Lake and Reservoir Management, 28:10–26, 2012
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ISSN: 0743-8141 print / 1040-2381 online
DOI: 10.1080/07438141.2011.646458

Factors determining the distributions of total phosphorus, total nitrogen, and chlorophyll *a* in Florida lakes

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Abstract

Bachmann RW, Bigham DL, Hoyer MV, Canfield DE Jr. 2012. Factors determining the distributions of total phosphorus, total nitrogen, and chlorophyll *a* in Florida lakes. Lake Reserv Manage. 28:10–26.

Using data from 1387 lakes collected over 3 decades, we found a wide range in the concentrations of total phosphorus (TP), total nitrogen (TN) and chlorophyll (Chl-*a*) in Florida lakes, and that edaphic factors as outlined by the United States Environmental Protection Agency's Florida Lake Regions were dominant in determining the concentrations of plant nutrients in the state's lakes. The hypothesis that the majority of the eutrophic lakes in Florida without known point source pollution are the result of nonpoint source nutrient pollution was tested in several ways and rejected. There was no correlation between the Landscape Development Intensity index and the concentrations of TP, TN, and Chl-*a* examined in Florida lakes. Several of Florida's 30 benchmark lakes (lakes with minimal human impact and meeting designated uses) were eutrophic, and there was no significant difference between the mean concentrations of TP and TN in these lakes versus all remaining Florida lakes. Paleolimnological studies also showed that several lakes were eutrophic to hypereutrophic prior to 1900, a time before significant population growth in the State of Florida. To help develop numeric nutrient criteria for Florida lakes that take regional differences into account, we grouped similar lakes into 6 TP zones and 5 TN zones.
[Supplemental materials are available for this article. Go to the publisher's online edition of Lake and Reservoir Management *to view the supplemental file.]*

Key words: chlorophyll, eutrophication, Florida, Lake Regions, nitrogen, paleolimnology, phosphorus

The United States Environmental Protection Agency (USEPA) has established numeric nutrient criteria for Florida lakes (USEPA 2010a) stipulating that any lake with an alkalinity >20 mg/L as $CaCO_3$ is out of compliance if the water has an average chlorophyll concentration >20 μg/L, which would classify the lake as eutrophic by the USEPA's definition. For Florida lakes with an alkalinity ≤ 20 mg/L, the lake must meet oligotrophic criteria, with an average chlorophyll concentration no greater than 6 μg/L, to be in compliance. Applying these criteria, about 44% of Florida's lakes are deemed "impaired." When a lake is placed on the impaired list, an expensive series of monitoring and planning actions are initiated, so a good scientific basis is important for the numeric nutrient criteria being enforced.

There are several implied assumptions behind USEPA's rules. Because the law regulates alterations in the nutrient concentrations of lakes and not their natural, unaltered concentrations, it is assumed that in the past all Florida lakes were either oligotrophic or mesotrophic. This means that all current eutrophic lakes have been subject to sufficient anthropogenic increases in nutrient loading to shift them into the eutrophic state, and that regulation of nutrient inputs will shift them to a mesotrophic state. It further implies that oligotrophic lakes in Florida can be identified as those with an alkalinity of 20 μg/L or less.

The objective of our study was to test the above assumptions. We started by examining the current geographic distributions of total phosphorus (TP), total nitrogen (TN) and chlorophyll *a* (Chl-*a*) in Florida lakes to determine if significant differences from one part of the state to another could be related to natural factors like soils and geology. Three approaches were used to examine the potential

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anthropogenic increases in nutrient concentrations in Florida lakes. We compared the nutrient concentrations of a large sample of Florida lakes to those in a group of lakes with no or few human activities in their watersheds (i.e., benchmark lakes) to determine if the 2 groups were different. We looked for correlations between nutrient concentrations in lakes using the Landscape Development Intensity index (LDI), a GIS-based index of human development around the lakes. Finally, we looked at the results of paleolimnological studies of lake sediments done by others to determine if there were eutrophic lakes in Florida prior to 1900.

General background on Florida lakes

With about 7700 lakes with surface areas of 4 ha (10 ac) or larger, Florida is one of the United States' major lake states along with Maine, Michigan, Wisconsin, and Minnesota. Unlike those northern states where glaciers have been the major geological force in shaping the landscape, Florida has a different and limnologically important geological history. Florida's complex geology and physiogeography relative to lakes has been discussed elsewhere (e.g., Canfield 1983, Canfield and Hoyer 1988), so we present only a brief summary here.

The bedrock beneath Florida consists of thick layers of limestone covered by sediments eroded from the Appalachian Mountains to the north that were reworked as cycles of significant sea level changes redistributed these materials and produced a diverse landscape. Some lake basins represent former marine lagoons and bays, while others were formed when sinkholes developed in the underlying limestones, causing the overlying materials to collapse and form depressions that hold water. In most cases, the lakes on sandy soils of northern Florida and on the ridges in the center of the peninsula are filled with waters of low dissolved solids, pH, hardness, and alkalinity. In the broad valleys of peninsular Florida, the groundwaters feeding the lakes are more likely to have contact with the carbonate rocks, so the lakes have waters with higher levels of dissolved solids, pH, hardness, and alkalinity (Canfield and Hoyer 1988).

Of particular importance to the numeric nutrient criteria issue facing Florida, and a major factor making Florida lakes different from others in the United States, is the presence of vast deposits of phosphorus-containing minerals that underlie several areas of the state (Lane 1994). These phosphatic deposits were laid down in ancient shallow seas by processes that are not fully understood. Chen and Ma (2001) have shown that these deposits are not uniformly distributed across the state but are related to differences in the TP content of the various orders of soils found in Florida. Likewise, Terziotti et al. (2010) presented maps showing an uneven geographic distribution of phosphorus in the sediments of first-order Florida streams, reflecting differences in the parent materials in their watersheds. In some areas of Florida, phosphatic deposits are of sufficient richness to support mining for agricultural fertilizers, and 75% of the phosphorus used for commercial fertilizers in the United States each year comes from Florida (FGS 2010). Surface or ground waters that flow over these deposits dissolve some of the phosphorus and contribute to elevated concentrations of phosphorus in Florida's springs, rivers, and lakes (Odum 1953). In setting nutrient criteria for Florida streams, the USEPA (2010c) established 6 different Nutrient Water Regions to recognize the considerable regional variability in the concentration of TP and TN in Florida streams related to soils and geology.

With the exception of some sinkhole lakes with depths of up to 30 m, most Florida lakes are shallow (<5 m) and well mixed. At least 70% of the lakes have no surface inlet or outlet, making it difficult to determine the surface watershed area or to calculate inputs of water and/or nutrients. The lakes are generally warm all year, but some of the northern Florida lakes have rare instances of overnight surface freezing during record colds. The trophic states of Florida lakes range from oligotrophic to hypereutrophic, with a broad range of chemical compositions (Canfield and Hoyer 1988, Griffith et al. 1997).

Human impacts on most Florida lakes are relatively recent. The 1900 census shows a state population of only 529,000 people, with most of the population concentrated on the coasts. The population in 2000 was 15,982,000, an increase of almost 3000%, or 30 times. Point source pollution has been a problem in the past because some lakes were once recipients of sewage effluents or other organic materials such as waste from citrus processing, although those point sources have been largely controlled. Another major impact is the extensive drainage projects carried out during Florida's development to make swampy areas habitable for agricultural and settlement projects. Many lakes were drained or their water levels lowered over the past century (e.g., Lake Apopka and Lake Okeechobee). At some lakes subject to heavy withdrawals from surface aquifers, deep injection wells along with structures help stabilize water levels.

What is not clear due to a lack of documentation is the overall impact of nonpoint source nutrient enrichment on Florida lakes. Florida has been aggressive in nonpoint control activities. For example, there currently are large nutrient reduction programs at lakes Apopka, Okeechobee, and others as a part of the total maximum daily load (TMDL) program. The Florida Department of Environmental Protection (FDEP) administers a statewide Stormwater Permitting Program adopted in 1979 that requires the treatment of stormwater from all new developments. The success of some of these efforts has been questioned (Canfield et al.

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2000, Bachmann et al. 2003); however, Terrell et al. (2000) showed that in recent years nutrient levels in a large sample of Florida lakes have not increased as the human population of Florida has grown. Terrell et al. (2000) examined trends in TP concentrations in a sample of 127 Florida lakes during a 27-year period (1970–1996) and found that, in spite of a 116% increase in the human population of Florida in that time period, in-lake TP levels decreased.

The Florida Lake Regions

The USEPA along with many Florida scientists recognized that USEPA's level III Ecoregions for the United States (Omernik 1987) were too broad to encompass the diversity of Florida lakes and that subregions were needed for water quality management purposes. Consequently, a collaborative project between the USEPA, the FDEP, and the University of Florida's LAKEWATCH program was initiated in the 1990s, resulting in the establishment of 47 Florida Lake Regions (Griffith et al. 1997). The multiple regions were established as a basis for managing Florida lakes based on their natural characteristics (Appendix Fig. A1) and are important for developing nutrient criteria because they allow differentiation in expected trophic states. The lakes within a specific region were grouped together because there is homogeneity in the types and quality of lakes and their associations with landscape characteristics, or there is a particular mosaic of lake types and quality (Griffith et al. 1997). The boundaries between the regions also generally followed those on soil maps. Thus, the different regions represented a manifestation of the differences in geology, soils, and hydrology from one part of the state to another, resulting in a patchwork appearance when the lake regions are represented on a map (Griffith et al. 1997). The USEPA (2010a) considered using the Florida Lake Regions in their approach for establishing numeric nutrient criteria, but rejected their use because of the high number of regions and the limited data for some regions.

Methods

Data collection

We analyzed TP, TN, and Chl-*a* data collected from 1387 Florida lakes covering all 47 lake regions (Appendix Fig. A2). Supplemental data on some lakes included measurements of Secchi disk depths, mean depths, total alkalinity, and color. Some lake regions have only a few lakes, so 10 or fewer lakes represent 11 of the lake regions (Appendix Table A1). Most of our data (1293 lakes) were collected by the Florida LAKEWATCH program, but they also included all lakes used by Griffith et al. (1997) in formulating the Florida lake regions. For most of the LAKEWATCH lakes, 3 stations were sampled each month, and the station values were averaged to find a monthly average. The monthly averages were used to find an annual average, and the annual averages were used to find an overall average for the lake. The dataset covers about 65,500 monthly samplings. We also included lake data collected by the FDEP and the water management districts for lakes that were not in the LAKEWATCH dataset. While the LAKEWATCH and FDEP protocols are not exactly the same, the LAKEWATCH procedures are subject to a quality control process, and the results have been verified in comparisons between volunteer samplers and professionals (Canfield et al. 2002). The 2 sampling approaches have been shown to produce results that are not statistically different from each other (Appendix C; Canfield and Bachmann 2010). Florida LAKEWATCH has not made the phaeophytin correction for its chlorophyll *a* measurements since its inception in 1986 because of reported errors in the correction procedure (Stich and Brinker 2005) of errors in the correction procedure (Riemann 1978). Because the proposed numeric nutrient criteria are based on corrected chlorophyll *a*, we developed an empirical correction factor to convert LAKEWATCH measurements to corrected chlorophyll *a*. We combined our adjusted measurements (93% of the 1387 lakes) with the corrected chlorophyll *a* values of FDEP and refer to them as Chl-*a* (see Appendix C).

Comparison with lakes from the National Lake Assessment

To compare the trophic states of Florida lakes with those nationwide, we examined the diatom-inferred values for TP as determined from the bottoms of short sediment cores taken from a probabilistic sample of lakes across the United States in 2007 (USEPA 2010b). Protocols from 293 cores indicated that the sediments at the bottom of the cores represented predisturbance conditions. We ranked the estimated TP values and determined the percents that fell into the various trophic state categories using the guidelines of Forsberg and Ryding (1980).

Size distribution of lakes in our sample

We tested to see how closely the distribution of lake surface areas of our sample lakes matched the distribution of lake surface areas of lakes across the State of Florida. Lake size can be important in determining lake chemical characteristics, so it is often taken into account in regional surveys of lakes such as the National Lake Assessment (USEPA 2010b) to determine how many waters in the area have certain limnological characteristics. The study of Schafer et al. (1986) listed 3298 named lakes in Florida. We compared the frequency distributions of surface areas of the named lakes with the lakes sampled in this study.

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Data analysis

Unless noted otherwise, we used common logarithms of the variables in our analyses. To find geometric means, we took the antilogarithms of the averages of the transformed variables. The lake data were subsequently sorted by lake regions, and frequency distributions for TP, TN, and Chl-*a* were developed for each lake region. We then characterized each of the lake regions by their geometric mean TP, TN, and Chl-*a* concentrations. For some analyses, we calculated the deviations of individual lake values for TP, TN, and Chl-*a* from the lake region mean values. The distribution of these deviations represents the frequency distributions for TP, TN, and Chl-*a* in Florida lakes with the effect of lake regions removed.

Establishment of nutrient zones for phosphorus and nitrogen

From the standpoint of the FDEP, using 47 lake regions was unwieldy for regulation; therefore, we grouped together lake regions with similar chemical characteristics into a smaller number of nutrient zones for TP and TN. To establish the TP zones, we ran an analysis of variance (ANOVA) using the JMP statistical package to find the geometric mean concentrations of TP in each of the lake regions and then compared all means with each other using the Tukey-Kramer HSD test. The results of these comparisons were used to group the lake regions into 6 trial TP zones. We then used ANOVA and Tukey-Kramer HSD to determine if the geometric mean values for TP in each of trial TP zones were significantly different from each other and also to determine if the geometric mean values for TP in the lake regions within each trial TP zone were different from each other. In an iterative process, lake regions were moved from one trial TP zone to another until we developed 6 phosphorus zones where the distributions of TP in each lake zone were significantly different from the distributions in each of the other zones. The same process resulted in 5 TN zones that were very similar to but not the same as the phosphorus zones, thus providing a statistical basis for zone establishment. Because the original lake regions were delimited in part in conjunction with information on soils, physiography, geology, vegetation, climate, and land use/land cover, as well as relying on the expert judgment of local limnologists (Griffin et al. 1997), these factors would carry over to the larger nutrient zones.

Effects of color and alkalinity

Separate analyses were made on subsets of our data, such as the 537 lakes with data on water color and total alkalinity, the 1001 lakes with color data, and the 359 lakes with mean depth measurements. We made statistical analyses to determine relationships between TP, TN, and Chl-*a* and independent variables such as mean depth, color, and alkalinity.

Use of benchmark lakes

The lakes in our sample currently have no known direct point sources of pollution, but they may have important nonpoint sources of nutrients like municipal stormwater; therefore, we wished to determine the possible extent of such enrichment (i.e., potential impairment) for our lakes as a group. We compared our sample of lakes with a group of 30 lakes established by the FDEP as "benchmark lakes" (FDEP 2009). In their report they stated:

> The nutrient benchmark distributional approach builds on methodologies originally developed to quantify human disturbance for biocriteria development purposes. By FDEP's definition, nutrient benchmark sites are only influenced by low levels of human disturbance, enabling full support of the most sensitive designated use—i.e., support of a healthy, well-balanced population of fish and wildlife. FDEP intends to use the upper end of nitrogen and phosphorus frequency distributions from benchmark sites to define nutrient thresholds that FDEP expects to be both defensible and reliable for protection of aquatic life in Florida waters.

In selecting these lakes, FDEP biologists examined aerial photos, conducted an onsite watershed survey to ensure there were no adverse human influences undetected by the LDI, and performed a whole-lake habitat assessment. Candidate benchmark lakes were excluded if they had current or a history of adverse human activity (FDEP 2009). Thus, the benchmark lakes are as close to natural conditions as we might find in Florida, and the nutrient concentrations in these lakes do not impair their most sensitive designated use of supporting a healthy, well-balanced population of fish and wildlife (FDEP 2009). The nonbenchmark lakes are representative of Florida lakes with a wide variety of human activities that could potentially raise lake nutrient concentrations to higher levels, resulting in use impairment. Therefore, if significant nutrient pollution were associated with human activities, the benchmark lakes should on average have significantly lower nutrient concentrations than nonbenchmark lakes. The difference between the 2 lake groups should then be representative of the degree of cultural eutrophication in Florida lakes.

We initially compared the nutrient levels in the benchmark lakes with those in the larger sample of Florida lakes. We used t-tests to compare the geometric mean TP and TN values of the benchmark lakes with the geometric mean values

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for TP and TN in all lakes sampled. For a more precise test that took into account the variations in the geometric mean values for TP and TN in the different lake regions, we also placed each benchmark lake in its respective USEPA Florida Lake Region. For TP we used a one-way ANOVA with TP as the dependent variable and the lake region category and the category of benchmark lake or nonbenchmark lake as independent variables. A similar analysis was made for TN.

We also found the difference between the benchmark lake concentrations of TP and TN and the geometric mean value for the lake region in which it was located. These represented deviations from the geometric mean value for that lake region. A negative value for the deviation indicates that the benchmark lake had a lower concentration of TP or TN than the geometric mean of the other lakes within that lake region, while a positive deviation indicates that the benchmark lake had a higher concentration of TP or TN than the geometric mean of the other lakes in that lake region. This process effectively removes the effects of differences between lake regions or zones and allows us to test for the effects of whether a lake is classified as a benchmark or nonbenchmark lake. A t-test was used to compare the mean deviations in the benchmark lakes with those in the nonbenchmark lakes. Paired t-tests also were used to compare benchmark lake TP concentrations with their lake region mean TP concentration. A similar test was made for TN.

We also used models to estimate the European presettlement average TP concentrations starting with the current TP values in our sample lakes on the assumption that originally only 5% of the lakes were eutrophic based on a threshold of 30 μg/L of TP. The rest of the currently eutrophic lakes (38%) were assumed to have been mesotrophic prior to cultural eutrophication. For one model we reduced the TP concentrations in each of the current eutrophic lakes by the same proportion until 95% of all of the lakes in the modeled population had TP concentrations no greater than 30 μg/L. In the second model we carried out similar proportional reductions in TP for both the current mesotrophic and eutrophic lakes. We also made power analyses to determine the minimum difference between the geometric mean concentrations for the benchmark lakes and nonbenchmark lakes that could be detected given the same sample size, geometric mean, and variance for the nonbenchmark lakes and the same sample size and variance for the benchmark lakes.

The Landscape Development Intensity index

To provide another estimate of the potential impact of human activities on Florida lakes, we used the LDI, a GIS-based measurement of human activities within 100 m of the shoreline of a lake (Brown and Vilas 2005). The method was developed for watersheds of wetlands in Florida and was evaluated by Mack (2006) for land areas surrounding wetlands in Ohio. He found the LDI was significantly related to other human disturbance gradient metrics used on watersheds around the same wetlands and also was correlated with their Vegetation Index of Biotic Integrity for those watersheds. The LDI has also been used by the USEPA (2010c) to estimate nutrient inputs to Florida streams from surrounding watersheds as a part of their process to develop numeric nutrient criteria for Florida streams. The LDI method recognizes 27 different land uses, and each land use has a coefficient ranging from 1 for natural open water to 10 for high intensity central business district uses. The fractional area of each land use in the band is multiplied times the respective energy coefficient for that land use, and the sum of the products is taken as the LDI. The higher the LDI values, the higher the inferred anthropogenic impact on the lake. The developers (Brown and Vilas 2005) found that a 200 or 500 m band was no better than a 100 m band for the use of the LDI around wetlands. Whether a 100 m band is sufficient to assess human impacts on lakes can be questioned, but many professionals and everyday lake users assess human impacts based on shoreline development. The FDEP has been using the LDI approach and was able to supply the LDI values for 1131 lakes in our sample.

We initially calculated correlations between the LDI and the TP, TN, and Chl-*a* concentrations in all lakes to examine the potential effects of human activities. We also calculated correlations between the LDI and the deviations of lake TP, TN, Chl-*a* concentrations and Secchi disk depths from their lake region geometric means for all lakes. The LDI values for those lakes were also included in a multiple regression analysis along with the nutrient zone means. Finally, we determined an average LDI value for all the lakes in our sample for each lake region and correlated it with the average lake TP, TN, Chl-*a* concentrations and Secchi depths for the respective lake regions.

Paleolimnological analyses

We used the results of previous paleolimnological studies to determine if eutrophic lakes occur naturally in Florida. Some of the past studies in Florida involved the use of long sediment cores where examinations are made of the physical, chemical, and biological properties of the sediments at several depths below the modern sediment surface to reveal the past history of the lakes. We reviewed studies on Florida lakes where past trophic states were determined through the examination of sediments at several different depths in a core (Riedinger-Whitmore et al. 2005, Brenner et al. 2006).

We also analyzed data from sediment cores for 32 Florida lakes summarized by Whitmore and Brenner (2002).

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Diatom-inferred concentrations of TP were reported for the surface sediments and for a deeper sample (about 1 m) with an average date of 1881. The bottom sample represents a time when the population of Florida was small, so anthropogenic influences are assumed to be minimal. From 1 to 4 cores were analyzed for each lake, and the TP values were inferred using the WACALIB computer program by Line et al. (1994).

A second dataset contains lakes sampled in 2007 as a part of the National Lake Assessment (USEPA 2010b). Diatoms were used to infer both TP and TN for their cores. A protocol was established to determine if the sediments at the bottoms of their cores represented predisturbance conditions, and the cores from 9 lakes met their criteria. Two of the lakes, Yale and Griffin, were in both datasets, so we have 39 different lakes in our sample.

We used these data to find the pre-1900 concentrations of TP in the sample lakes. We also made comparisons of the conditions represented by the sediments at the top of the core with those deposited prior to 1900 to detect changes (Hall and Smol 1996). We used paired t-tests to look for differences between the top and bottom values for all lakes as a group.

To determine which lakes had statistically significant differences ($\alpha = 0.05$) between the top sample and the deeper sample, we used the root mean square error of prediction (RMSEP) term to calculate 95% confidence limits for the differences between the diatom-inferred TP concentrations published by Riedinger-Whitmore et al. (2005) for the 32 lakes reported by Whitmore and Brenner (2002). The error was reported as 0.387 ln units, which we converted to 0.168 Log_{10} units to conform to our calculation units. For the 9 lakes sampled by the USEPA (2010b), the error terms for TP were 0.36 Log_{10} units and for TN 0.29 Log_{10} units, which represent the standard deviation for the diatom-inferred value for a TP or TN concentration when expressed in logarithmic units. Next we determined the standard error of the difference between the means of the paired diatom-inferred values (equation 1) following Sokal and Rohlf (1995). Appendix B has a detailed explanation of the statistical tests used.

$$S_{\text{diff}} = s\sqrt{2/n} \tag{1}$$

where S_{diff} = the standard error of the difference between the top and bottom values; S = the standard deviation for a single diatom-inferred value; and n = the number of cores being averaged.

Also following Sokal and Rohlf (1995), the 95% confidence limits for the difference between the top and deeper values are given by

$$\text{Confidence limits} = \pm t_{05} S_{\text{diff}} \tag{2}$$

where t_{05} = the value for t found in a 2-sided table of t with a probability of 95% and with $(N_c - 1)$ degrees of freedom; and N_c = the number of lakes in the calibration dataset, which was 69 for the Whitmore and Brenner (2002) lakes and 500 for the USEPA (2010b) lakes.

Lakes where the difference between the diatom-inferred values at the top and deeper levels of the cores exceeded the 95% confidence limits had a statistically significant difference. While several paleolimnologists have used the RMSEP itself to determine significant differences between 2 diatom-inferred values (Bennion et al. 2004, 2005, Hall and Smol 1996, Ramstack et al. 2002, 2004, Reavie et al. 2002, Leira et al. 2006), we chose not to because the effective confidence level of that approach is only 48%, and we wanted to use the same 5% confidence level for this analysis as for all other analyses in the study. Brenner et al. (1993) also used the 95% confidence interval to determine significant differences between pairs of diatom-inferred TP concentrations.

We used the JMP Statistical package (Version 7) for all of our statistical analyses. In every case we used the 5% level of probability for significance, and all coefficients of determination presented in the results are adjusted R^2 values.

Results

There is a large variation in the concentrations of TP, TN, and Chl-*a* in lakes across Florida (Appendix Tables A1–A4). All lakes in our sample show that each of the 3 trophic state variables has a range of at least 2 orders of magnitude (Fig. 1). They also show a broad range of other physical and chemical variables, such as water color, alkalinity, specific conductance, and pH as well as the degree of anthropogenic activity near the lakes as measured by the LDI (Table 1). Applying the trophic classification system of Forsberg and Ryding (1980) to the TP concentrations we found in Florida lakes, 39% of the lakes would be classified oligotrophic (TP < 15 μg/L), 23% mesotrophic (TP 15–24.9 μg/L) and 38% eutrophic to hypereutrophic (TP > 25 μg/L). Using the proposed numeric nutrient criteria for clear alkaline lakes (USEPA 2010a), we found 36% of these lakes exceed 30 μg/L TP, 26% exceed 1050 μg/L TN, and 23% exceed a Chl-*a* level of 20 μg/L. Using the proposed numeric nutrient criteria for clear acid lakes, we found 77% of these lakes exceed 10 μg/L TP, 64% exceed 510 μg/L TN, and 42% exceed a Chl-*a* level of 6 μg/L.

Our analysis of the diatom-inferred concentrations of TP in the sediments representing undisturbed conditions

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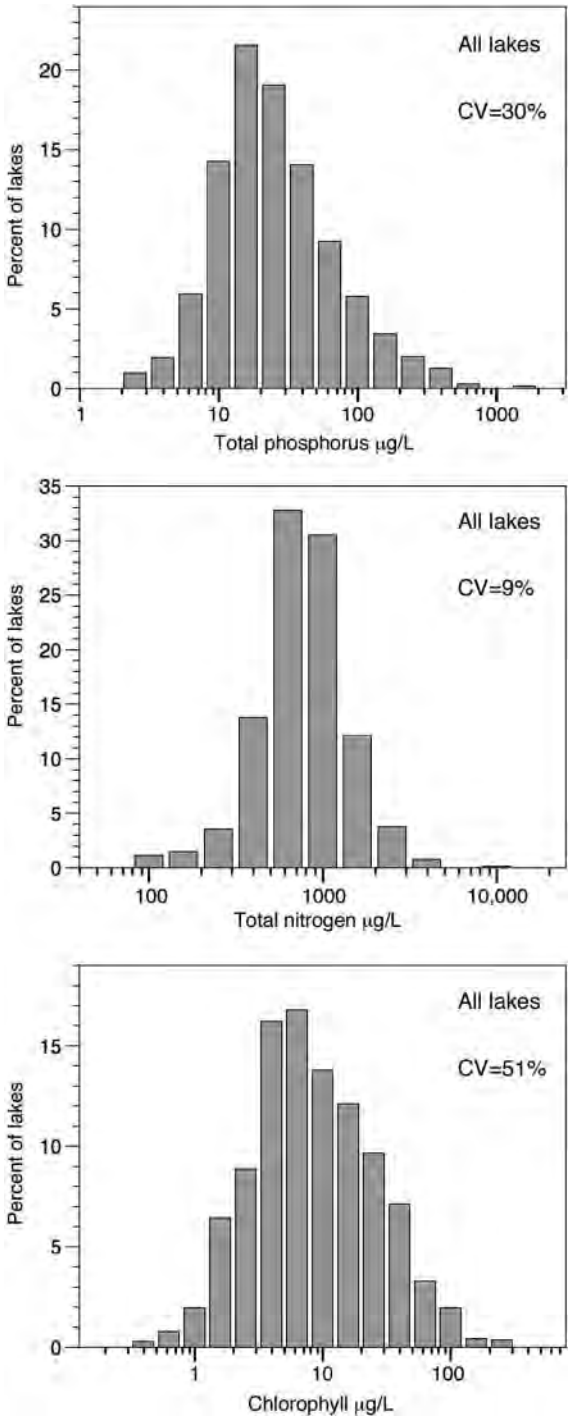

Figure 1.-Distribution of total phosphorus, total nitrogen, and chlorophyll *a* in the 1387 lakes in our sample. The coefficient of variation (CV) is based on the logarithmic values.

in 293 lakes in the National Lake Assessment (USEPA 2010b) showed that 52% of the lakes in the United States would be classified oligotrophic, 18% mesotrophic, and 28% eutrophic to hypereutrophic. These would represent the trophic states of undisturbed lakes in the United States.

When we compared the frequency distributions of surface areas of the named lakes with the lakes sampled in this study (Appendix Fig. A3), we found that the size distributions were similar, with our sample of lakes having surfaces areas slightly larger than the surface areas of all the named lakes as a whole. The geometric mean of the surface areas of our sample lakes was 18 ha and the named lakes 11 ha. The total surface area for the lakes in our sample (470,942 ha) was 91% of the total surface area (613,000 ha) of the named lakes listed by Schafer et al. (1986) and 77% of the total surface area of all lakes in Florida with surface areas of 0.1 ha or greater as determined by Lazzarino et al. (2009). These data indicate the size distribution of our sample lakes is representative of Florida lakes in general.

Relationships among the trophic state variables

The strongest relationship among the trophic state variables is between TP and Chl-*a*, with an R^2 of 0.68 (Table 2); TN is not as strongly related to Chl ($R^2 = 0.53$). These findings are similar to Brown et al. (2000), but as is often the case, TP and TN are so closely related ($R^2 = 0.51$ Table 3) that neither can be established as the limiting nutrient with this type of analysis. Nitrogen limitation is known for a number of Florida lakes (Brown et al. 2000). In a multiple regression analysis with Chl-*a* as the dependent variable and TP and TN as independent variables, the R^2 equaled 0.71 (Table 2). A similar estimate of Chl-*a* in this large set of Florida lakes involves TP and color ($R^2 = 0.72$), and when TN is added to TP and color as the third variable, the R^2 increases to 0.77. Color itself is weakly related to TP and TN (Table 3), while alkalinity only explained 13–22% of the variance in TP, TN, and Chl-*a* and 1% of the variance in color.

Geographic distribution of nutrients and chlorophyll

In 1998, the USEPA proposed a national strategy for developing nutrient criteria based on aggregations of Level III ecoregions (USEPA 1998). A set of nutrient criteria was established for each ecoregion, and those criteria were recommended for the portions of each state within an aggregated ecoregion. We found that the 3 Level III Ecoregions established by the USEPA for the United States (Omernik 1987) that are found in Florida (Ecoregions 65, 75, and 76) are poor predictors of plant nutrients in our large sample of Florida lakes. An analysis of variance found that the percents of the total variance in TP, TN, and Chl-*a* that can be explained by USEPA's major Ecoregions were only 1, 3, and 1% respectively. Because of the large sample size, these

Table 1.-Mean values, coefficients of variation, and percentile distributions for several variables in the sample of Florida lakes used in this study. Geometric means were used for all variables except the Landscape Development Intensity index (LDI) and pH where arithmetic means were used.

				Percentiles				
Variable	**Number of lakes**	**Mean**	**CV**	**10**	**25**	**50**	**75**	**90**
TP μg/L	1386	25.2	30	8.2	13.0	21.7	43.4	95.4
TN μg/L	1385	761	9	397	549	764	1078	1530
Chl-*a* μg/L	1381	8.5	51	2.1	3.9	7.7	18.3	36.6
Secchi depth m	1245	1.5	40	0.6	0.9	1.5	2.3	3.2
Color Pt-Co units	1001	25.8	13	8.3	12.9	23.3	49.1	104.0
Alkalinity as mg/L $CaCO_3$	632	12.1	57	0.3	3.2	17.7	42.4	93.9
Specific conductance μS/cm @ 25 C	633	152	19	49	86	157	241	377
pH	596	7.0	16	5.5	6.3	7.1	7.7	8.3
LDI	1127	4.7	43	1.7	3.1	4.8	6.5	7.4

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were statistically significant, but for all practical purposes the 3 Level III Ecoregions are of little use in explaining the differences among lakes.

The Florida Lake Regions established by USEPA (Griffin et al. 1997) differ from each other in their chemical characteristics (Appendix Tables A1–A4) and are important in determining the TP, TN, and Chl-*a* values of the lakes. The ANOVA of the TP, TN, and Chl-*a* concentrations for the individual lakes against the lake region category explains 45% of the variance in TP, 40% of the variance for TN and 35% of the variance for Chl-*a* for all the Florida lakes in our sample. An ANOVA analysis of the 359 lakes with data on mean depth showed that once lake regions were accounted for, mean depth only accounted for an additional 1–3% of the variance for TP, TN, and Chl-*a*. Thus, knowing the lake region where a lake is located is the best predictor of its trophic state, although the confidence limits are broad (Fig. 2).

The proposed nutrient zones for phosphorus and nitrogen (Fig. 3; Appendix Tables A5 and A6) had distributions of TN and TP that were significantly different from each other respectively as determined by a one-way analysis of variance and a Tukey-Kramer HSD comparison of all geometric means; however, the zone ranges overlapped (Fig. 4). The average TP values had an approximate doubling from one zone to the next while the TN average is increased by a factor of about 1.6 in progressing from the lowest to highest zone average. Consequently, the percent of lakes in a nutrient zone that are classified as eutrophic on the basis of Chl-*a* > 20 μg/L (Table 4) shows a progressive increase from 0% in zone TP1 up to 70% in zone TP6. Results are similar if the lake's classification is made on the basis of TP or TN.

Benchmark lakes

Concentrations of TP in the benchmark lakes ranged from 3 to 241 μg/L, and TN concentrations ranged from 98 to 1953 μg/L (Appendix Table A7). In reference to the USEPA (2010a) criteria for alkaline lakes, 46% of the benchmark lakes exceeded 30 μg/L of TP and 32% exceeded 1050 μg/L of TN. Thus, a substantial number of lakes chosen by FDEP as meeting their designated use and having minimal human disturbance would be classified as impaired by the USEPA.

Table 2.-Empirical relationships between several variables and chlorophyll *a* based on 1001 lakes with color data. The best fit was found with TP, TN, and untransformed color data. All of the relationships are statistically significant at the 5% level of probability.

Log_{10} Chl-*a* $= -3.09 + 1.38\ \text{Log}_{10}$ TN	$R^2 = 0.53$
Log_{10} Chl-*a* $= -0.377 + 0.929\ \text{Log}_{10}$ TP	$R^2 = 0.68$
Log_{10} Chl-*a* $= -1.58 + 0.708\ \text{Log}_{10}$ TP $+ 0.515$ Log_{10} TN	$R^2 = 0.71$
Log_{10} Chl-*a* $= -0.403 + 1.001\ \text{Log}_{10}$ TP-0.00168 color	$R^2 = 0.72$
Log_{10} Chl-*a* $= -1.84 + 0.747\ \text{Log}_{10}$ TP-0.00203 color $+ 0.627\ \text{Log}_{10}$ TN	$R^2 = 0.77$

Table 3.-Relationships between the logarithms of TP, TN, Chl-*a*, color, and total alkalinity for 537 lakes. Adjusted R^2 values for the bivariate relationships are shown in the body of the table. Except for pair color and alkalinity, the relationships are significant at the 5% level of probability.

Variables	**TP**	**TN**	**Chl**	**color**
TN	0.51			
Chl-*a*	0.68	0.52		
Color Pt-Co units	0.31	0.33	0.14	
Alkalinity as mg/L $CaCO_3$	0.13	0.22	0.13	NS

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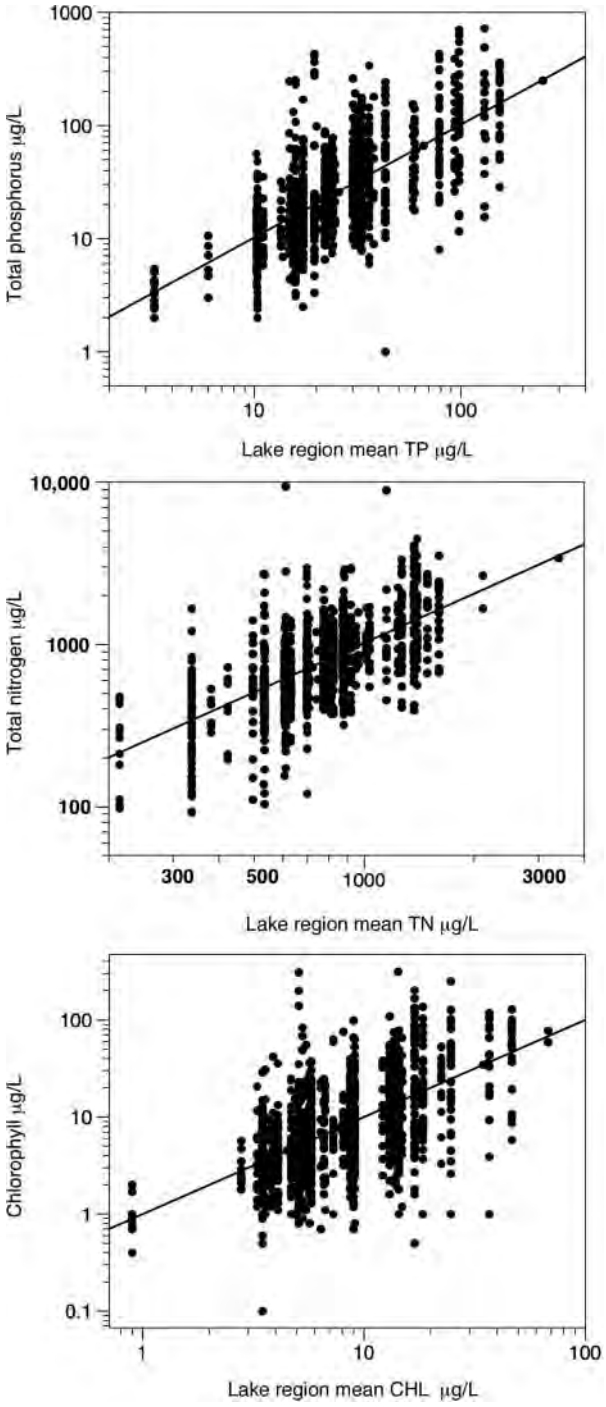

Figure 2.-Plots of total phosphorus (TP), total nitrogen (TN), and chlorophyll *a* (Chl-*a*) in individual lakes against the geometric mean values for the lake region in which they are located.

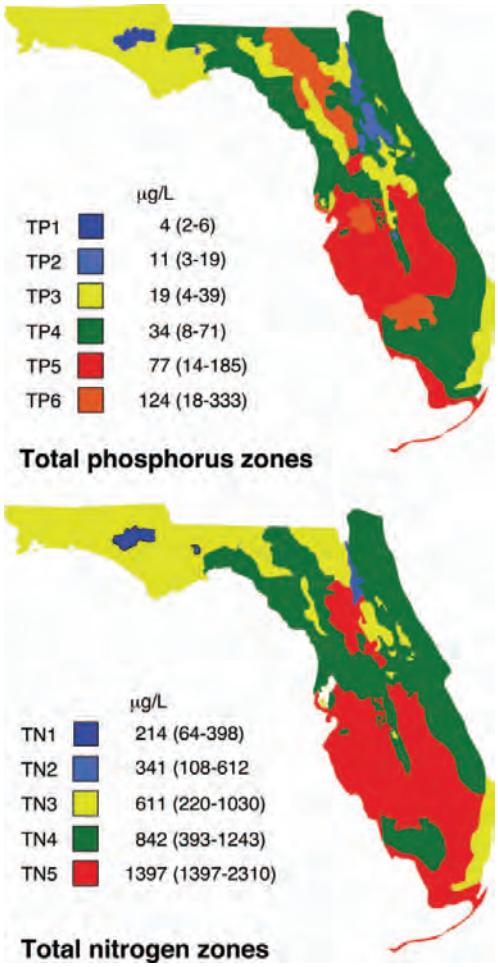

Figure 3.-Maps showing the 6 nutrient zones for TP and the 5 nutrient zones for TN. In the key the geometric mean for the zone is given followed by the 95% range for the lakes in that zone.

The concentrations of TP and TN in the benchmark lakes were related to the nutrient zone geometric means in the nutrient zones in which they were located. For each of the 30 benchmark lakes, the values for TP and TN were within the 95% confidence limits for the nonbenchmark lakes for their respective lake regions. Paired t-tests showed no significant difference between the mean TP values in the benchmark lakes and the mean TP values for the other lakes in the same lake region ($p = 0.71$). A similar test for TN also was not significant ($p = 0.47$).

When we looked at the means of the deviations from the lake region averages for TP in the benchmark versus nonbenchmark lakes (Fig. 5), the TP difference was not statistically significant using a t-test ($p = 0.70$) or the nonparametric Wilcoxon/Kruskal-Wallis test ($p = 0.46$). The same is true for TN using a t-test ($p = 0.53$) and the nonparametric Wilcoxon/Kruskal-Wallis test ($p = 0.96$). The ratio of the number of benchmark lakes that had TP concentrations greater than the mean for their region to the number of lakes with TP concentrations less than the mean was 16:14, and for TN the ratio was 17:13. Using a binomial test, neither of these ratios is significantly different from

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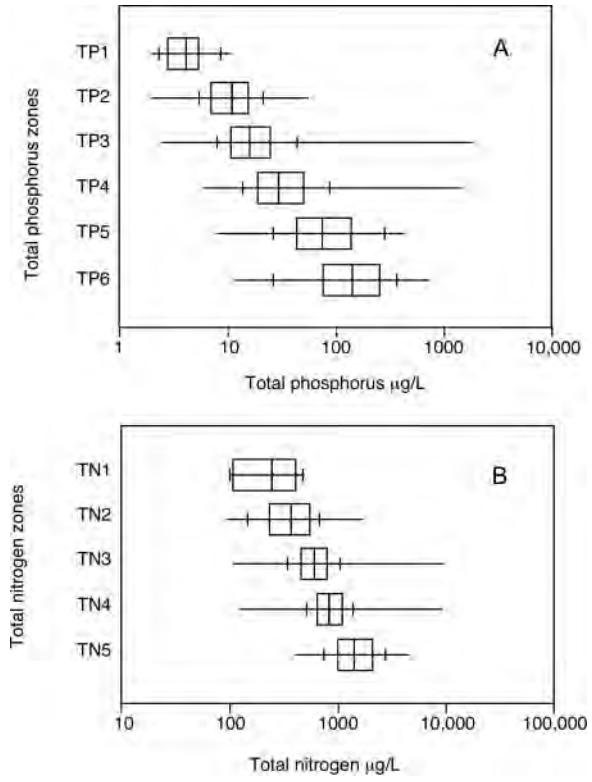

Figure 4.-A. Box and whisker plots of TP concentrations in lakes in 6 total phosphorus zones. The lakes in each zone come from multiple USEPA Florida Lake Regions with similar TP concentrations. The ends of the horizontal line indicate the maximum and minimum concentrations while the 2 boxes span the 25th to the 75th percentile and are joined at the median value. The short vertical lines represent the 10th and 90th percentiles. B. Similar plots for the 5 TN zones.

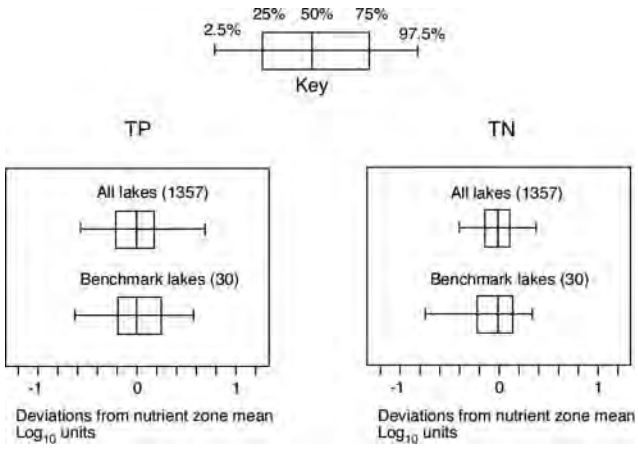

Figure 5.-Distributions of deviations of TP and TN measurements from nutrient zone geometric mean values comparing the benchmark lakes with all lakes in our sample. The ends of the horizontal lines represent the 2.5th percentile and the 97.5th percentile while the 2 boxes span the 25th to the 75th percentile and are joined at the median value. The TP difference is not statistically significant using a t-test ($p = 0.70$) or the nonparametric Wilcoxon/Kruskal-Wallis test ($p = 0.46$). The same is true for TN using a t-test ($p = 0.53$) and the nonparametric Wilcoxon/Kruskal-Wallis test ($p = 0.96$).

15:15, indicating that the benchmark lakes are members of the same distribution as the nonbenchmark lakes.

The results of our most conservative model, where only the lakes that became eutrophic were assumed to have had TP increases, estimated a presettlement average TP concentration of 13.5 μg/L compared to the current concentration of 25.2 μg/L, implying an 86% increase in TP. The other model that included increases in the TP content of mesotrophic lakes that did not reach eutrophic status estimated a presettlement average TP concentration of 5.8 μg/L, implying an increase of 333% from presettlement to modern times. The power analysis showed that our comparison of means could detect a difference of 65% with a confidence level of 5%, so our statistical test could detect the modeled differences.

Table 4.-Percent of lakes exceeding the total phosphorus (TP) criterion of 30 μg/L and the chlorophyll *a* (Chl-*a*) criterion of 20 μg/L in the 6 phosphorus zones, and the percent of lakes exceeding the total nitrogen (TN) criterion of 1050 μg/L in the 5 nitrogen zones.

Nutrient zone	TP > 30 μg/L	Chl-*a* > 20 μg/L	Nutrient zone	T > 1050 μg/L
TP1	0	0	TN1	0
TP2	5	2	TN2	2
TP3	19	10	TN3	11
TP4	43	35	TN4	28
TP5	87	45	TN5	68
TP6	90	70		

When we compared the geometric mean value for TP in the benchmark lakes (22.5 μg/L) with that in the larger sample of all lakes (25.3 μg/L), a t-test showed no statistically significant difference between the 2 groups of lakes ($p = 0.64$). A t-test showed no statistically significant difference ($p = 0.24$) when we compared the geometric mean value for TN in the benchmark lakes (625 μg/L) with that in the larger sample of all lakes (764 μg/L). The differences also were not statistically significant using the nonparametric Wilcoxon/Kruskal-Wallis test for TP ($p = 0.81$) and TN ($p = 0.66$).

The finding of no difference was unexpected because the benchmark lakes have minimal human disturbance. A more refined statistical analysis was used to make the comparison of benchmark versus nonbenchmark lakes because the benchmark lakes are not evenly distributed across all of the

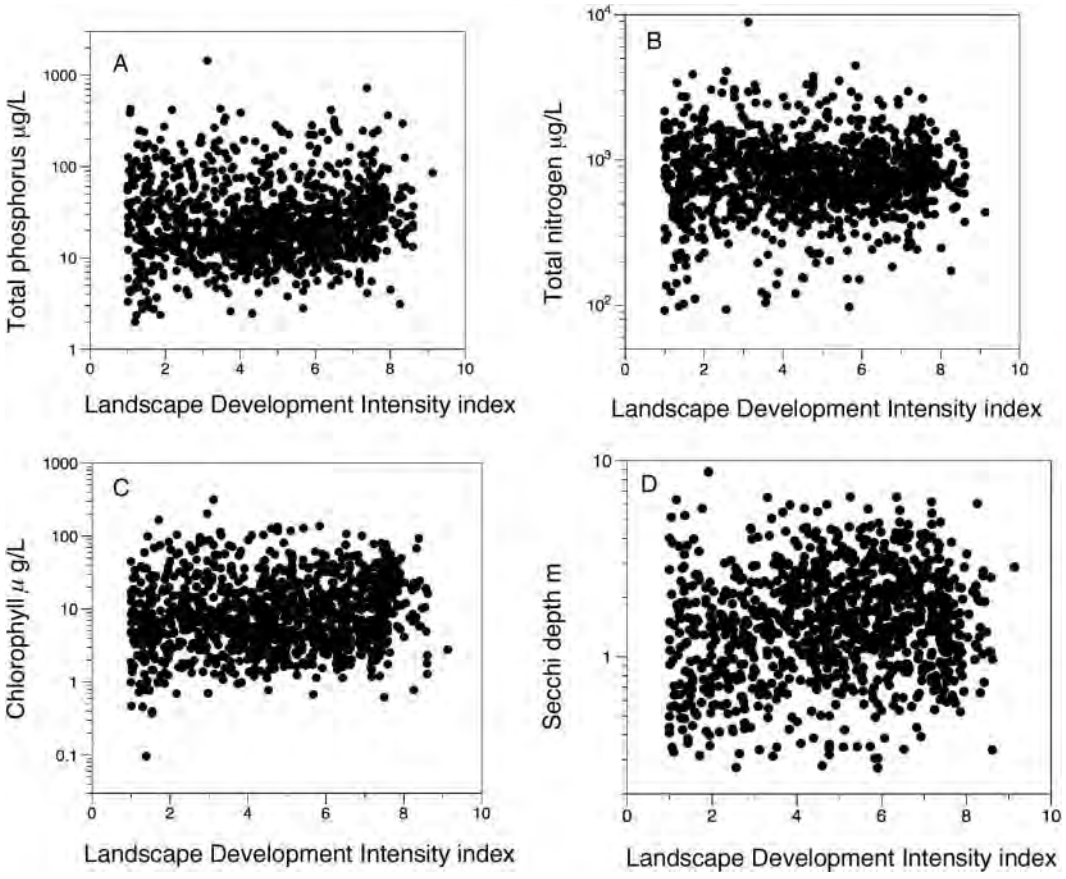

Figure 6.-Plots of total phosphorus, total nitrogen, chlorophyll *a* and Secchi disk depths versus the Landscape Development Intensity index (LDI) for our sample lakes. The LDI has a potential range of 1 to 10, where 1 represents undisturbed conditions.

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nutrient zones. An analysis of variance for TP found, once the variance due to nutrient zone was taken into account, there was no statistically significant effect of classifying the lakes into benchmark or nonbenchmark lakes ($p = 0.57$). The same was true for TN ($p = 0.36$). This means that as a group, the Florida lakes with a variety of human activities in their watersheds on average are not different from the group of benchmark lakes that have minimal human activity in their watersheds. Because the benchmark lakes were chosen in part because they met their designated uses, the idea is supported that most Florida lakes are meeting their designated uses despite being eutrophic.

Influence of land use using the Landscape Development Intensity index

Another way to examine the potential effects of human activities on limnetic nutrient concentrations and other measures of lake trophic state is to look for correlations between the LDI and the measured TP, TN, Chl-*a*, and Secchi disk depths. The underlying assumption behind establishing numeric nutrient criteria is that a direct correlation exists between in-lake nutrient concentrations and anthropogenic development. Our plots (Fig. 6A and B), however, do not indicate that the LDI is related to the trophic states of Florida lakes. The LDI had adjusted R^2 values of 0.002, <0.001, 0.02, and 0.03 for TP, TN, Chl-*a*, and Secchi disk depths, respectively. The TP and TN correlations were not significant at the 5% level, while those with Chl-*a* and Secchi disk depth were statistically significant at the 5% level but were not quantitatively important in explaining the observed variance. In the case of Secchi depths, the regression indicated increasing Secchi depths with increasing values for human development, which was unexpected. We also found no statistically significant correlations at the 5% level between the deviations of TP, TN, and Chl-*a* from their lake region means and the LDI. All 3 R^2 values were <0.004.

We found the same lack of correlation with the LDI when we took regional differences into account. In an ANOVA analysis with TP as the dependent variable, the category Lake Region made a significant reduction in the total variance, however there was no statistically significant variance

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reduction when the LDI was added as a second variable ($p = 0.13$). In a similar analysis the LDI had no significant effects on TN ($p = 0.13$) or on Chl-*a* ($p = 0.09$).

Paleolimnological findings

Paleolimnological studies did show evidence of eutrophication in lakes with past point-source pollution. For example, long cores were used to determine past conditions in 3 lakes (Conine, Haines, and May) in the Winter Haven Chain of Lakes (Riedinger-Whitmore et al. 2005). These lakes are in an urbanized area and have a history of past point-source pollution, including untreated municipal sewage effluent, chemical fertilizer plants, citrus packing plants, citrus and vegetable canning, and soft drink and milk bottling waste. The profiles of the sediments indicated increases in plant pigments and diatom-inferred TP consistent with the point sources of pollution.

Another group of lakes investigated for nutrient increases using long cores was located in Hillsborough County (Brenner et al. 2006). These lakes had low water levels and had been impacted by shallow groundwater pumping from deep wells in the Floridian Aquifer to maintain water levels. An unexpected consequence was that the added ground water had a different chemistry including concentrations of TP higher than those naturally in the lakes, which was reflected in the sediment cores from these lakes. This is also an example of point-source pollution resulting in an increase in the trophic state of the receiving lakes.

The short core data (Appendix Table B2) indicate that the inferred TP concentrations at the bottoms of the cores in 66% of the lakes in our sample were at 30 μg/L or more prior to major human settlement in Florida. While the lakes tested are not a random sample of Florida lakes, the core data do show that Florida has some naturally eutrophic lakes.

The paired t-test comparing the diatom-inferred concentrations of TN at the tops and bottoms of the 9 cores with TN data (USEPA 2010b) showed no significant difference ($p = 0.73$). A similar paired t-test for TP using all 39 lakes in our sample also showed no significant difference ($p = 0.08$). If we only use the 32 lakes reported by Whitmore and Brenner (2002), then there is a significant difference ($p = 0.001$) for the paired t-test for TP (Table 5). The justification for making the separate analysis without the lakes from the National Lakes Assessment was that the diatom-inferred concentrations from the Whitmore and Brenner (2002) study had a smaller error term than those from the National Lakes Assessment study.

To identify the individual lakes with a statistically significant change in diatom-inferred TP at the 5% level of confidence, we used the 95% confidence limits on the differences between diatom-inferred concentrations of TP in sediments from the tops and bottoms of short sediment cores (Fig. 7) and found that 6 of the 39 lakes in our sample showed statistically significant increases in TP, while the remaining 33 lakes showed no statistically significant change in TP. A paired t-test with the 26 lakes from the Whitmore and Brenner (2002) study that were within the 95% confidence limits showed no significant differences between the top and bottom diatom-inferred concentrations of TP ($p = 0.06$).

Table 5.-Results of paired t-tests for the logarithms of the diatom-inferred concentrations of TP and TN at the tops and lower depths of sediment cores for various combinations of samples include the number of lakes compared, the t statistic and the probability of exceeding this value of t. The data from the National Lakes Assessment (USEPA 2010b) are indicated by NLA, and the data from Brenner and Whitmore (2002) are indicated with B & W. For the last comparison, the 6 lakes with differences between the top and lower concentrations exceeding the 95% confidence limits were removed from the sample.

Variable	Dataset	N	t value	probability
TN	NLA	9	0.35	0.74
TP	NLA + B & W	41	1.83	0.08
TP	B & W	32	3.53	0.001
TP	B & W without 6 lakes	33	−1.99	0.06

Point sources of nutrient pollution seem to be responsible for most of the lakes with changes. Lake Parker in Polk County is one of the lakes showing a significant increase in TP. It is an urban lake that is underlain by phosphate-rich deposits of the Bone Valley Formation and Hawthorn Group (Canfield 1981), and phosphate has been mined on the lake's north and east shores (Brenner et al. 1999). It also has stormwater inflow, and 2 power plants that use its waters for cooling.

Lake Jessup in Seminole County is a large lake along the St Johns River. From the 1960s through the 1980s, 7 different wastewater discharge points were directed toward Lake Jessup (Cable et al. 1997), which contributed to a deterioration of its water quality. In addition, its connection to the river has been reduced in the last 50 years through the construction of berms and bridges.

Lake Dora in Lake County, part of a chain of lakes headed by Lake Apopka, has had significant increases in phosphorus loadings due to pumping from muck farms and some municipal wastewater discharges (Bachmann et al. 1999). The enriched waters from Lake Apopka are the major source of nutrients to Lake Dora (Fulton 1995). Lake Dora is one of the 2 lakes of the 39 that showed a statistically significant increase in TP from a mesotrophic to a eutrophic state (Appendix Table B2).

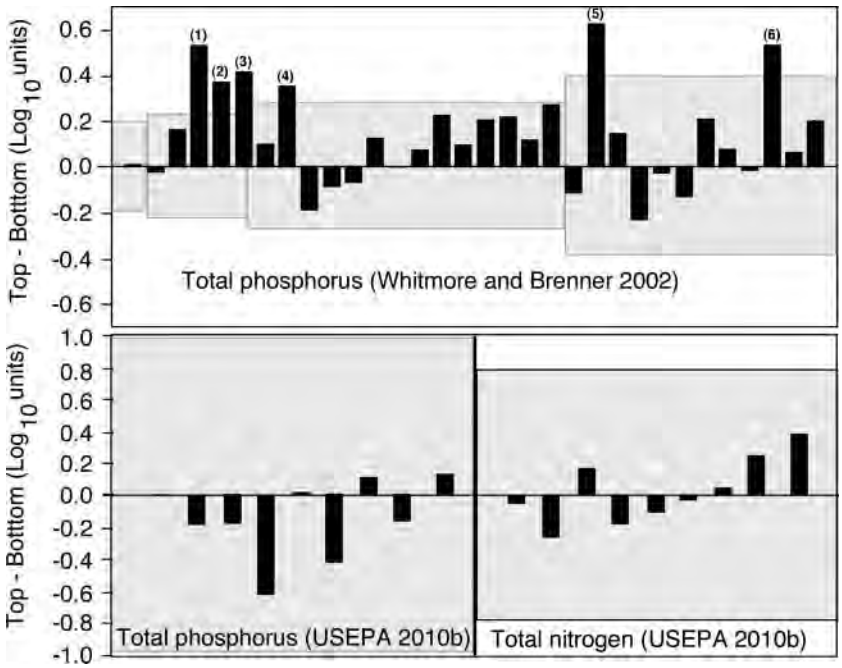

Figure 7.-Each bar represents the difference (Log_{10} units) between the diatom-inferred concentrations of total phosphorus or total nitrogen based on diatoms collected from the tops and deeper levels of sediment cores from several Florida lakes. For interpretation, the antilogarithm of a difference in Log_{10} units is equal to the value of the inferred concentration at the top of the core divided by the inferred concentration at the deeper level in the core. The shaded areas represent the 95% confidence limits for the differences. Bars that extend beyond the confidence limits represent lakes where the differences are significantly different from zero at the 5% level of probability. The upper panel represents 32 lakes summarized by Whitmore and Brenner (2002) who reported on 1–4 cores per lake. The top and bottom values were averaged for each lake, so the confidence limits become smaller as the number of cores increases. The lakes in the lower panels represent cores from 9 Florida lakes from the National Lake Assessment (USEPA 2010b) and include diatom-inferred values for both total phosphorus and total nitrogen. The lakes with significant differences between the top and bottom values are numbered (1) Lake Dora, (2) Lake Parker, (3) Lake Jessup, (4) Round Lake, (5) Lake Lucerne, and (6) Lake Washington.

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Round Lake, Hillsborough County, has been subjected to significant groundwater extraction for municipal water supplies. To maintain water levels, deep groundwater has been pumped directly into the lake, which Brenner et al. (2006) noted has a higher TP content (60 μg/L) than the lake water (8.2 μg/L). That would explain the increases in TP in this lake over time.

Lake Washington, Brevard County, is located in what was once a large wetland in the headwaters of the St Johns River. Since 1900, significant hydrological modifications have reduced the size of the wetland and reduced the flow of water in the river channel (Brenner et al. 1999). Discharges of nutrient-rich waters from urban, agricultural, and ranching areas along with the introduction and periodic herbicide treatment of the exotic macrophytes *Eichornia* and *Hydrilla* are also thought to contribute to accelerated nutrient accumulations in this lake (Brenner et al. 1999). Lake Washington is one of the 2 lakes in the sample that showed a statistically significant increase in TP from a mesotrophic to a eutrophic state (Appendix Table B2).

The sixth lake to show a statistically significant change in TP concentration is Lake Lucerne in Polk County. It is a small lake (0.18 km^2) in the sand hills of the Winter Haven Karst region in the Central Highlands (Lee et al. 1991) with a drainage area about 3 times the lake area. Because of the sandy surficial deposits around the lake, there are no inflowing streams, and groundwater inflow is the only significant source of water. The original pine forests were cleared for citrus groves starting in 1911 (Kass 1994). There were 14 houses around the lake in 1959 and 48 in 2011. Schelske et al. (1994) used 210_{Pb} dating to determine the organic matter content of the sediments at several depths in a 50 cm core in Lake Lucerne and found the percent organic matter was remarkably constant (34–37%) in the upper 36 cm of the profile, but then declined to 15% at 50 cm, indicating that a significant change had taken place in the lake. The major change in the sediment properties occurred prior to the date (1911 at 27 cm in the core) of land clearing and the date of the diatom-inferred TP (28 cm at 1899 according to Schelske et al. 1994). We have not been able to obtain further chemical data for the sediment core, so resolving

what happened in Lake Lucerne over the past century is not possible. The calculated difference in the diatom-inferred TP could be related to land use following 1911, although the major change in the organic content of the sediments occurred well before that time.

In summary, the various paleolimnological studies cited demonstrate that eutrophic lakes were present in Florida in the 1880s at a time when there was very little human development in the state. When statistical tests were used to compare the concentrations of diatom-inferred TP between the tops and lower levels of sediment cores, only 6 of 39 lakes in our sample showed statistically significant increases (Fig. 7). The remaining 33 lakes showed no statistically significant changes. Of the 6 lakes showing increases, 5 had documented point-source pollution in the past. None of the 9 lakes with diatom-inferred concentrations of TN showed statistically significant changes in TN. Only 2 lakes shifted from a mesotrophic to a eutrophic state, and both had documented point-source nutrient enrichment. Thus, a eutrophic lake state does not necessarily mean that a Florida lake is or has been polluted with nutrients.

Discussion and conclusions

The concentrations of TP and TN in Florida lakes and subsequent biological productivity as measured by Chl-*a* range from oligotrophic to hypereutrophic. The differences between lakes are best explained by geography and can be represented by the 6 TP nutrient zones and the 5 TN nutrient zones established in this study. We showed that the percent of lakes classified as eutrophic in the TP zones ranged from 0 to 70%, clearly showing the problem with setting a single standard for all lakes in the State of Florida. This is comparable to the situation in Minnesota, which has a gradient in nutrients and productivity from northeast to southwest (Moyle 1945). A major difference is that, while Minnesota has a gradient that can be described by the 3 major EPA Level III Ecoregions (MPCA 2005), the Florida nutrient zones (which are aggregations of the 47 USEPA Lake Regions) form a patchwork across the state. The Florida nutrient zones, however, were shown to account for a large fraction of the variance in TP, TN, and Chl-*a* in the lakes across the state and provide a basis for establishing quantitative numeric nutrient criteria that recognize natural variations in background concentrations of TP and TN. The differences between the nutrient zones are rooted in the complex geology, soils, and hydrology of Florida, especially the presence of extensive deposits of phosphatic rocks and soils across parts of the state. Limnologists have long recognized that trophic status of lakes in their natural state is determined primarily by edaphic (geology and soils), morphometric, and climatic factors (Naumann 1919, Chandler 1944, Moyle 1956). We note that the USEPA (2010c) independently established 6 different Nutrient Water Regions for Florida to set numeric nutrient criteria for Florida streams because they recognized the considerable regional variability in the concentration of TP and TN in Florida streams related to soils and geology.

While the nutrient zones explain about 40% of the variance in trophic states, unexplained factors control the remainder of the differences among lakes. Of natural factors, mean depth is known to play a role in some of our large shallow lakes, but for our group of lakes we could not find a large effect of mean depth alone, probably due to the lack of significant numbers of deep lakes in Florida. The relatively small number of Florida lakes with depth measurements would make it difficult to set criteria based on lake depths. Likewise, we found that total alkalinity was a poor predictor of lake trophic states in our sample of Florida lakes. Most Florida lakes have no surface inlets or outlets and have ill-defined watersheds, which precludes the use of traditional analyses of water and nutrient loadings for most of the lakes. Groundwater flow is important for many Florida lakes; however, data on how groundwater moves nutrients are lacking, except for a small number of lakes where there have been intensive studies. Understanding how groundwater might move TP and TN to lakes and the factors that account for the variability among lakes within the nutrient zones are fertile grounds for future research.

The alternative hypothesis is that cultural eutrophication is responsible for the eutrophic lakes in Florida. For a small but important group of lakes, past point-source pollution was shown to be responsible for increases in plant nutrients and other indicators of lake productivity. However, since the early 1970s, Florida has adopted strict controls on point-source pollution, so that it is no longer of major concern. This leaves nonpoint sources of nutrient enrichment as the potential causal factor for the eutrophic lakes in the state, and cultural eutrophication is often cited as a major lake management issue.

Several lines of evidence indicate that eutrophic lakes are a natural part of the Florida ecosystem and were present prior to European settlement. First, the distribution of eutrophic lakes as indicated by Chl-*a* concentrations was found to be dependent on geography, with eutrophic lakes making up 0% of the lakes in nutrient zone TP1, 2% in TP2, 10% in TP3, 35% in TP4, 45% in TP5, and 90% in TP6. Similar results were found for the TN zones with the percent of lakes classified as eutrophic increasing from 0 to 68% going from zones TN1 to TN5. The boundaries of the nutrient zones (Fig. 6) were based largely on soils and geology, and the high nutrient zones show a correspondence with areas of phosphatic deposits.

Our analysis of the benchmark lakes also supports the hypothesis that eutrophic lakes were natural components of the

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Florida landscape prior to European settlement. The benchmark lakes in our study were selected by the FDEP because they represented lakes with no or little development in their watersheds; yet we found 46% and 32% of those lakes were eutrophic based on the proposed EPA criteria of 30 μg/L of TP and 1050 μg/L of TN, respectively. Further, we found no statistically significant differences in the concentrations of TP and TN between the relatively undisturbed benchmark lakes and the concentrations of these nutrients in all other lakes with a range of human activities along their shores. This would indicate that most Florida lakes have not suffered significant cultural eutrophication since European settlement.

Another test was to look for the effects of human activities on lake nutrients and biological productivity using the LDI. No pattern of increased concentrations of TP and TN was found as the LDI values increased. Seemingly, the kinds of activities measured by the LDI were not having a significant impact on the adjacent lakes; however, the lack of an effect could reflect the success of vigorous programs to remove point sources of pollutions from Florida lakes and to control stormwater runoff to reduce nutrient loads to lakes. Also, the Florida Department of Agriculture and Consumer Services has a program of Best Management Practices for agricultural activities designed to protect both water quality and quantity.

Paleolimnological studies based on short sediment cores indicated that eutrophic lakes were present prior to 1900 when Florida was sparsely settled. The diatom-inferred TP concentrations at the bottoms of the cores in 66% of the lakes sampled exceeded 30 μg/L or more prior to major human settlement in Florida. Only 2 of the 39 sample lakes showed a change from a mesotrophic to eutrophic state, and they were subjected to documented point-source nutrient pollution. While the lakes tested are not a random sample of Florida lakes, our data show that eutrophic lakes are a natural component of the state's waters.

The major conclusion of our study is that geographic factors as represented by the proposed nutrient zones for TP and TN are the main determinants of nutrient concentrations in Florida lakes, and that eutrophic lakes are a natural part of the Florida landscape and do not always represent accelerated eutrophication. This is not unexpected because the paleolimnological results of the National Lake Assessment indicate that 28% of lakes in the United States were eutrophic in their undisturbed condition. Considering that Florida has large deposits of phosphatic rocks not equaled in other states, naturally eutrophic lakes in the state are expected. Laws governing the establishment of numeric nutrient criteria are concerned with changes in nutrient concentrations relative to their natural state; therefore, agencies establishing numeric nutrient criteria for Florida or any other state or province must take regionalized information, especially the influence of geology and physiography on lake nutrients, into account before assuming a present-day eutrophic lake is due solely to cultural eutrophication.

Because we have been looking at the characteristics of large groups of lakes, we recognize some lakes may have been culturally eutrophied; however, our studies indicate that the number is much smaller than the current number of eutrophic lakes. Only detailed investigations of the lakes most likely to fall in this category will determine how many there might be and where they are located. To account for this uncertainty, in another study we propose a program of adaptive management with regard to nutrient criteria that would prioritize investigations toward lakes with concentrations of TP and TN that deviate the most from those expected for their respective nutrient zones.

Acknowledgments

We benefited from discussions with Jack Jones and Karl Havens and from the comments of Tom Whitmore and the anonymous reviewers of the manuscript. Russell Frydenborg, Kenneth Weaver, and Nia Wellendorf of the Florida Department of Environmental Protection assisted us in obtaining lake data collected by their agency.

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Appendix A. Supplemental tables and figures.

Table A1. The distribution of total phosphorus concentrations (µg/L) by lake region with the number of lakes in our sample.

Region Number	Number of Lakes	Mean	Percentile 10	25	50	75	90
65-01	8	15	5	6	23	29	42
65-02	15	15	6	8	14	28	43
65-03	12	3	2	3	3	4	5
65-04	62	36	16	20	40	54	97
65-05	6	6	3	4	6	9	11
65-06	17	131	18	65	160	241	529
75-01	44	20	7	10	16	22	285
75-02	3	24	12	12	29	39	39
75-03	10	20	12	14	17	23	86
75-04	84	10	5	7	11	15	22
75-05	2	14	7	7	14	28	28
75-06	4	38	9	12	36	132	180
75-07	2	132	76	76	132	230	230
75-08	51	43	14	21	38	83	156
75-09	22	10	3	7	10	16	37
75-10	103	33	11	17	31	58	98
75-11	77	17	7	11	15	25	56
75-12	19	22	11	16	23	35	44
75-13	14	59	20	27	39	110	473
75-14	11	13	9	10	12	18	29
75-15	20	11	7	7	10	14	22
75-16	62	22	10	15	20	32	59
75-17	6	25	13	14	26	39	46
75-18	1	61	61	61	61	61	61
75-19	45	15	8	11	14	23	28
75-20	19	15	8	10	13	14	86
75-21	135	32	15	21	32	48	67
75-22	13	33	14	22	36	56	67
75-23	41	16	8	11	16	20	25
75-24	94	24	13	17	24	32	45

75-25	15	94	29	44	96	197	312
75-26	1	34	34	34	34	34	34
75-27	28	24	14	17	21	39	61
75-28	32	98	16	45	112	210	517
75-29	2	31	18	18	31	53	53
75-30	25	154	53	83	165	305	353
75-31	56	30	16	21	27	38	66
75-32	24	17	8	11	17	24	30
75-33	38	10	5	7	10	16	18
75-34	32	31	10	16	25	51	107
75-35	14	60	21	38	64	101	127
75-36	45	79	23	42	75	155	340
75-37	1	250	250	250	250	250	250
76-01	2	38	23	23	38	63	63
76-02	1	26	26	26	26	26	26
76-03	68	16	5	8	12	28	62
76-04	1	66	66	66	66	66	66

Table A2. The distribution of total nitrogen concentrations (µg/L) by lake region with the number of lakes in our sample.

			Percentile				
Region Number	Number of Lakes	Mean	10	25	50	75	90
65-03	12	214	98	105	238	396	472
65-04	62	629	368	473	660	828	905
65-05	6	382	287	306	375	476	533
65-06	17	1033	654	745	1091	1308	1567
75-01	44	534	239	373	521	781	1376
75-02	3	533	283	283	609	879	879
75-03	10	704	490	601	628	873	1264
75-04	84	338	141	224	362	541	673
75-05	2	749	611	611	749	917	917
75-06	4	964	752	772	851	1364	1588
75-07	2	2110	1671	1671	2110	2663	2663
75-08	51	1368	645	837	1425	2119	3153
75-09	22	496	163	321	553	757	1103
75-10	103	879	466	577	883	1359	1690
75-11	77	698	355	555	733	1018	1215
75-12	19	1015	853	898	1011	1082	1159
75-13	14	1152	507	758	951	1534	5069
75-14	11	708	419	577	740	865	1348
75-15	20	630	432	520	572	750	1216
75-16	62	775	483	578	748	967	1269
75-17	6	878	643	682	902	1084	1201
75-18	1	816	816	816	816	816	816
75-19	45	823	525	693	851	1035	1222
75-20	19	532	392	459	517	568	645
75-21	135	777	503	639	765	940	1151
75-22	13	950	615	826	994	1089	1244
75-23	41	606	355	475	650	784	987
75-24	94	813	504	679	833	1017	1179
75-25	15	1491	745	978	1698	2377	2626
75-26	1	1130	1130	1130	1130	1130	1130
75-27	28	809	515	643	799	1022	1327
75-28	32	1266	586	870	1346	1891	2759

75-29	2	741	460	460	741	1193	1193
75-30	25	1601	704	1224	1749	2295	2426
75-31	56	874	517	686	813	1253	1662
75-32	24	883	530	601	836	1021	1913
75-33	38	697	412	483	572	892	1972
75-34	32	921	454	648	889	1330	1674
75-35	14	1237	706	903	1315	1596	1990
75-36	45	1397	822	1022	1337	1868	2805
75-37	1	3409	3409	3409	3409	3409	3409
76-01	2	1362	904	904	1362	2053	2053
76-02	1	724	724	724	724	724	724
76-03	68	610	266	439	638	827	1089
76-04	1	1239	1239	1239	1239	1239	1239

Table A3. The distribution of chlorophyll concentrations (µg/L) by lake region with the number of lakes in our sample.

			Percentile				
Region Number	Number of Lakes	Mean	10	25	50	75	90
65-01	8	6.7	1.1	2.2	11.7	16.7	21.1
65-02	15	4.9	1.0	1.8	6.5	10.1	15.6
65-03	12	0.9	0.4	0.7	0.8	1.5	1.9
65-04	62	13.1	4.0	6.6	13.1	24.7	37.2
65-05	6	2.8	1.8	2.0	2.6	4.0	5.7
65-06	17	22.4	5.4	16.9	26.4	35.3	52.7
75-01	44	5.3	1.6	2.8	4.8	7.4	33.4
75-02	3	2.8	1.8	1.8	2.5	4.7	4.7
75-03	10	7.3	2.8	5.1	6.3	8.1	49.1
75-04	84	3.5	1.3	2.2	3.7	6.0	9.5
75-05	2	5.2	1.8	1.8	5.2	14.7	14.7
75-06	4	7.3	1.0	1.5	6.6	39.1	63.0
75-07	2	67.9	59.3	59.3	67.9	77.7	77.7
75-08	51	17.0	3.8	5.4	15.4	56.8	114.9
75-09	22	3.6	1.3	2.3	3.4	4.8	13.4
75-10	103	9.0	2.6	4.9	8.2	19.0	29.0
75-11	77	5.8	1.9	3.3	5.4	10.8	18.8
75-12	19	8.1	3.7	5.4	7.1	10.6	17.0
75-13	14	14.3	2.8	4.3	16.6	31.2	119.4
75-14	11	3.8	1.2	1.9	5.6	6.9	8.8
75-15	20	3.3	1.4	1.9	3.1	5.4	10.9
75-16	62	9.1	2.7	4.6	8.9	18.9	31.8
75-17	6	6.4	0.7	2.2	8.1	20.8	22.9
75-18	1	4.5	4.5	4.5	4.5	4.5	4.5
75-19	45	4.7	1.7	3.0	4.7	6.0	12.1
75-20	19	3.9	1.7	2.3	2.9	5.5	11.0
75-21	135	14.4	4.1	8.0	16.4	27.4	37.4
75-22	13	12.2	2.9	6.0	12.1	32.7	37.2
75-23	41	5.5	2.2	3.1	5.1	10.3	17.8
75-24	94	8.7	3.0	5.0	9.4	14.9	23.9
75-25	15	37.0	6.0	17.7	44.1	91.7	112.8

75-26	1	7.9	7.9	7.9	7.9	7.9	7.9
75-27	28	5.7	1.8	3.4	5.0	8.6	27.1
75-28	32	24.8	4.5	11.9	33.7	53.0	100.6
75-29	2	15.0	9.8	9.8	15.0	23.2	23.2
75-30	25	46.6	9.4	37.0	57.2	88.1	95.8
75-31	56	13.9	4.8	7.7	14.3	26.3	42.4
75-32	24	6.6	2.2	3.5	6.0	14.5	22.7
75-33	38	4.1	1.5	2.6	3.8	7.3	9.2
75-34	32	13.7	4.8	6.3	12.4	31.4	56.9
75-35	14	14.9	4.4	6.4	15.2	28.9	53.4
75-36	45	18.5	6.5	9.3	16.6	30.5	73.7
75-37	1	36.6	36.6	36.6	36.6	36.6	36.6
76-01	2	14.2	11.5	11.5	14.2	17.6	17.6
76-02	1	7.7	7.7	7.7	7.7	7.7	7.7
76-03	68	5.1	1.3	2.0	4.2	9.7	19.7
76-04	1	34.7	34.7	34.7	34.7	34.7	34.7

Table A4. The distribution of Secchi disk depths (m) by lake region with the number of lakes in our sample.

Region Number	Number of Lakes	Mean	Percentile 10	25	50	75	90
65-01	8	1.7	1.2	1.4	1.8	1.9	2.4
65-02	15	1.8	1.0	1.4	1.6	2.2	4.7
65-03	12	4.3	2.7	3.7	4.1	5.7	6.1
65-04	62	1.2	0.7	0.8	1.0	1.5	2.2
65-05	6	2.3	1.8	1.8	2.6	2.6	2.6
65-06	17	1.2	0.8	0.9	1.1	1.5	2.0
75-01	44	1.2	0.7	0.7	1.2	1.6	2.3
75-02	3	0.9	0.9	0.9	0.9	0.9	0.9
75-03	10	1.3	0.6	1.1	1.5	1.6	2.2
75-04	84	2.3	1.2	1.6	2.3	3.1	4.5
75-05	2	1.6	1.1	1.1	1.6	2.5	2.5
75-06	4	1.5	0.5	0.6	1.5	3.7	4.6
75-07	2	0.7	0.6	0.6	0.7	1.0	1.0
75-08	51	0.9	0.3	0.6	0.9	1.4	1.8
75-09	22	2.1	1.0	1.4	2.2	2.6	4.3
75-10	103	1.0	0.5	0.7	1.1	1.6	2.1
75-11	77	1.7	0.8	1.5	1.8	2.4	3.0
75-12	19	1.4	0.9	1.1	1.5	1.9	2.2
75-13	14	1.1	0.4	0.9	1.4	1.4	2.1
75-14	11	1.9	0.5	1.7	2.2	3.0	3.4
75-15	20	3.0	1.2	2.5	3.4	3.6	4.5
75-16	62	1.7	0.8	1.2	1.7	2.6	3.2
75-17	6	1.3	0.9	0.9	1.4	1.9	2.2
75-18	1	1.0	1.0	1.0	1.0	1.0	1.0
75-19	45	1.7	0.8	1.1	2.0	2.9	3.5
75-20	19	3.0	1.5	2.7	3.2	3.9	4.1
75-21	135	1.4	0.8	1.0	1.3	2.0	2.5
75-22	13	1.1	0.7	0.7	1.1	1.5	2.6
75-23	41	2.2	1.3	1.7	2.1	2.8	4.0
75-24	94	1.6	1.0	1.3	1.7	2.1	2.7

75-25	15	0.8	0.3	0.4	0.7	1.3	2.3
75-26	1	0.8	0.8	0.8	0.8	0.8	0.8
75-27	28	1.0	0.5	0.6	0.8	1.5	1.9
75-28	32	0.9	0.3	0.6	1.0	1.6	2.3
75-29	2	1.6	1.3	1.3	1.6	2.0	2.0
75-30	25	0.7	0.4	0.5	0.6	0.8	2.0
75-31	56	1.3	0.6	0.8	1.4	2.0	2.5
75-32	24	2.0	0.9	1.1	2.1	3.0	4.6
75-33	38	3.0	1.5	2.2	3.0	4.1	5.5
75-34	32	1.0	0.5	0.7	1.0	1.6	2.4
75-35	14	0.8	0.5	0.6	0.7	1.2	1.4
75-36	45	1.0	0.4	0.7	1.0	1.3	1.7
75-37	1	0.4	0.4	0.4	0.4	0.4	0.4
76-01	2	1.0	0.9	0.9	1.0	1.0	1.0
76-02	1	2.0	2.0	2.0	2.0	2.0	2.0
76-03	68	2.2	0.8	1.4	2.4	4.0	4.9
76-04	1	NA	NA	NA	NA	NA	NA

Table A5. Summary of the concentrations of total phosphorus (µg/L) and total nitrogen (µg/L) in their respective nutrient zones.

Nutrient zones	Number of lakes	mean	Percentiles 10	25	50	75	90
Total phosphorus							
TP1	18	4	2	3	4	5	9
TP2	175	11	5	7	11	15	22
TP3	556	19	8	12	17	27	45
TP4	485	34	14	20	33	53	93
TP5	76	77	26	43	73	139	280
TP6	77	124	27	76	141	250	359
Total nitrogen							
TN1	12	214	98	105	239	400	472
TN2	90	341	143	229	362	535	663
TN3	427	611	341	459	616	822	1099
TN4	670	842	510	647	830	1086	1375
TN5	188	1397	726	969	1394	2032	2708

Table A6. The constituent EPA Lake Region numbers for the proposed nutrient zones.

Zones	Constituent USEPA Lake Regions
Total phosphorus zones	
TP1	65-03, 65-05
TP2	75-04, 75-09, 75-14, 75-15, 75-33
TP3	65-01, 65-02, 75-01, 75-03, 75-05, 75-11, 75-12, 75-16, 75-19, 75-20, 75-23, 75-24, 75-27, 75-32, 76-03
TP4	65-04, 75-02, 75-06, 75-08, 75-10, 75-13, 75-17, 75-21, 75-22, 75-26, 75-29, 75-31, 75-34, 76-01, 76-02
TP5	75-18, 75-25, 75-35, 75-36, 76-04
TP6	65-06, 75-07, 75-28, 75-30, 75-37
Total nitrogen zones	
TN1	65-03
TN2	65-05, 75-04
TN3	65-01, 65-02, 65-04, 75-01, 75-02, 75-03, 75-09, 75-11, 75-15, 75-20, 75-23, 75-33, 76-03
TN4	65-06, 75-05, 75-06, 75-10, 75-12, 75-13, 75-14, 75-16, 75-17, 75-18, 75-19, 75-21, 75-22, 75-24, 75-26, 75-27, 75-29, 75-31, 75-32, 75-34, 76-02
TN5	75-07,75-08, 75-25, 75-28, 75-30, 75-35, 75-36, 75-37, 76-01, 76-04

Table A7. Average values for total phosphorus and total nitrogen in the 30 benchmark lakes established by the Florida Department of Environmental Protection (FDEP). Some of the data were collected by Florida LAKEWATCH (LW).

Lake	Study	Mean TP µg/L	Mean TN µg/L
Annie	LW	7	394
Ashby	LW	100	883
Blue Cypress	LW	115	1390
Buck	FDEP	20	569
Cassidy	LW	4	137
Charles	LW	59	1748
Disston	LW	30	1229
Dunford	FDEP	3	479
Eaton	LW	38	1233
Fox	LW	52	1493
Gore	LW	12	657
Harney	LW	58	1285
Iamonia	LW	20	590
Russell	FDEP	105	1303
Lowe	LW	241	962
Merial	FDEP	4	102
Miccosukee	LW	26	822
Norris	LW	40	1409
Ocean Pond	FDEP	29	609
Orange	LW	81	1923
Otter	LW	9	648
Palestine	LW	12	283
Pate	FDEP	8	295
Rattlesnake	LW	3	98
Redwater	FDEP	127	1692
Sellers	LW	4	111
Swift Creek Pond	FDEP	39	879
Turkey Pen Pond	FDEP	3	212
Wauberg	LW	127	1953
Wildcat	LW	7	334

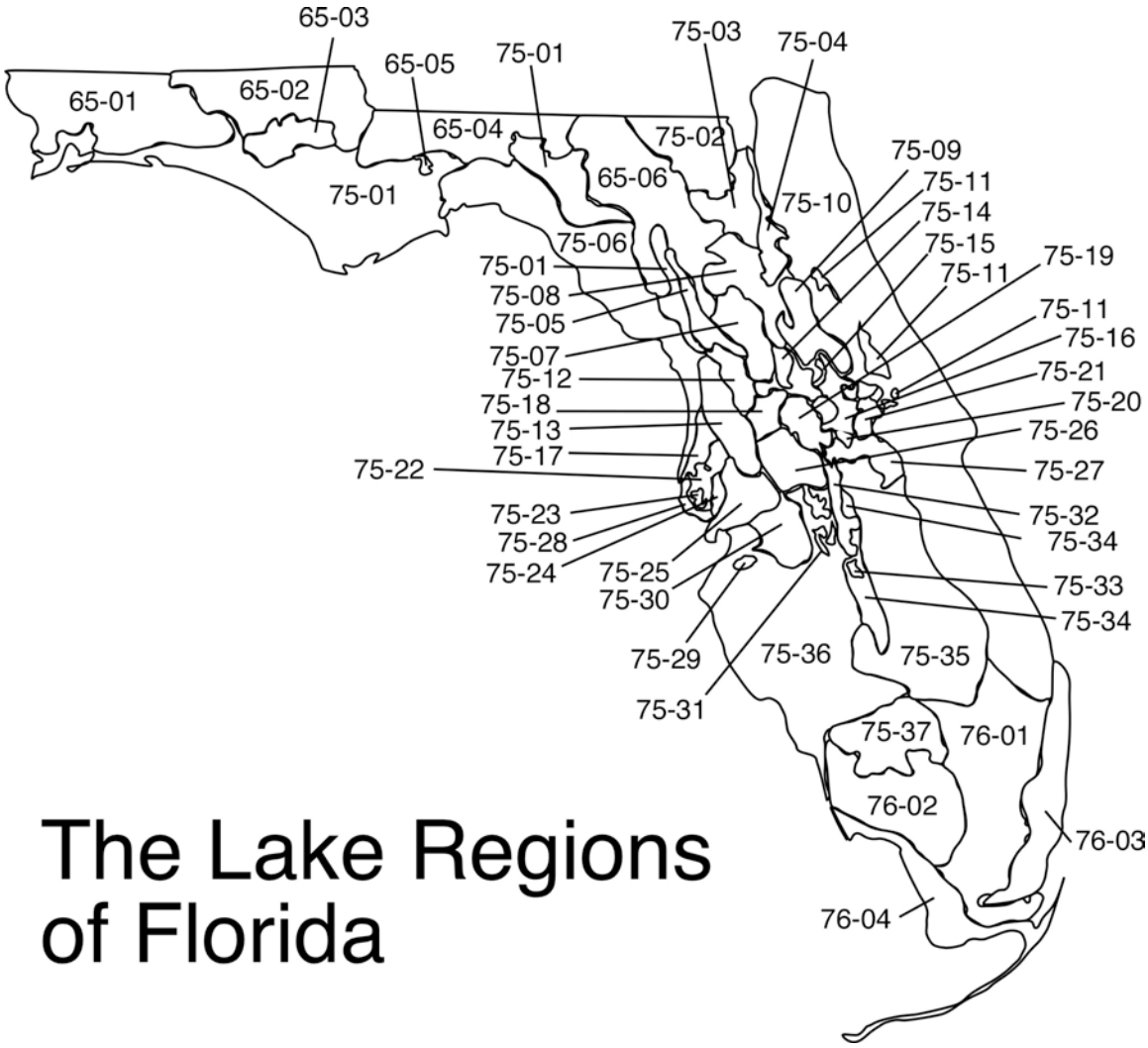

Figure A1. USEPA Lake Regions for Florida as redrawn from Griffith et al. (1997).

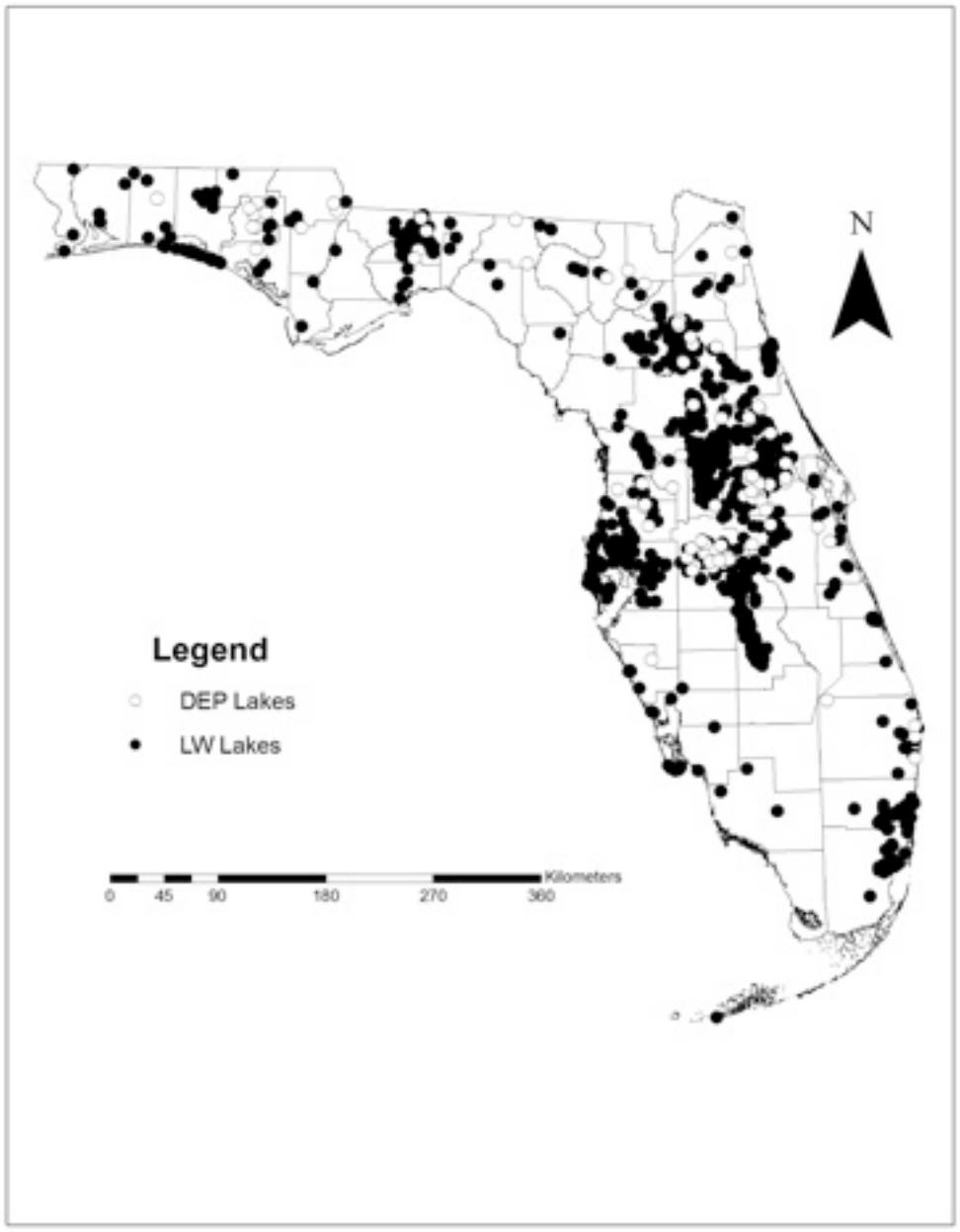

Figure A2. Location of lakes sampled by Florida LAKEWATCH and by the Florida Department of Environmental Protection.

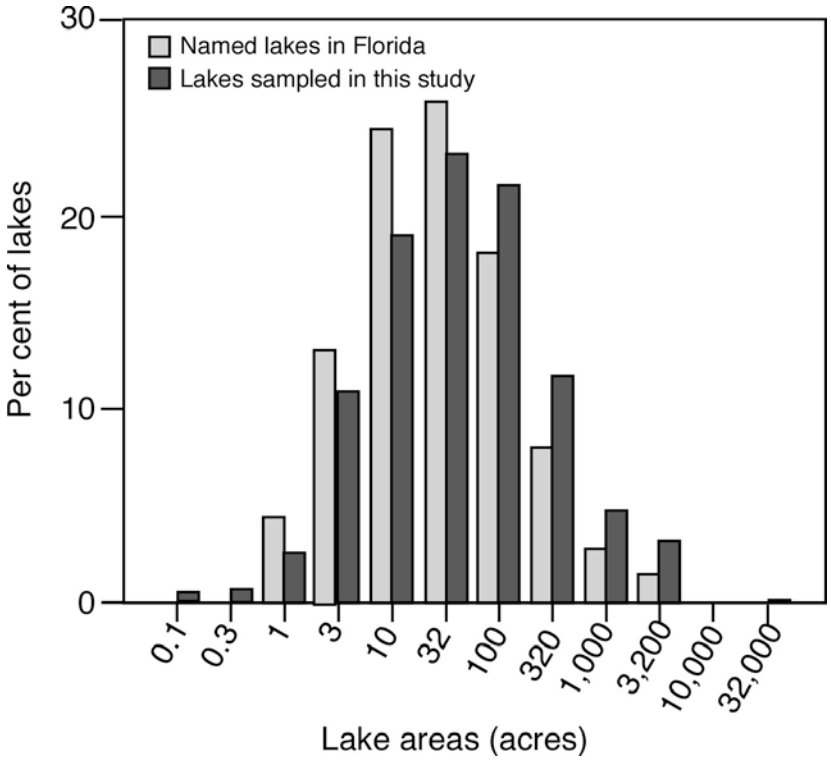

Figure A3. Frequency distributions of the lakes used in this study compared with the distribution of the named lakes of Florida from Schafer et al. (1986).

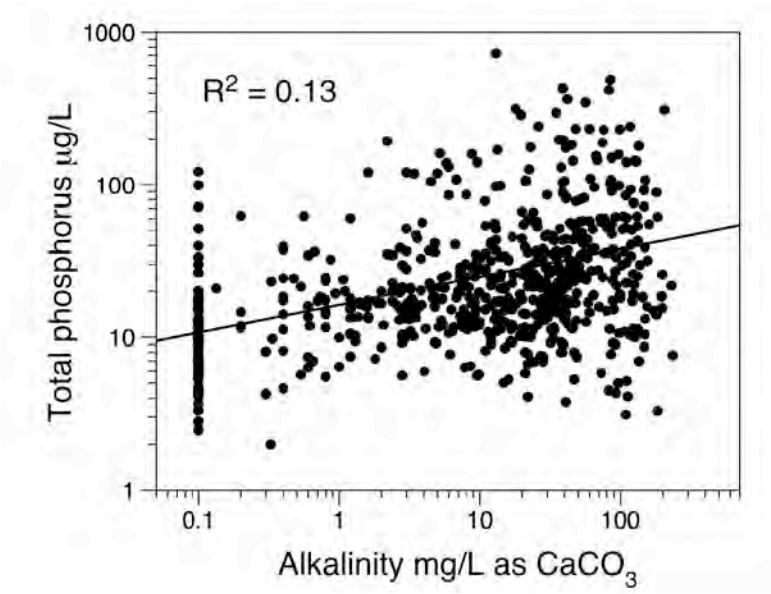

Figure A4. Concentrations of total phosphorus in 1001 lakes versus alkalinity.

Appendix B. Finding confidence limits for diatom-inferred total phosphorus and total nitrogen concentrations for lakes

Paleolimnologists have developed complex statistical models and computer programs such as WACALIB that allowed them to use the species composition of the diatoms found in a thin layer of lake sediments to calculate the concentrations of total phosphorus (TP) or other chemical variables in the lake water at the time those diatoms were produced (Hall and Smol 1996). The models that use the diatom community structures to calculate TP values are called transfer functions, and the calculated value for TP would be called the diatom-inferred total phosphorus concentration (DI-TP). The basis for this method is that the different diatom communities exist at different nutrient concentrations.

Many studies (e.g. Dixit et al. 1998, Hall and Smol 1996) have used short sediment cores (about 1-m in length) where the diatoms are examined at the top of the core and at the bottom of the core to examine potential changes over time. The DI-TP at the top of the core represents the current condition, and the DI-TP at the bottom of the core represents conditions assumed to be 100 years or more ago. The goal is to determine if there has been a change in TP in the lake over time by comparing the inferred values for the DI-TP at the top of the core and the DI-TP at the bottom of the core. Increases in concentration are considered to indicate eutrophication has taken place. Since each inferred value has a statistical error associated with it, we wished to determine the 95 % confidence limits on the inferred concentrations in order to determine when the changes are statistically significant.

A weighted-averaging calibration such as WACALIB that has been programmed by Line el al (1994) uses a large sample of lakes with measurements of the species composition of the diatom community at the top of the sediment cores as well as current measurements of TP in the lake water for each lake to develop and calibrate the model (transfer function) relating the diatoms to DI-TP. For nutrients like TP and total nitrogen (TN), logarithms of the concentrations are often used to develop the model and the resulting inferred values are also in logarithmic units. For our examples we will use total phosphorus and common logarithms, though the results would be the same for TN and/or natural logarithms.

In deriving the transfer function the root mean squared error (RMSE) is calculated as:

$$RMSE = \sqrt{\Sigma (Log\ TP_{obs} - LogTP_T)^2/(N_C - 1)} \quad (1)$$

Where TP_T = the inferred total phosphorus concentration at the top of the core (µg/L), TP_{obs} = the measured TP concentration in the lake water in the same lake (µg/L), N_C = the number of lakes in the calibration data set.

The best-fit model has the smallest value for the RMSE, and its value is an indicator of how closely the diatom-inferred TP matches the measured TP in the lake. Since the same lakes that are used to calibrate the model are used to derive the error term, there is a bias towards underestimating the error. To produce a more realistic estimate of the error of prediction, procedures such as bootstrapping or jackknifing (Smol 2002) are used to produce an unbiased estimate. The resulting value is usually somewhat larger than the original RMSE.

Let RSMEP= the root mean squared error of prediction obtained by bootstrapping or jackknifing. The RSMEP is equivalent to the standard deviation of the predicted TP concentration, so it is used in determining 95 % confidence limits on diatom-inferred concentrations. We will also need to find the value for Student's t.

t_{05} = the value for t found in a two-sided table of t with a probability of 95% and with (N_C -1) degrees of freedom.

We will outline here how we determined the 95% confidence limits for the results of short sediment cores. The statistical equations are from Sokal and Rohlf (1995). We will start by making all of the calculations in logarithmic units and then show how to interpret them in the original units of measurement. We will start with confidence limits on the diatom-inferred values for the logarithms of total phosphorus at the top of a core (TP_T). The same procedure would apply to the diatom-inferred concentrations at the bottom of the core (TP_B).

Let
S_e = the standard error for $\overline{TP}$

Where $\overline{TP}$ = equals the average DI-TP based on n samples, so from Sokal and Rohlf (1995):

$$S_e = s/\sqrt{n} \quad (2)$$

Where
s = the standard deviation for the predicted values = RSMEP

The 95 % confidence limits are given by:

$$L_1 \text{ (lower limit)} = \overline{TP} - t_{05}\, S_e \quad (3)$$

L_2 (upper limit) = $\overline{\overline{TP}} + t_{05}\, S_e$ (4)

Finding confidence limits for the difference between the top and bottom of the cores

In the usual situation with short cores, the logarithm of the diatom-inferred value for TP at the bottom of the core is subtracted from the logarithm of diatom-inferred value for TP at the top of the core. We are interested in the confidence limits on the difference between the two values to see if they are significantly different. The calculation will not be the same as the previous case, for we are finding the difference between two values, each of which has a statistical error.

The starting point is the calculation of the standard error for the difference between the two means (S_{diff}). Since in our case we have equal numbers of top and bottom samples for each core and the standard deviations for the two measurements are the same, we can calculate the standard error of the difference between them as:

$$S_{diff} = s\sqrt{2/n} \quad (5)$$

To find the confidence limits on the difference let

$\overline{\overline{TP}}_T$ = the mean inferred value at the top of the column

$\overline{\overline{TP}}_B$ = the mean inferred value at the bottom of the column

As before the 95% confidence limits are given by

L_1 (lower limit) = (Log $\overline{\overline{TP}}_T$ - Log $\overline{\overline{TP}}_B$)- $t_{05}\, S_{diff}$ (6)

and

L_2 (upper limit) =(Log $\overline{\overline{TP}}_T$ - Log $\overline{\overline{TP}}_B$) + $t_{05}\, S_{diff}$ (7)

Since the null hypothesis is that the difference between the top and bottom values is 0, Equations 6 and 7 reduce to:

L_1 (lower limit) =0 - $t_{05}\, S_{diff}$ (8)

And

L_2 (upper limit) =0 + $t_{05}\, S_{diff}$ (9)

Finding untransformed confidence limits for the diatom-inferred concentrations

When the calculations are made with logarithmically transformed units, the error term is added to and subtracted from the mean so the confidence limits will be symmetrical about the mean. The situation is different when we untransform the confidence limits by taking antilogs of Equations 3 and 4.

The lower 95% confidence limit = $\overline{\overline{TP}}$ / antilog ($t_{05}\, s_e$) (10)

The upper 95% confidence limit = $\overline{\overline{TP}}$ x antilog ($t_{05}\, s_e$) (11)

Now the error term is multiplied times the mean to get the upper limit and divided into the mean to get the lower limit. The result is that the confidence limits are asymmetrical about the mean.

Finding untransformed confidence limits for the difference between top and bottom of the core diatom-inferred concentrations

Since the difference between two logarithms is equal to the logarithm of their ratio, Equations 6 and 7 can be rewritten as:

L_1 (lower limit) = Log ($\overline{\overline{TP}}_T$ / $\overline{\overline{TP}}_B$) - $t_{05}\, S_{diff}$ (12)

and

L_2 (upper limit) = Log ($\overline{\overline{TP}}_T$ / $\overline{\overline{TP}}_B$) + $t_{05}\, S_{diff}$ (13)

When we take the antilogarithms of Equations 12 and 13, we find the confidence limits on the ratio of the diatom-inferred concentration at the top of the core to the diatom-inferred concentration at the bottom of the core.
The lower 95% confidence limit is given by

($\overline{\overline{TP}}_T$ / $\overline{\overline{TP}}_B$) / antilog ($t_{05}\, S_{diff}$) (14)

The upper 95% confidence limit is given by

($\overline{\overline{TP}}_T$ / $\overline{\overline{TP}}_B$) antilog ($t_{05}\, S_{diff}$) (15)

Since the null hypothesis is that the ratio is equal to 1, Equations 14 an15 become:

The lower 95% confidence limit is given by
1 / antilog ($t_{05}\, S_{diff}$) (16)

The upper 95% confidence limit is given by
1 x antilog ($t_{05}\, S_{diff}$) (17)

These confidence limits are on the ratio of the diatom-inferred concentration (µg/L) at the top of the core to the diatom-inferred concentration (µg/L) at the bottom of the core.

Examples of calculations using our data

Starting with the RSMEP values and the numbers of lakes used in the calibration data set, we show how we made our calculations in Table B1. The values for Students t with (N_C – 1) degrees of freedom were found from standard two-sided tables of t for a probability of 0.05. The standard error for the difference (S_{diff}) was calculated using Equation 5. The confidence limits on the difference between the tops and bottoms of the cores in logarithmic units were calculated with equations 8 and 9 and the confidence limits on the ratios of the top values divided by the bottom values were calculated with equations 14 and 15. The basic data for the lakes analyzed in this study are presented in Table B2.

Myrtle Lake was sampled as a part of the National Lake Assessment (USEPA 2010). The diatom-inferred TP concentrations at the top and bottom of the single core were 43 µg/L and 117 µg/L respectively. The ratio of the top to bottom concentrations was 0.36. From the top line in Table B1 the 95% confidence limits on the ratio cover the range from 0.10 to 9.95, so the ratio in Myrtle Lake was not significantly different from one.

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Table B1. The calculation of 95% confidence limits on comparisons between the diatom-inferred concentrations of TP and TN at the tops and bottoms of short cores from two studies. N_c = the number of lakes in the calibration of the transfer function, t_{05}= the t value for 5% probability, n = the number of cores per lake, and S_{diff} = the standard error of the difference between the common logarithms of the diatom-inferred concentration of TP and/or TN at the tops and bottoms of short cores.

Variable	RSMEP	N_c	t_{05}	n	S_{diff}	95% Confidence limits Log units	Ratios $\overline{TP}_T$ / $\overline{TP}_B$
TP[1]	0.36	500	1.96	1	0.51	-1.00 to 1.00	0.10 to 9.95
TN[1]	0.29	500	1.96	1	0.41	-0.80 to 0.80	0.16 to 6.37
TP[2]	0.168	69	1.67	1	0.24	-0.40 to 0.40	0.40 to 2.49
TP[2]	0.168	69	1.67	2	0.17	-0.28 to 0.28	0.52 to 1.91
TP[2]	0.168	69	1.67	3	0.14	-0.23 to 0.23	0.59 to 1.69
TP[2]	0.168	69	1.67	4	0.12	-0.20 to 0.20	0.63 to 1.58

1 Data from National Lakes Assessment (USEPA 2010)

2 Data from Whitmore and Brenner (2002)

Table B2. Average concentrations of diatom-inferred total phosphorus and total nitrogen from the tops and bottoms of short sediment cores used in this study. W&B indicates the data are from Whitmore and Brenner (2002) and NLA indicates the data are from the National Lake Assessment (USEPA 2010). The number of cores used for both and top estimates are given by n. The ratios of the inferred values are found by dividing the top value by the bottom value for both total phosphorus and total nitrogen. Lakes whose ratios that fall outside the 95% confidence limits are presented in bold type indicating that there has been a statistically significant change in the inferred values over time.

		Total phosphorus µg/L				Total nitrogen µg/L			
Lake	Study	n	Top	Bottom	Ratio	n	Top	Bottom	Ratio
Beauclaire	W&B	1	86	54	1.6				
Clear	W&B	1	21	15	1.4				
Conine	W&B	2	70	42	1.7				
Dora	**W&B**	**3**	**94**	**27**	**3.4**				
Dosson	W&B	1	34	21	1.6				
Eustis	W&B	2	49	29	1.7				
Floral City	W&B	1	32	34	0.9				
Francis	W&B	1	33	45	0.7				
Griffin	NLA	1	55	43	1.3	1	2255	929	2.4
Griffin	W&B	3	59	41	1.5				
Haines	W&B	2	65	40	1.6				
Halfmoon	W&B	1	12	16	0.8				
Harris	W&B	2	47	40	1.2				
Hartridge	W&B	2	29	45	0.6				
Hollingsworth	W&B	2	91	69	1.3				
Horseshoe	NLA	1	73	108	0.7	1	412	624	0.7
Howard	W&B	2	47	47	1.0				
Jackson	W&B	2	45	53	0.9				
Jessup	**W&B**	**3**	**126**	**48**	**2.6**				
Lucerne	**W&B**	**1**	**17**	**4**	**4.3**				
Marianna	W&B	2	30	37	0.8				
May	W&B	2	62	50	1.2				
McClellan	NLA	1	16	25	0.7	1	406	274	1.5
Minnehaha	W&B	1	34	29	1.2				
Myrtle	NLA	1	43	117	0.4	1	474	605	0.8
Newnans	W&B	4	61	60	1.0				
Orange	W&B	3	45	48	0.9				
Palestine	NLA	1	25	105	0.2	1	392	720	0.5
Parker	**W&B**	**3**	**105**	**44**	**2.4**				
Persimmon	W&B	1	24	41	0.6				

Reedy	NLA	1	16	24	0.7	1	654	704	0.9
Round	**W&B**	**2**	**23**	**10**	**2.3**				
Sawgrass	W&B	1	42	43	1.0				
Seminole	W&B	1	72	62	1.2				
Sheeler	NLA	1	10	10	1.0	1	135	152	0.9
Tarpon	NLA	1	81	61	1.3	1	2123	1193	1.8
Thonotosassa	W&B	2	95	50	1.9				
Washington	**W&B**	**1**	**61**	**18**	**3.5**				
Weir	W&B	2	23	18	1.3				
Yale	NLA	1	28	27	1.0	1	754	679	1.1
Yale	W&B	2	46	35	1.3				

Appendix C. Corrected versus uncorrected chlorophyll a measurements and a comparison of LAKEWATCH measurements of total phosphorus, total nitrogen, and chlorophyll with those of the Florida Department of Environmental Protection

Measurements of chlorophyll pigments in suspended particles in lakes have long been used by limnologists as an approximate measure of the amounts of plankton algal biomass in the water because the chemical measurement is easier to carry out than making algal-cell counts and measuring biovolumes of planktonic cells. For example, Canfield et al. (1985) found a significant correlation (r=0.80) between chlorophyll and phytoplankton biomass. It is, however, an approximate measure of algal biomass because the correlation between chlorophyll measurements and the biomass of living algal cells has a lot of scatter due to varying concentrations of chlorophyll pigments per dry weight of algal biomass and also because of the presence of other pigments in both living and dead algal cells that can interfere with the measurement. In Florida, it was found that the chlorophyll per unit phytoplankton biomass ranged over two orders of magnitude (Canfield et al. 1985).

Originally, measurements of light extinction for extracted pigments were made at a wavelength of 665 nm and were reported as chlorophyll *a*. Then, Lorenzen (1967) pointed out that phaeophytin pigments also absorbed at the same wavelength and could interfere with the calculation of the chlorophyll *a* concentration. He proposed that after the initial spectrophotometer reading was made on the extract of the filtered seston, the sample be acidified to convert the chlorophyll *a* to phaeophytin pigments. A second reading was made as a measure of the total phaeophytin and the difference between the second and the first reading was used to calculate what was termed phaeophytin corrected chlorophyll *a*. This method was then adopted in Standard Methods (APHA 1989) and adopted by many government laboratories. If the correction was not made, what used to be called chlorophyll *a* is now referred to as uncorrected chlorophyll *a*. To some, this inferred that it was an inferior measurement compared to a measurement reported as corrected chlorophyll *a*.

Not all research limnologists adopted the new procedure. They were influenced by Riemann (1978) who described laboratory experiments on the effects of acidification on all algal pigments. He found that the degradation of other pigments in the particles could falsely indicate more phaeophytins than were initially present and thus give an incorrect value for chlorophyll *a* when the phaeophytin correction was made. He cautioned: "Corrections for chlorophyll degradation productions should be made with care to ensure that the presence of phaeopigment *a* is legitimate and not a function of the ratios of epoxidic carotenoids to chlorophyll *a*." None of the standard methods follow that caution.

The choice to use uncorrected chlorophyll *a* measurements for the LAKEWATCH program was influenced by the Riemann (1978) findings and also the fact that David Schindler, Director of the landmark Experimental Lakes Program in Canada, agreed with the conclusions of Riemann (1978) and chose to use uncorrected chlorophyll *a* in their research program that established the importance of accelerated phosphorus loading in lake eutrophication in whole lake experiments. We also recognized that we were not using the chlorophyll measurement the way a plant physiologist might use a chlorophyll *a* measurement. Instead we were interested in using chlorophyll as an index of algal populations, so a chlorophyll measurement without the acidification step was just as useful. We refer to our measurement as total chlorophyll or just chlorophyll and have used it in peer-reviewed publications on Florida lakes that established relationships between fish and trophic state (Bachmann, et al 1996), chlorophyll-phosphorus relationships (Brown et al 2000), seasonal variations in chlorophyll concentrations (Brown et al 1998, chlorophyll-biomass nutrient relations (Canfield et al 1985), frequencies of algal blooms (Bachmann et al 2003), trophic states and aquatic plants (Bachmann et al 2002), and chlorophyll and Secchi disk transparency (Bachmann et al 1999). Using uncorrected chlorophyll measurements, LAKEWATCH has built the largest statewide record of long term measurements of chlorophylls and associated measurements of total phosphorus (TP), total nitrogen (TN) and Secchi disk depths with tens of thousands of measurements in over 1200 lakes. The South Florida Water Management District has used uncorrected chlorophyll measurements in their long-term studies of Lake Okeechobee, and the St Johns River Water Management District used uncorrected chlorophyll measurements for monthly sampling program on Lake Apopka since 1986. There is essentially no difference between our chlorophyll-phosphorus curves and those of others using corrected chlorophyll. The other sources of variation are much more important than the details of the chlorophyll method used. By eliminating the acidification step, the efficiency of our laboratory analyses of chlorophyll is increased and this allows us to sample more lakes with the available resources.

Since 1978, there have been other studies that have shown problems with the acidification step in both marine and freshwaters. A recent paper (Stich and Brinker 2005) compared photometric determinations of uncorrected chlorophyll *a* and pheophytin-corrected chlorophyll *a* concentrations with high-pressure liquid chromatography (HPLC) determinations on the same water samples. They found that pheophytin-corrected chlorophyll *a* concentrations differed significantly from the HPLC-determinations, whereas the uncorrected photometric measurements did not differ. Based on their findings, they recommended the abandonment of the acidification of sample extracts for the determination of pheophytin-corrected chlorophyll *a* concentrations. The reasons for these recommendations were the methodological procedure for the photometric determination of uncorrected chlorophyll *a* concentrations is less time-consuming, the accuracy of the resulting data was better and there was a better comparison of the measurements obtained with different photometers.

For the purposes of this study, we wished to join our chlorophyll data with those collected by the Florida Department of Environmental Protection (FDEP) in order to have the largest possible sample of Florida lakes. We wanted to convert our measurements to the equivalent corrected chlorophyll *a* value, since that was the value used by the USEPA (2010a) in setting the chlorophyll a criterion for Florida lakes. The Minnesota Water Pollution Control Agency (MPCA 2005) also joined corrected and uncorrected chlorophyll *a* measurements in setting their criteria. We did not have a side by side set of measurements, so we compared our LAKEWATCH database with lake data provided by the FDEP and found 8 lakes where we both sampled the same lakes in the same month (not necessarily the same day) on several different occasions. They reported their results as corrected chlorophyll *a* and we reported ours as chlorophyll. When we ran a regression on the 205 pairs of monthly data, we found a significant relationship ($R^2 = 0.82$) between the two variables. The slope of the line was 0.78, reflecting the expected lower values for the pheophytin-corrected chlorophyll *a* concentrations. We then made an empirical adjustment to our data by multiplying each of our chlorophyll values by the factor 0.78. The resulting plot (Figure C1) shows that our adjustment provided a good estimate of the pheophytin-corrected chlorophyll *a* of the FDEP. Most likely the agreement would be even better if the samples had been taken side by side at the same lake stations on the same days rather than within the same months. We ran a paired t-test using the pheophytin-corrected chlorophyll *a* of the FDEP versus our adjusted LAKEWATCH samples collected in the lake in the same month. There was no significant difference (p=0.05) between the paired values. This shows that we have good agreement with both protocols. This was the basis for the adjustment of the LAKEWATCH chlorophyll data used in the study being reported.

We used another way to compare our adjusted LAKEWATCH chlorophyll *a* measurements with corrected chlorophyll *a* values. The USEPA (2010b) developed a linear regression equation with corrected chlorophyll *a* measurements as the dependent variable and total phosphorus as the independent variable. The data set included 417 Florida lakes with color values of 40 PCU or less. The equation was:

$$\text{Ln (Chla)} = 1.11 \ln (\text{TP}) + 6.25$$

Where Chla = the corrected chlorophyll *a* concentration (ug/L), TP = the total phosphorus concentration (µg/L).

We used data on TP and Chla from 631 Florida lakes in our study that had color data of 40 PCU or less. We plotted our adjusted chlorophyll a measurements (0.78 x uncorrected chlorophyll) against corrected chlorophyll *a* concentrations calculated from the USEPA (2010b) regression equation relating corrected chlorophyll *a* concentration to total phosphorus concentrations in clear Florida lakes (Figure C2). In general, the agreement is quite good indicating that our empirical adjustment provided a good estimate of the corrected chlorophyll *a* values.

We made similar comparisons of TP and TN measured on water samples from the same lakes and the same times as the chlorophyll samples (Figure C1). Again, paired t-tests showed there was no significant difference (p=0.05) between the measurements made with the LAKEWATCH protocols and those made by the Florida Department of Environmental Protection using their protocols.

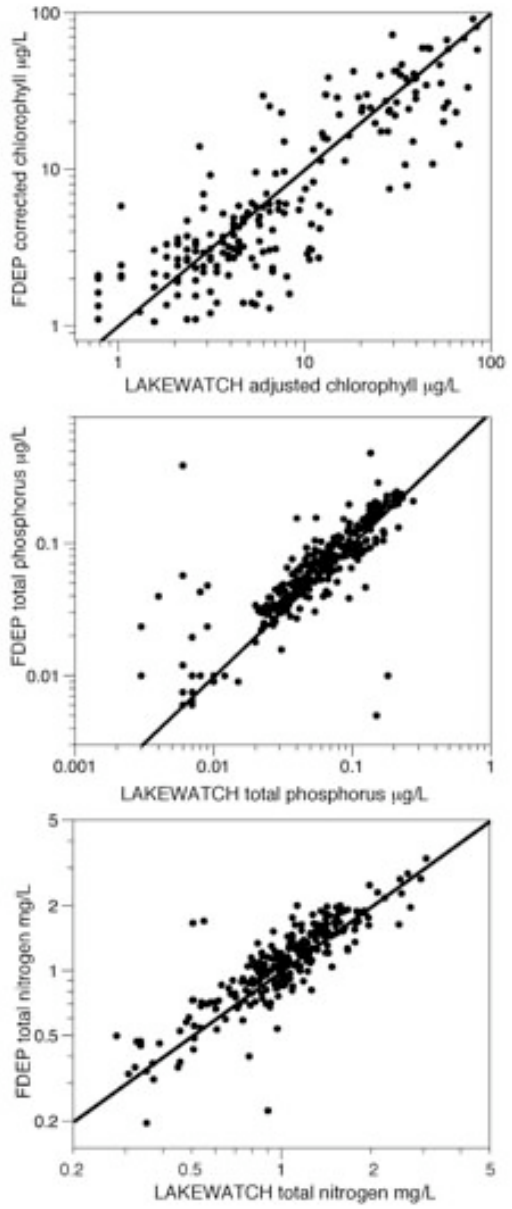

Figure C1. Plots of adjusted chlorophyll, total phosphorus, and total nitrogen concentrations as measured by Florida LAKEWATCH using their protocols versus corrected chlorophyll a, total phosphorus and total nitrogen concentrations as measured by the Florida Department of Environmental Protection using their protocols. The samples were taken from 8 Florida lakes during the same months but not the same days. The lines represent a 1:1 ratio.

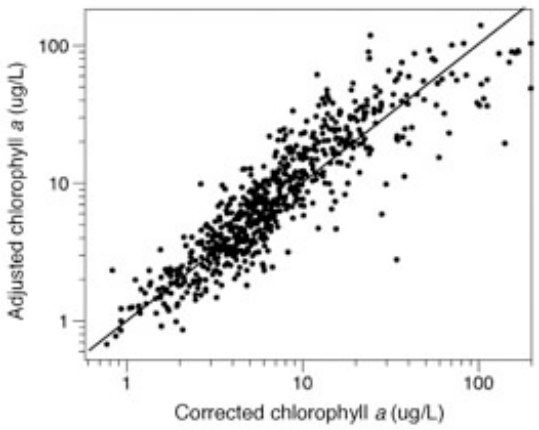

Figure C2. The LAKEWATCH adjusted chlorophyll *a* concentrations are plotted against corrected chlorophyll *a* concentrations calculated from a regression equation (USEPA 2010b) relating corrected chlorophyll *a* concentration to total phosphorus concentrations in clear Florida lakes. A 1:1 line is shown.

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