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Comment on Bachmann et al. (2013): A nonrepresentative sample cannot describe the extent of cultural eutrophication of natural lakes in the United States

In their recent paper, Bachmann et al. (2013) evaluate the extent to which natural lakes in the contiguous United States have been affected by cultural eutrophication since European settlement, using paleolimnological data collected during the 2007 National Lakes Assessment (NLA; U.S. Environmental Protection Agency [USEPA] 2009). The NLA sites were selected using a statistically valid sampling design that allows for the overall ecological condition of the nation's lakes to be accurately characterized (USEPA 2009). Given the current consensus among limnologists regarding the prevalence of culturally eutrophic lakes (Finlayson and D'Cruz 2005; Carpenter et al. 2011), the conclusion of Bachmann et al. that "in the United States of America, the extent that natural lakes have been changed by cultural eutrophication does not seem to be large" (Bachmann et al. 2013, p. 950) is surprising. The findings of Bachmann et al. supporting this statement are not based on the entire NLA sample of natural lakes but rather on a subset of them. We demonstrate below that not only is this subset not representative of the entire population of natural lakes in the United States, but that it is biased toward lakes in regions with less anthropogenic activity and substantially lower nutrient concentrations. Consequently, we argue that the conclusions drawn by Bachmann et al. (2013) at the national scale are based upon a statistically flawed analysis.

Historical water quality trends used in Bachmann et al. (2013) were inferred from diatom species composition in samples from the top and bottom of sediment cores (USEPA 2010). Because the sediment cores were not dated, a conservative set of criteria was applied to determine whether the bottom of the core represented conditions prior to European settlement. Cores "were accepted for analysis if they occurred in nutrient ecoregions 2 and 8 where sedimentation rates are known to be relatively low, based on previous studies; lakes with undisturbed, or relatively undisturbed watersheds, and at least moderately long cores for the region; lakes in the Northeast US greater than 25 cm in length were generally considered sufficiently long […]" (USEPA 2010). On the other hand, lakes "were rejected if: cores less than 20 cm in length, except a few reference lakes that seemed clearly undisturbed […]; all lakes in nutrient ecoregion 6 with percent watershed disturbance (usually Ag) greater than 50% […]; and all cores in this ecoregion, regardless of percent watershed disturbance, that were less than 30 cm long were not considered for analysis" (USEPA 2010). A number of cores were also classified as "Uncertain." The analysis of long-term change in nutrient concentrations of natural lakes presented by Bachmann et al. relies on cores from the first category, for which the USEPA was relatively certain that the bottom of the core represented pre-European settlement conditions (Bachmann et al. 2013, p. 946).

Probability surveys, such as the NLA, are designed to assess a survey sample of a target population in an unbiased manner (Peck et al. 2013). These designs differ from a simple random subsample by having variable inclusion probabilities (i.e., all sites do not have the same probability of being part of the sample). In the case of the NLA, for example, lakes in different size classes were assigned different probabilities of inclusion to avoid having the smallest size class (4–10 ha) dominate the sample. Population-level estimates, therefore, can be made only if the data analyses make proper use of sample weights, yet Bachmann et al. failed to do this. Further, their use of only a subset of the data, as detailed below, precluded appropriate application of sample weights. Whereas this alone suggests that their conclusions about all natural lakes in the conterminous United States were not drawn in a statistically valid manner, there are several more substantial sources of bias in their analysis. Therefore, their exclusion of sample weights will not be considered further in this comment (i.e., our analysis will also be based on an unweighted analysis of the sampled lakes).

In addition to probability sites, which have weights, NLA used data from a set of hand-selected lakes, which do not have weights (they are not part of the probability design). These lakes are hand-chosen because they are likely to be low in nutrients, and therefore these should not be combined with probability data to draw conclusions. Bachmann et al. purportedly analyzed the probability lakes and reference lakes separately. However, a careful analysis of the data used by Bachmann et al. reveals that they incorrectly sorted data into these two categories, resulting in the exclusion of 34 probability lakes from the random sample of natural lakes with good presettlement core data and inclusion of 40 nonrandomly selected lakes (i.e, hand-picked potential reference lakes) in their "probability" data set. If the data are correctly sorted, there are 234 probability lakes with valid cores and inferred nutrient concentrations, not the 240 reported by Bachmann et al. (the 234 correct lakes not being a simple subset of the 240 Bachmann et al. lakes). In the following analysis, therefore, the data used by Bachmann et al. and the "corrected" version of the data will be considered separately.

The application of the criteria quoted above to determine core confidence necessitated the systematic omission of lakes that have been most subjected to watershed disturbance (i.e., those lakes most likely to be affected by cultural eutrophication) from the Bachmann et al. data. Based on the (unweighted) NLA probabilistic dataset, the majority of natural lakes in the United States are located in the Western Forested Mountains (nutrient ecoregion 2), the South Central Cultivated Great Plains (5), the Corn Belt and Northern Great Plains (6), the Mostly Glaciated Dairy Region (7), and the Nutrient Poor Largely Glaciated Upper Midwest and Northeast (8; Figs. 1, 2). However, the NLA sediment core data used by Bachmann et al. (corrected to represent only lakes in the probability

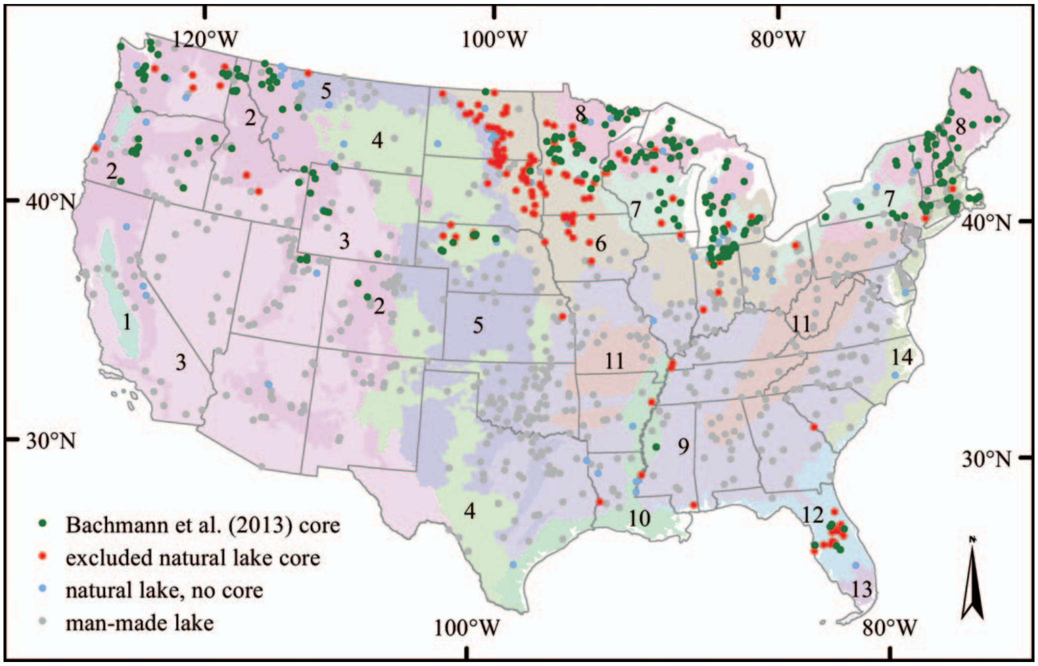

Fig. 1. Map of NLA dataset showing sediment cores included in and excluded from the analysis of Bachmann et al. (2013), corrected as described in the text, as well as noncored lakes and reservoirs in the NLA probability sample. Numerals identify ecoregions (*see* Fig. 2).

sample) are clearly not similarly distributed (Figs. 1, 2). Nutrient ecoregions 2, 7, 8, and 13 are overrepresented relative to all NLA natural lakes, with the majority of core samples from lakes in ecoregions 2, 7, and 8. In ecoregions 2 and 8, mountains and forests are dominant and agriculture and urban development is relatively scarce; and, thus, these are the regions of the nation where cultural eutrophication is expected to be least evident (Dillon and Kirchner 1975; Clesceri et al. 1986). On the other hand, the two most highly agricultural regions where natural lakes are abundant and cultural eutrophication might be expected (5 and 6) are severely underrepresented. Further, of the few natural lakes that were sampled in the Southeast and highly agricultural Mississippi River Alluvial Plain, nearly all sediment cores were excluded from analysis (Figs. 1, 2). The land uses typically associated with eutrophication (e.g., agriculture) are also typically associated with higher rates of erosion—and consequently, sedimentation—yet, due to core-length limitations, lakes with high sedimentation rates were generally excluded from the data set (USEPA 2010).

Analysis of water chemistry and land use and land cover (LULC) data further indicate that the subset of natural lakes used in Bachmann et al. are biased toward systems with lower nutrient concentrations and reduced amounts of anthropogenic LULC compared to the lakes excluded from their analysis. This is especially true given the inadvertent inclusion of 40 hand-selected reference lakes in the random sample combined with the omission of 34 probabilistic lakes. Water chemistry variables (Table 1; Fig. 3) and basin LULC (Table 2) were compared among the lakes included in Bachmann et al., the corrected version of their data (*see* above), and the lakes excluded from the corrected data using standard analysis of covariance. Pairwise comparisons were made using Tukey's Honestly Significant Difference (HSD) test. Water chemistry data were $\log_{10}$-transformed to approximate normality and to maintain consistency with Bachmann et al. LULC variables were arcsine square root transformed. Notably, results from these comparisons indicate that median total phosphorus (TP) concentrations are approximately an order of magnitude (OM) higher in lakes excluded from Bachman et al. than those included in their analysis (Table 1; Fig. 3). A similar pattern was observed for total nitrogen (TN, 0.6 OM), chlorophyll *a* (Chl *a*, 0.8 OM), dissolved organic carbon (DOC, 0.4 OM), and specific conductance (SC, 0.7 OM). Further, comparison of basin LULC metrics indicate that the lakes excluded by Bachmann et al. have greater amounts of developed and agricultural land and substantially less forested land in their basins than the lakes that were included (Table 2). The large differences in basin LULC between included and excluded lakes is not surprising given the spatial distribution of the lakes examined by Bachmann et al. (Fig. 1). Although Bachman et al. incorrectly included 40 reference lakes in their analysis ($\sim$ 17%), this inclusion did not have a significant effect on median water chemistry or LULC values relative to the correct set of natural lakes with presettlement core data (Tables 1, 2).

The 2007 NLA data are not sufficient to determine for certain whether the set of lakes excluded by Bachmann et al. have in fact been largely influenced by human activity post-European settlement. However, the causes and consequences of eutrophication in lakes have been extensively studied for more than five decades (Vollenweider 1968; Smith et al. 1999; Schindler 2006), and it has become abundantly clear that eutrophication of surface waters is primarily caused by their enrichment in phosphorus and nitrogen originating from agricultural and urban areas

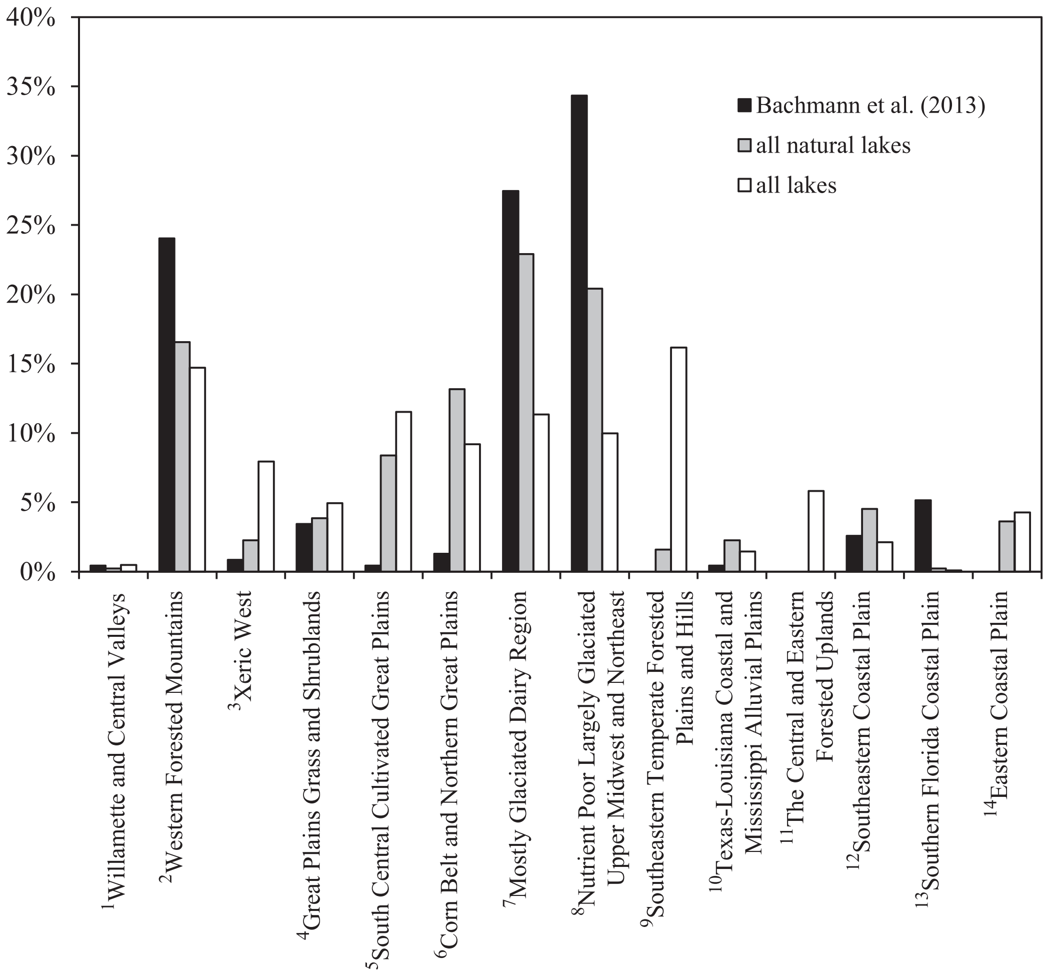

Fig. 2. Distribution of the Bachmann et al. (2013) data, corrected as described in the text, excluded data, and all lakes and reservoirs among the EPA nutrient ecoregions (shown in Fig. 1). The "all lakes" category includes man-made water bodies.

(Carpenter et al. 1998). The current characteristics of the systems excluded from the Bachmann et al. analysis (e.g., large nutrient concentrations and disturbed watersheds) suggest that they would, at a minimum, be more likely to have experienced eutrophication than those that were included. Consequently, their conclusions regarding the extent of cultural eutrophication in the United States are not supported by appropriately representative data and likely underrepresent culturally eutrophic natural lakes.

Bachmann et al. acknowledged that there are "individual lakes or groups of lakes that have been altered due to anthropogenic nutrients," but say that "their numbers are just not large enough to make a significant difference in the averages" (Bachmann et al. 2013, p. 950). In their summary report, the USEPA (2010) notes a related finding: "The difference between the top and bottom of the sediment cores suggests that many lakes may have lower total phosphorus and total nitrogen levels now than they once did […] Without dating the cores, however, more information and

Table 1. Comparison of median values of measured chemical characteristics of natural lakes from the Environmental Protection Agency (EPA) National Lake Assessment with presettlement cores used in Bachmann et al. 2013 (Bachmann lakes; $n = 240$), corrected lakes with presettlement data (corrected lakes; $n = 234$), and natural lakes with rejected cores ($n = 143$; core confidence of either "no" or "uncertain"). Values were log transformed prior to analysis and mean values back-transformed here. Superscript letters denote statistically significant groups (Tukey HSD; $p < 0.05$).

Parameter	Bachmann lakes	Corrected lakes	Excluded lakes
Total phosphorus (μg L^{-1})	11.04 [a]	11.46 [a]	102.8 [b]
Total nitrogen (μg L^{-1})	430.5 [a]	429.9 [a]	1787 [b]
Chlorophyll *a* (μg L^{-1})	4.24 [a]	4.39 [a]	27.3 [b]
Dissolved organic carbon (μg L^{-1})	5.25 [a]	5.28 [a]	13.3 [b]
Specific conductance (μS cm^{-1})	127 [a]	128 [a]	701 [b]

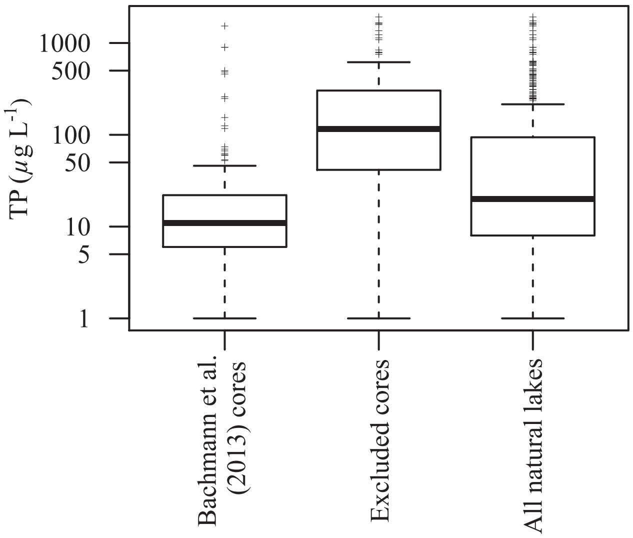

Fig. 3. Boxplots of total phosphorus (TP) for lakes used by Bachmann et al. (2013), corrected as described in the text, NLA natural lake cores excluded from the analysis, and all natural lakes in the NLA.

analysis are needed in explaining these results'' (USEPA 2009). Exclusion of those lakes most likely affected by cultural eutrophication might explain the apparently large fraction of lakes that exhibited a decline in TN and TP. Similarly, exclusion of these lakes might explain why there are not ''large enough'' numbers of lakes that have clearly been affected by anthropogenic nutrient inputs in the data to make a significant difference in the averages.

Bachmann et al. (2013) clearly state that their analysis is limited to natural lakes (defined as those that existed pre-European settlement), as we have also done here for comparison. However, it is worth noting that the areas of the nation that generally contain the most pristine waters (e.g., nutrient ecoregions 2 and 8) are dominated by natural lakes, whereas many highly agricultural areas (e.g., the Ohio River basin and the central Great Plains) are populated by mostly artificial water bodies (Fig. 1). Further, reservoir-dominated areas comprise approximately the southern half of the county (Fig. 1). Therefore, we argue that limiting a study of the extent of cultural eutrophication in the United States to natural water bodies essentially weights the analysis toward regions in which cultural eutrophication is less expected.

In conclusion, the fact that Bachmann et al. ''did not find significant increases since presettlement times in TP and TN'' (Bachmann et al. 2013, p. 950) may simply be due to the nonrepresentative nature of the data they analyzed; and, whereas the finding may be true for their set of selected lakes, it cannot be extended to all natural lakes in the United States. Bachmann et al. stated that ''The assumption of widespread cultural eutrophication for setting nutrient criteria in lakes is not supported'' (Bachmann et al. 2013, p. 950). Here we have shown that the analysis supporting that statement was critically flawed. Given the overwhelming weight of several decades' worth of evidence documenting the occurrence and exploring the causes of cultural eutrophication (Smith et al. 2014), a continued regulatory focus on reducing nutrient inputs to lakes remains the only scientifically defensible way to protect and restore our lakes.

Table 2. Comparison of mean values and difference of major watershed land use characteristics (%) among natural lakes with presettlement cores used in Bachmann et al. 2013 (Bachmann lakes; $n = 240$), corrected lakes with presettlement data (corrected lakes; $n = 234$), and natural lakes with rejected cores ($n = 143$; core confidence of either ''no'' or ''uncertain'') from the EPA National Lake Assessment. Values arcsine square root transformed prior to analysis. Results are back-transformed. Superscript letters denote statistically significant groups (Tukey HSD; $p < 0.05$).

Parameter	Bachmann lakes	Corrected lakes	Excluded lakes
Developed	4.6 [a]	4.5 [a]	7.7 [b]
Forested	44.6 [a]	43.0 [a]	5.3 [b]
Agricultural	7.0 [a]	6.4 [a]	30.3 [b]
Wetlands	6.2 [a]	5.9 [a]	5.9 [a]
Open water	10.5 [a]	10.4 [a]	12.6 [a]

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