 90% of the time. The mean for mortar and pestle grinding was 42.48 ug/L and for bath sonication was 37.93 ug/L. Results of simple linear regression analysis with sonication as the independent variable indicated that mortar and pestle (ground) chlorophyll values and sonicated chlorophyll values are significantly related ($p<0.0001$, $r^2 = 0.95$) and expressed by the following model: mortar and pestle chlorophyll = 0.97(sonicated chlorophyll) + 5.32ug/L. For this study, mortar and pestle grinding was the chosen method due to the better yield, although sonication could be employed and approximate ground chlorophyll values calculated with the linear regression equation.

To further test whether a significant relationship exists between the two data sets a Pearson Product Moment correlation analysis was done. The correlation coefficient (r) was 0.9784 with p=0.0001. Thus, a highly significant correlation exists between chlorophyll *a* values obtained by grinding and chlorophyll a values obtained by sonication. These results are presented in Figure 1. It would seem that in cases where simplicity and speed are desired over a high rate of recovery, sonication could be a viable alternative to mortar and pestle grinding.

Short Term Correlation Study

To determine the degree of correlation between extracted chlorophyll *a* and YSI chlorophyll that could be obtained within a short period of time (approximately 4 hr.) but taken at different sites around the lake, samples were collected at five different sites around Lewisville Lake on April 9, 2000. Samples were obtained in triplicate from a

depth of 1 meter. Sites included those in coves, near the dam and in the middle of the central body of the lake. Simple linear regression with extracted chlorophyll as the independent variable yielded the equation, YSI Chlorophyll = 1.36(Extracted chlorophyll) + 0.66ug/L (p=0.0104) with an R-square of 0.9173. For this study, the YSI fluorescence probe was calibrated the previous day using a water sample of known concentration as the second standard. The correlation coefficient was 0.96 (p=0.0104) for extracted chlorophyll a and multiprobe chlorophyll. Figure 2 is a plot of extracted chlorophyll *a* and multiprobe chlorophyll by site number.

Precision and Linearity

To test the precision and linearity of the assay, studies were done with samples containing high concentrations of chlorophyll a. A sample was taken from ODELA pond and 7 replicates of 500 ml each were filtered and analyzed using mortar and pestle to disrupt cells. A mean of 22.73 ug/L chlorophyll a was obtained with an S.D. of 1.68 ug/L and C.V. of 7.4%. Samples with high chlorophyll *a* concentrations were diluted to yield solutions of 90% to 30% of original concentration. Values of percent full-scale fluorescence (%FS) versus extracted chlorophyll yielded correlation coefficients of 0.92 to 0.95. An example of a dilution study done on 2-10-00 is shown in Figure 3.

To determine the relative accuracy of the spectrophotometric analysis of samples, a commercial chlorophyll *a* standard was obtained. Dilutions of the standard were made to yield concentrations of 25, 20, 15, 10, 5, and 1 ug/ml. These samples were read on the spectrophotometer and chlorophyll *a* concentrations calculated. Calculated values were 20.26, 15.83, 13.54, 9.02, 4.54 and 0.88 ug/ml to yield a correlation coefficient of 0.99. Readings of higher concentrations were not linear and it was determined that absorbance

readings greater than approximately 1.9 should be avoided. Figure 4 is a plot of theoretical values of the chlorophyll *a* standard solution versus the calculated value from the spectrophometric readings.

Laboratory Spectrophotometric Analysis vs. YSI Chlorophyll Readings

Initial data collection from the YSI multiprobes have shown periods of extreme fluorescence spiking which clearly doesn't represent the actual amount of chlorophyll in the water. This and other factors have led to poor correlation between laboratory analysis and multiprobe readings. A unique feature of the YSI 6600 multiprobe is the addition of small wiper pads over the optical surface of the chlorophyll and turbidity probes. These wipers are activated automatically during unattended sampling operations. One factor leading to erratic chlorophyll readings was that strands of filamentous algae could be caught on the probe wiper arms leading to spiking of readings. Also, the wiper may become worn and not function properly. Another factor to consider is, of course, calibration. Calibration, preferably 2-point, is required at one to two week intervals. A Pearson Product Moment correlation analysis was performed between extracted chlorophyll and YSI chlorophyll on the data collected at the Lake Lewisville site from 9-9-99 to 12-29-99. Ignoring three of the values where obvious spiking occurred in the chlorophyll/fluorescence readings, the correlation coefficient was 0.52 for 11 observations. The plot of YSI chlorophyll versus extracted chlorophyll contains two points, which appear to be outliers (Figure 5). A Cook's D statistical assessment was performed to determine if these Y values were unusual with respect to the model determined by all the other observations, except the unusual Y values. The YSI chlorophyll reading of 198.6 had a Cook's D value of 0.943 indicating a probable outlier

(Table 2, Figure 6). After this point was removed, the correlation coefficient was 0.61. After deleting this value, Cook's D was run again and the value of 81.1 had a Cook's D value of 1.761, again indicating an outlier (Table 3, Figure 7). After deleting these two observations, the correlation coefficient was 0.79 (Figure 8) and no value had Cook's D greater than 0.417 (Table 4, Figure 9). The next highest YSI chlorophyll value was 24.94. When this value was deleted, no further improvement was obtained in the correlation coefficient. From Figure 8, it appears that extracted chlorophyll values are approximately twice as high as YSI values. This is due primarily to the calibration procedure employed. Probes were calibrated by simply zeroing the fluorescence reading using de-ionized water as a zero standard. This calibration method involves setting zero fluorescence with de-ionized water and using the default sensitivity for calculation of chlorophyll concentration in ug/L.

In an effort to generate a mathematical model of the relationship between extracted chlorophyll values and multiprobe values, linear regression analysis was performed using the 'no intercept' option. This option forces the regression line to pass through zero. Results from this analysis are shown in Figure 8. With extracted chlorophyll as the independent variable and YSI chlorophyll as the dependent variable, R-square was 0.9811 with a slope of 0.49. Thus, the model is YSI chlorophyll = 0.49 (extracted chlorophyll). If the "no intercept" option is not used, R-square is 0.6286 with a slope of 0.44 and a Y-intercept of 1.94 ug/L. Figure 10 shows predicted YSI chlorophyll and upper and lower 95% confidence limits versus extracted chlorophyll a based on data for extracted chlorophyll and multiprobe chlorophyll shown in Table 4. Figure 11 is a plot of residuals versus extracted chlorophyll. A significant difference was found between the

observed distribution of residuals and a normal distribution (Shapiro-Wilk test for normality, $W = 0.82$, $p = 0.036$).

CHAPTER 5

RESULTS OF FURTHER STUDIES

Calibration with a Phytoplankton Suspension

In order to correct the problem of erratic spiking of the chlorophyll sensor, multiprobes were cleaned and calibrated at least bi-weekly when possible. Fluorescence and chlorophyll readings were monitored on the ECOPLEX website in order to detect problems. Multiprobes were brought into the lab for calibration and maintenance when required. The Operations Manual offers two options for probe calibration: simply zeroing the generic fluorescence parameter or performing a 1, 2 or 3-point calibration in ug/L chlorophyll. Two formulations of standards may be used for the second option. The most accurate is a phytoplankton suspension whose chlorophyll content has been determined by the extractive analysis procedure described in Standard Methods. The second and easier is to prepare and use a very dilute solution of the fluorescent dye, acridine orange.

During the period from Feb. 8, 2000 to May 8, 2000, multiprobes were calibrated with a phytoplankton suspension taken from Lake Lewisville, which had been previously assayed by the spectrophotometric method. Results of linear regression analysis with no intercept yielded an R-square of 0.9579 and a slope of 1.43 ($p<0.0001$) when extracted chlorophyll is the independent variable and multiprobe chlorophyll is the dependent variable. Correlation analysis yielded a correlation coefficient of 0.74 ($p<0.0001$), Pearson Product Moment). If the no intercept option is not used, R-square is 0.5464 with

a slope of 1.08 and an intercept of 8.48 ug/L. A scatterplot of extracted chlorophyll *a* vs. YSI chlorophyll with the no intercept option is shown in Figure 12.

When extracted chlorophyll is compared to fluorescence with extracted chlorophyll again as the independent variable, linear regression with no intercept yielded an R-square of 0.9358 with a slope of 0.16 ($p<0.0001$). If the no intercept option is not used, the R-square is 0.4206 with a slope of 0.12 and an intercept of 0.98. Correlation analysis yielded a correlation coefficient of 0.65 ($p=0.0003$). A scatterplot of extracted chlorophyll vs. percent full-scale fluorescence is shown in Figure 13.

Calibration with Acridine Orange

During the period of May 9, 2000 to August 22, 2000, multiprobes were calibrated with a 0.2 mg/L acridine orange solution. Results of linear regression with no intercept yielded an R-square of 0.9443 ($p<0.0001$) with a slope of 0.49 when extracted chlorophyll was the independent variable and multiprobe chlorophyll was the dependent variable. If the no intercept option is not used the R-square is 0.4712 with a slope of 0.41 and an intercept of 2.46 ug/L. The correlation coefficient in this case is 0.69 ($p=0.0002$). A plot of extracted chlorophyll vs. multiprobe chlorophyll is shown in Figure 14.

When extracted chlorophyll was compared to fluorescence and the no intercept option used for linear regression, R-square was 0.9288 with a slope of 0.12 (Figure 15). If the no intercept option is not used, the R-square is 0.3954, with an intercept of 0.69 and a slope of 0.10. Correlation analysis yielded a correlation coefficient of 0.63 ($p=0.0008$).

Comparison of the Turner Fluorometer with the YSI Multiprobe and the Acetone Extraction Procedure

To further assess the performance of the multiprobe, a comparison was done with the Turner Fluorometer. Percent full-scale fluorescence readings taken in the lake by the multiprobe were compared to raw fluorescence readings taken in the lab with the Turner Fluorometer. The Turner Fluorometer readings were also compared to the acetone extraction values. Table 5 and 6 list the results of correlation analysis with the above variables. Figure 16 compares percent full-scale fluorescence and Turner fluorometer raw fluorescence readings. When linear regression with no intercept was performed on the above data, the model was Turner raw fluorescence = 0.39(YSI fluorescence) with $p=<0.0001$ and an R-square of 0.9667. If the no intercept option was not used the model was Turner raw fluorescence = 0.16(YSI fluorescence) + 0.79 ($p=0.0139$) with an R-square of 0.4085. It appears that the YSI multiprobe readings correlate well with the Turner Fluorometer readings considering the difference in instrumentation and ambient conditions ($r = 0.6392$, $p = 0.0139$).

CHAPTER 6

DISCUSSION

Sources of Error

Obviously, there is a less than perfect correlation between the laboratory acetone extraction procedure and the multiprobe readings for chlorophyll or fluorescence. Other researchers have found this to be the case, in general, for fluorometric measurements of chlorophyll, since there are potentially multiple sources of error (YSI Operations Manual 1999, Trees, et al. 1985, Arar and Collins 1997, Schloss 2000).

As far as the acetone extraction procedure, error can be introduced in several ways. Variation in pressure used to grind the filter and length of time of grinding could cause variability. Also, all spectrophotometers may not have the same extinction coefficient for chlorophyll (Lee, et al.1995). When a commercial preparation of chlorophyll *a* was analyzed by this procedure, actual values differed from theoretical values by approximately 10% for absorption readings of 1.25 or less. Since none of the analyzed samples had absorption readings greater than 1.0, this would be the maximum error for the spectrophotometer. In addition to chlorophyll *a*, diatoms and dinoflagellates contain chlorophyll *c* and green algae contain chlorophyll *b*. Some spectrophotometric interference will occur when these pigments are present in significant amounts (Marker et al. 1980). Some researchers believe, however, that neither chlorophyll *b* nor chlorophyll *c* occur in sufficient concentrations to affect the determination of chlorophyll *a* in natural populations of phytoplankton (Marker, et al. 1980).

When considering fluorescence readings from the multiprobe, several sources of error can be identified. Variable amounts of inorganic turbidity in the water column can contribute to light scattering and absorption (Lee, et al. 1995). Although the YSI Manual states that turbidity can be a problem, it recommends a turbidity interference factor of only 0.03 ug/L chlorophyll per NTU. The highest turbidity recorded during the study period was 59.5 NTU, which would have introduced an error of 1.8 ug/L chlorophyll. Also, fluorescence of phytoplankton can show significant temperature dependence (YSI Operations Manual 1999, Arar and Collins, 1997). Although the 6600 multiprobe has a temperature compensation routine in the software, YSI representatives recommended that it not be employed (personal communication, Randy Rushin, YSI Incorporated, unreferenced). One problem in using temperature compensation is that each species of phytoplankton is likely to be unique with regard to the temperature dependence of its fluorescence (YSI Operations Manual 1999). Another potential source of error is the variation among probes. When zeroing fluorescence is the calibration method, the default sensitivity is used for calculation of chlorophyll in ug/L. The manual states that the default sensitivity is within 25% for any probe, thus probes could vary as much as 25% when this calibration method is used. Although in vivo fluorescence has often been used to estimate chlorophyll concentration and thus primary productivity, it is not a conservative property of chlorophyll *a*. Fluorescence is a physiological variable that is strongly affected by variations of the photosynthetic characteristic of phytoplankton (Nieke et al. 1997).

Conclusions

Two methods of algal cell disruption were examined and the method involving mortar and pestle grinding was found to be superior to bath sonication. Bath sonication could be used for cell disruption but with a slight loss of recovery.

The primary purpose of the study was to determine the relationship of the YSI 6600 multiprobe chlorophyll sensor readings to the laboratory acetone extraction procedure. This study seems to bear out the observations of others with regards to temperature dependence of fluorescence. Figure 17 is a plot of date versus temperature and Figure 18 is a plot of date versus the ratio of fluorescence to extracted chlorophyll *a*. These data indicate that ratios of fluorescence to extracted chlorophyll *a* are much higher in winter when water temperature is lower. Figure 19 illustrates the relationship between the ratio of fluorescence to extracted chlorophyll *a* compared to temperature. There is significant negative correlation between these two factors ($r=-0.6132$, $p<0.0001$). Table 7 lists the data used in these observations. Other factors discussed above also contributed to the variation between extracted chlorophyll *a* and values of fluorescence and chlorophyll from the YSI 6600 multiprobe. If linear regression was applied to the data and the line of best fit forced through the origin, the regression coefficients were very good. When this option is used and the intercept is forced through zero, the regression line is heavily influenced by the zero point and the values at higher concentrations. There is a great dichotomy between the results obtained when percent fluorescence or YSI chlorophyll is regressed against extracted chlorophyll and the regression line is forced through 0,0 and when it is not. In every case the regression fit is much better when the line is forced through zero. By using the “no intercept” option and forcing the regression

line through 0,0 the resulting R-square is higher, with the data behaving as two points. Had there been data collected with lower concentrations of chlorophyll, R-square would have increased but possibly not to the values obtained with the "no intercept" option. When the data collected from the field are examined there are few values below about 10 ug/L of chlorophyll *a*, therefore the database for low values of extracted chlorophyll *a* and percent fluorescence or YSI chlorophyll is limited. The question to be answered is; what does the relationship between extracted chlorophyll and percent fluorescence or YSI chlorophyll look like at low concentrations of chlorophyll? Do these data suggest that it is reasonable to assume a linear relationship and for the intercept to be zero? To investigate this relationship, a sample of water from the golf course duck pond was taken and a dilution series of 5, 7, 15, 20, 25, 40, 50, 75, and 100% was prepared. Extractions for each level were performed in duplicate. The results of this study are shown in Figure 20 which shows percent fluorescence graphed against extracted chlorophyll *a*. In addition to analyzing the dilution series with the YSI multiprobe and the spectrophotometric method, dilutions were also analyzed with the Turner Fluorometer. Figure 21 shows all data points for all methods graphed against percent solution.

These data, while limited, suggest that the relationship between percent fluorescence or YSI chlorophyll and extracted chlorophyll is linear and that regressions for which a 0,0 intercept is used are probably reasonable expressions of this relationship. Additional samples and dilution series should be analyzed to increase the confidence level that this indeed is the appropriate relationship.

Results of these studies indicate that it should be possible to monitor chlorophyll concentrations and estimate primary productivity using the YSI 6600 multiprobe in situ for extended periods of time. Several caveats should be considered when evaluating multiprobe results. Readings for all parameters should be monitored closely and probes cleaned and calibrated whenever readings show abnormal fluctuations. The calibration method found to be most efficient in this study was the acridine orange method. It should also be pointed out that the results derived from the Lake Lewisville platform cannot be extrapolated to other aquatic systems or even to other locations on the lake due to the variations in environments.

As far as calibration is concerned, no one method offered a distinct advantage and the choice is dependent on considerations of convenience and reproducibility. The acridine orange standard has the advantage of being easy to prepare and can be used to check the reproducibility and stability of probes. Since individual probes can vary considerably in the response of the chlorophyll sensor, this would be an important consideration. As far as possible, the same probe should be deployed at the study site to reduce variability. The tremendous amount of data that can be collected by the multiprobes deployed at the ECOPLEX site and displayed on-line can expand our knowledge of conditions on Lake Lewisville and perhaps improve lake management in the future.

TABLES AND FIGURES

Table 1. Mortar and pestle grinding vs. bath sonication for cell disruption

		Mortar and pestle					Bath sonication				
Date	Site	Chl.a mean	Rep1	Rep 2	Rep3	C.V.	Chl.a mean	Rep 1	Rep 2	Rep 3	C.V.
9/9/99	LL	31.59	30.97	32.21		2.7	26.7	27.76	25.63		5.6
9/14/99	GC	101.74	96.67	106.80		7.0	105.20	105.73	104.65		0.7
9/15/99	LL	37.98	37.75	38.2		0.8	33.07	29.58	36.56		14.9
9/21/99	GC	80.91	77.57	84.25		5.8	86.45	89.00	83.91		4.2
9/21/99	OD	3.47	3.47	3.47		0	2.14	1.34	2.94		52.8
9/22/99	LL	31.60	31.15	32.04		2.0	28.48	28.03	28.93		2.2
9/28/99	GC	93.55	89.44	97.65		6.2	76.29	76.29	76.29		0
9/29/99	LL	46.76	48.23	45.28		4.5	33.96	34.60	33.32		2.7
10/7/99	LLP	41.65	46.46	39.52	38.98	10.0	31.33	30.97	31.51	31.51	1.0
10/12/99	LLP	28.04	30.26	25.81	28.04	7.9	24.92	25.37	23.59	25.81	4.7
10/14/99	LLP	25.81	26.70	25.63	25.10	3.2	24,74	25.10	24.03	25.10	2.5
10/26/99	LLP	48.06	46.99	45.92	51.26	2.3	41.30	42.19	40.58	41.12	2.0
10/28/99	LLP	45.75	46.99	44.86	45.39	6.2	40.23	40.05	40.58	40.05	0.8
11/2/99	LLP	22.07	21.89	22.43	21.89	1.4	19.94	20.29	20.83	18.69	5.6
11/4/99	LLP	41.48	44.86	38.45	41.12	7.8	36.84	38.98	35.24	36.31	5.2
11/11/99	LLP	45.12	43.25	47.79	44.32	5.3	39.16	42.72	34.71	40.15	10.4
11/18/99	LLP	31.68	30.97	30.97	33.11	3.9	26.88	26.70	28.30	25.63	5.0
11/30/99	LLP	26.32	24.48	28.30	26.17	7.3	20.47	20.29	21.89	19.22	6.6
12/2/99	LLP	33.92	25.94	40.05	35.78	21.3	31.15	30.44	33.11	29.90	5.5
12/29/99	LLP	30.26	28.30	32.04	30.44	6.2	27.06	25.10	28.84	27.23	6.9
1/24/00	LLP	29.73	32.04	27.77	29.37	7.3	26.53	28.30	28.30	23.00	11.5
2/1/00	LLP	27.77	26.70	28.84	27.77	3.9	25.81	24.03	28.30	25.10	8.6

Chlorophyll *a* is in ug/L LL = Lake Lewisville OD = ODELA
GC = Golf Course Pond LLP = Lake Lewisville Platform

Figure 1: Comparisons of Bath Sonication to Mortar and Pestle Grinding for Cell Disruption

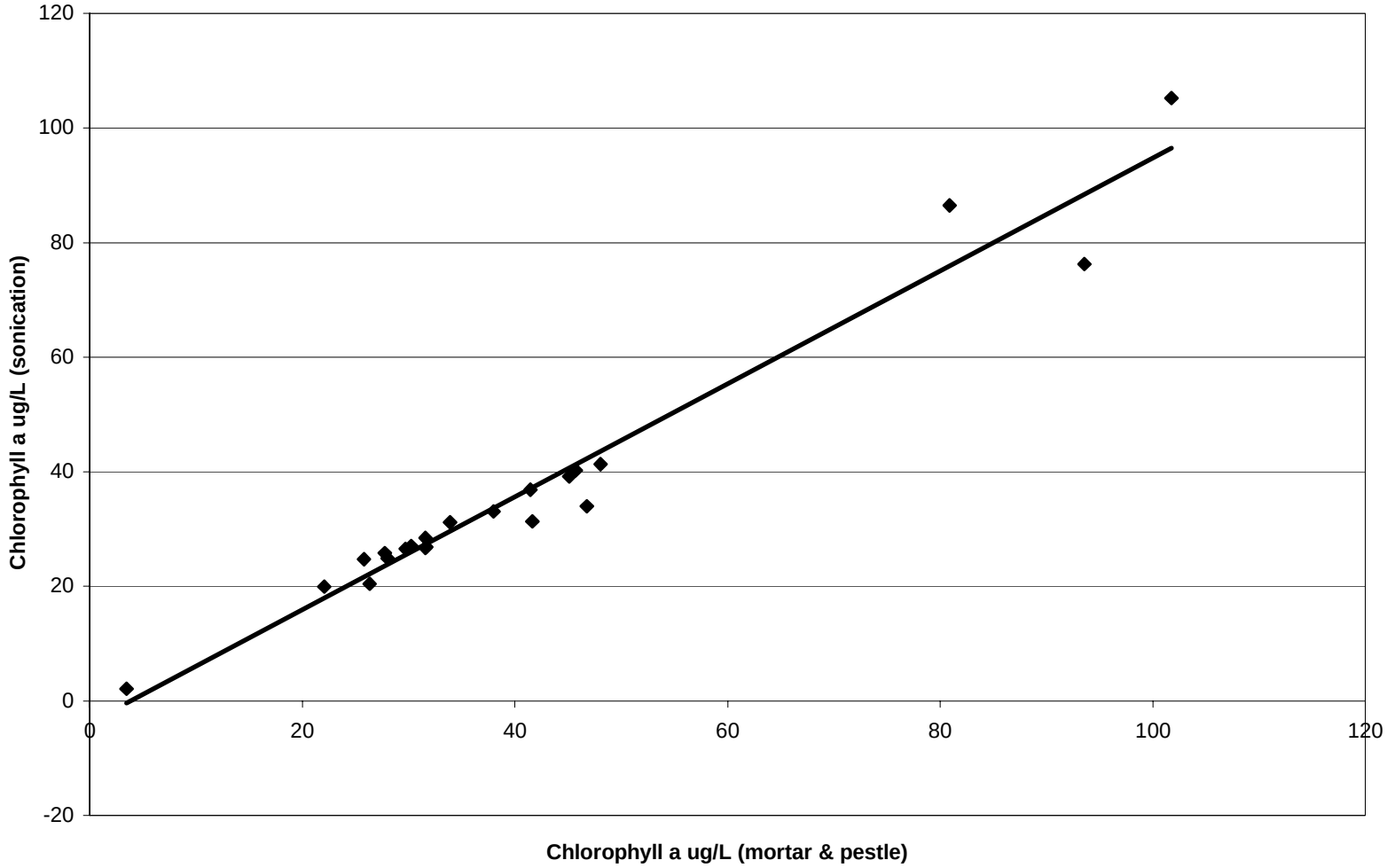


Figure 2: YSI Chlorophyll and Extracted Chlorophyll on Lake Lewisville: Short -Term Correlation Study

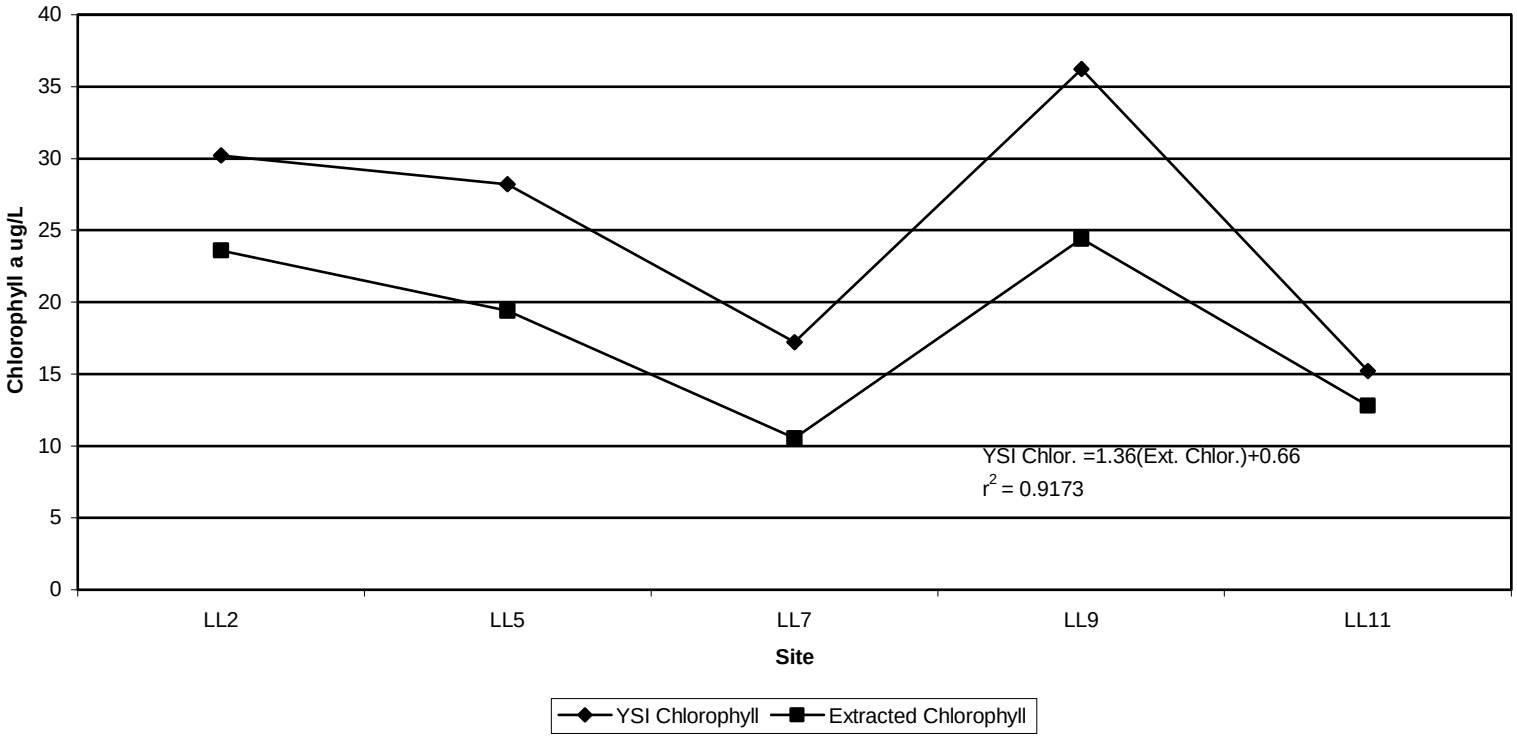


Figure 3: Typical Dilution Study

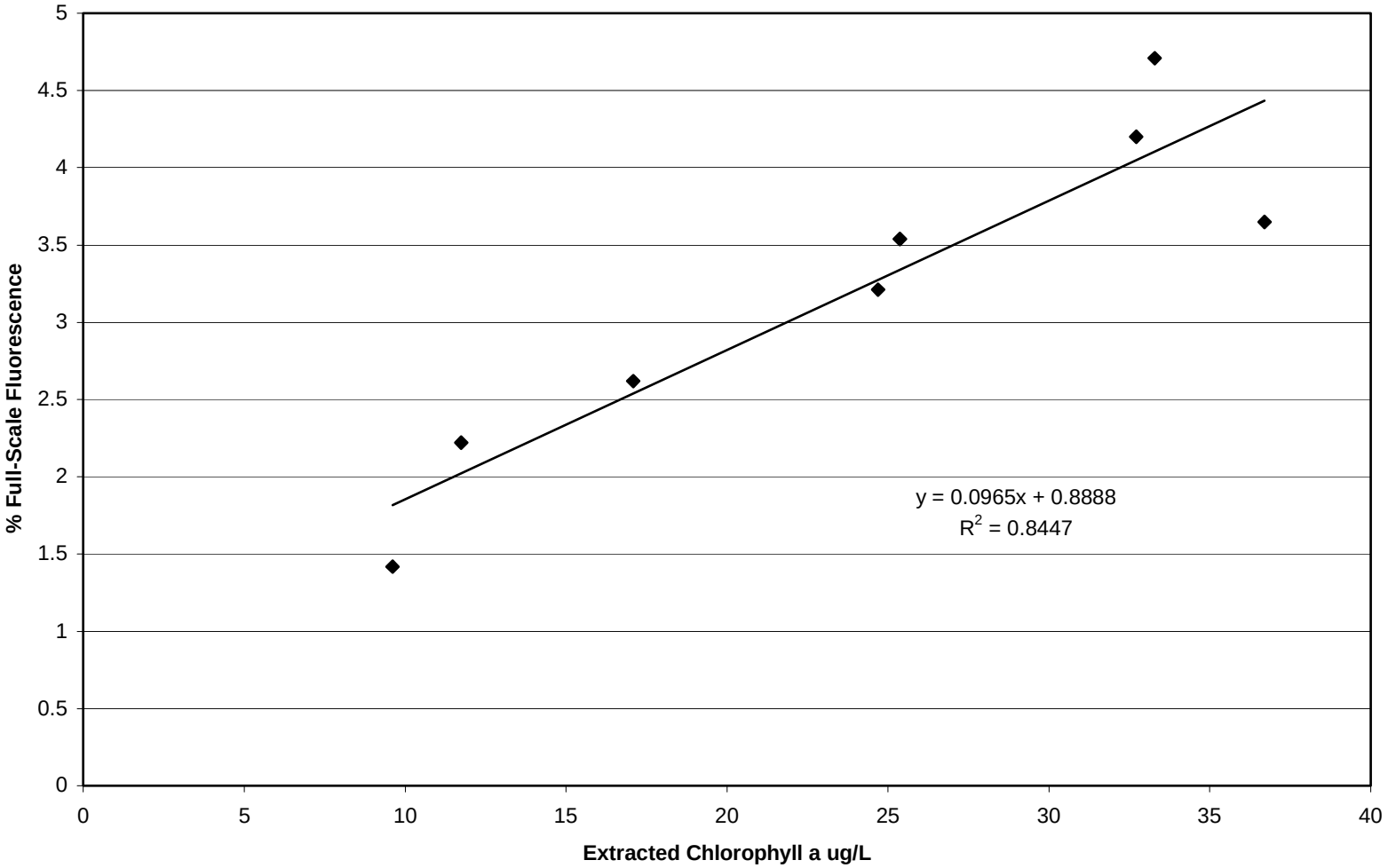


Figure 4: Comparison of a Standard Solution of Chlorophyll *a* with Spectrophotometric Readings

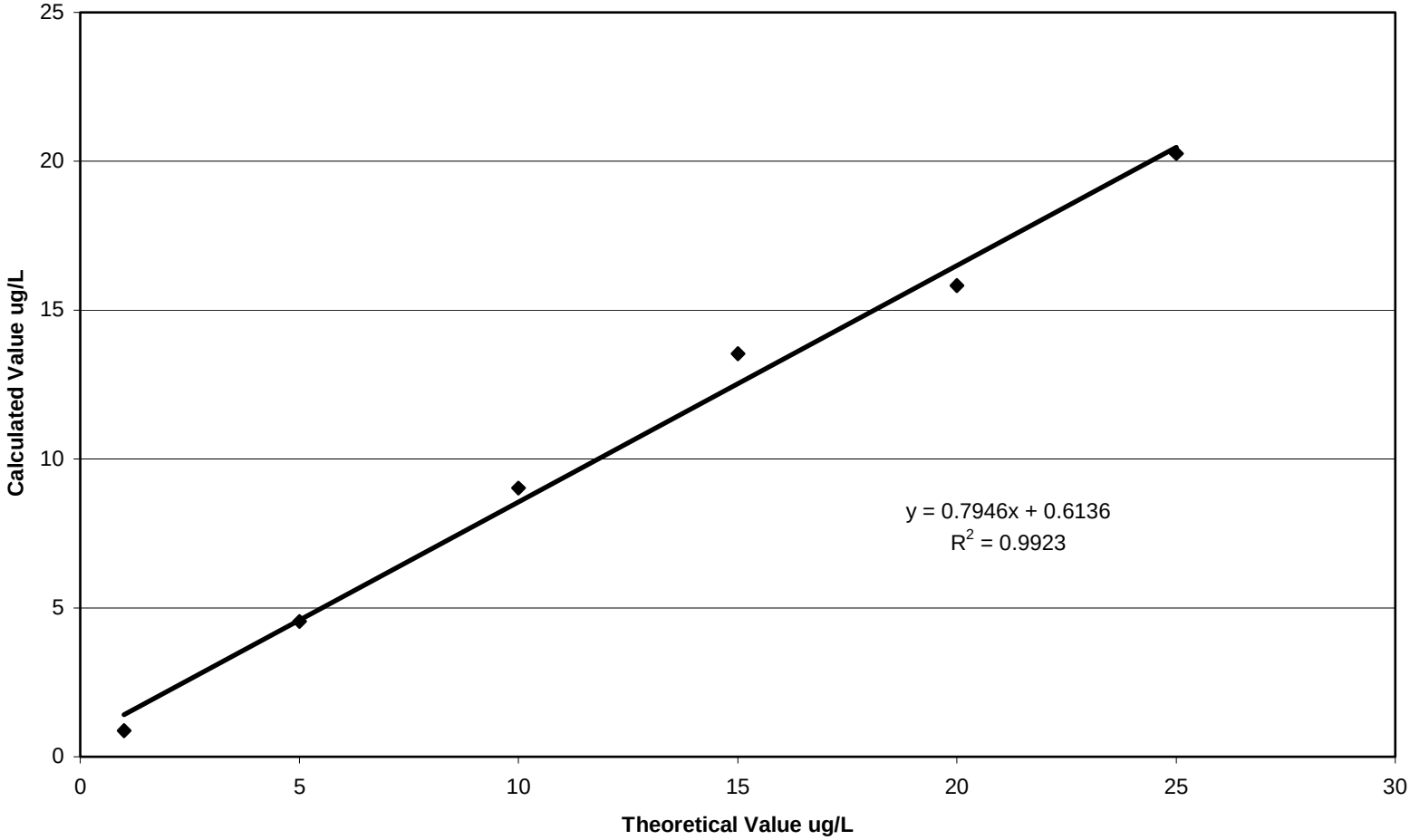


Figure 5: Extracted Chlorophyll vs. YSI Chlorophyll 9/29/99 to 12/29/99

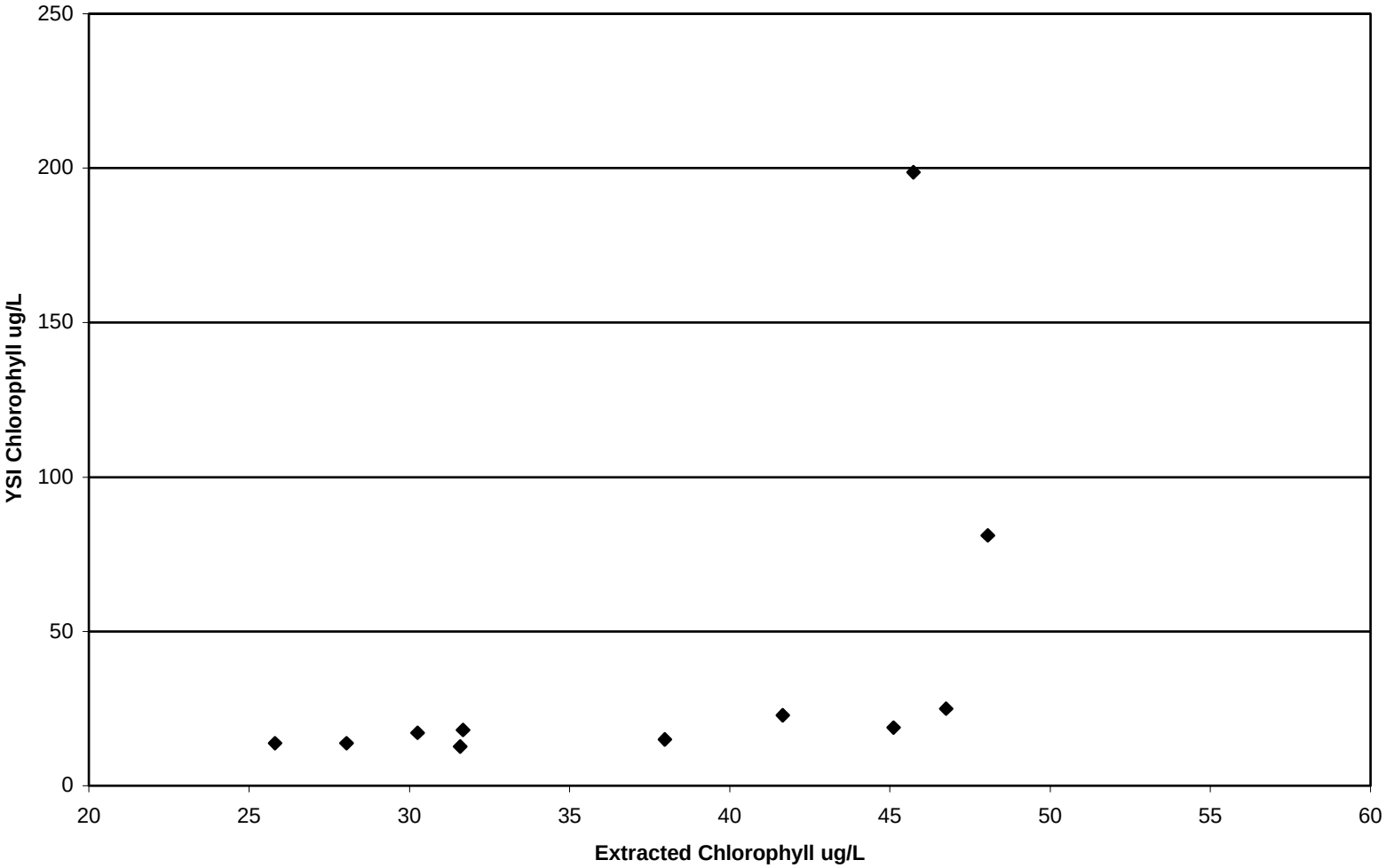


Table 2. Cook's D Influence Statistic on all data from 9-9-99 to 12-29-99. Dependent variable: multiprobe chlorophyll.

Obs. #	Date	Extracted Chlorophyll	Multiprobe Chlorophyll	Residuals	Cook's D
1	9-9-99	31.59	12.71	-6.6234	0.002
2	9-15-99	37.98	14.96	-26.3506	0.015
3	9-29-99	46.76	24.94	-46.5677	0.145
4	10-7-99	41.65	22.90	-31.0328	0.027
5	10-12-99	28.04	13.8	6.6762	0.003
6	10-14-99	25.81	13.8	14.3459	0.023
7	10-26-99	48.06	81.1	5.1212	0.002
8	10-28-99	45.74	198.6	130.6004	0.943
9	11-11-99	45.12	18.9	-46.9672	0.109
10	11-18-99	31.68	18.1	-1.5429	0.000
11	12-29-99	30.26	17.1	2.3409	0.000

Figure 6. Cook's D Influence Statistic for data from 9/9/99 to 12/29/99

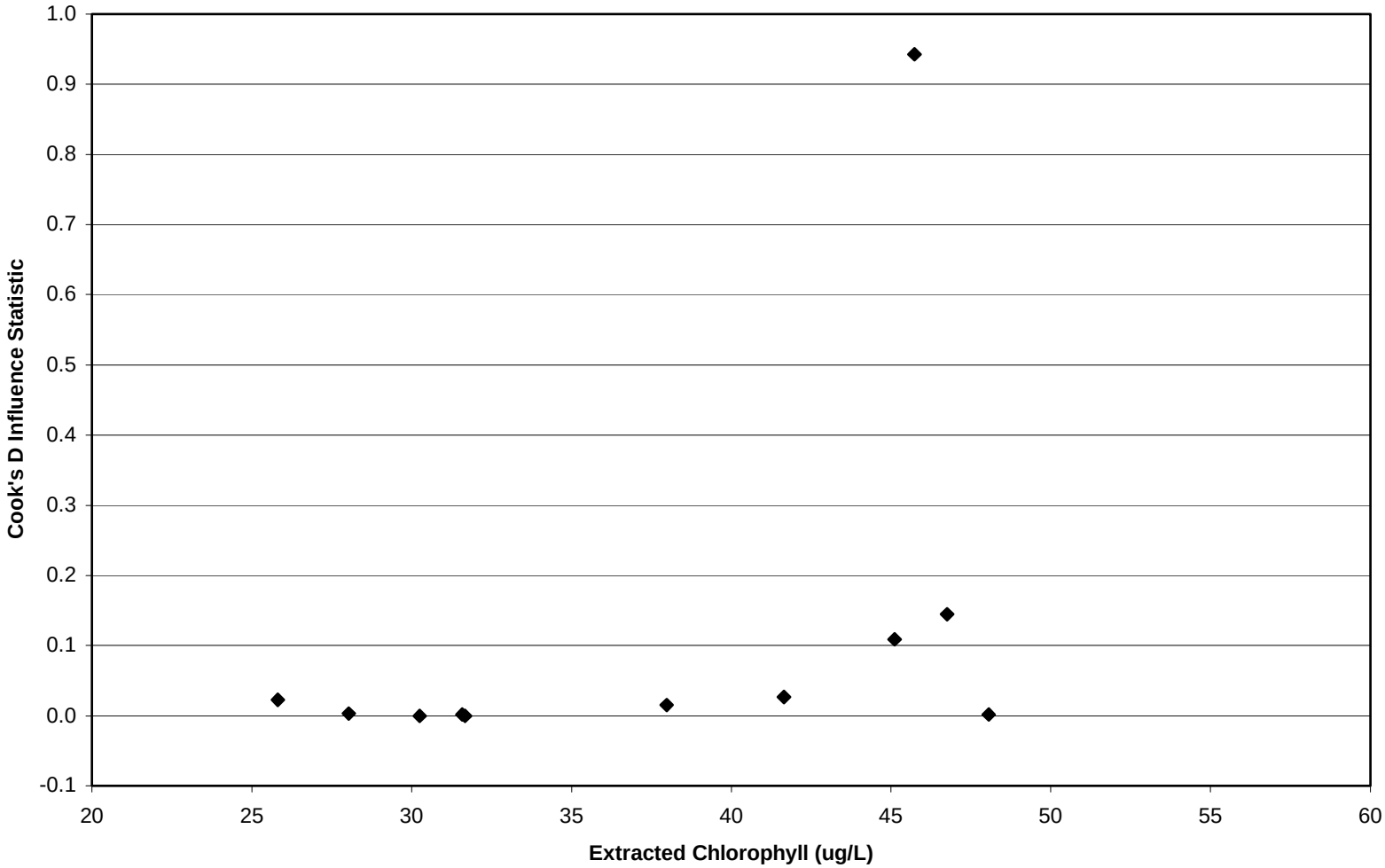


Table 3. Cook's D Influence Statistic after deletion of first high outlier. Dependent variable: multiprobe chlorophyll.

Obs. #	Date	Extracted Chlorophyll	Multiprobe Chlorophyll	Residuals	Cook's D
1	9-9-99	31.59	12.71	-3.3902	0.004
2	9-15-99	37.98	14.96	-10.8169	0.025
3	9-29-99	46.76	24.94	-14.1329	0.165
4	10-7-99	41.65	22.90	-8.4346	0.023
5	10-12-99	28.04	13.8	3.0757	0.006
6	10-14-99	25.81	13.8	6.4527	0.041
7	10-26-99	48.06	81.1	40.0584	1.761
8	11-11-99	45.12	18.9	-17.6894	0.184
9	11-18-99	31.68	18.1	1.8635	0.001
10	12-29-99	30.26	17.1	3.0138	0.004

Figure 7. Cook's D Influence Statistic for 9/9/99 to 12/29/99, First High Outlier Deleted

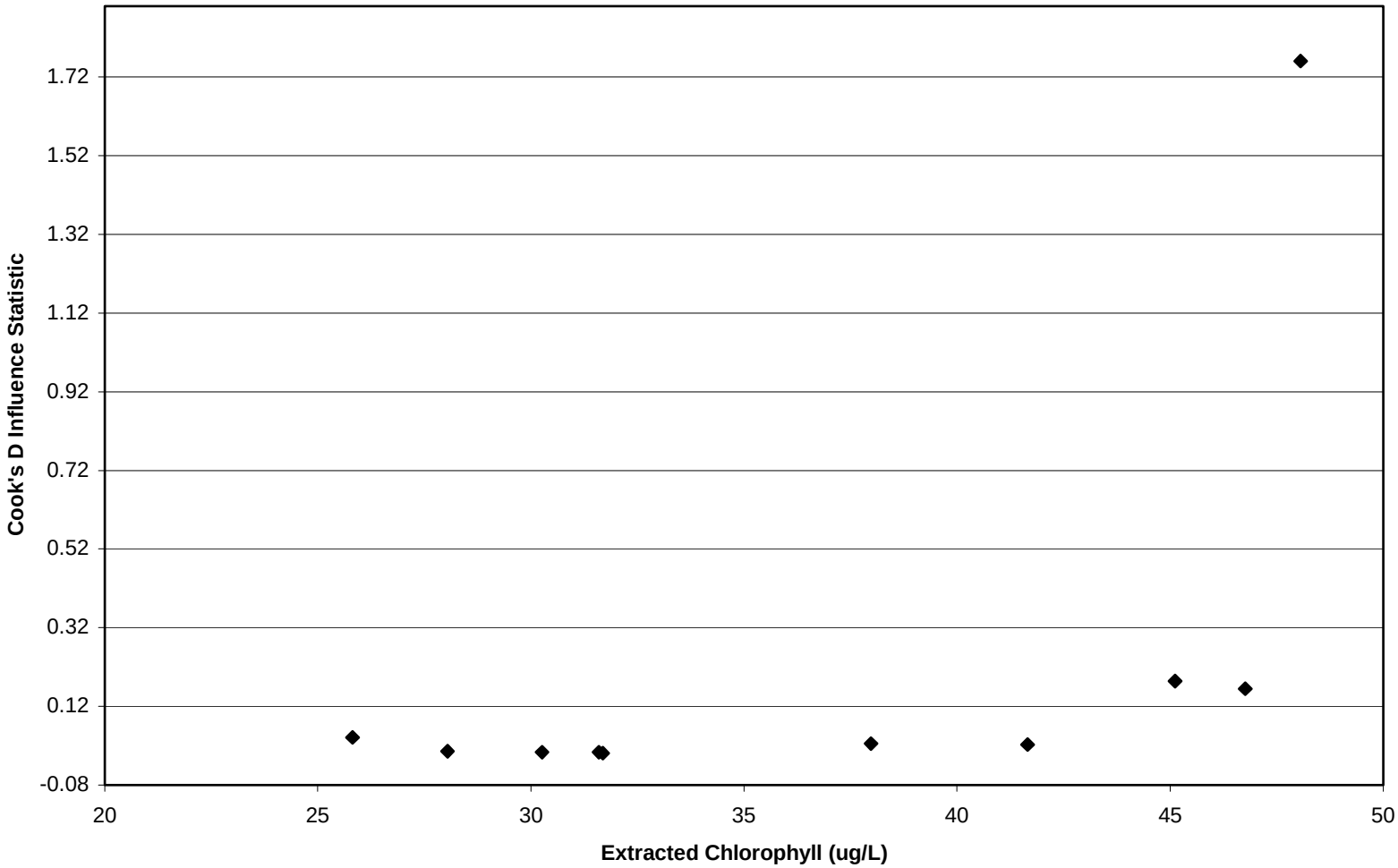


Figure 8: Extracted Chlorophyll vs. YSI Chlorophyll: Calibration by Zeroing Fluorescence

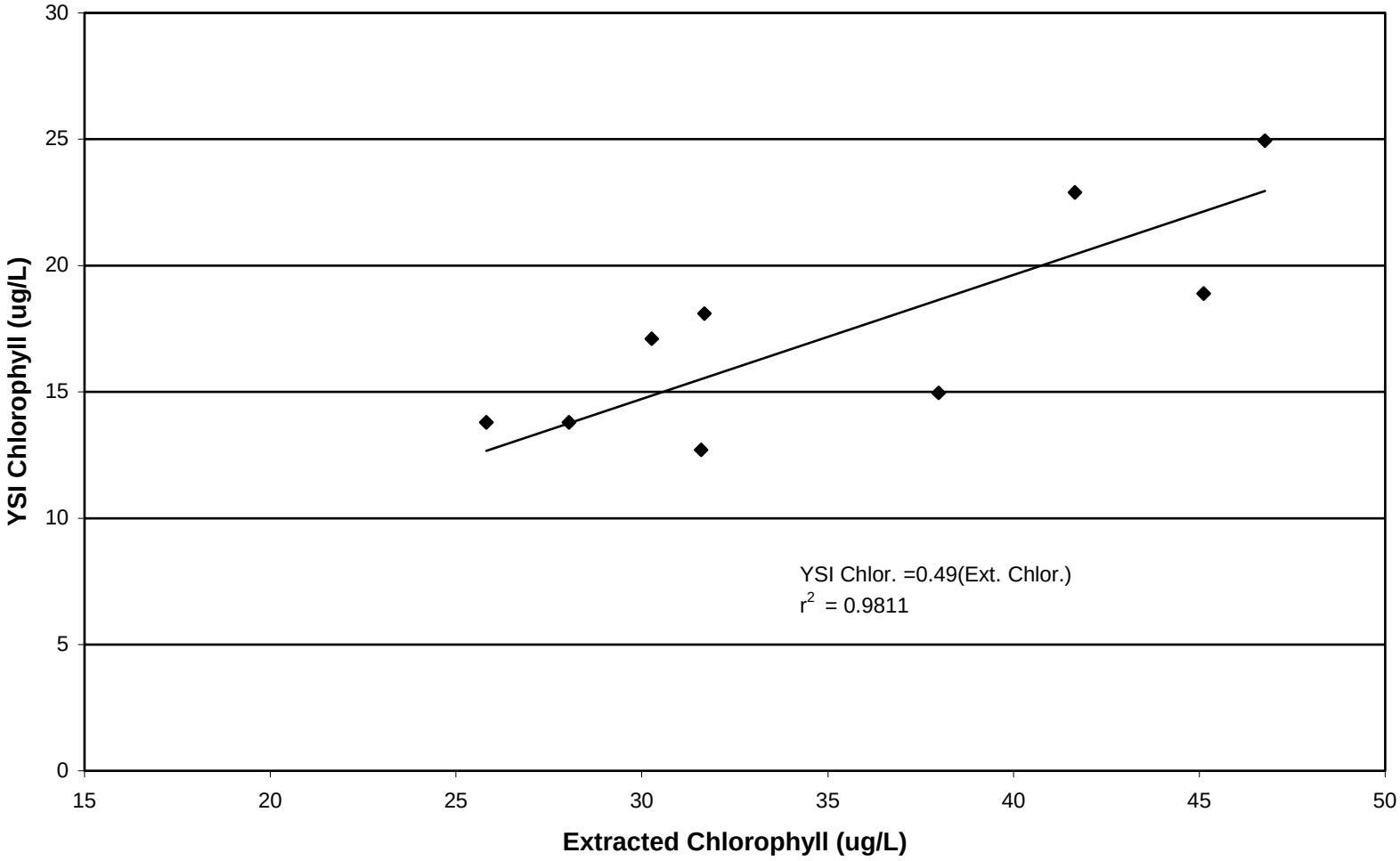


Table 4. Cook's D Influence Statistic after deleting second high outlier.
Dependent variable: multiprobe chlorophyll.

Obs. #	Date	Extracted Chlorophyll	Multiprobe Chlorophyll	Residuals	Cook's D
1	9-9-99	31.59	12.71	-3.0733	0.120
2	9-15-99	37.98	14.96	-3.6248	0.140
3	9-29-99	46.76	24.94	2.5059	0.417
4	10-7-99	41.65	22.90	2.7062	0.143
5	10-12-99	28.04	13.8	0.4269	0.005
6	10-14-99	25.81	13.8	0.5508	0.013
7	11-11-99	45.12	18.9	-2.8151	0.340
8	11-18-99	31.68	18.1	2.2773	0.065
9	12-29-99	30.26	17.1	1.8998	0.057

Figure 9. Cook's D Influence Statistic after deleting second high outlier

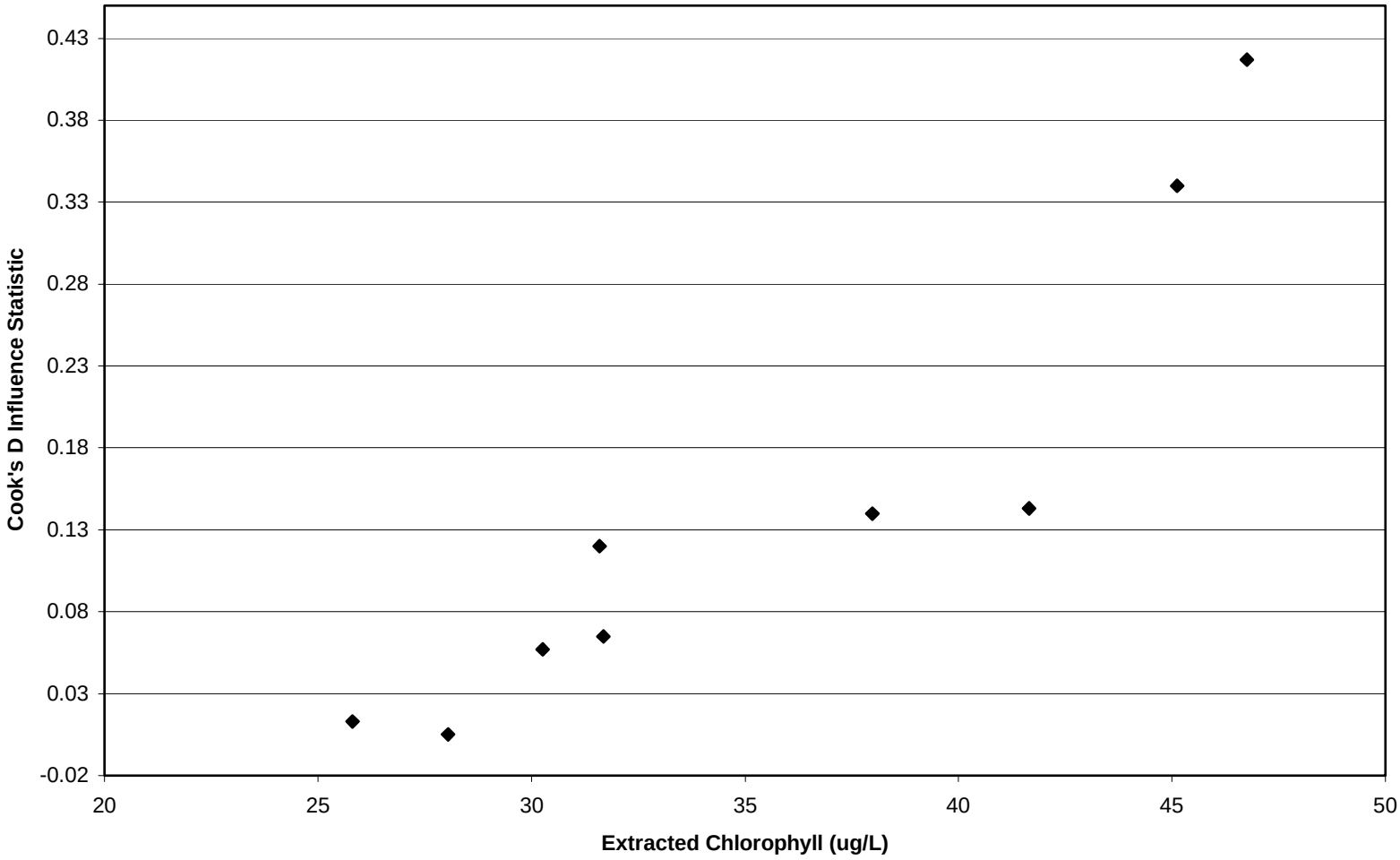


Figure 10: Predicted Values of YSI Chlorophyll with Upper and Lower 95% Confidence Limits

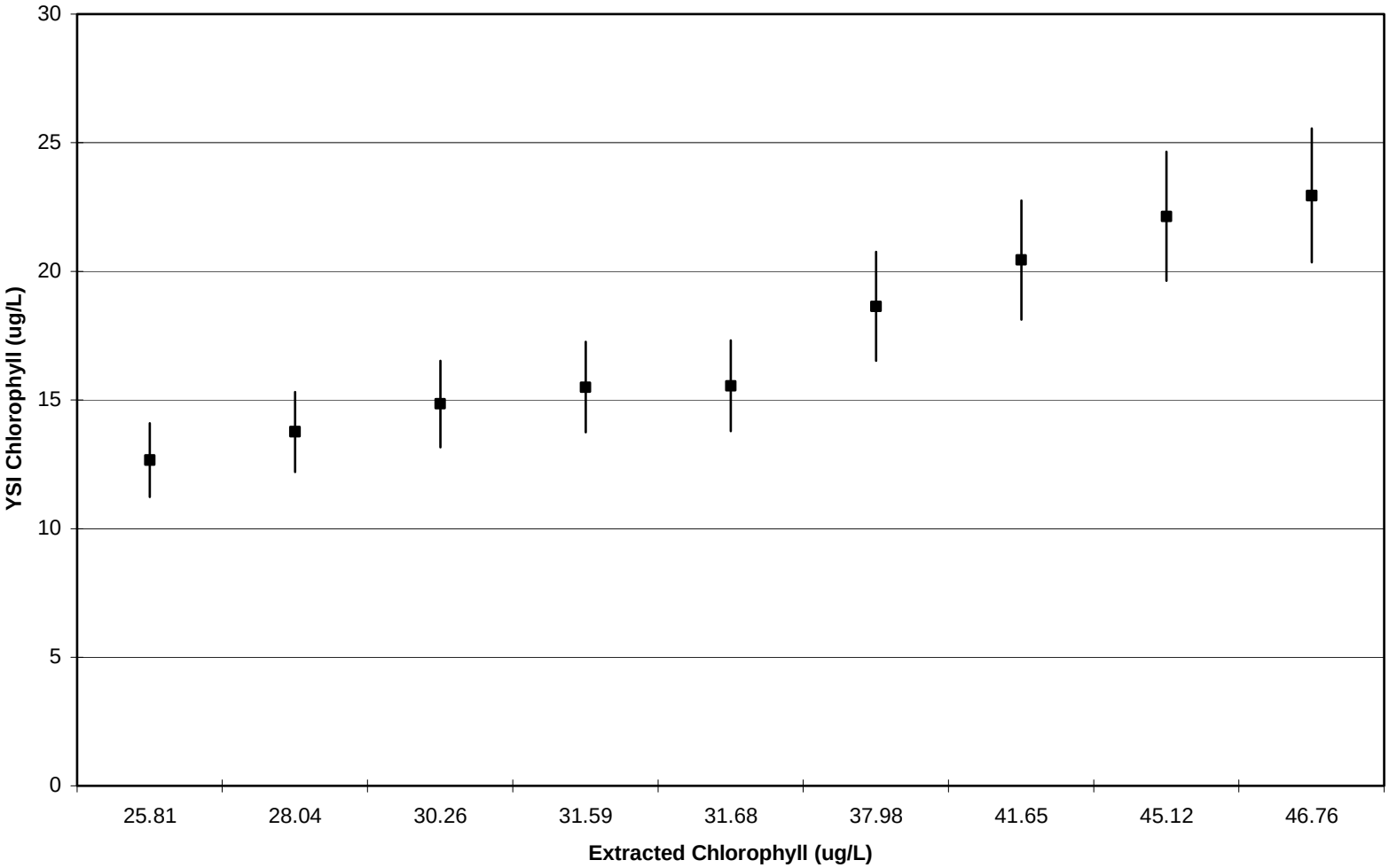


Figure 11. Plot of Residuals vs. Extracted Chlorophyll

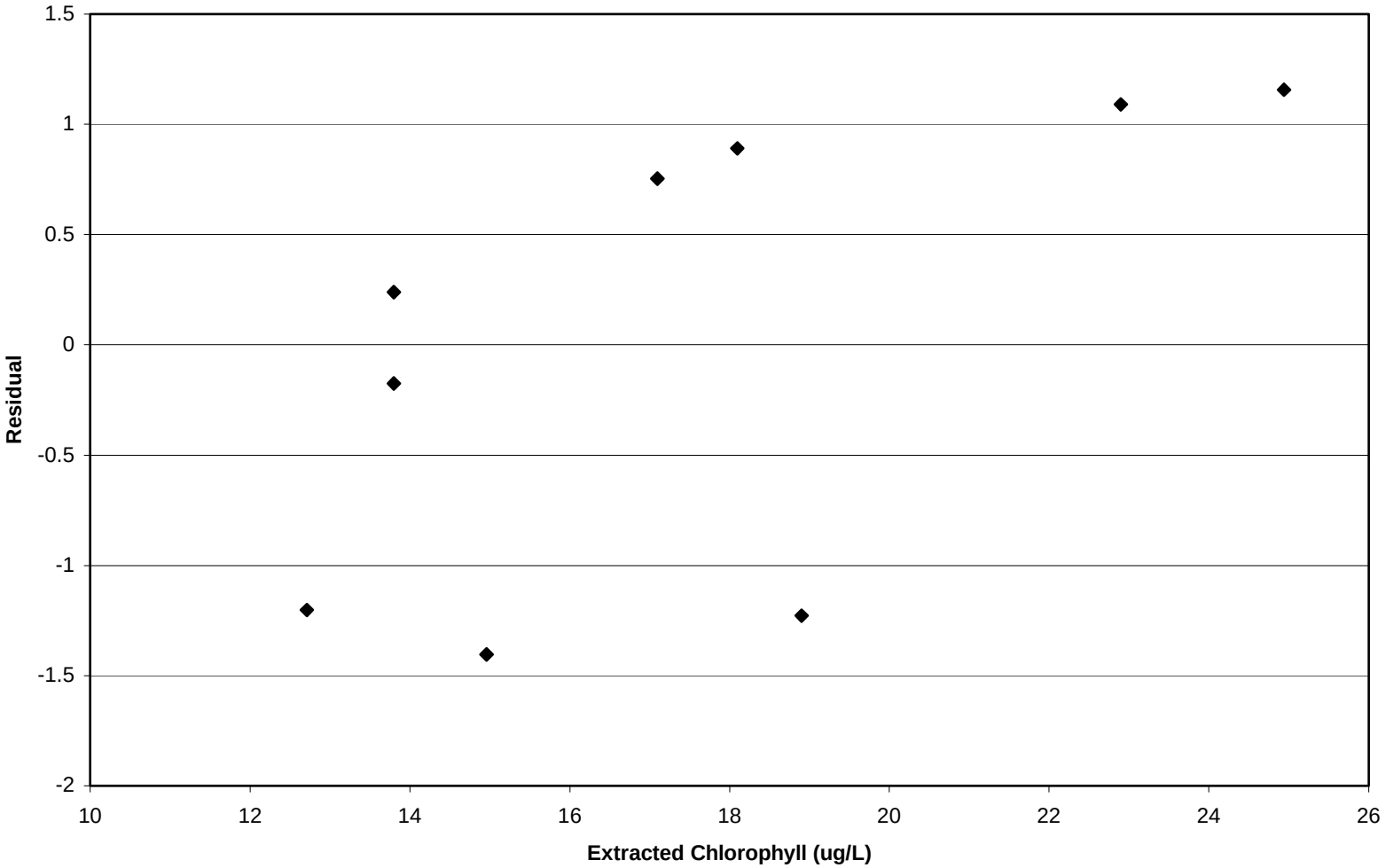


Figure 12: Multiprobe Chlorophyll vs. Extracted Chlorophyll 2-8-00 to 5-8-00: Calibration with a Phytoplankton Suspension

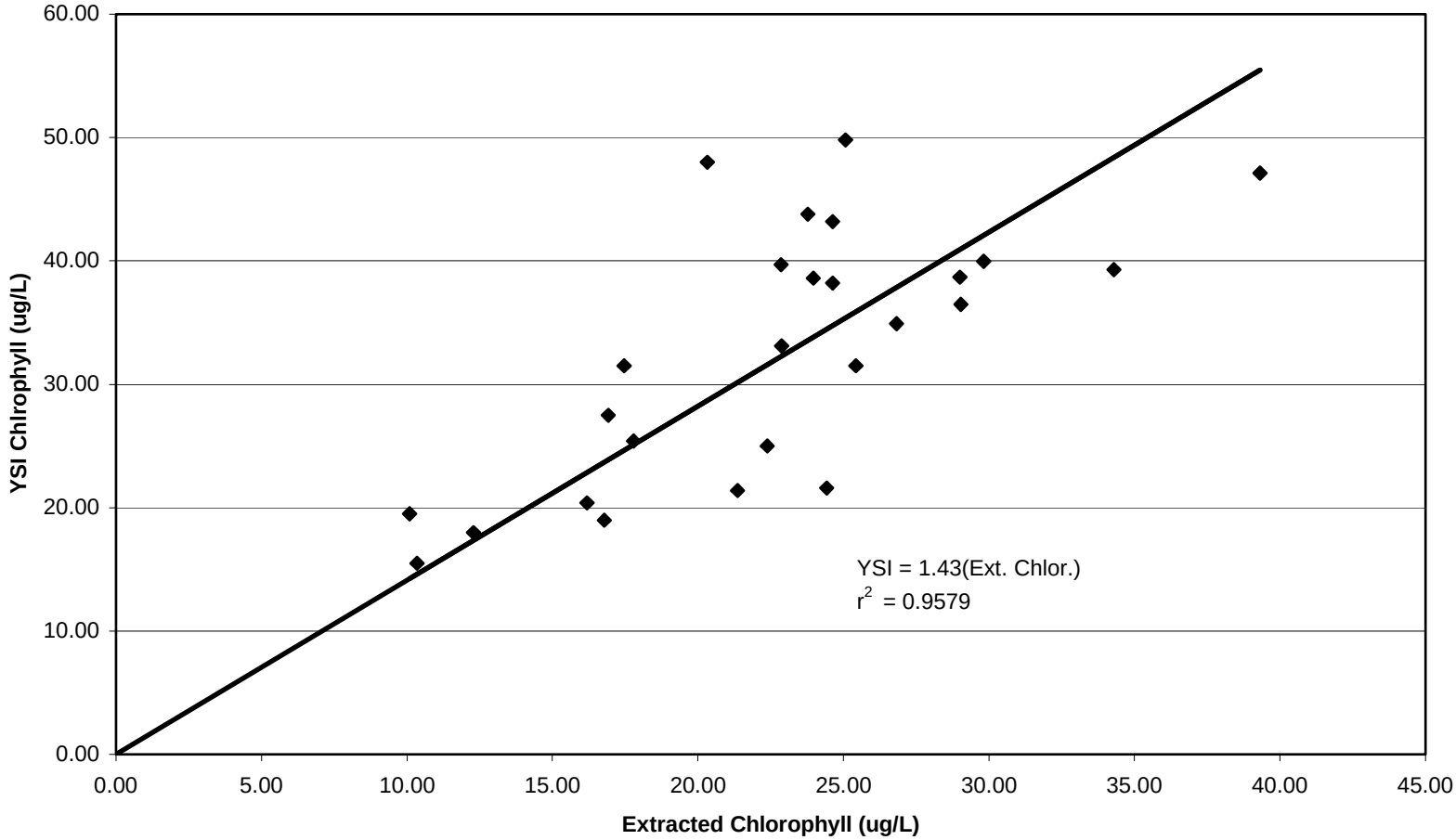


Figure 13: Percent Full-Scale Fluorescence vs. Extracted Chlorophyl 2-8-00 to 5-8-00

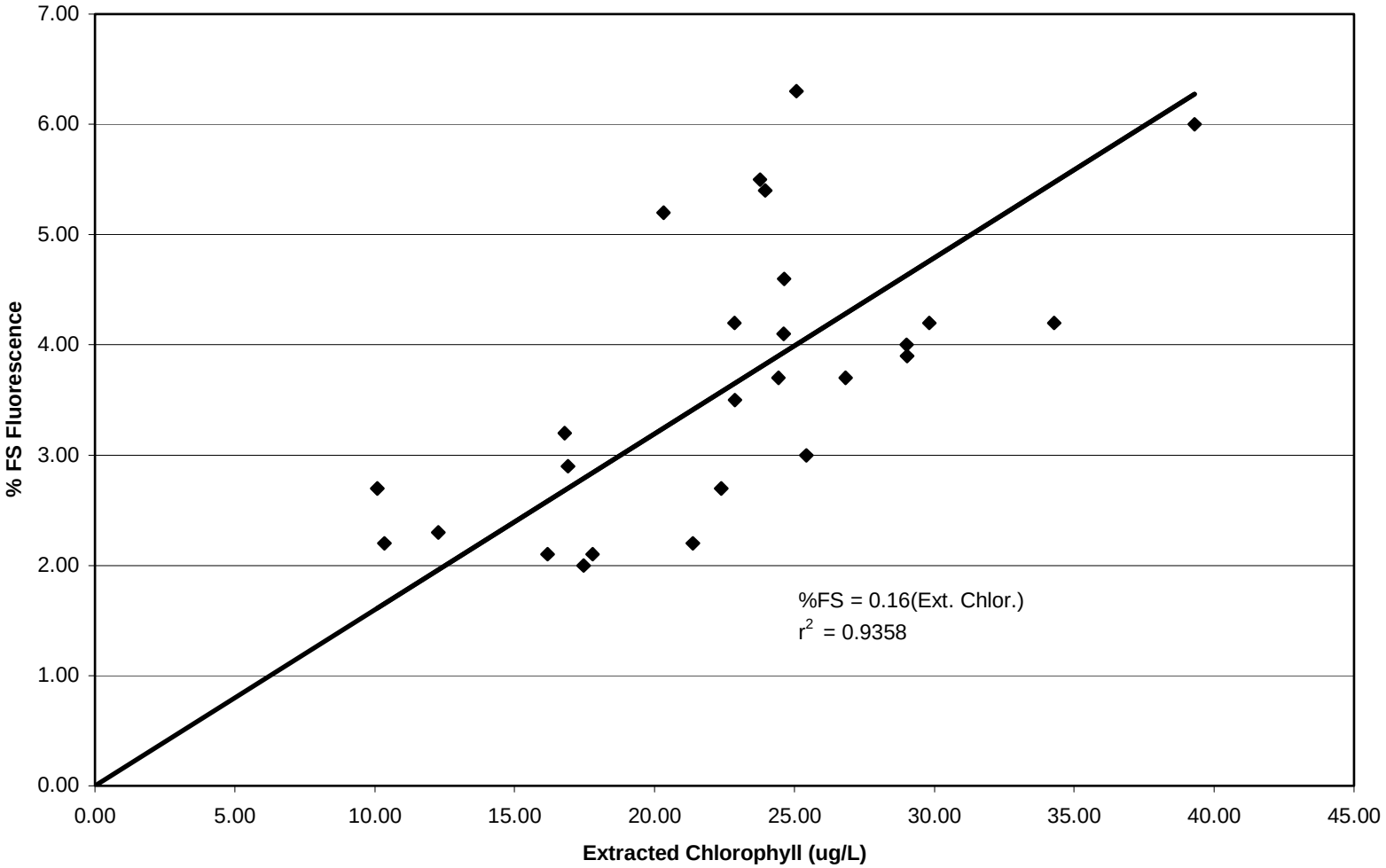


Figure 14: Multiprobe Chlorophyll vs. Extracted Chlorophyll data from 5-9-00 to 8-22-00:Calibration with Acridine Orange

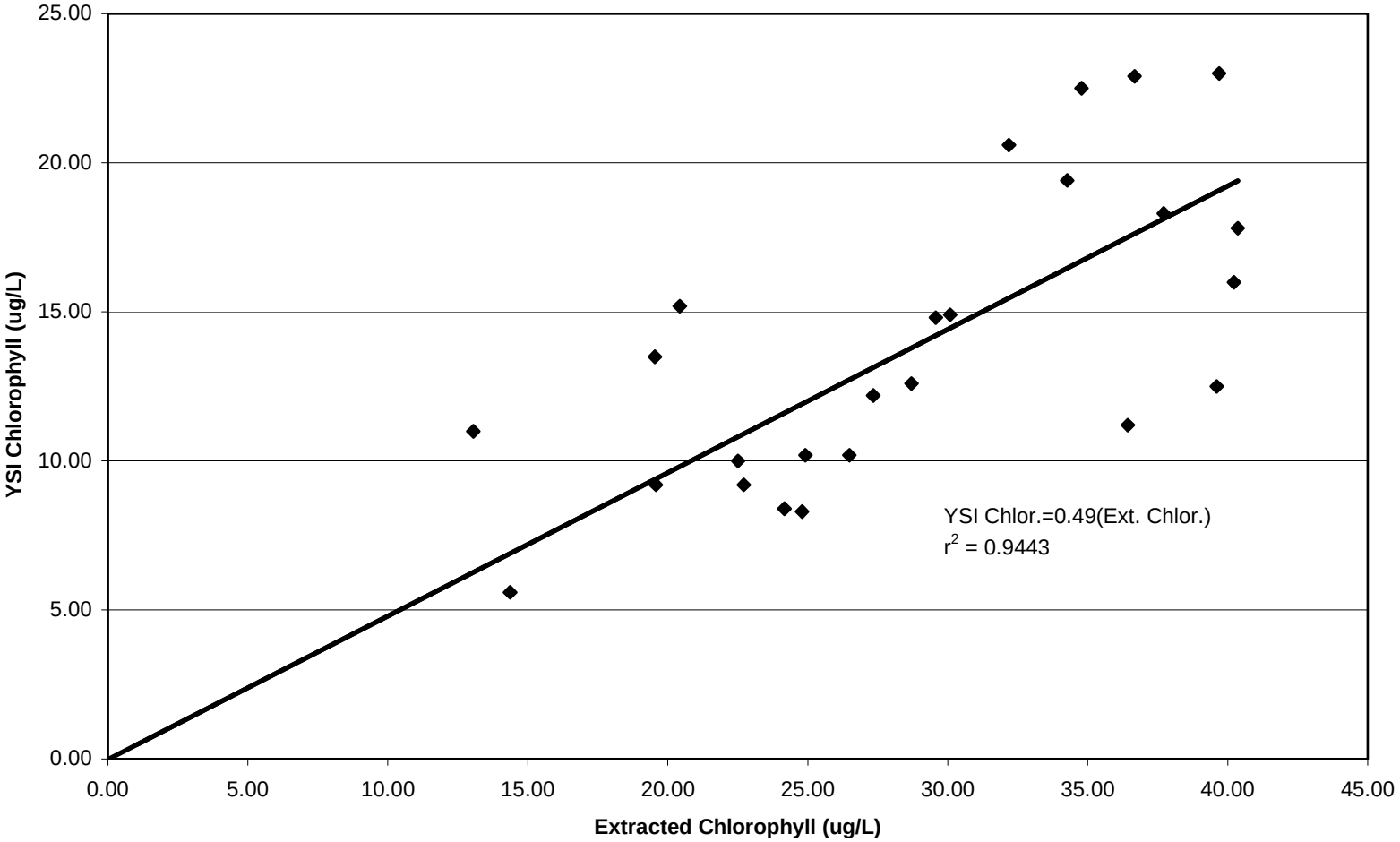


Figure 15: Percent Full-Scale Fluorescence vs. Extracted Chlorophyll data from 5-9-00 to 8-22-00

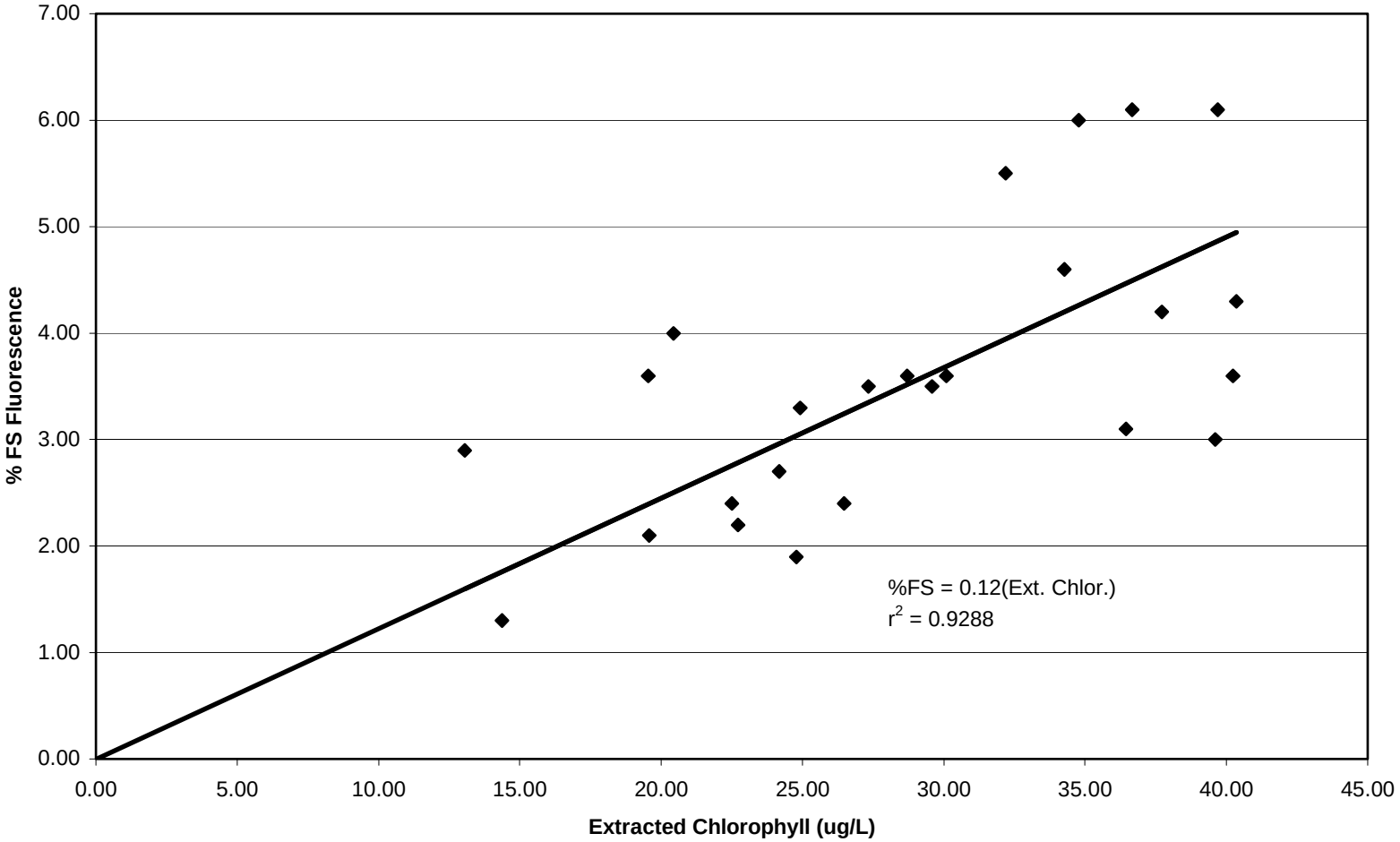


Table 5. Data for Turner Fluorometer, YSI multiprobe and Acetone Extraction Comparison Study.

Observation	Date	YSI %FS Fluorescence	YSI Chlorophyll (ug/L)	Turner Fluorometer Raw Fluor.	Extracted Chlorophyll (ug/L)
1	6/21/00	3.6	14.9	1.56	30.09
2	6/22/00	4.6	19.4	1.45	34.26
3	6/27/00	3.5	14.8	1.38	29.58
4	6/29/00	3.5	12.2	1.32	27.33
5	6/30/00	3.6	12.6	1.18	28.70
6	7/5/00	2.4	10.0	1.13	22.50
7	7/6/00	2.4	10.2	1.10	26.48
8	7/11/00	2.2	9.2	1.20	22.72
9	7/19/00	2.5	11.1	1.13	36.44
10	7/27/00	3.6	16.0	1.81	40.23
11	8/3/00	2.1	9.2	1.04	19.58
12	8/15/00	4.2	18.3	1.30	37.72
13	8/17/00	3.0	12.5	1.35	39.60
14	8/22/00	4.3	17.8	1.43	40.35

Table 6. Comparison of Turner Fluorometer Raw Fluorescence, YSI Fluorescence and Extracted Chlorophyll using Correlation and Linear Regression with No Intercept.

Independent Variable	Dependent Variable	R-square	P value	Correlation Coefficient	P value
YSI Fluorescence	Turner Fluorometer	0.9667	<.0001	0.6392	0.0139
YSI Chlorophyll	Turner Fluorometer	0.9692	<.0001	0.6876	0.0066
Extracted Chlorophyll	Turner Fluorometer	0.9720	<.0001	0.6237	0.0171

Figure 16: % Full-Scale Fluorescence vs. Turner Fluorometer Raw Fluorescence

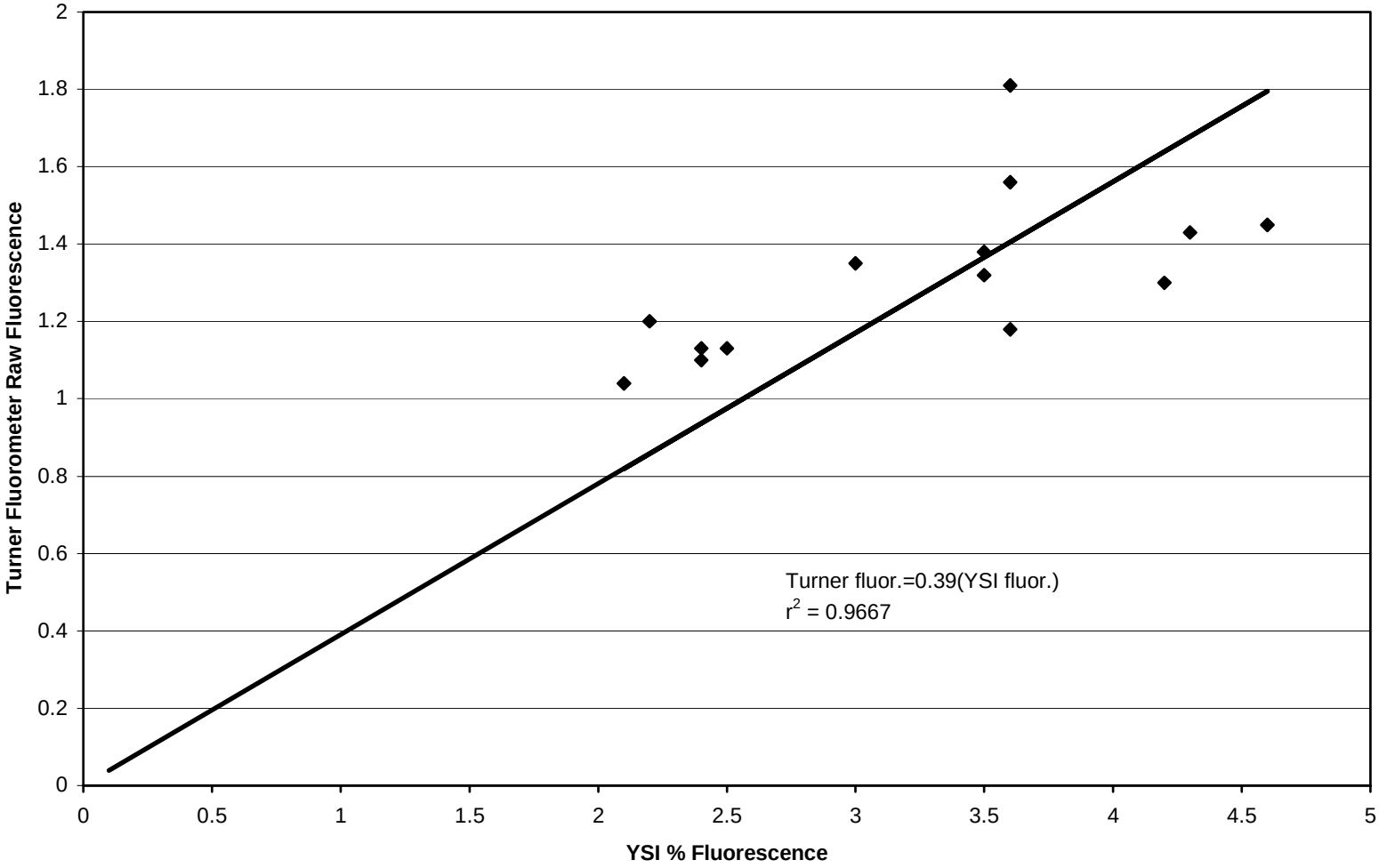


Figure 17: Plot of Date vs. Temperature for All Data

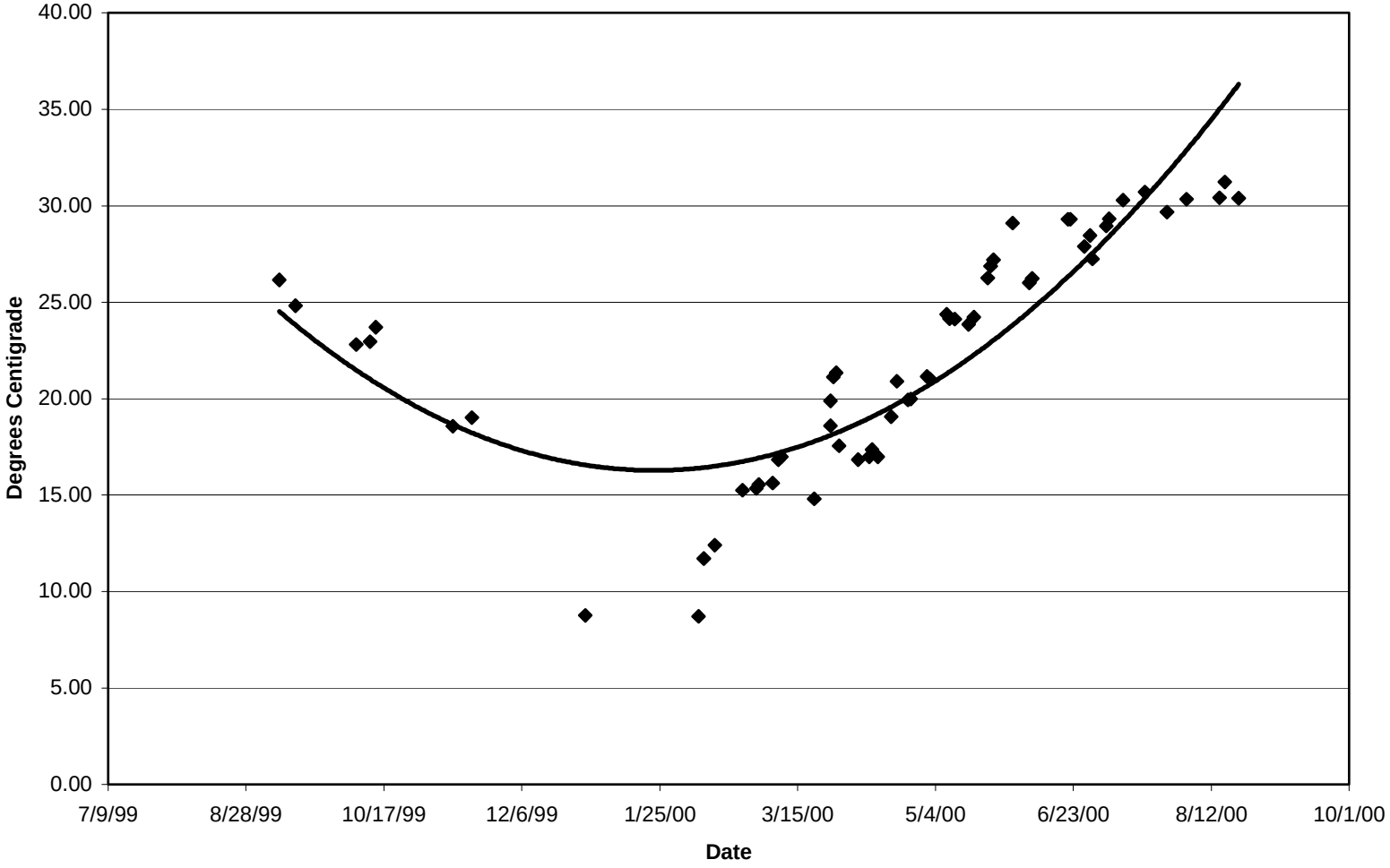


Figure 18: Date vs. Ratio of Fluorescence to Extracted Chlorophyll for All Data

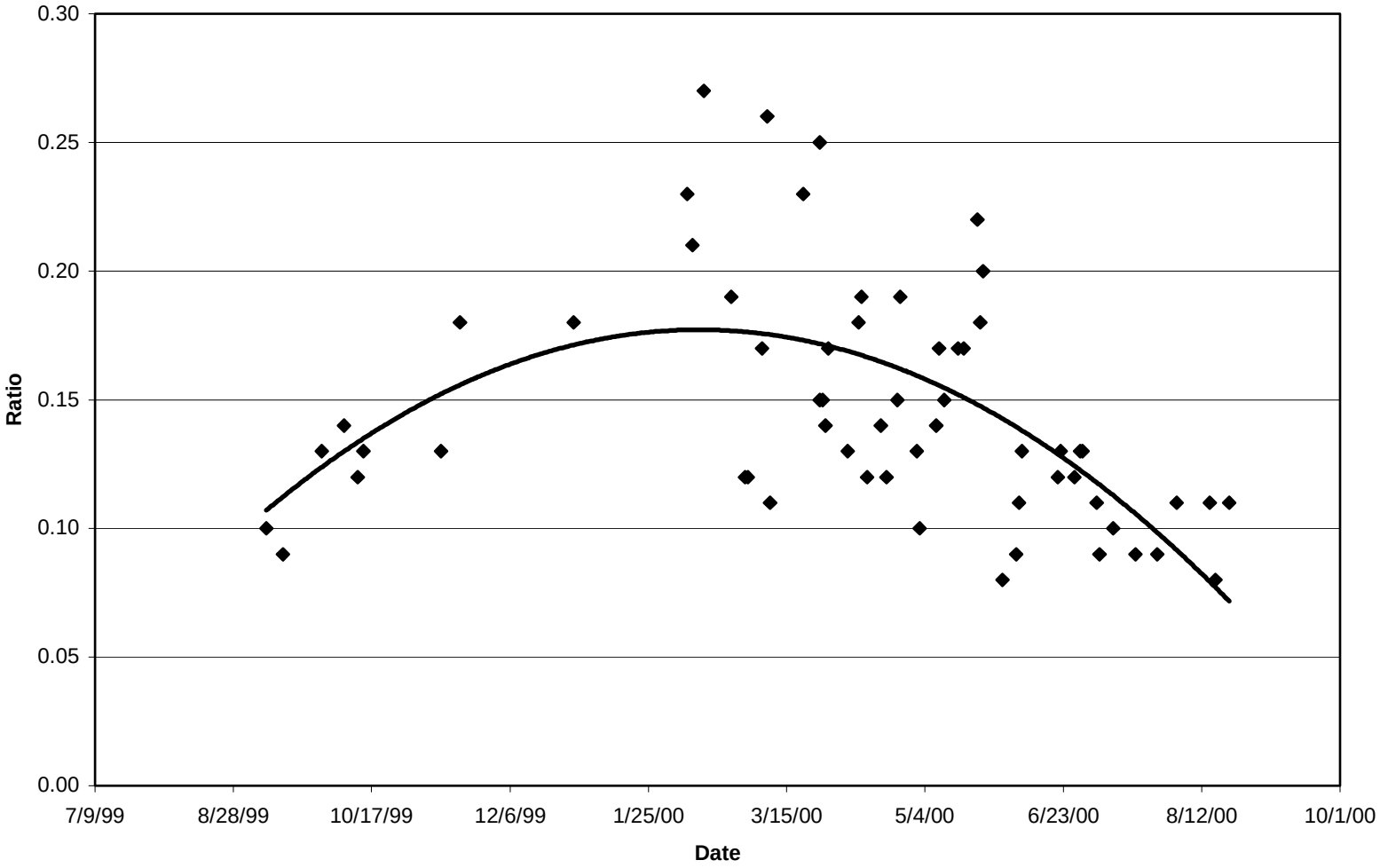


Figure 19: Temperature vs. Ratio of Fluorescence to Extracted Chlorophyll for All Data

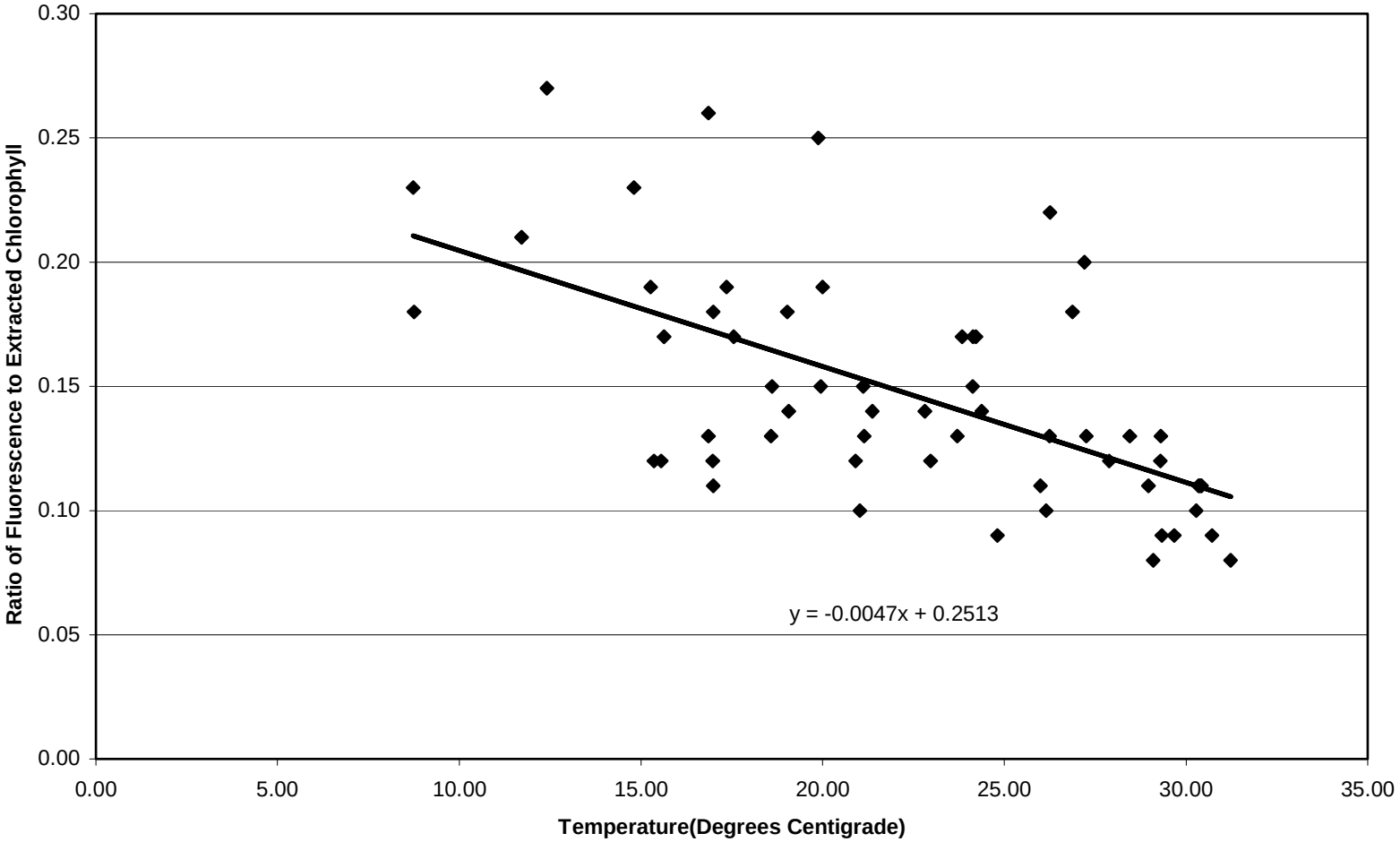


Table 7. Data from 9/9/99 to 8/22/00.

Date	Temp.	Fluor.	YSI Chlorophyll	Ext. Chlorophyll	Ratio
9/9/99	26.15	3.06	12.71	31.59	0.10
9/15/99	24.81	3.60	14.96	37.98	0.09
9/29/99		6.00	24.94	46.76	0.13
10/7/99	22.81	5.77	22.90	41.65	0.14
10/12/99	22.97	3.30	13.80	28.04	0.12
10/14/99	23.70	3.30	13.80	25.81	0.13
11/11/99	18.58	6.00	18.90	45.12	0.13
11/18/99	19.02	5.70	18.10	31.68	0.18
12/29/99	8.76	5.40	17.10	30.26	0.18
2/8/00	8.73	5.40	38.60	23.96	0.23
2/10/00	11.71	2.20	15.50	10.34	0.21
2/14/00	12.41	2.70	19.50	10.09	0.27
2/24/00	15.26	2.30	18.00	12.28	0.19
2/29/00	15.36	2.70	25.00	22.38	0.12
3/1/00	15.55	3.00	31.50	25.42	0.12
3/6/00	15.63	4.10	38.20	24.62	0.17
3/8/00	16.85	5.20	48.00	20.32	0.26
3/9/00	16.99	2.00	31.50	17.46	0.11
3/21/00	14.80	5.50	43.80	23.77	0.23
3/27/00	18.61	6.00	47.10	39.31	0.15
3/27/00	19.88	6.30	49.80	25.07	0.25
3/28/00	21.12	3.50	33.10	22.88	0.15
3/29/00	21.36	4.20	39.99	29.82	0.14
3/30/00	17.55	2.90	27.50	16.91	0.17
4/6/00	16.85	3.90	36.50	29.03	0.13
4/10/00	16.99	4.20	39.70	22.85	0.18
4/11/00	17.36	4.60	43.20	24.63	0.19
4/13/00	16.98	2.10	25.40	17.78	0.12
4/18/00	19.06	3.70	34.90	26.83	0.14
4/20/00	20.91	4.20	39.30	34.29	0.12
4/24/00	19.95	3.70	21.60	24.42	0.15
4/25/00	20.00	3.20	19.00	16.78	0.19
5/1/00	21.14	2.10	20.40	16.18	0.13
5/2/00	21.03	2.20	21.40	21.36	0.10
5/8/00	24.38	4.00	38.70	29.01	0.14
5/9/00	24.14	5.50	20.60	32.19	0.17
5/11/00	24.13	6.10	23.00	39.70	0.15
5/16/00	23.84	6.10	22.90	36.67	0.17

Date	Temp.	Fluor.	YSI Chlorophyll	Ext. Chlorophyll	Ratio
5/18/00	24.22	6.00	22.50	34.77	0.17
5/23/00	26.26	2.90	11.00	13.05	0.22
5/24/00	26.87	3.60	13.50	19.54	0.18
5/25/00	27.20	4.00	15.20	20.43	0.20
6/1/00	29.09	1.90	8.30	24.79	0.08
6/6/00		1.30	5.60	14.37	0.09
6/7/00	26.00	2.70	8.40	24.17	0.11
6/8/00	26.24	3.30	10.20	24.92	0.13
6/21/00	29.30	3.60	14.90	30.09	0.12
6/22/00	29.31	4.60	19.40	34.26	0.13
6/27/00	27.89	3.50	14.80	29.58	0.12
6/29/00	28.45	3.50	12.20	27.33	0.13
6/30/00	27.25	3.60	12.60	28.70	0.13
7/5/00	28.96	2.40	10.00	22.50	0.11
7/6/00	29.33	2.40	10.20	26.48	0.09
7/11/00	30.28	2.20	9.20	22.72	0.10
7/19/00	30.72	3.10	11.20	36.44	0.09
7/27/00	29.67	3.60	16.00	40.23	0.09
8/3/00	30.34	2.10	9.20	19.58	0.11
8/15/00	30.42	4.20	18.30	37.72	0.11
8/17/00	31.22	3.00	12.50	39.60	0.08
8/22/00	30.38	4.30	17.80	40.35	0.11

Figure 20: Extracted Chlorophyll and % FS Fluorescence 11-27-00

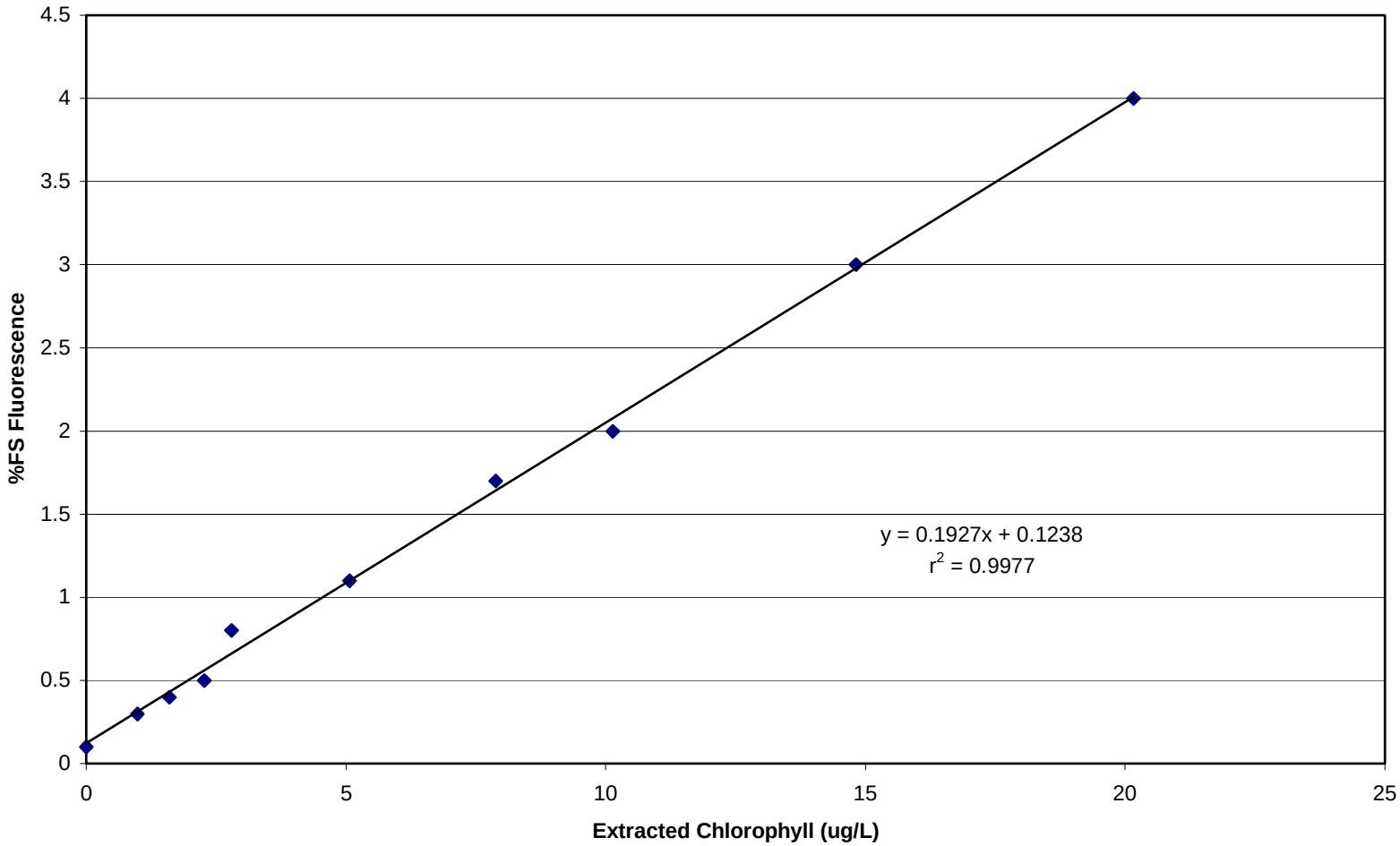
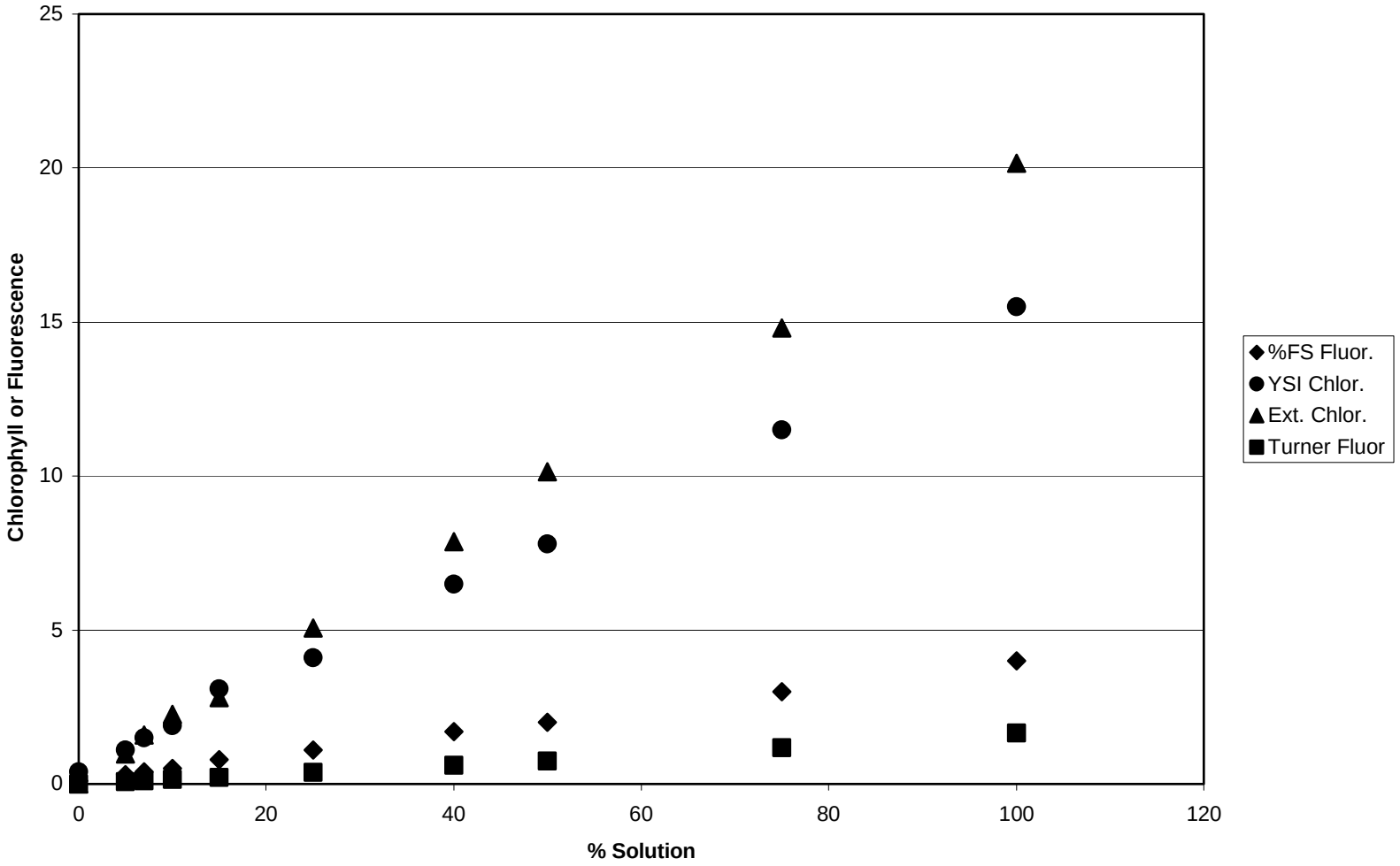


Figure 21: Dilution Study 11-27-00



REFERENCE LIST

Arar, EJ, and Collins, GB 1997. Method 445.0, In vitro determination of chlorophyll *a* and pheophytin *a* in marine and freshwater algae by fluorescence. National Exposure Research Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Cincinnati, Ohio 45268.

APHA. 1992. Standard methods for the examination of water and wastewater. 18th edition. American Public Health Association, American Water Works Association, Water Environment Federation, Washington D.C.

Axler, RP, and Owen, CJ. 1994. Measuring chlorophyll and phaeophytin: whom should you believe? Lake and Reservoir Management, Washington, D. C.; North American Lake Management Society 8 (2): 143-151.

Carpenter, SR, Cole, JJ, Kitchell, JF and Pace, ML. 1998. Impact of dissolved organic carbon, phosphorous and grazing on phytoplankton biomass and production in experimental lakes, Limnol. Oceanogr 43(1): 73-80.

ECOPLEX Homepage. www.ecoplex.unt.edu. Accessed 2000 Nov. 20.

Golterman, HL, Clymo, RS, Ohnstad, MAM. 1998. IBP handbook no. 8. Methods for physical and chemical analysis of fresh waters. 2nd edition. Oxford: Blackwell Scientific Publications.

Lee, GF, Jones-Lee, AJ and Rast, W. 1995. Secchi depth as a water quality parameter. March 1995, G. Fred Lee and Associates, El Macero, CA.

Marker, AF, Nusch, EA, Rai, H, and Riemann, B. 1980. The measurement of photosynthetic pigments in freshwaters and standardization of methods: Conclusions and Recommendations. Archiv. Hydrobiologie. Beih. (Ergebn. Limnol.) 14: 91-106.

Nieke, B, Vincent, WF, Therriault, JC. Legendre, L, Berthon, JF, and Condal, A. 1997. Use of a ship-borne laser fluorosensor for remote sensing of chlorophyll *a* in a coastal environment. Remote Sens. Environ. 60:140-152.

Palmer, CM. 1959. Algae in water supplies. U. S. Department of Health, Education , and Welfare Public Health Service. Cincinnati, OH.

Sartory, DP and Grobbelaar, JU. 1984. Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. Hydrobiologia 114: 177-187.

Schloss, J. 2000. Streamlined *in-situ* chlorophyll monitoring technology. LakeLine 20(3): 16-18.

Simon, D. and Helliwell, S. 1998. Extraction and quantification of chlorophyll *a* from freshwater green algae; Technical Note. Wat. Res. 5: 2220-2223.

Trees, CC, Kennicutt II, MC and Brooks, JM, 1985. Errors associated with the standard fluorometric determination of chlorophylls and phaeopigments, Marine Chemistry 17:1-12.

YSI 6-series Environmental Monitoring Systems Operations Manual. 1999. YSI Incorporated, Yellow Springs, OH.

Zar, JH, 1999. Biostatistical analysis. Fourth edition. Upper Saddle River, N. J. Prentice Hall.