SPATIAL DISTRIBUTION AND HISTORICAL RECORD OF CHLOROPHYLL *A* IN MIRROR LAKE, NEW HAMPSHIRE

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Abstract. Chlorophyll filters were collected at 0.5-m depths in one location in Mirror Lake since 1996 and frozen for storage. The stored filters were analyzed to determine any temporal trends in Chlorophyll *a* concentrations. Viability of stored filters was determined from the Chlorophyll *a*: phaeophytin ratio. This ratio remained stable as far back as 1999, indicating little if any detrimental storage effects. Chlorophyll *a* concentrations were typically lower than 2 μg L^{-1} for the entire record, with some notable exceptions. There was a clear seasonal fluctuation in concentration with peaks observed during fall and winter. Concentrations have remained approximately the same (under 2 μg L^{-1}) throughout the years except for an increase since 2006.

Spatial distribution of Chlorophyll *a* was measured across the lake during July 2010 to determine whether concentrations differed between the pelagic, littoral, and inlet areas. Fluorescence measurements were recorded at 3 mid-lake stations, 8 shoreline sites, and 6 locations near the 3 inlets. Vertical profiles were determined at the 3 mid-lake stations as well. The vertical profile at the deepest region of the lake revealed a nearly 5-fold increase of Chlorophyll *a* with depth, with less variable concentrations in deeper waters. Chlorophyll *a* concentrations, which were usually under 2 μg L^{-1}, were approximately 53% higher at shoreline and inlet locations than at mid-lake stations (for the surface, 0.5-m, and 1-m depths).

INTRODUCTION

Mirror Lake is a clear-water, oligotrophic lake located in the White Mountains of New Hampshire and has been under continuous monitoring and research since 1965 (Likens 1985). It is 15 ha in area with an average depth of 5.75 m and a maximum depth of 11.0 m (Likens 2009). The lake has three inlets, West, Northwest, and Northeast, which drain 85 ha of the surrounding watershed, and a single outlet.

Previous studies on phytoplankton were conducted in the early 1970s and focused mostly on species composition. In 1972, 1974, and 1979, vertical profiles of Chlorophyll *a* (Chl-*a*) concentrations (mg m^{-3}) were determined for various months. The profiles for July showed that the surface water concentrations were typically between 1 and 3 mg m^{-3} and that these concentrations increased at greater depths, reaching anywhere between 3 and 18 mg m^{-3} (DeNoyelles and Likens 1985).

No studies, however, have been done on the horizontal distribution of phytoplankton, which prompted the question of whether there is spatial variation in Chl-*a* across the lake. Previous studies on the Laurentian Great Lakes have revealed variations in Chl-*a* between shoreline and offshore locations (Millie et al. 2002, Hall et al. 2003, Depew et al. 2006). Although Mirror Lake is a much smaller body of water, concentrations of different ions and nutrients vary substantially between the three different inlets and the lake (Buso et al. 2009) as does the composition of the zooplankton community between the limnetic and littoral regions (Makarewicz 1985). Thus, it

would be useful to know whether such differences would result in different concentrations of phytoplankton between the inlets, shorelines, and pelagic regions of Mirror Lake.

The low concentrations of algae found in Mirror Lake are co-limited by both nitrogen and phosphorus (Gerhart 1975; Gerhart and Likens 1975; Bade et al. 2009). The relatively pristine condition of the lake is important for maintaining ecosystem health and local aesthetic enjoyment. These conditions also enhance the opportunities to support short-term and long-term research studies. Monitoring of Chl-*a* of the lake has been continued since 1996 by filtering water at the 0.5-m depth at the deepest region of the lake and storing the filters in a freezer without further analysis. The second aim of this study was to assess the viability of the stored filters and obtain a historical record of Chl-*a* values from the past 14 years.

METHODS

Historical record

Sampling for Chl-*a* was begun in 1996 as part of regular monitoring of Mirror Lake and has been continued by Don Buso and Tammy Wooster of the Hubbard Brook Ecosystem Study. Filters were collected seasonally, approximately every 2 months, and 6 replicates were collected consecutively each time. The filters used for sampling were 25-mm diameter Whatman GF/F glass microfiber filters. All filter sets were stored in Petri dishes in the freezer. To determine if the Chl-*a* on the filters had remained viable, the ratio of Chl-*a* to phaeophytin (phaeo) was compared for all sets. Because phaeo is a breakdown product of Chl-*a*, a chronological data sequence should not show any decline in this ratio if Chl-*a* had not degraded in the stored samples throughout the years. If however, Chl-*a* in the older filters had degraded, this ratio should exhibit a decrease. The relative proportions of pigments and their breakdown products have previously been used to study the impacts of zooplankton grazing on phytoplankton (Head and Harris 1992; Landry et al. 1995) and the concentrations of phaeo are added in calculations of pigment budgets to account for degradation of Chl-*a* (Landry et al. 1994). Here the ratio of Chl-*a* to phaeo was used to detect any changes in the proportion of degradation products extracted from the stored filters.

The chl-*a* was extracted from the collected set of filters using a method without grinding. Instead, filters were soaked in 5 ml of basic methanol (0.5 N NaOH) for 24 h. The resulting extractions were read on a Turner 700 fluorometer (Welschmeyer 1994). The samples were then acidified with 50 µL of 0.3 N hydrochloric acid to convert Chl-*a* pigments into phaeo (by removing the magnesium atom from Chl-*a* molecules) and another fluorometer reading was made after 1 h. The first fluorometer reading gave a combined value of Chl-*a* and phaeo concentrations and the second reading was only of phaeo. Therefore, from the pre- and post- acidification readings, the concentrations in µg L^{-1} of Chl-*a* and phaeo were determined individually.

Spatial distribution

A Turner Designs Self-Contained Underwater Fluorescence Apparatus (SCUFA) was used to measure the in situ, spatial distribution of Chl-*a* in Mirror Lake on 8 separate days in July 2010. Sampling locations included 3 mid-lake stations, 8 shoreline sites, and 6 locations near the 3 inlets (Figure 1). Shoreline and inlet locations were chosen so they were as close to the shoreline as possible while still being 1-m deep (typically 2-4 m from the shoreline). An exception was the Northwest inlet, which was approximately one-third-m deep at the sampling site and 10 m from inlet/lake boundary. Fluorescence measurements were taken at the surface, 0.5-m, and 1-m depths for all locations and at every meter to the bottom for the mid-lake locations. Due to depth

limitation, only one measurement was recorded for the Northwest inlet. The GPS coordinates were noted for the major locations (Table 1). An additional measurement was made for each inlet that was closer toward the shore than the 1-m depth location.

To convert the relative fluorescence units into μg L^{-1} of Chl-*a*, grab samples of water were filtered from a vertical profile at the deepest mid-lake location. The Chl-*a* from these filters was extracted using the same methodology as for the stored filters and these values were compared to fluorescence measurements taken at the same time. The regression (Figure 2) was then used to calculate actual Chl-*a* units for all subsequent fluorescence readings.

Effects of turbidity were not accounted for in calculations of Chl-*a* values from florescence measurements for two reasons: (1) the water in the lake is very clear (Likens 2009) and naturally has low turbidity; and (2) because the regression line correlating the in situ and extracted values had a very high correlation ($R^2 = 0.95$) showing that any turbidity at the deepest depths (where it would have the greatest influence on measurements) is minimal.

RESULTS

Historical Record

The chl-*a* values were obtained for each sampling date from 1996 to 2010 (Figure 3). Because each sample typically contained 6 filters, the values were expressed as averages with one standard deviation. Years 1998 and 1999 show unusually high concentrations of Chl-*a*; it should, however, be noted that only 2 sampling dates in 1998 (7 January and 23 November) and one in 1999 (4 January) account for the striking difference between those years and the rest of the data set. For all subsequent years, concentrations of Chl-*a* remain below 2 μg L^{-1}.

The averages of Chl-*a* to phaeo ratios were also recorded for the period from 1996 to 2010 (Figure 4). The decreasing ratio indicated by the slope of the regression line is supported by the high ratios of 1998 and 1999. By excluding these early values, the stability of the Chl-*a* to phaeo ratio in the stored filters may be better represented (Figure 5). There is no significant increase or decrease of the ratio from 26 March 1999 onwards (p=0.92).

Focusing on the years from 2006 to 2010 for Chl-*a* concentrations (Figure 6) gives a clearer view of the regular seasonal variation exhibited in the lake. The chl-*a* remains low in the summer and then spikes up during the fall, typically after the fall turnover when nutrients in the deep water are mixed throughout the water column. Some years, such as 2008, display a peak during the winter (late December or early January). Winter peaks occurred about as often as fall peaks. The concentrations take a sharp dip during the late-March or early-April sampling dates before stabilizing at low concentrations for the summer. The period from 2006 to 2010 also exhibits an increase in Chl-*a* concentrations. Yearly high values increased more sharply than the yearly low values, ≈0.26 μg L^{-1}-yr and ≈0.07 μg L^{-1}-yr respectively.

Spatial distribution

The chl-*a* concentrations in μg L^{-1} were recorded for each location during July 2010 (Table 2). Concentrations were typically under 2 μg L^{-1} and showed appreciable variability across locations and sampling dates. For example, while the average of all dates at the 0.5-m depth for location 5 (Figure 1) was 0.48 ± 0.09 μg L^{-1}, the average at location 2 was 0.57 ± 0.48 μg L^{-1}. The chl-*a* concentrations were on average 53% higher at shoreline and inlet locations than at mid-lake stations (for the surface, 0.5-m, and 1-m depths). A notable exception was location 11, which

usually had the same or lower Chl-*a* values when compared to mid-lake locations. Concentrations at the inlets did not differ much from other shoreline locations (0.63 µg L^{-1} and 0.72 µg L^{-1}, respectively) but the concentrations at the West and Northwest inlet locations closer to the inlet were on average 53% higher than other shoreline locations.

The chl-*a* concentrations measured at location 1 (Figure 7) show a nearly 5-fold increase with greater depths. The epilimnetic waters (above 5 m) had an average concentration of 0.67 µg L^{-1} in 2 July, which decreased to 0.24 µg L^{-1} by 17 July, and increased to 0.52 µg L^{-1} by 30 July. The average concentration was 0.42 µg L^{-1} (± 0.17 SD). In contrast, the concentrations in the hypolimnetic depths (≥8 m) were higher and remained more constant throughout the month (≈1.97 µg L^{-1}, ±0.13 SD). The lowest variability in Chl-*a* was observed at 6 m (≈0.96 µg L^{-1}, ±0.08 SD). Mid-lake location 2 also showed decreasing variability with depth, with the lowest standard deviation also at 6 m (≈1.00 µg L^{-1}, ±0.06 SD). Although Chl-*a* at mid-lake location 3 increased with depth from 0.44 µg L^{-1} at the 1-m depth to 1.54 µg L^{-1} at the 7-m depth, there was no trend in variability.

DISCUSSION

Historical Record

The potential to obtain a solid long-term data set of Chl-*a* concentrations would provide useful information about Mirror Lake. Any observable patterns or trends could indicate not only changes in primary productivity, but also changes in lake physical or chemical properties. To verify the validity of the data obtained from the stored filters, the ratio of Chl-*a* to phaeo was plotted against time (Figure 4). The decreasing regression line for Chl-*a* to phaeo ratios seems to imply that breakdown products had accumulated throughout the years. This simple analysis, however, may be misleading due to the presence of a few high ratios in the early years (7 January and 23 November of 1998 and 4 January of 1999). From March 1999 onwards, however, the ratio actually remained very stable (Figure 5). It was expected that Chl-*a* breakdown product accumulation in filters would have shown an increase throughout the years, but most of the data exhibited no decrease or increase in the Chl-*a* to phaeo ratio. Thus, it was assumed that Chl-*a* data from all years, even the early ones, were valid.

It is possible that sampling during January and November of 1998 and January of 1999 was done during the early or middle stages of phytoplankton blooms. If that were the case, it would indicate that peaks in the grazing community lag behind peaks in the phytoplankton community as is common in many systems (Sommer et al. 1986). Because phaeo is an in situ product of grazing (Daley 1973), it would be found in minimal concentrations before an increase in grazing impact occurs, and sampling would, therefore, have revealed a high Chl-*a* to phaeo ratio. Skjoldar and Lännergren (1978) have also found this ratio to be high during a bloom in a Norwegian fjord as compared to the lower ratio during the collapse of the bloom. They, however, contribute this decline to the sinking of debris caused by nutrient starvation. In either case, it is plausible that the production of live phytoplankton biomass would exceed the production of breakdown products during bloom formation, which would lead to higher proportions of Chl-*a* than phaeo in the water.

The seasonal variation in Chl-*a* concentrations (Figure 6) followed a similar pattern throughout the years. Low summer values are suggestive of the nitrogen and phosphorus limitation characteristic of Mirror Lake (Bade et al. 2009). More than 50% of the input of nitrogen is retained in the lake and less than 50% of phosphorus (Buso et al. 2009). A pronounced spike in Chl-*a* normally occurred after fall turnover when mixing of the water column uplifts nutrients

from the hypolimnion and sediments. Even though the lake is dimictic, with complete fall and spring turnover (with the occasional spring meromixis), the same spike was not observed during the spring. The length of the mixing period may play an important role because spring mixing in Mirror Lake usually lasts for only a few days whereas fall mixing occurs over a longer period, typically lasting from August to October (Johnson et al. 1985). On the other hand, the 0.5-m sampling depth does not give a complete picture of the distribution of phytoplankton throughout the water column. If the winter peak observed at this depth were offset by very little phytoplankton biomass at other depths, then the total biomass could actually have been lower in the winter than summer. If this were the case, then less organic matter may have settled to the bottom during the winter and the uplifting of hypolimnetic water at the end of winter would have provided less nutrients than it would have after summer.

Winter Chl-*a* concentrations remained high at the 0.5-m depth. Contrary to what was expected due to limited irradiance reaching the water surface because of snow and ice cover, many winter Chl-*a* values exceeded those of the fall values. Although species composition was beyond the scope of this study, it is possible that the change in biomass in winter may have been linked to seasonal variations in species abundance. During a study from 1968 to 1971, Chrysophyceae, Diatomeae, Peridineae, and Cryptophycaeae dominated the phytoplankton community of Mirror Lake during both the summer and winter months (DeNoyelles and Likens 1985). Out of the 53 most common species recorded, 52 were most abundant during the ice-free season, 20 were found year round, and 29 during the winter season.

The 0.5-m sampling depth may also have been conducive to error caused by melt water. Warmer weather and melt water under the ice were observed during many of the winter sampling periods when the Chl-*a* peak for that time had occurred during the previous fall. The Chl-*a* concentrations from those samples may have been diluted by melt water. Dilution may also have played a role in producing the low spring Chl-*a* values.

There has been an increase in Chl-*a* concentrations since 2006 (Figure 6). Values increased more sharply for seasonal highs (≈0.26 μg L^{-1}-yr) than for seasonal lows (≈0.07 μg L^{-1}-yr). If this trend continues, it is possible that the increase in biomass during phytoplankton blooms will be more pronounced than any increase in yearly average biomass. This increase in Chl-*a* values suggests that Mirror Lake, though oligotrophic, has the capacity to support more productivity.

Spatial Distribution

Concentrations of Chl-*a* increased with greater depth at mid-lake locations. The same trend was also observed at the deepest region of the lake in July of 1972, 1974, and 1979 (DeNoyelles and Likens 1985), though the recorded values were much higher, ranging from about 3 to 18 μg L^{-1}. Deep water peaks in phytoplankton have also been observed in other lakes such as oligotrophic Lake Tahoe (Kiefer et al. 1972) and it is suggested that the sinking of phytoplankton from the euphotic zone is the contributing factor to the greater biomass observed in the aphotic zone. It is also noted that the sinking and accumulation of phytoplankton biomass in the deeper waters of Lake Tahoe may be responsible for reducing the rates of nutrient regeneration in the upper layers. In Mirror Lake, the accumulation of phytoplankton in the hypolimnion due to sinking from the epilimnion may have prevented eutrophication during the summer stratification period. Because nutrients are continuously relocated to the hypolimnion without regeneration in the upper waters from mixing, deep-water peaks and shallow-water lows would be plausible during the summer season. However, this mechanism may have been responsible for generating blooms in the fall after the turnover redistributed these nutrients throughout the water column.

The higher Chl-*a* concentrations observed at the shorelines and inlets may suggest a higher availability of nutrients in these regions perhaps by inputs from the streams, groundwater seeps, or allochthonous material from the shore. Though nutrient concentrations were not measured at the sampling sites, it seemed that there were mechanisms at play that determined the variability of Chl-*a* at different regions across the lake. Because the shoreline locations were only a meter deep, it is also reasonable that data at these locations showed the same variability as the epilimnetic layers in the pelagic regions. During the summer stratification period, water mixes horizontally across the epilimnion of the lake more than it does vertically with the hypolimnion (Johnson et al. 1985). Therefore, any changes in the condition of surface water at one location that could potentially influence primary production would have a greater impact on the surface water elsewhere than on the relatively isolated hypolimnion.

Comparison of changes in water chemistry with the dynamics of primary productivity could give more insight into the stability and functioning of aquatic ecosystems. Studies of Chl-*a* distribution patterns could be an important avenue of future research at Mirror Lake, especially considering the potential for changes in land use and associated nutrient export to the lake.

CONCLUSION

Historical Record

- Data from 14 years of stored Chl-*a* filters provided valid estimates of Chl-*a* concentrations in Mirror Lake.
- Chl-*a* concentrations at the 0.5-m depth fluctuated seasonally with highs in the fall and winter and lows in spring and summer.
- Chl-*a* concentrations remained approximately the same after 1999 except for an increase during the period from 2006 to 2010 (≈0.26 μg/L-yr for seasonal highs and ≈0.07 μg/L-yr for seasonal lows).

Spatial Distribution

- Chl-*a* concentrations were on average 53% higher at shoreline and inlet locations than at mid-lake stations (for the surface, 0.5-m, and 1-m depths), suggesting differences in nutrient availability.
- Chl-*a* concentrations increased with depth and were more stable in deeper waters.

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APPENDIX

TABLE 1. GPS coordinates of sampling locations on Mirror Lake (Figure 1). UTM format NAD83. Locations 7, 9, and 12 (inlets) had an additional sampling location nearby (a few meters closer to the inlet/ lake boundary).

#	UTM zone 19 north
1	0283941mE 4869201mN ± 5m
2	0284019mE 4869017mN ± 9m
3	0283832mE 4869152mN
4	0283930mE 4868977mN ± 6m
5	0283794mE 4869044mN ± 8m
6	No coordinates.
7	0283719mE 4869132mN ± 2m
8	0283706mE 4869261mN ± 5m
9	0283720mE 4869360mN ± 5m
10	0283837mE 4869373mN ± 5m
11	0284020mE 4869256mN ± 6m
12	0284085mE 4869200mN ± 7m
13	0284160mE 4869029mN ± 9m
14	0284036mE 4868704mN ±17m

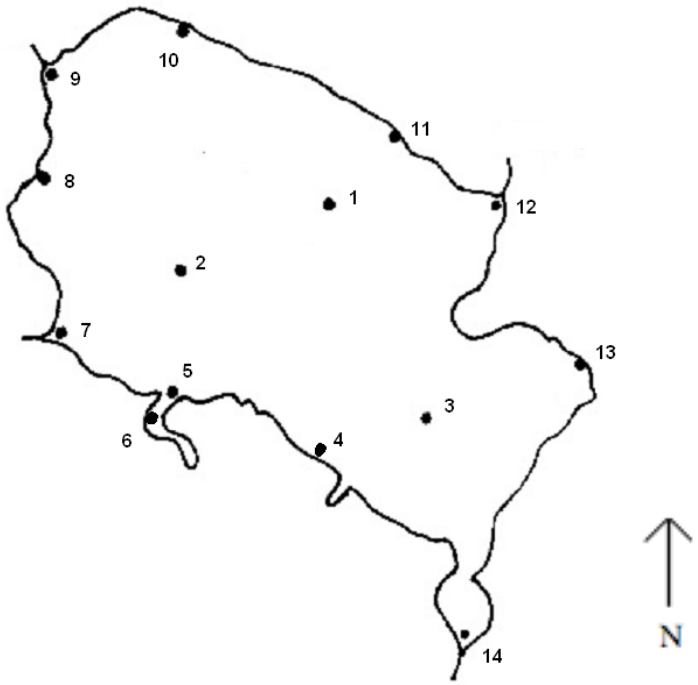

FIGURE 1. Sampling locations in Mirror Lake. Locations 7, 9, and 12 (inlets) had an additional sampling location nearby (a few meters closer to the inlet/ lake boundary).

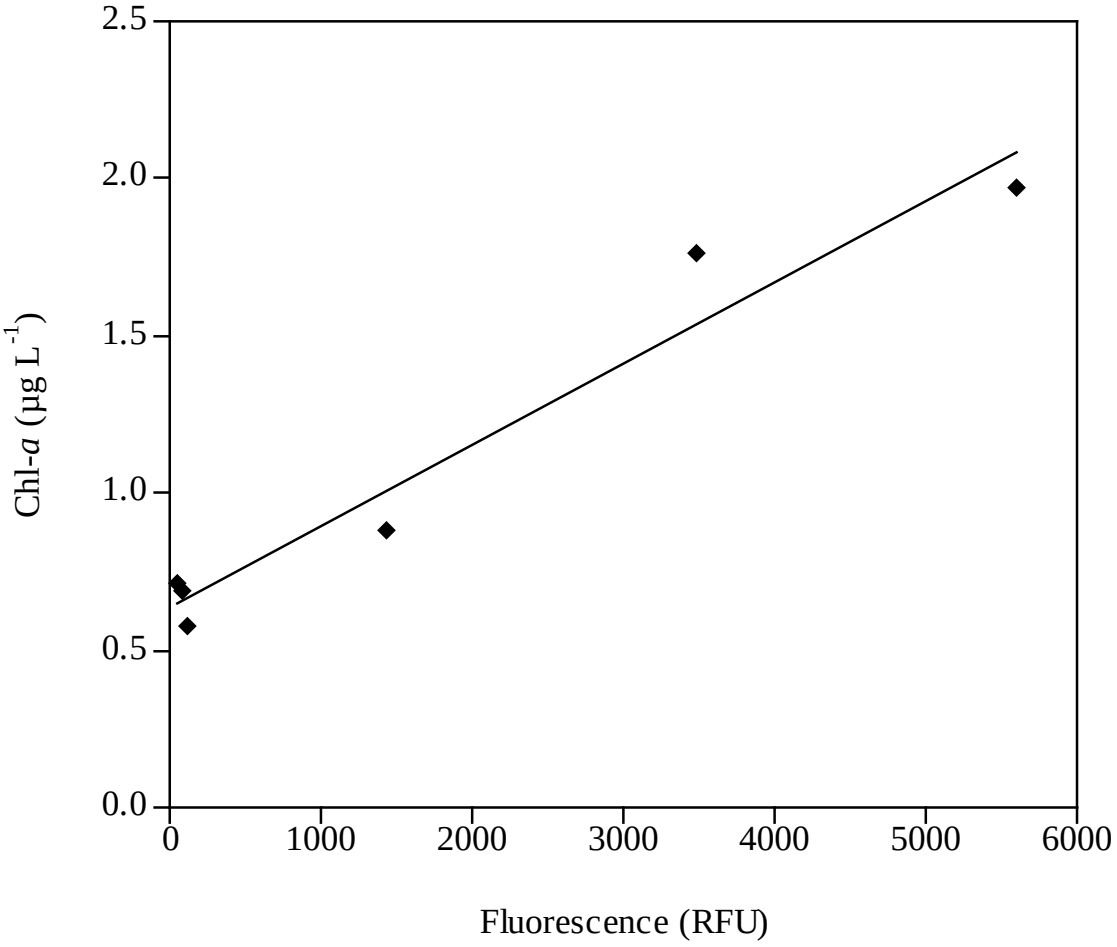

FIGURE 2. Calibration of SCUFA measurements using extractions of Chl-*a* from filters from 0, 0.5, 2, 4, 6, and 8 m depths at sampling location 1 (deepest region of the lake). Fluorescence measurements are in relative fluorescence units (RFU). Regression: $y = 0.6371 + 0.0002581x$; $R^2 = 0.95$; $p < 0.01$.

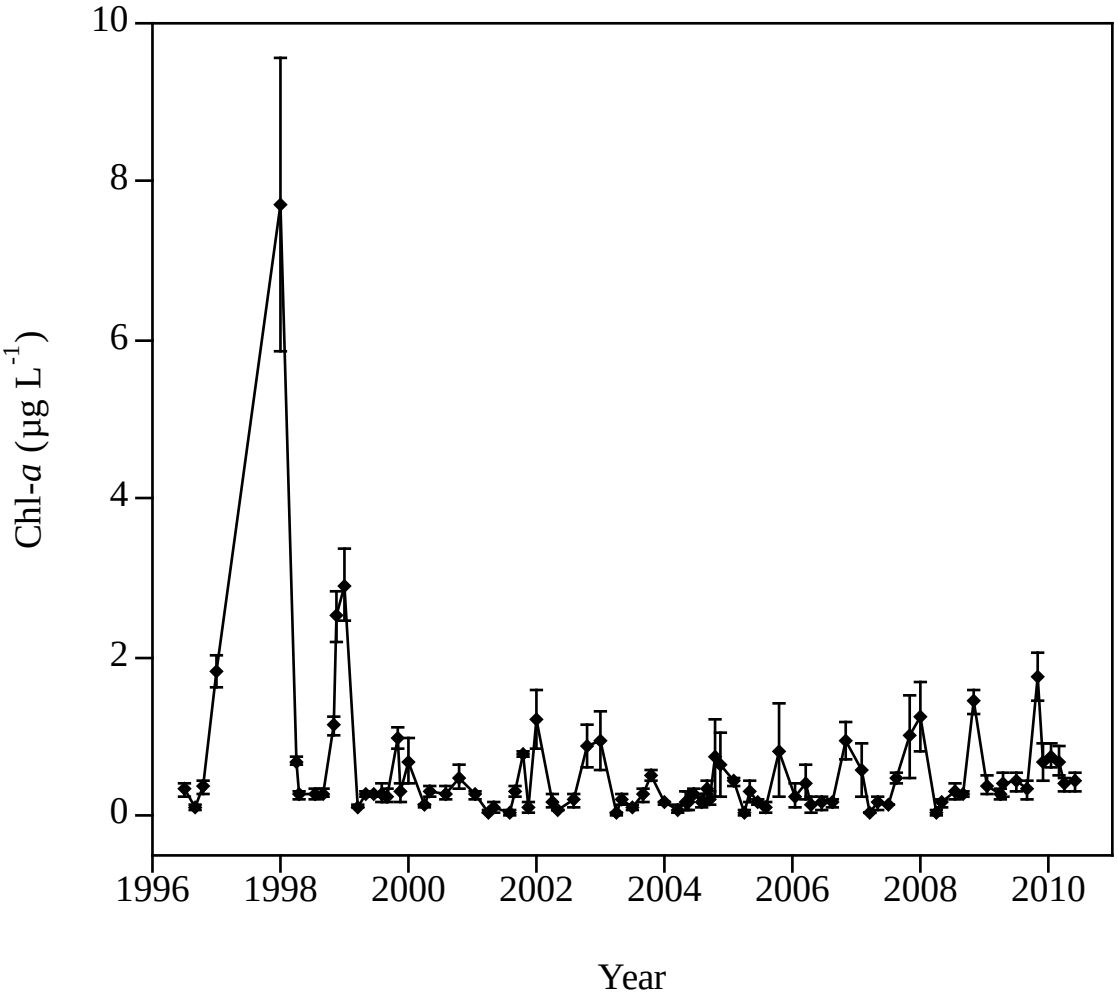

FIGURE 3. Average Chl-*a* concentrations with one standard deviation from 1 July 1996 to 4 June 2010. Each value represents the average from 6 filters collected at the 0.5 m depth at the deepest region of the lake.

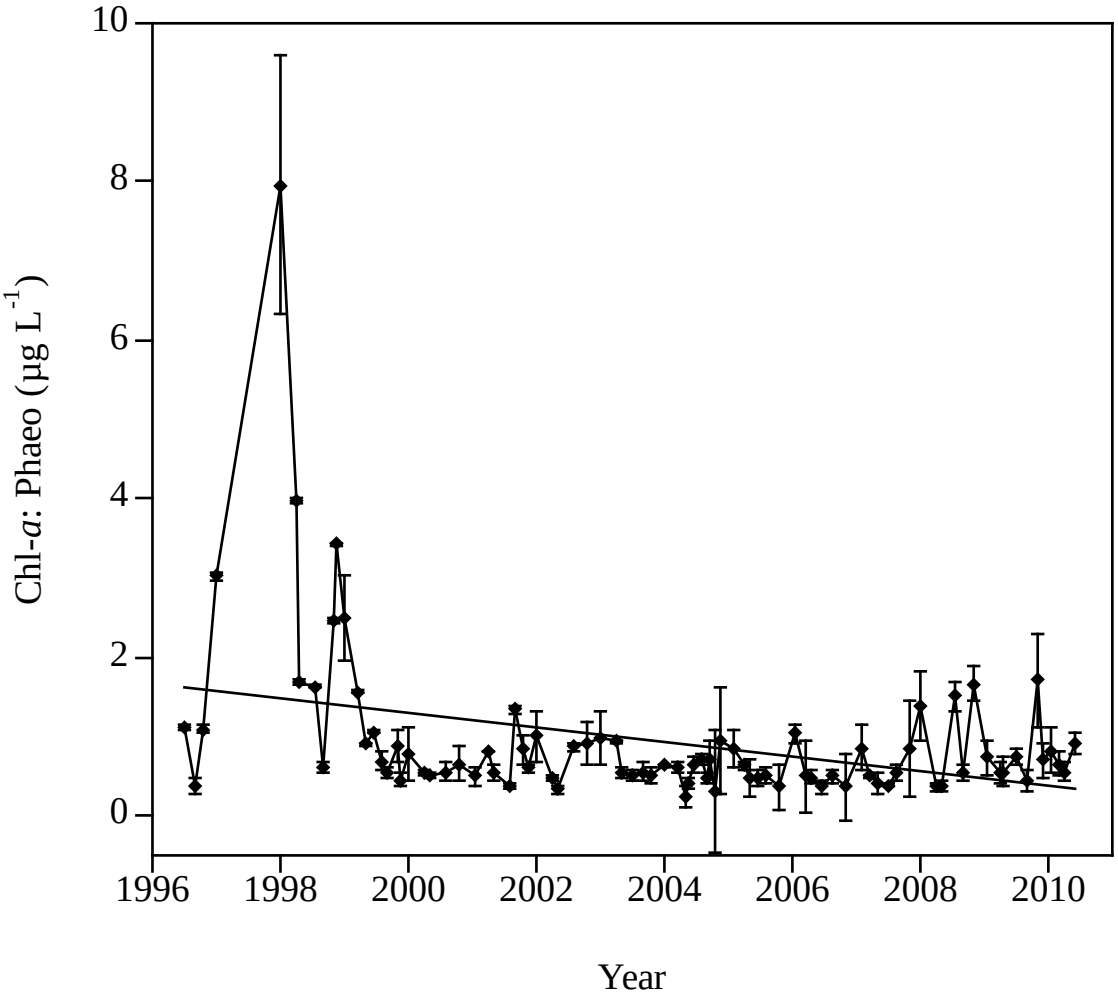

FIGURE 4. Average Chl-*a* to phaeo ratios with one standard deviation from 1 July 1996 to 4 June 2010. Each value represents the average from 6 filters collected at the 0.5 m depth at the deepest region of the lake. Regression: $y = 10.131 – 2.9164e\text{-}9x$; $R^2 = 0.13$; $p < 0.01$.

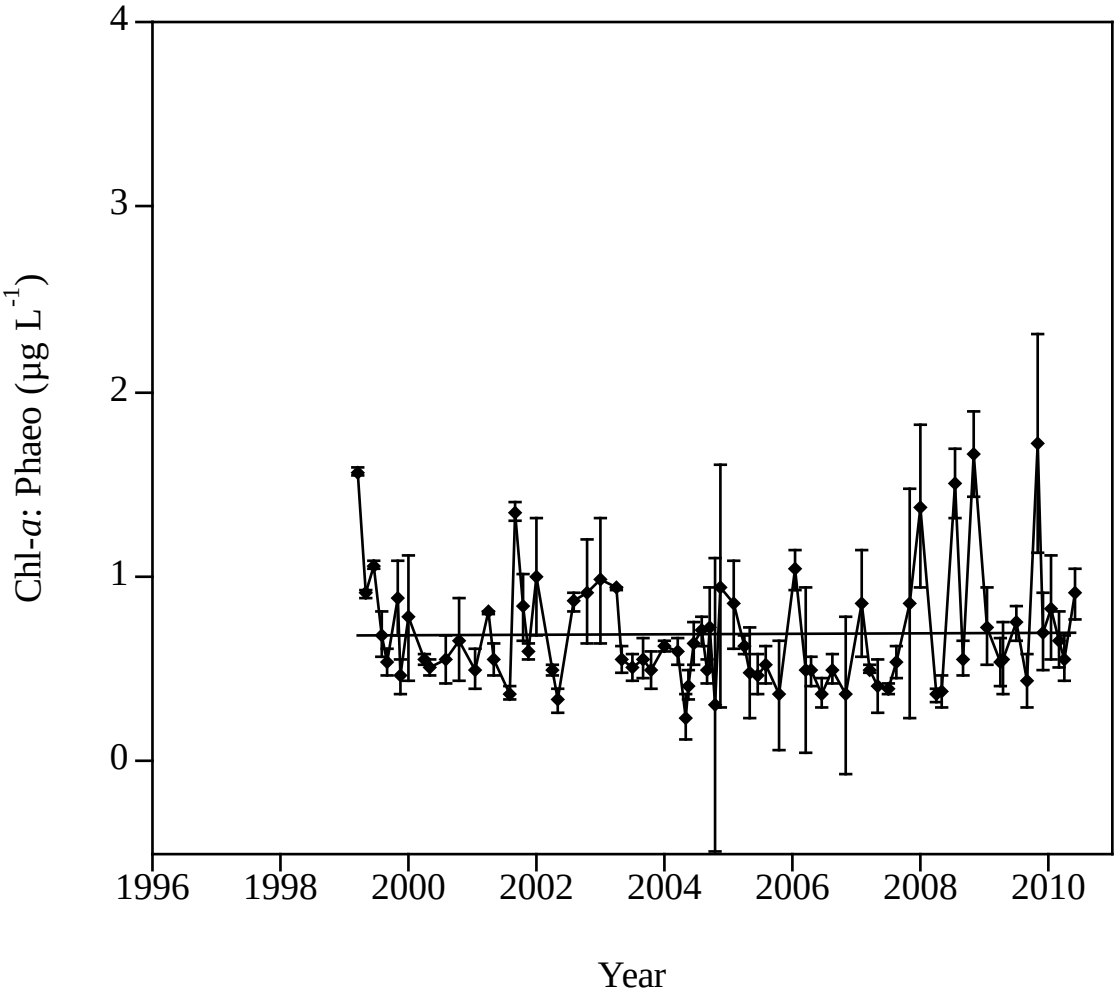

FIGURE 5. Average Chl-*a* to phaeo ratios with one standard deviation from 26 March 1999 to 4 June 2010. Each value represents the average from 6 filters collected at the 0.5 m depth at the deepest region of the lake. Regression: y = 0.57661 + 3.6398e-11x; $R^2 < 0.01$; p = 0.92. The slope of the regression indicates a more stable ratio when the outliers of the early years are excluded.

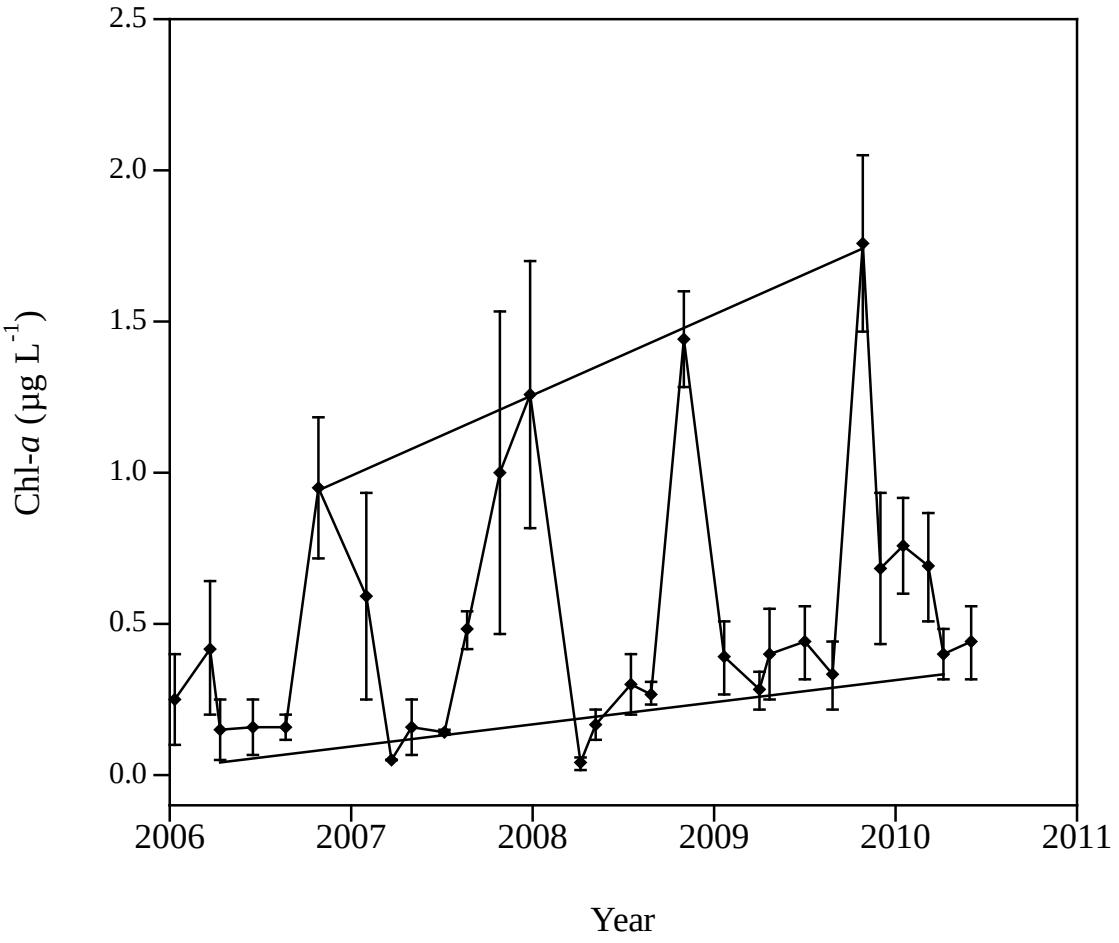

FIGURE 6. Average Chl-*a* concentrations with one standard deviation from 13 January 2006 to 4 June 2010. Each value represents the average from 6 filters collected at the 0.5 m depth at the deepest region of the lake. Regression lines are drawn for both the high and low trends. Regression for peak values: y = -26.271 + 8.3873e-9x; R^2 = 0.99; p < 0.01. Regression for low values: y = -7.4725 + 2.3271e-9x; R^2 = 0.56; p = 0.09.

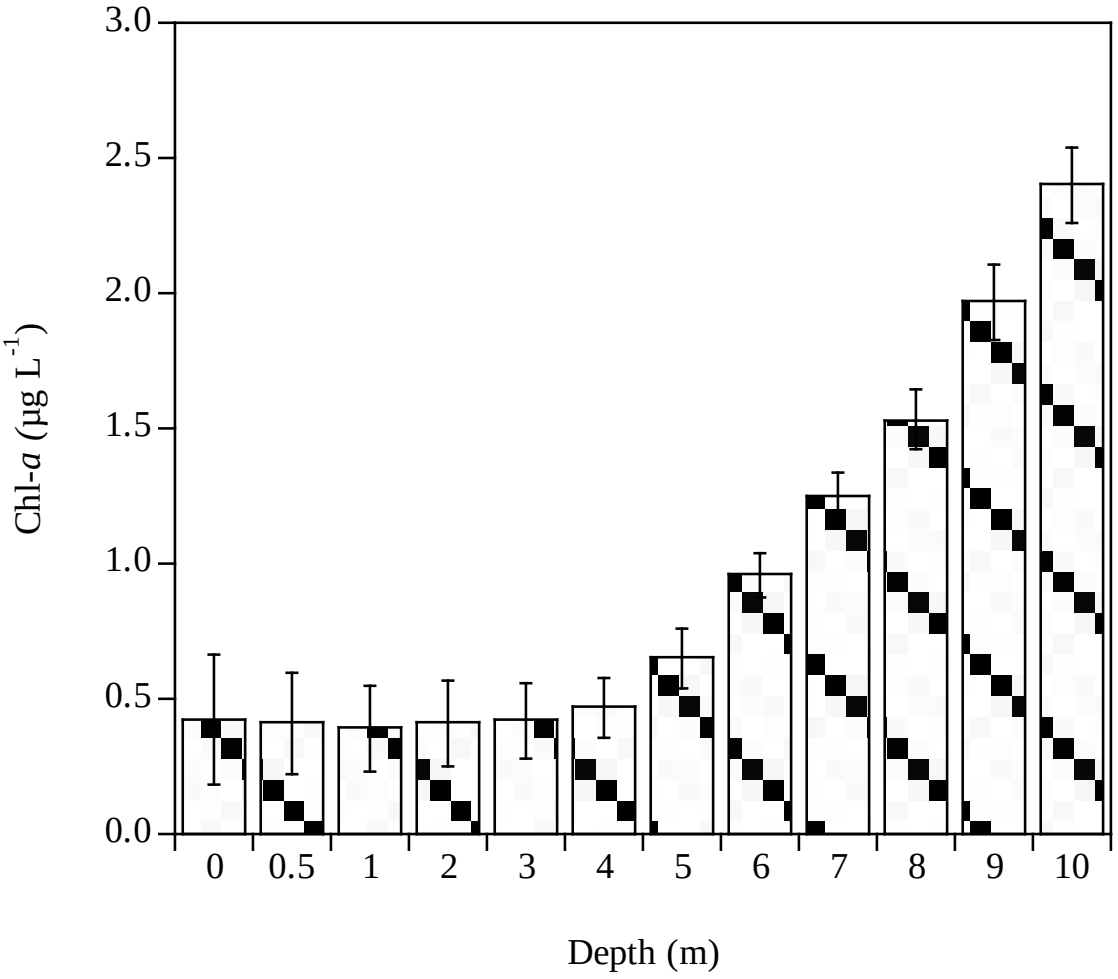

FIGURE 7. Average Chl-*a* concentrations with one standard deviation at various depths at location 1 (deepest region of the lake). Each bar represents the average from 8 sampling dates during 2-30 July 2010.