A Comparison of Chlorophyll Collection and Analysis Methods: The New England Lakes and Ponds Project

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Introduction

One of the most problematic aspects of cooperative regional studies is the standardization of the various sampling and analysis methods used by each of the participating groups. This is most prevalent in the analysis of chlorophyll, which is collected and analyzed in a unique manner by each of the state and regional labs within New England. In an effort to understand the variation resulting from each lab's techniques, chlorophyll samples were collected from lakes sampled by the EPA in 2006 as part of the New England Lakes and Ponds Project.

Methods

Ten replicate samples were collected from most lakes: one each for the state labs of Connecticut (CT), Maine (ME), Massachusetts (MA), New Hampshire (NH), one for the Watershed Watch lab at the University of Rhode Island (URI), one for the Lakes Lay Monitoring Program Lab at the University of New Hampshire (UNH), and three for the Vermont (VT) state lab for replicate analysis.

A composite sample was collected then sub-sampled according to the appropriate agency's standard methods and delivered to the respective lab for analysis (Table 1). Given the amount of time invested in the collection and processing of these samples, each lab was only provided with one filter, thus sacrificing replication. The lack of replicate samples excluded the use of common methods such as ANOVA in the analysis of the data. To look for trends in the data, I examined the percent deviation of each lab's result for each lake from the mean value as measured by all 7 labs. This analysis was also performed on lakes grouped by trophic status as provided by each lake's state monitoring program.

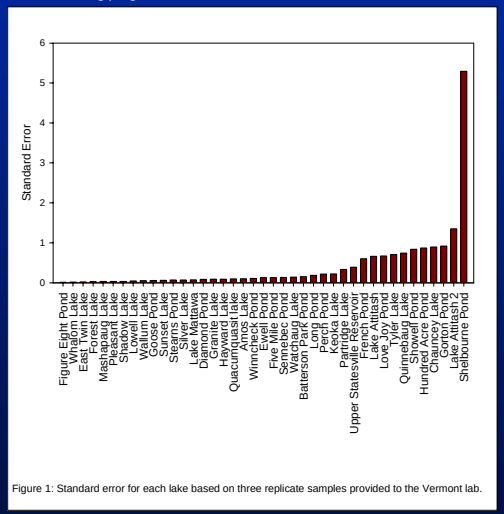

Figure 1: Standard error for each lake based on three replicate samples provided to the Vermont lab.

Table 1: Summary of collection and analysis methods of state labs as well as the Lakes Lay Monitoring Program lab at the University of New Hampshire (UNH) and the Watershed Watch lab at the University of Rhode Island (URI).

Lab	Sample Volume	Filter Type (pore size)	Preservation	Extraction	Analysis
CT	250 mL	GF/F (0.7 μm)	Filtered and Frozen	90% acetone	Fluorometric
MA	15 mL	GF/C (1.5 μm)	Filtered and Frozen	90% acetone	Fluorometric
ME	100 mL	Membrane (0.45 μm)	$CaCO_3$; Filtered and Frozen	90% acetone	Trichromatic
NH	100 mL	Membrane (0.45 μm)	Filtered and Frozen	90% acetone	Spectrophotometric
URI	50 mL	GF/F (0.7 μm)	MgCO3; Filtered and Frozen	90% acetone	Fluorometric
UNH	100 mL	Membrane (0.45 μm)	Filtered and Frozen	90% acetone	Spectrophotometric
VT	250 mL	GF/F (0.7 μm)	Filtered and Frozen	90% acetone	Fluorometric

Figure 2a: Oligotrophic Lakes

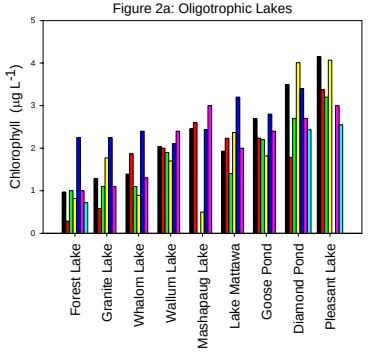

Figure 2b: Mesotrophic

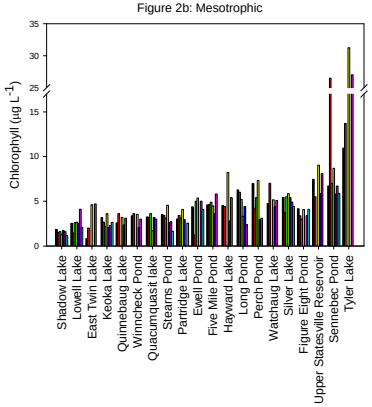

Figure 2c: Eutrophic Lakes

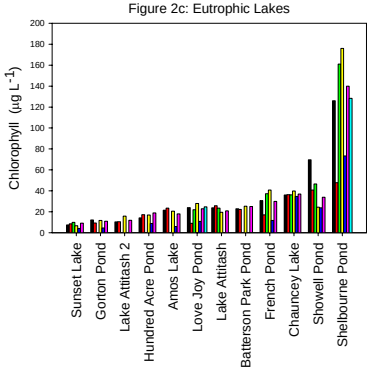

Figure 2: Chlorophyll a values (μg L^{-1}) reported by each lab for each lake in the study based on trophic status provided by each state. Bar colors are as follows: Vermont: black, Connecticut: red; Massachusetts: green; New Hampshire: yellow; Lakes Lay Monitoring Program, University of New Hampshire: blue; Maine: pink; Watershed Watch lab, University of Rhode Island: cyan

Figure 3a: All Lakes

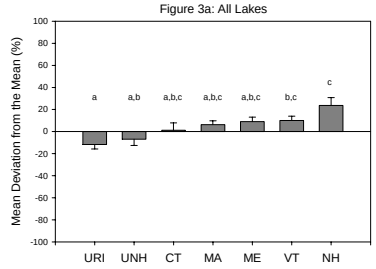

Figure 3b: Oligotrophic Lakes

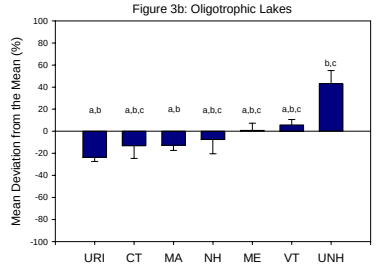

Figure 3c: Mesotrophic Lakes

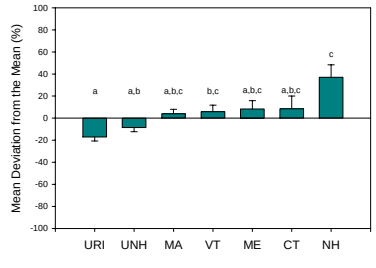

Figure 3d: Eutrophic Lakes

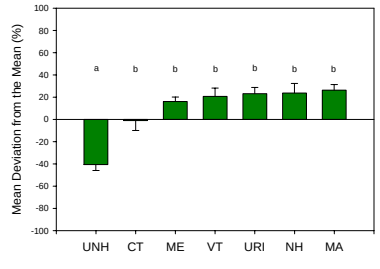

Figure 3: Average percent deviation from the mean Chlorophyll a value for all labs in the study. Different letters indicate statistically significant differences based on ANOVA or ANOVA on Ranks when appropriate. Abbreviations are as follows: Vermont: VT; Connecticut: CT; Massachusetts: MA; New Hampshire: NH; Lakes Lay Monitoring Program, University of New Hampshire: UNH; Maine: ME; Watershed Watch Lab, University of Rhode Island: URI.

Results

Analysis of the three replicate samples provided to Vermont (Fig. 1) indicates a high level of consistency between each of the sub-samples collected, which would imply the same level of consistency for each of the labs.

Examination of the raw data shows a great deal of variability in results for each lab (Fig. 1.) As previously stated, there were no statistical analyses performed on the data in this format; this has been presented simply to show the inter-lab variability for each lake.

The average deviation from the mean has been presented for each lab for all lakes, as well as by trophic status of the lakes (Fig 3a-d). When looking at the entire data set, we see that NH state results exhibit the greatest average deviation from the mean, 23.2% higher than the mean. Vermont, ME, and URI have similar deviations (9.7%, 10.0%, and -12.3%, respectively.) The deviations for UNH and MA are lower still at -5.2% and 5.5%, respectively. Connecticut, however, nearly matches the mean with a deviation of only 1.0%. Deviations at opposite ends of the spectrum differ significantly, with URI differing from NH state and VT, while UNH differs from NH only (Kruskal-Wallis ANOVA on Ranks; $p<0.001$.)

Interestingly, these same relationships are not maintained when the data is divided by trophic status. In oligotrophic lakes (Fig. 3b) UNH exhibits the greatest deviation (about 40%). Rhode Island's results are still below average, but so are CT, MA, and NH's. Maine's results nearly match the average in this group of lakes. In this case UNH differs only from URI and MA (Kruskal-Wallis ANOVA on Ranks; $p=0.004$.)

In Mesotrophic lakes NH has the greatest deviation and UNH's results are closer to, yet below, the average. Rhode Island's results are the only others below average. Massachusetts, ME, VT, and CT are all above average and deviate by less than 10%. New Hampshire's results are significantly different from URI and UNH (Fig 3c; Kruskal-Wallis ANOVA on Ranks; $p<0.001$.) Eutrophic lakes exhibit the most variability and deviation (Fig 3d). For eutrophic lakes, UNH is below average by approximately 40% and significantly different from each of the other states (ANOVA; $p<0.001$). Connecticut is also below average, but only slightly, especially when compared to the deviation exhibited by the others. Each of the other states' results are approximately 20% above average.

Discussion

It is evident that the specified methods for each of the labs involved in this study are capable of producing drastically different results (Figs. 2 & 3.) The results produced by UNH are the most variable across trophic conditions, yielding relatively high results for oligotrophic lakes, low (but within 10%) results for mesotrophic lakes, and relatively low results for eutrophic lakes (Fig. 3). Similarly, NH produced deviations that were greater than the others' for lakes in the mesotrophic range. Rhode Island, as well, consistently deviates from the mean, always by more than 10%. Massachusetts, ME, CT, and VT often yielded statistically similar results (within 10% of the mean), but not in all cases. In the absence of replicate samples, we can merely infer, based on the precision of VT's results (Fig. 1), that this deviation is the result of differences in filtration and analysis techniques.

Statistically different results were only generated by comparing the labs with the greatest positive deviation to those with the greatest negative deviation (Fig. 3). Perhaps more conclusive evaluations could be made if replicate samples could be collected. Adding replication to this relatively large sample set of lakes of various trophic status from around the region would expand the number of statistical tests that could be performed and greatly improve the power of the study.

Acknowledgements

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