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INVESTIGATING ERROR IN CALCULATION OF AREAL CHLOROPHYLL *a* CONCENTRATION IN TWIN LAKES, COLORADO

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16. ABSTRACT

Analysis of chlorophyll *a* data collected at Twin Lakes, Colorado, from 1977 to 1981 indicated possible error in areal chlorophyll concentrations extrapolated from six-point samples collected along a 0-15 m depth profile.

Field experiments to identify the sources of error in areal chlorophyll *a* concentration were conducted during the ice-free seasons of 1982 and 1983. Two different methods of sample collection were used. The first was a six-point depth profile; the second, an integrated (composite) 0-15 m sample collected with a flexible PVC hose.

Two probable sources of error were identified. First, a standard sampling depth (9 m) sometimes coincided with the bottom of the thermocline during summer stratification. Algae tend to settle at the density interface along the bottom of the thermocline, which can result in a concentrated layer of algae of variable thickness. Second, some algal layers occurred at depths not routinely sampled by the 6-point depth profile method.

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Applied Sciences Branch
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Denver, Colorado

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CONTENTS

TABLES

FIGURES

APPLICATION

Results of this study will be combined with other preoperational data on the physical, chemical, and biological limnology of Twin Lakes for comparison with postoperational conditions to assess the impact of the Mt. Elbert Pumped-Storage Powerplant. Information from these studies is already being used by the USBR (Bureau of Reclamation) in preparing designs and plans for other pumped-storage facilities.

Anyone assessing the environmental effects of pumped-storage powerplants will find the data from these studies useful. The results of this study will also be valuable to anyone studying lake or reservoir ecosystems because a sampling method that gives a more accurate estimate of trophic status or standing crop is described.

INTRODUCTION

Lack of correlation among chlorophyll *a*, carbon fixation rate, and net-sized phytoplankton population parameters at Twin Lakes, Colorado, led to speculation that collection methods may have caused a bias in the data. Both chlorophyll *a* and carbon fixation rate areal values were extrapolated from five or six discrete samples collected along a 0-15 m depth profile, while net-sized phytoplankton population values were averaged from samples collected by 5-m incremental hauls over the same 0-15 m water column.

The working hypothesis for the lack of correlation among these parameters was that the depth profile method for collecting chlorophyll *a* and carbon fixation rate samples could have introduced a bias in areal estimations. This bias may have been caused by the patchy distribution of phytoplankton populations, which is well documented in the literature (Platt and Denman, 1980) [1]*. A layer of phytoplankton that coincided with a discrete sampling depth could have caused an overestimation of areal values; while a layer at a depth not sampled could have caused an underestimation of areal values.

The relationships among the various biotic and abiotic factors at Twin Lakes are critical to a long-term study evaluating the effects of the pumped-storage powerplant operation on the limnology of the lakes. Clarification of data artifact phenomena will help biologists examine the data for the final report.

Field experiments to verify the suspected bias in chlorophyll *a* and carbon fixation rate data were conducted at Twin Lakes during the ice-free seasons of 1982 and 1983. Experiments were conducted only on the chlorophyll *a* parameter, which is less expensive and time consuming to collect and process for results. Two sampling methods were compared. First, the standard six-point depth profile method, and second, an integrated sampling method that collected a composite sample over the entire 0-15 m stratum. Areal estimations of chlorophyll *a* concentration from the two sampling methods were compared using the chi square distribution analysis. The null hypothesis for this analysis was that the two methods sampled the same population and, therefore, the resulting areal estimations of chlorophyll *a* concentration would be statistically the same.

STUDY AREA

Twin Lakes are a pair of oligotrophic, dimictic, montane lakes located in central Colorado, about 24 km southwest of Leadville (fig. 1). The lower lake is the largest natural mountain lake in Colorado (Pennak, 1966) [2]. The lakes are 2802 m above mean sea level. Maximum water surface areas are about 736.5 ha for the lower lake and 263.4 ha for the upper lake, with depths of about 27 and 28 m, respectively. The maximum area capacity at 2802 m elevation is 112 653 088 m^3 in the lower lake and 41 078 107 m^3 in the upper lake (fig. 2).

Limnological sampling has been ongoing since 1971, as part of a major study evaluating the effects of pumped-storage powerplant operation on the ecology of the lakes. Preoperational studies ended in 1981, when the Mt. Elbert Pumped-Storage Powerplant (fig. 2) began operating. Postoperational studies now being performed are scheduled to end in September 1985.

MATERIALS AND METHODS

Limnological sampling is performed at Twin Lakes at approximately 2-week intervals. A complete survey includes sampling of physical-chemical parameters such as temperature, dissolved oxygen concentration, pH (hydrogen ion concentration), conductivity, and oxidation-reduction potential. Samples are also collected for water chemistry, nutrients, trace metals, chlorophyll *a*, carbon fixation rate, plankton, and benthos. Light attenuation and water clarity measurements complete the standard limnological survey at Twin Lakes. Methods for all sampling at the lakes can be found in LaBounty and Sartoris (1982) [3].

Chlorophyll *a*

Chlorphyll *a* samples are routinely collected at six discrete depths: 0.1, 1.0, 3.0, 5.0, 9.0, and 15.0 m.

* Numbers in brackets refer to entries in the bibliography.

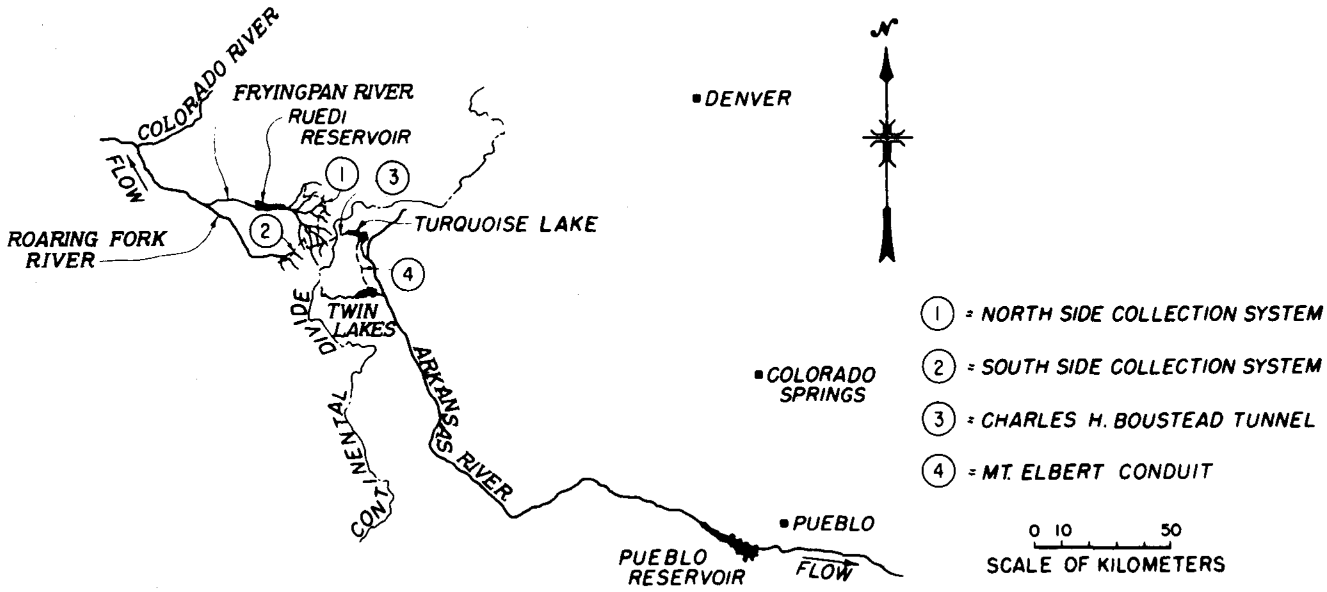

Figure 1. – General location map of Twin Lakes, Colorado.

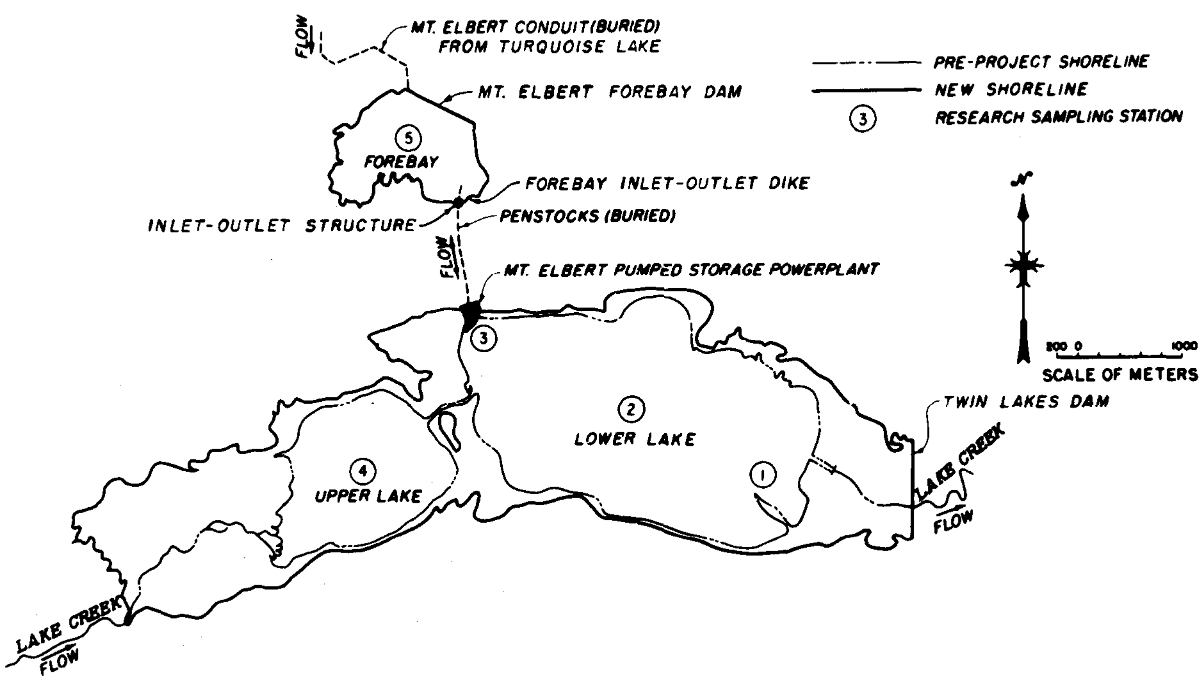

Figure 2. – Twin Lakes shoreline.

Using a Van Dorn-type sampler, 4.1 L of water are collected, and a 1.9 L aliquot is decanted into a nalgene container. This container is immediately stored in a darkened, insulated box, until the samples are filtered at the field laboratory facility, usually within 2-4 hours after collection. The composite sample was collected using a 15-m long, 3.8-cm diameter PVC hose (fig. 3). The contents of the hose are emptied into a large nalgene bucket and thoroughly mixed. Two 1.9 L aliquots are taken from the bucket and placed in the darkened, insulated box with the depth profile samples.

Field processing of samples consists of vacuum filtering two 750-mL replicate subsamples from each sample container through 0.45 micron (0.45 micrometer) pore-size fiberglass filter pads. Each filter is suction dried and folded in half, sample surface inside. The samples are frozen until analyzed in our Denver laboratory.

Figure 3. – Composite water sampler used to collect water for chlorophyll analysis at Twin Lakes. P801-D-80955

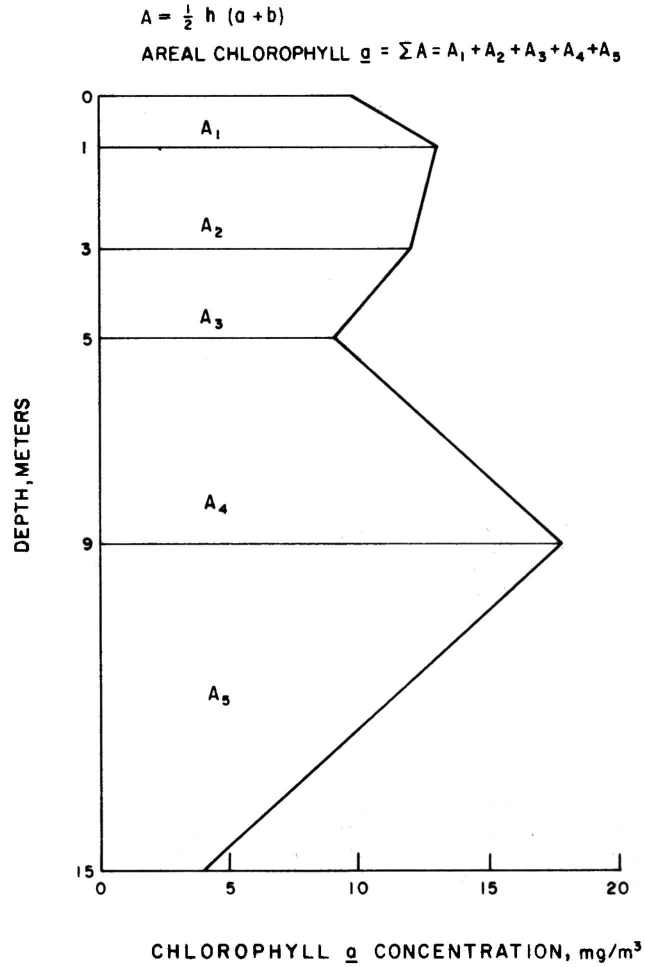

Figure 4. – Example of the method used to extrapolate areal chlorophyll *a* concentration from depth profile samples.

Laboratory processing and spectrophotometric analysis are performed according to Holm-Hansen and Riemann (1978) [4]. Chlorophyll *a, b,* and *c* concentrations are calculated using trichromatic equations reported in Jeffrey and Humphrey (1975) [5]. Areal chlorophyll *a* concentration is calculated using the standard formula for the area of a trapezoid. An example of the calculation is displayed on figure 4.

During the ice-free season in 1983, classic thermal stratification was evident in both lakes. Thermal and transmissivity (water clarity) profiles were measured to document the presence, thickness, and relative position of any layers of decreased clarity relative to the thermocline. Additionally, on two occasions in the lower lake and on three occasions in the upper lake, point samples of water were taken from areas of reduced transmissivity. Chlorophyll analysis and net-sized algae counts and identification were performed on subsamples collected in reduced transmissivity areas.

RESULTS AND DISCUSSION

Depth profile and composite areal chlorophyll *a* concentrations from sample collections during 1982 and 1983 are summarized in table 1 and displayed on figure 5. Areal estimations of chlorophyll *a* concentration were very similar in both depth profile and composite sampling results during the ice-free season of 1982 (fig. 5). But the results of depth profile and composite sampling conducted in 1983, start to differ in July 1983 in the lower lake and in August 1983 in the upper lake (fig. 5).

The two areal chlorophyll *a* estimates differed during classic thermal stratification in the lakes. The definition of classic thermal stratification originated by

Table 1. – Areal chlorophyll *a* concentrations (mg/m²) extrapolated from data collected using two different sampling methods at Twin Lakes, 1982 and 1983.

Sample date	Lower lake		Upper lake	
	Profile areal chlorophyll *a*	Composite areal chlorophyll *a*	Profile areal chlorophyll *a*	Composite areal chlorophyll *a*
1982				
May 19	48.53	49.25	33.19	30.95
June 2	41.59	42.50	28.06	31.40
June 15	47.06	43.65	13.13	11.70
June 30	45.56	44.75	6.04	5.55
July 14	38.71	33.95	5.61	7.10
July 28	45.97	40.90	14.54	15.25
Aug. 11	58.89	59.75	25.03	20.45
Aug. 26	47.52	48.70	24.62	22.15
Sept. 8	48.79	45.95	28.14	30.60
Sept. 22	61.32	55.90	36.46	29.85
Oct. 6	67.73	57.25	40.28	39.05
Oct. 20	85.84	86.55	50.46	49.35
Nov. 4	103.53	106.88	62.21	64.80
Nov. 17	102.12	106.05	72.39	74.70
1983				
May 23	31.67	31.50	34.26	32.70
June 8	49.10	46.50	29.43	27.00
June 21	52.51	53.40	12.45	11.25
July 6	46.04	45.30	4.35	5.25
July 21	53.94	*43.05	7.05	8.70
Aug. 4	69.41	*49.80	38.61	28.50
Aug. 17	57.68	*45.45	69.69	*77.40
Sept. 1	45.21	*37.35	62.19	*84.15
Sept. 15	35.20	*37.35	124.65	*53.40
Sept. 29	59.83	*71.25	87.88	*70.20
Oct. 11	80.86	78.90	90.19	90.15
Oct. 27	81.36	75.98	77.35	62.93
Nov. 8	90.25	65.33	52.70	42.98
Nov. 22	81.20	78.08	42.62	37.50

* Data set deleted from linear regression analysis.

E. A. Birge in 1897, and quoted from Hutchinson (1957) [6] is "that layer in which the fall in temperature exceeds one degree Centigrade per meter." This classic thermal stratification is the major difference between conditions at the lakes in 1982 and 1983. Temperature profiles in Twin Lakes during the ice-free seasons of 1982 and 1983 are displayed on figure 6. A classic thermocline was observed on just one occasion in 1982: August 11, in the upper lake. But in 1983, classic thermal stratification was documented in both lakes beginning in July and continuing through September.

A major factor contributing to the absence of classic thermal stratification in the lakes during 1982, was the operation of the Mt. Elbert Pumped-Storage Powerplant (LaBounty et al., 1985) [7]. The mean daily hours of operation during each month of 1982 and 1983, are displayed on figure 7. Powerplant activity was considerably greater in 1982 than in 1983 (fig. 7). The first unit of the powerplant came on-line in late 1981, and considerable testing continued through September 1982. Operation of the powerplant resulted in induced mixing of the profile, especially in the lower lake, which effectively prevented formation of a classic thermocline. In 1983, the powerplant did not operate until September and then only for annual maintenance and performance verification procedures (fig. 7).

Profiles of transmissivity in the lakes during the ice-free seasons in 1982 and 1983 are displayed on figure 8. The transmissometer is used at Twin Lakes to measure water clarity during annual spring runoff and to monitor decreases in water clarity that may be caused by algal layering. Transmissometer profiles measured in both lakes during 1982 show a relatively homogeneous condition from the surface to the bottom, throughout the ice-free season. In 1983, sharp decreases in water clarity were noted in both lakes in August and September. The relative depth and the thickness of the areas of low transmissivity were recorded. Point samples for chlorophyll *a* analysis and plankton identification and enumeration were collected on two occasions in the lower lake and on three occasions in the upper lake. A summary of the algal layering documentation data is shown in table 2.

Layering was first observed on the August 17, 1983, sampling date in both lakes (table 2). The layers were very thin, especially in the upper lake. Point samples collected within the low transmissivity areas were taken with a Van Dorn-type water sampler, 82.5 cm long. Because the length of this water sampler exceeded the thickness of the observed layers on all sampling dates, the concentrations of chlorophyll and plankton found in the layers may have been diluted. Baker and Brook, 1971 [8] noted a similar problem in their study of stratified phytoplankton populations in 10 Minnesota lakes and devised a microsampler to collect point samples within thin layers of algae. Nevertheless, the information we gathered indicates the relative difference in water clarity and chlorophyll *a* concentration in areas immediately above and below the layers (table 2).

Beginning with the August 17, 1983 sampling date, differences in extrapolated areal chlorophyll *a* concentration between the two sampling methods were accompanied by observation of layers of reduced transmissivity. The documentation of these layers provided evidence supporting the working hypothesis for the methods comparison experiments.

As previously discussed, extrapolation of areal chlorophyll *a* concentrations from 6-point samples collected by the depth profiling method might result in

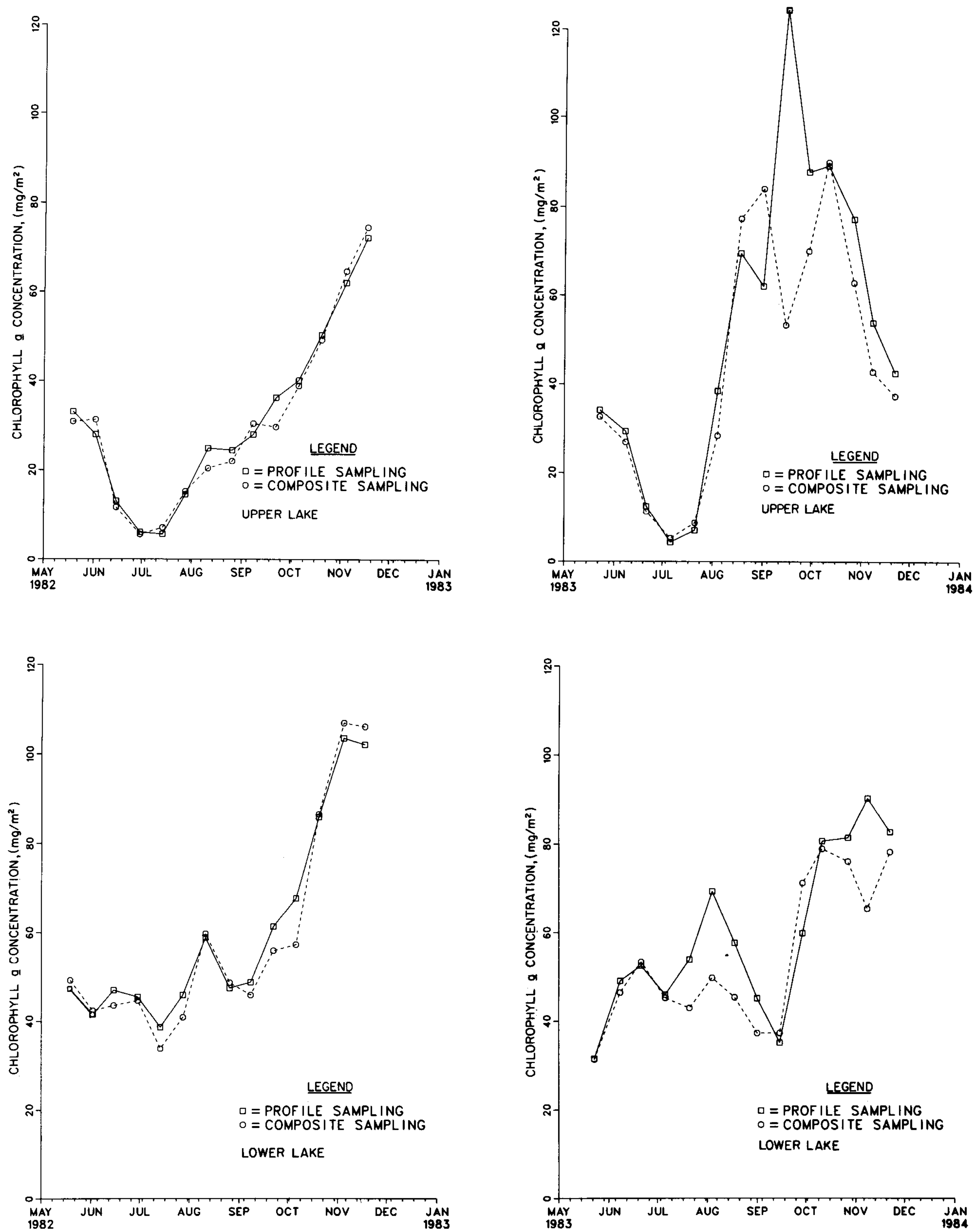

Figure 5. – Comparison of depth profile and composite sampling areal chlorophyll *a* concentrations (mg/m²) collected in Twin Lakes 1982-83.

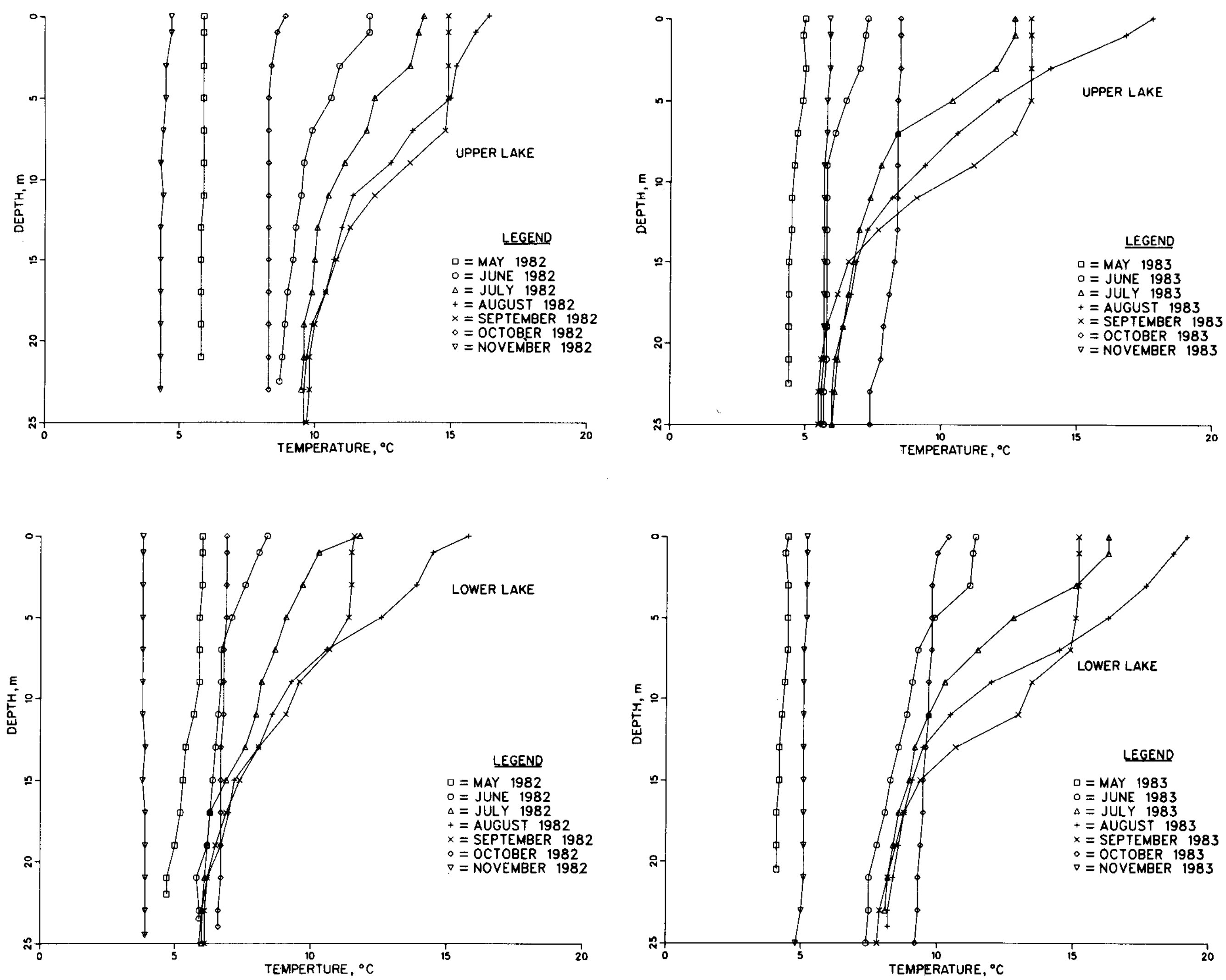

Figure 6. – Temperature profiles in Twin Lakes during the ice-free seasons in 1982 and 1983.

both an underestimation and an overestimation of areal values. A layer of reduced transmissivity was observed at 7.6 m in the upper lake on August 17, 1983. The difference in areal chlorophyll *a* concentration between the two sampling methods indicated that an underestimation of areal values might be occurring because the layer at 7.6 m was not sampled by the depth profiling method. A layer of reduced transmissivity at 8.7 m in the lower lake on the same sampling date nearly coincided with the routinely sampled 9 m depth interval for the depth profile sampling method. The difference in areal chlorophyll *a* concentration between the two sampling methods indicated that overestimation of areal values might be occurring because the layer at 8.7 m was being sampled by the depth profiling method.

Evidence provided by documenting layers of reduced transmissivity throughout the stratification period in 1983 confirmed the working hypothesis. Algal layers that did not coincide with point sampling depth resulted in underestimation of areal chlorophyll *a* concentration in the upper lake on two occasions in August 1983. Algal layers that coincided with point sampling depths resulted in overestimation of areal chlorophyll *a* concentration in the lower lake on three occasions in August-September 1983, and on two occasions in the upper lake in September 1983 (table 2).

Table 2 shows the change in depth of the observed layers from one sampling date to another. Short-term changes in both thermocline depth and location of algal layers have also been observed in Twin Lakes. In 1979, a 24-hour diurnal study was conducted at the lakes, with physical-chemical profiles and plankton sampling performed at 4-hour intervals. Thermal profiles indicated that the thermocline changed position in the water column as much as 2 m in the 4 hours between measurements. Thermal stratification

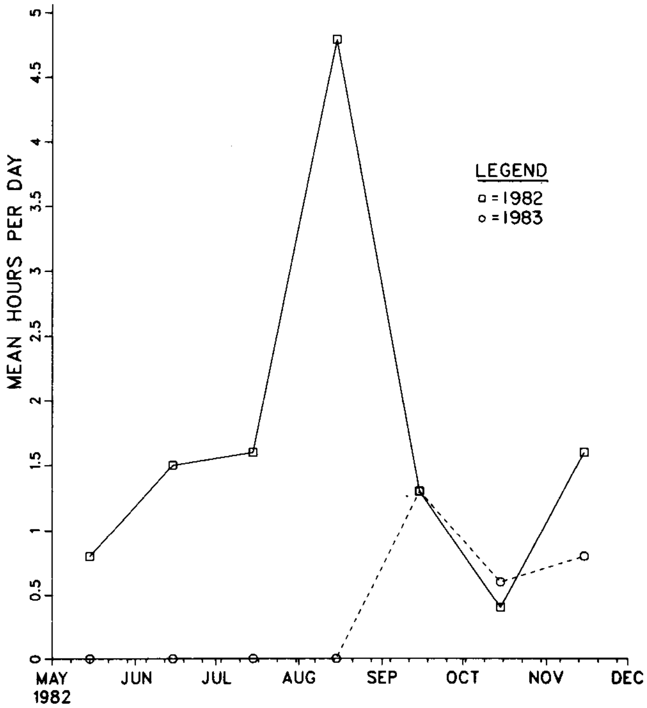

Figure 7. – Mt. Elbert Pumped-Storage Powerplant operation at Twin Lakes during the ice-free seasons in 1982 and 1983.

in Twin Lakes has always been rather weak, and the lakes experience considerable wind mixing daily. Summer thunderstorms occur frequently, particularly in July and August. Wind mixing of the lakes causes the thermocline to tilt downward with the prevailing wind.

During this 24-hour diurnal study, a concentrated layer of the chrysophycean alga *Dinobryon* was observed in the lower lake. On four of the times sampled, this layer coincided with a point sample collected at 9 m, the point at which the temperature change exceeded the 1 °C/m decrease criteria for classic thermal stratification. On the three other times sampled, neither the thermocline nor the algal layer was at the 9 m depth sampled. Obviously, at Twin Lakes, the position of both the thermocline and algal layers is transitory. They must be located using the transmissometer and thermal profiles performed at a specific time.

Two other items of interest in the summary shown in table 2 are the shift in the dominant net-sized algal species that occurred between the August 31 and September 14 samplings and the change in the chlorophyll *a* concentration when the species shift occurred. The shift from diatoms such as *Synedra* to chrysophycean algae such as *Dinobryon* is typical of species succession at Twin Lakes. The difference in chlorophyll *a* concentration results from the form in which these algae occur. *Dinobryon* occurs as a multicellular, branching colony, while *Synedra* occurs as single cells. Therefore, the total cell density when *Dinobryon* dominates the algal population is approximately 8 to 12 times greater than when *Synedra* dominates the algal population.

DATA ANALYSIS

Statistical analysis of the two areal chlorophyll *a* data sets collected in 1982 and 1983 was performed using chi square probability and linear regression analyses. The null hypothesis for probability of chi square was that the two sampling methods measured the same population of algae and, therefore, the results of depth profile and composite areal chlorophyll *a* estimations would be statistically the same.

Chi square analysis of data collected in 1982, indicated that the probability of X^2 is 0.98 in both lakes. As previously discussed, classic thermal stratification was not observed in the lakes in 1982, probably because the Mt. Elbert Pumped-Storage Powerplant operated enough to cause induced mixing of the water column. The null hypothesis could not be rejected, and thus the two sampling methods were not statistically different in 1982.

Chi square analysis of data collected in 1983 indicates that the probability of X^2 is 0.0, after the onset of classic thermal stratification in the lakes. Therefore, the null hypothesis must be rejected. The two sampling methods were statistically different in 1983, after the onset of thermal stratification.

Linear regression analysis of the two data sets was performed. Composite areal chlorophyll *a* estimates were regressed on depth profile areal chlorophyll *a* estimates. Regression of data sets collected in 1982, yielded an *r* (correlation) of 0.99 in both lakes. This supports the chi square analysis and, again, indicates that the two sampling methods were not statistically different in 1982. The regression of data sets collected in 1983 yielded a correlation of 0.86 for data collected in the lower lake and 0.80 for data collected in the upper lake. Although the 1983 data shows a strong positive relationship, the fit is not as good as the 1982 fit.

If the 1983 sample date corresponding with documented classic thermal stratification periods are eliminated from the regression analysis, $r = 0.98$ for the upper lake data sets and $r = 0.93$ for the lower lake data sets. The sampling dates eliminated from the regression analysis are indicated by an asterisk in table 1. Note that 6 of 14 sets were eliminated from the regression analysis on data sets from the lower lake, but only 4 of 14 sets were eliminated from the

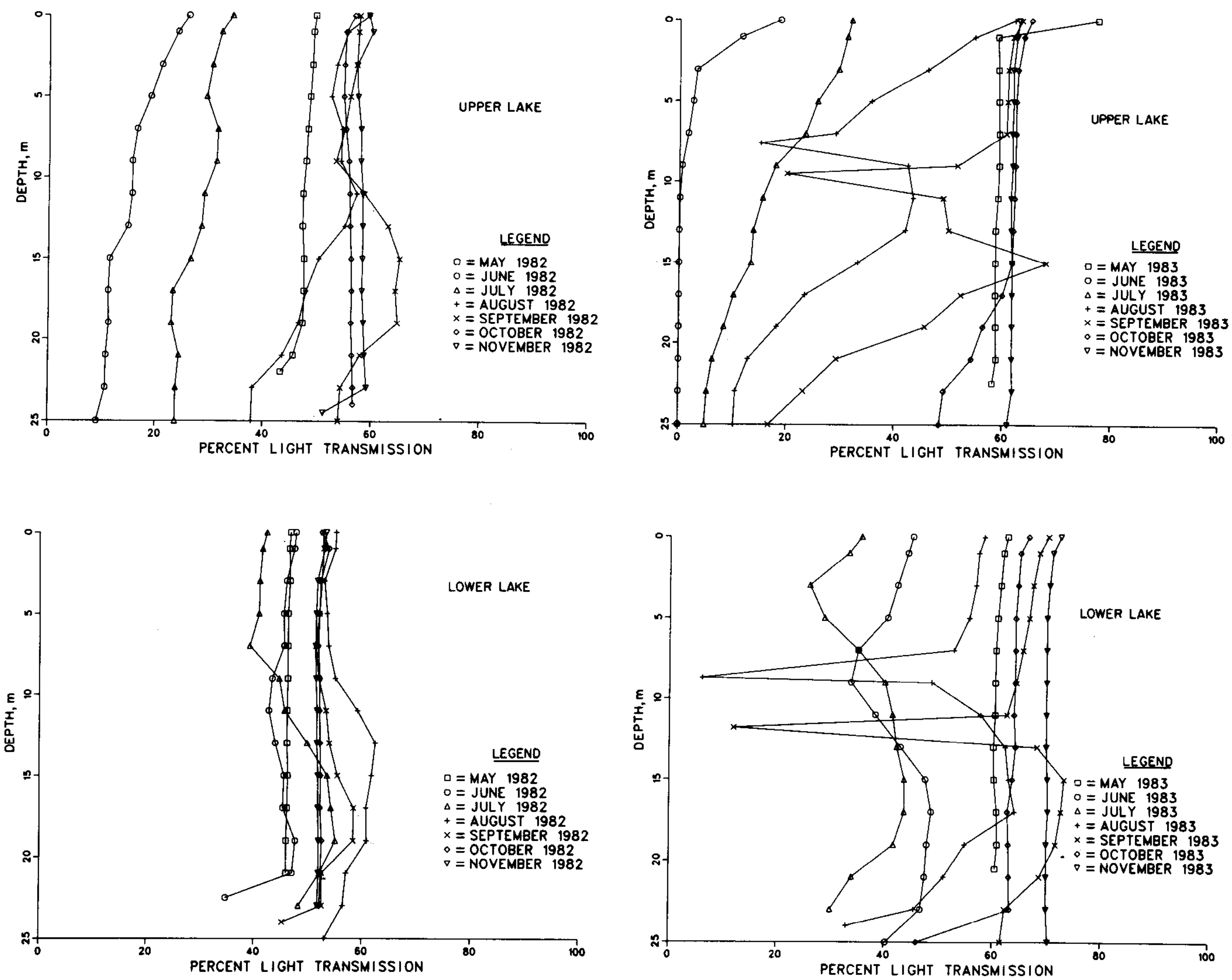

Figure 8. – Transmissivity (water clarity) profiles in Twin Lakes during the ice-free seasons in 1982 and 1983.

Table 2. – Results of algal layer monitoring during the thermal stratification periods at Twin Lakes, 1983.

Lake	Date	Depth of layer, m	Thickness of layer, cm	% Transmissivity of layer	Adjacent transmissivity, above/below	Chlorophyll *a* concentration in layer, mg/m³	Chlorophyll *a* concentration composite, mg/m³	Dominate plankton genus	Net-sized plankton abundance, No. cells
Lower	Aug. 17	8.7	20.3	6.1	52.8/48.7	–	3.03	–	–
	Aug. 31	9.5	40.0	28.8	49.3/66.3	12.51	2.49	*Synedra*	2 456 000
	Sept. 14	11.9	17.8	12.0	64.4/62.7	56.14	2.49	*Dinobryon*	14 192 000
Upper	Aug. 17	7.6	5.0	15.1	29.4/42.4	–	5.16	–	–
	Aug. 31	7.1	60.0	7.3	54.4/52.4	8.73	5.61	*Synedra*	570 667
	Sept. 15	9.5	40.6	20.0	51.6/49.0	25.99	3.56	*Dinobryon*	2 790 400
	Sept. 27	9.2	20.0	29.8	59.8/65.0	7.78	4.68	*Dinobryon*	311 200

upper lake data. The data collected in the upper lake on July 21 and August 4, 1983, were not eliminated from the regression analysis, although the technical criteria for thermal stratification were met on both sampling dates because the effects of annual spring runoff; i.e., increased turbidity and flushing rate, result in decreased chlorophyll *a* concentrations, particularly in the upper lake (Campbell and LaBounty, 1985) [9]. Chlorophyll *a* concentrations and the associated algal population in the upper lake in late July and early August, 1983, did not yet reflect an established algal assemblage.

It is not intended that the data sets be discarded from the regression analysis to make the statistical results show a better fit. The data sets where classic thermal stratification criteria could be applied were eliminated to verify the hypothesis that thermal stratification periods were associated with reduced correlation between the two sampling methods for estimating areal chlorophyll *a* concentration. The two methods seem to correlate well when the lakes are not classically stratified, but are not as closely correlated when the lakes are classically stratified.

CONCLUSION

A depth profile and a composite sampling method for extrapolating areal chlorophyll *a* concentrations were compared during the ice-free seasons in 1982 and 1983, at Twin Lakes, Colorado. Statistical analysis of data collected in 1982, indicated that the two methods were not statistically different. The probability of chi square was 0.99 for data from both lakes. But statistical analysis of data collected in 1983 indicated that the two methods were statistically different. The probability of chi square was 0.0, beginning with the onset of classic thermal stratification in the lakes. The major difference in conditions between these years was the absence of thermal stratification during 1982, when operation of the Mt. Elbert Pumped-Storage Powerplant resulted in induced mixing of the water column.

The working hypothesis for the comparison of the two methods was that the depth profile method could overestimate or underestimate areal chlorophyll *a* concentration. An overestimation could result when a thin, intense layer of algae occurred at a point routinely sampled in the depth profile collections; an underestimation could result when a layer occurred at a point not routinely sampled.

By monitoring the presence of algal layers and their depths in 1983, it was possible to see both parts of the working hypothesis verified. Underestimations of areal chlorophyll *a* concentration resulted when algal layers occurred at 7.6 and at 7.1 m in the upper lake. Overestimations resulted when algal layers occurred at the 9 m depth routinely sampled in depth profile collections.

Two important findings resulted from comparison of two sampling collection methods discussed in this report. The first is the ability to identify outlying data points that result from sample collection methods. When the effects of operating the Mt. Elbert Pumped-Storage Powerplant are assessed, the ability to sift the data base for such anomalies will be invaluable. The second, and perhaps more important finding, is the effectiveness of an alternative method for estimating trophic status. The composite method of collecting chlorophyll *a* samples seems to give a better estimation of trophic status or standing crop than do point samples collected in a depth profile manner.

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The Bureau's original purpose "to provide for the reclamation of arid and semiarid lands in the West" today covers a wide range of interrelated functions. These include providing municipal and industrial water supplies; hydroelectric power generation; irrigation water for agriculture; water quality improvement; flood control; river navigation; river regulation and control; fish and wildlife enhancement; outdoor recreation; and research on water-related design, construction, materials, atmospheric management, and wind and solar power.

Bureau programs most frequently are the result of close cooperation with the U.S. Congress, other Federal agencies, States, local governments, academic institutions, water-user organizations, and other concerned groups.