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Determining Ecoregional Reference Conditions for Nutrients, Secchi Depth and Chlorophyll *a* in Kansas Lakes and Reservoirs

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Abstract

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Baseline environmental conditions are a critical consideration in the development of scientifically defensible aquatic nutrient criteria. We applied three methods to ecoregionally stratified data to determine reference conditions in Kansas lakes and reservoirs with respect to total phosphorus, total nitrogen, Secchi depth, and planktonic chlorophyll *a* (chl *a*). First, minimally developed lake/watershed units were identified based on existing geographical databases and visual basin surveys. Lakes and reservoirs in these watersheds were considered minimally-to-least impacted "reference" waters. Second, median nutrient, Secchi depth, and chl *a* values were determined for the best one-third of lakes and reservoirs and applied as indicators of reference condition (trisection). Third, a regression-based extrapolation method was applied to estimate water quality conditions in the absence of anthropogenic influences. The first method suggested no ecoregional effect on the trophic status of minimally impacted reference water bodies, whereas the other two methods indicated some significant ecoregional differences. Lack of ecoregional effect in reference bodies could indicate that differences were driven by anthropogenic influences rather than natural regional characteristics. Reference conditions, as determined by these three methods, broadly agreed for all parameters and were generally at or less than literature values for the mesotrophic-eutrophic threshold for lakes and reservoirs worldwide. Reference values for total phosphorus were primarily less than levels commonly associated with cyanobacterial blooms. Overall, the data suggest that multiple methods can be used to determine reference condition, and that in Kansas lakes and reservoirs reference condition corresponds to mesotrophic state.

Key Words: nitrogen, phosphorus, nutrient criteria, ecoregion, eutrophication

Controlling eutrophication in lakes and reservoirs has been a consistent focus of lake managers around the world for decades (Vollenweider 1976, OECD 1982, Rast and Holland 1988, Banens and Davis 1998, Jeppesen *et al.* 2003). Eutrophication is a global problem and shows no signs of abating in the near future (Smith 2003). Even in man-made impoundments, avoidance of fish kills, taste and odor problems, toxic algal contamination of drinking waters, loss of recreational uses and revenues, and aesthetic and property-value impacts make control of trophic state desirable (Smith *et al.* 1999, Dodds 2002).

Limits on nutrients in lakes and reservoirs in any given region will not be obtainable if they are set lower than the values occurring in relatively pristine watersheds of the region. Different regions (Omernik 1995) and lake or reservoir types (Kennedy 2001) can be characterized by different reference nutrient concentrations. Variations in trophic status can occur across several hundred kilometers (Jones and Knowlton 1993) possibly limiting the applicability of trophic status scales for broad geographic areas (Carlson 1977, OECD 1982, Nürnberg 1996).

In defining the trophic status of lakes and reservoirs, nutrients such as total nitrogen (TN) and total phosphorus (TP) are generally described as driver variables and planktonic chlorophyll *a* (chl *a*) and Secchi depth as response variables. Although some emphasize the role of phosphorus as a primary limiting nutrient (Correll 1999), empirical results suggest that both nitrogen and phosphorus can control primary production in lakes and reservoirs (Smith 1982, Dodds *et al.* 1989, Elser *et al.* 1990, Carney 1996 to 2003), so we consider both. Non-algal turbidity can decouple the relationships between nutrient concentration and algal biomass (Knowlton and Jones 1993). Accordingly, water bodies exhibiting high levels of inorganic suspended material are excluded from our present analysis.

The United States Environmental Protection Agency (USEPA) has directed states to set nutrient criteria for lakes, reservoirs, rivers, streams and wetlands. The initial step for setting criteria for lakes and reservoirs is determination of appropriate baseline reference conditions (Gibson *et al.* 2000). Several approaches have been suggested for delimiting reference conditions (Gibson *et al.* 2000, USEPA 1998a, USEPA 1998b). Here, we identify lakes and reservoirs that have relatively little development in their watersheds or along their shores. The resulting frequency distributions for TN, TP and mean chl *a* concentration can be used to characterize the reference condition.

In areas where few or no minimally impacted lakes are identified, the lower 25th percentile of the frequency distribution of the entire lake database has sometimes been equated with the reference condition (Gibson *et al.* 2000). Some states more recently have adopted the trisection method recommended for biotic integrity indices (USEPA 1998b) as a better alternative to this approach. Using the trisection method, median values derived from the best one-third of the data are considered indicative of the reference condition. The problem with this approach is its sensitivity to the proportion of impacted sites and the degree of regional impact.

An additional approach previously applied to rivers and streams considers the statistical relationships among land uses and nutrient concentrations in rivers and streams (Dodds and Oakes 2004). These relationships are used to estimate nutrient concentrations occurring in the absence of measurable anthropogenic impact on the landscape.

Kansas lakes and reservoirs span a variety of ecoregions (Chapman *et al.* 2001) and offer insight into the identification of the reference conditions and the development of appropriate criteria within this and other regions of central North America. We use data from lakes and reservoirs across the state to test three techniques for determining reference condition.

Materials and Methods

Data for TP, TN, chl *a*, Secchi depth, and algal cell counts were collected from lakes and reservoirs across the state as part of the ambient monitoring network maintained by the Kansas Department of Health and Environment (KDHE 2000). A total of 220 water bodies were selected for this analysis from 1985 to 2002. Most water bodies were reservoirs, but a few smaller ones were oxbows and sink hole lakes. Morphometric characteristics (Table 1) of these reservoirs and lakes indicated a wide variety of watershed and lake areas with relatively shallow median depth. Data were included from reservoirs and lakes that had at least four surveys in separate years over this time interval, and that did not have chronic high turbidity or widespread and dense macrophyte communities that might decouple nutrient/chl relationships. Approximately 112 water bodies were sampled on a rotational schedule as part of a fixed ambient water quality network. The remaining 108 were sampled as part of several synoptic surveys, special projects and other nonroutine sampling efforts.

During each survey, duplicate 0.5 m depth grab samples were taken at an integrator station located at the deepest part of the water body near the dam or outlet. Results from analyses of these samples for each water body were compiled into long-term, epilimnetic summer mean values for each parameter. Although chl *a* data were available for all 220 lakes and reservoirs, some water bodies lacked data for one or more of the other trophic state parameters. Analysis of algal cell counts was limited to the last three years of the time interval (2000-2002), which included 115 lakes and reservoirs.

Total N concentration was estimated by summing Kjeldahl nitrogen values (EPA Method 351.1) with nitrate and nitrite values determined by ion chromatography (EPA Method 300.0). Total P was measured by colorimetric analysis after acid hydrolysis (EPA Method 365.1). During the time period used for this analysis, minimum reporting limits were 100 and 10 $\mu g \cdot L^{-1}$ for TN and TP, respectively. Concentrations lower than the reporting limits were encountered infrequently, and values were set to one-half the detection limit when this occurred.

Chl *a* concentration was determined spectrophotometrically and corrected for the presence of phaeophytin (Standard Method 10200H; APHA-AWWA-WPCF 1989). Samples collected for phytoplankton cell counts were preserved with Lugol's Iodine and counted using Standard Method 10200 F, with a modified Sedgwick-Rafter counting cell (APHA-AWWA-WPCF 1989). Algal samples were obtained from an aliquot of the water collected for nutrient and chl *a* analyses. Prior to counting, samples were concentrated five-fold using settling tubes (1-2 weeks duration), after which the top 80% of each sample was gently drawn off with a suction apparatus and the remainder stored for later counting (KDHE 2000).

Table 1.-Morphometric characteristics of lakes and reservoirs used in this study by ecoregion. CGP = Central Great Plains, CIP = Central Irregular Plains, SWT = Southwest Tablelands, WCP = Western Cornbelt Plains, WHP = Western High Plains.

Ecoregion Number	CGP 55	CIP 103	FH 29	SWT 5	WCP 19	WHP 9	State 220
Watershed Area (km²)							
Maximum	32178	1535	1786	105	2788	91	32178
75th %ile	239	11	36	66	21	21	23
Median	17	3	14	41	7	2	6
25th %ile	3.64	0.72	3.81	8.44	2.56	0.06	1.27
Minimum	0.008	0.004	0.222	0.069	0.263	0.012	0.004
Lake Area (ha)							
Maximum	6464	2828	1131	101	4929	32	6464
75th %ile	86	44	105	35	47	12	55
Median	16	12	46	31	25	4	18
25th %ile	4	3	30	15	4	1	4
Minimum	0.4	0.4	0.4	0.4	1.6	0.4	0.4
Maximum Depth (m)							
Maximum	19	16	15	6.5	16	6	19
75th %ile	6	8	10	5	9.5	3	8
Median	3.5	5	7	4	4.5	2	4.75
25th %ile	2	3	5	2	4	1.5	3
Minimum	1	0.5	1	1	1.5	1	0.5
Mean Depth (m)							
Maximum	7.8	7.9	6.1	2.8	6.5	3.3	7.9
75th %ile	3	3.2	3.4	2.5	3.3	1.3	3.2
Median	1.7	2	2.7	1.7	1.9	0.8	2
25th %ile	0.8	1.3	2	0.8	1.7	0.5	1.3
Minimum	0.1	0.1	0.1	0.1	0.5	0.1	0.1

Table 2.-BPJ method reference values (median and upper quartile) for chl *a*, Secchi depth, total phosphorus, and total nitrogen. Number of lakes and reservoirs included for each analysis is given as "n." Values are derived from the mean summer epilimnetic values for each water body from 1985 to 2002.

Parameter	Median	75th Percentile	n
Chl *a* ($\mu g \cdot L^{-1}$)	8	10	58
Secchi Depth (cm)	129	155	55
Total Phosphorus ($\mu g \cdot L^{-1}$)	23	33	58
Total Nitrogen ($\mu g \cdot L^{-1}$)	625	861	47

Algal biovolume was calculated based on the cell counts combined with the average cell volume for each species encountered.

Three methods were used to determine the reference trophic conditions for Kansas lakes and reservoirs. The first involved identification of a reference water-body population, composed of lakes and reservoirs with minimally impacted watersheds, determined by examination of available land use databases and visual surveys of the watersheds (hereafter also referred to as the best professional judgment, or BPJ method). Reference water bodies were identified as those that had no more than 20% cropland and/or urban land in their drainages, very little (if any) of that area in immediate contact with the main inflow and/or shoreline, and no obvious in-lake characteristics strongly influencing water quality (*e.g.*, sediment resuspension problems in shallow lakes, or very large and dense macrophyte communities). The number of lakes and reservoirs meeting these criteria ranged from 47 to 58, depending on the parameter of interest (Table 2). Both the median and the upper quartile were calculated for these reservoirs (Gibson *et al.* 2000). Ecoregions were combined if analysis of variance indicated no significant effect of ecoregion on a water quality constituent ($p>0.05$).

The trisection method initially considered all the sampled lakes and reservoirs in Kansas but retained only that third with the lowest nutrient or chl *a* concentrations or with the greatest Secchi depths, assuming that the least-impacted water bodies were represented by the best one-third of the distribution (USEPA 1998a). The 50th percentile (median) value was calculated from this subpopulation. Ecoregions were combined if analysis of variance indicated no significant effect of ecoregion on a water quality constituent before trisection ($p>0.05$).

Table 3.-Reference chl *a* values ($\mu g \cdot L^{-1}$) and Secchi depths (cm) derived from the trisection and extrapolation methods, as applied to lake and reservoir data from selected EPA Level III Ecoregions in Kansas. The number of water bodies for each analysis is given under "n." "NA" = not analyzed because method did not provide a statistically significant model.

Ecoregion	Chl *a*				Secchi Depth			
	Trisection	n	Extrapolation	n	Trisection	n	Extrapolation	n
Central Great Plains	11	18	NA		117	17	66	44
Central Irregular Plains	8	34	11	100	130	31	109	92
Flint Hills	5	9	9	26	149	9	112	25
Western Corn Belt	13	6	NA		114	5	93	14

The final method for determining the reference condition was based on analysis of watershed characteristics, similar to the method proposed previously for use in rivers and streams (Dodds and Oakes 2004). This technique incorporated data on the anthropogenic impacts in the watershed, created a regression model with those data, and used the y-intercept to predict nutrient concentration in the absence of any anthropogenic land uses in the watershed. To determine land use in each watershed, KDHE personnel drove through the watersheds, coded general land use categories on copies of topographic maps of each watershed and used a gridded section template to tally totals for each. This model initially used analysis of co-variance (ANCOVA) to detect a specific ecoregional effect. If an ecoregional or interaction effect with ecoregion was deemed insignificant ($p>0.05$), data were combined across ecoregions. If a significant ecoregional effect was found, data from each ecoregion were analyzed separately.

Multiple regression analysis using Mallow's CP information criteria (an index that controls for the increase in r^2 that results when additional parameters are added to multiple-regression models and allows determination of the best regression model of all possible models) was used to identify the best regression models (Helsel and Hirsch 2002). These models were then used to partition anthropogenic effects in the estimation of reference condition. Percentages of cropland, other agricultural land, urban land and feedlots documented within the watersheds of these lakes and reservoirs were used as indicators of anthropogenic influence.

Results

Preliminary determination of chl *a*, Secchi depth, TP and TN data distributions indicated that none of the four variables were normally distributed. Log transformations successfully normalized most of these distributions (Kolmogorov-Smirnov test, $p>0.05$), and all subsequent statistical analyses were undertaken on log-transformed data.

Analysis of variance revealed no significant effect of ecoregion on chl *a*, Secchi depth, TP or TN when data were considered from the BPJ reference sites, so these data were combined across ecoregions. However, analysis of variance indicated a significant ecoregional effect when considering the entire data set ($p>0.05$), so trisection analyses were done by ecoregion. In the extrapolation model application, analysis of covariance also established significant ($p>0.05$) ecoregional effects, so those data were analyzed separately by ecoregion as well.

In general, median reference values derived from the BPJ method (Table 2) were bracketed by values derived from the other two methods, even though these methods were analyzed by ecoregion. For example, median reference chl *a* values were 8 $\mu g \cdot L^{-1}$ for the BPJ method (Table 2), 5-13 $\mu g \cdot L^{-1}$ for the trisection method and 9-11 $\mu g \cdot L^{-1}$ for the extrapolation method (Table 3). Reference Secchi depths were 129 cm for the BPJ method, 114-149 cm for the trisection method and 66-112 cm for the extrapolation method (Table 3). Reference TP values had a median of 23 $\mu g \cdot L^{-1}$ for the BPJ method, 19-44 $\mu g \cdot L^{-1}$ for the trisection-method and 23-62 $\mu g \cdot L^{-1}$ for the extrapolation method (Table 4). Finally, reference TN concentrations had a median value of 625 $\mu g \cdot L^{-1}$ for the BPJ method and 201-695 $\mu g \cdot L^{-1}$ for the trisection method (Table 4). The extrapolation method yielded significant models between land use and TN for only a single ecoregion (Table 4).

Even the upper quartile values derived from the BPJ method were bracketed by the other two methods, suggesting that the BPJ method lends itself to more conservative estimates of reference condition. The extrapolation method generally failed for TN prediction because few significant relationships existed between TN and land use for the lakes and reservoirs sampled in this study. The trisection method consistently generated lower values for TP and larger values for Secchi depth than the extrapolation method, suggesting that the extrapolation method is the least conservative approach considered in this study.

A significant ($p<0.05$) positive relationship existed between TP and planktonic chl *a* (values in $\mu g \cdot L^{-1}$) for the115 lakes and reservoirs used for analysis of phytoplankton count data

Table 4.-Reference values for total phosphorus and total nitrogen for reservoirs, rivers and streams in selected U.S. Environmental Protection Agency level III Ecoregions in Kansas. Dodds and Oakes (2004) and Smith *et al.* (2003) values are for rivers and streams in aggregated nutrient ecoregions corresponding to the Level III Ecoregion. Values for Smith *et al.* (2003) are corrected for atmospheric deposition. All values in $\mu g \cdot L^{-1}$. "NA" = not analyzed because method did not provide a statistically significant model. Note aggregated ecoregions were used in the two literature sources.

Parameter	Ecoregion	Trisection (Lakes)	n	Extrapolation (Lakes)	n	Dodds and Oakes (Streams)	Smith *et al.* (Streams)
Total Phosphorus	Central Great Plains	44	16	62	40	23	58
	Central Irregular Plains	20	33	27	97	31	48
	Flint Hills	19	9	23	25	59	60
	Western Corn Belt	25	6	27	17	23	54
Total Nitrogen	Central Great Plains	695	8	NA		566	258
	Central Irregular Plains	362	17	NA		370	150
	Flint Hills	301	13	NA		659	95
	Western Corn Belt	201	4	658	10	215	355

(Fig. 1). This relationship is represented by the following equation:

$$\log_{10} \text{chl } a = (\log_{10} \text{TP} * 0.960) - 0.421$$
$$r^2 = 0.65$$

Further analysis of this data set revealed that a greater proportion of planktonic cell volume and cell count was composed of cyanobacteria when TP was abundant, as evidenced by the cluster of points in the lower left panel of Fig. 1. When data were subjected to a two-dimensional Kolmogorov-Smirnov test for bivariance (Garvey *et al.* 1998), the relationship was bivariate ($p<0.004$), with breakpoints in the relationship at 55 $\mu g \cdot L^{-1}$ TP and 51% cyanobacterial volume. Another way to view this relationship is with frequency distributions (Fig. 1C). Values up to 100 $\mu g \cdot L^{-1}$ TP yield a constant probability of having at least 25% cyanobacterial biovolume. The probability of 50% and 75% biovolume appears to increase at 50-100 $\mu g \cdot L^{-1}$ TP.

Discussion

Why should nutrient criteria be established for reservoirs and other water bodies that are not a part of the natural landscape but are, instead, a product of human civilization? This question may be particularly relevant in a state such as Kansas where the prevailing surface geology, climate and limited extent of glaciation mean there are few natural lakes. Eutrophic conditions in reservoirs should be avoided, however, particularly in those used for drinking water and recreation.

Arruda and Fromm (1989) used a taste-testing panel to identify a significant correlation between the trophic state of selected Kansas reservoirs and taste and odor problems in drinking water derived from those reservoirs. The City of Wichita recently installed a $7 million pretreatment system for drinking water to remove tastes and odors associated with algal blooms, and Emporia, Kansas, spent $0.8 million for the same reason. Over the past few years, many water bodies in Kansas have suffered eutrophication-related fish kills or impacts on water supply and recreational uses. Among those with severe eutrophication problems during 2003 and 2004 were Marion Lake and Cheney Reservoir (water supply and recreational impacts due to massive cyanobacterial blooms), Winfield City Lake (taste and odor complaints due to algae), Lake Waltanna (eutrophication-related fish kills), Lake Meade State Park (recreational and potential fish-kill concerns associated with massive cyanobacterial blooms) and numerous small municipal lakes and reservoirs around the state. These situations illustrate the need to establish appropriately protective nutrient criteria for Kansas lakes and reservoirs.

We assessed the published literature on lake and reservoir trophic state to help put our reference values in a broader geographic perspective. Nürnberg (1996) reviewed trophic classifications throughout the world and regionally (Table 5). She based her thresholds between TP-based trophic-state categories on prior classification schemes and the observation that these limits were approximately evenly spaced on a logarithmic scale. A logarithmic scale seems necessary to capture the wide variety of lake trophic states (Carlson 1977). Nürnberg then calculated expected TN, chl *a*, Secchi depth, and hypolimnetic anoxia on the basis of relationships developed with data from a large number of water bodies. Her calculated boundaries agreed fairly well with other published boundaries.

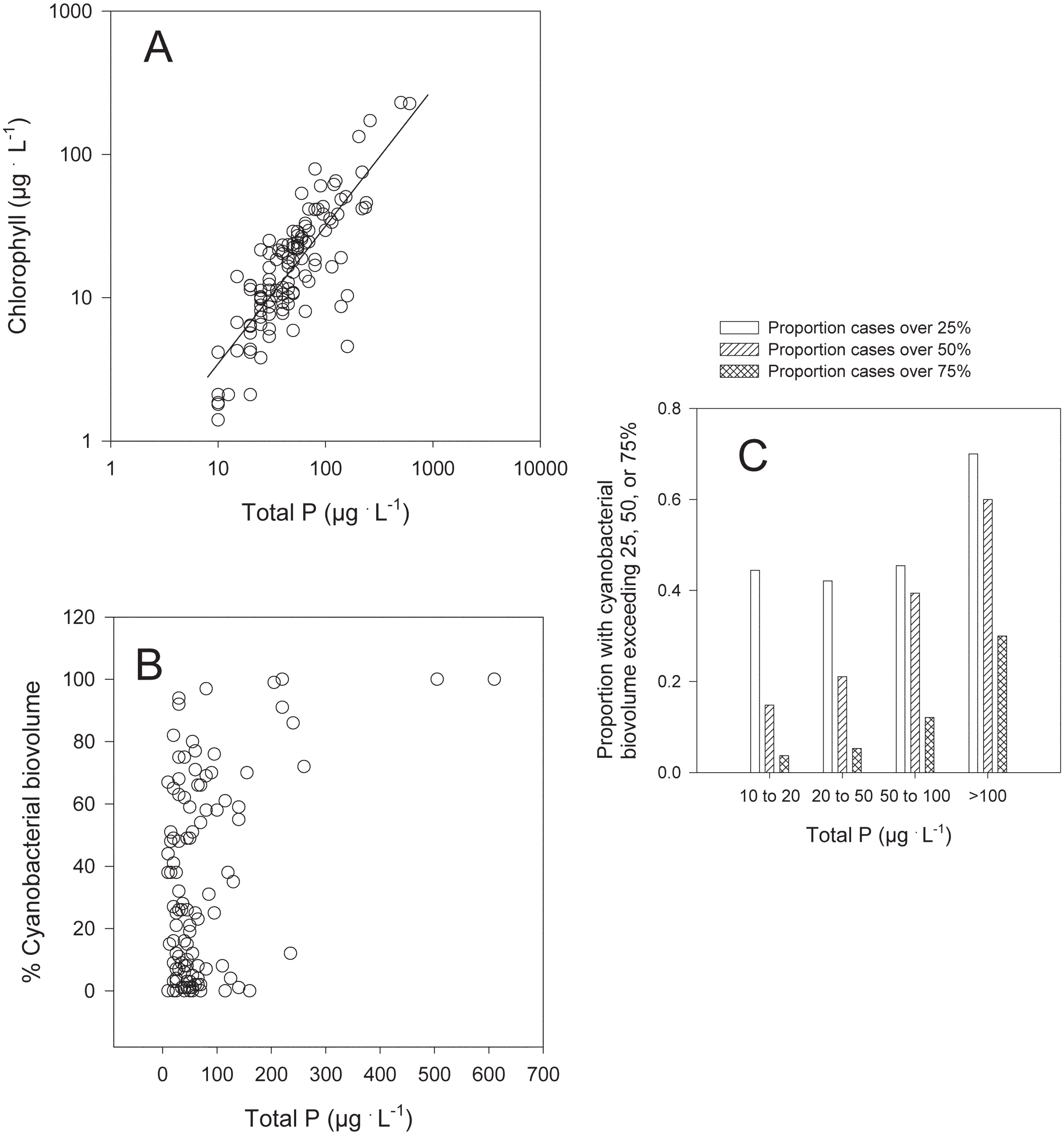

Figure 1.-Relationships among TP and chl *a* (A), TP and percent of planktonic biovolume that is cyanobacterial (B), and TP and proportion of cases with cyanobacterial biovolume exceeding 25, 50 and 75% (C), for 115 Kansas lakes and reservoirs.

Table 5.-Trophic thresholds for lakes and reservoirs worldwide as reviewed by Nürnberg (1996). O-M is the oligotrophic-mesotrophic boundary, M-E is the mesotrophic-eutrophic boundary, and E-H is the eutrophic-hypertrophic boundary.

Attribute	Source	O-M	M-E	E-H
Total Phosphorus ($\mu g \cdot L^{-1}$)	Recommended	10	30	100
	Literature minimum	10	18	50
	Literature maximum	15	45	1500
Total Nitrogen ($\mu g \cdot L^{-1}$)	Recommended	350	650	1200
	Literature minimum	140	180	1200
	Literature maximum	499	920	2940
Chl *a* ($\mu g \cdot L^{-1}$)	Recommended	3.5	9	25
	Literature minimum	2	5	18
	Literature maximum	4.3	10	40
Secchi (m)	Recommended	4	2	1
	Literature minimum	3.9	2	1
	Literature maximum	6	3	1.5
Hypolimnetic O_2 demand ($mg\ m^{-2}\ d^{-1}$)	Recommended	252	398	550

More important, the computed limits for hypolimnetic O_2 demand in Nürnberg's study (1996) suggest that at the mesotrophic-eutrophic boundary, in a 7.5-m-deep hypolimnion with 8 $mg \cdot L^{-1}$ of dissolved O_2 at the time of stratification, the complete hypolimmion would become anoxic within 150 days. Similar calculations suggest that at least 238 days would be required to reach anoxia at the oligotrophic-mesotrophic boundary under the same starting conditions. In lakes situated above the mesotrophic-eutrophic boundary there is a high probability that their hypolimnia would become anoxic during the summer stratification period (about 100-150 days in Kansas). These findings are relevant to lake management in that taste and odor problems as well as internal nutrient loading are enhanced by hypolimnetic anoxia.

Our suggested ranges for reference values fall mostly below the mesotrophic-eutrophic boundaries proposed by Nürnberg (1996). However, our boundaries for Secchi are considerably shallower than her proposed mesotrophic-eutrophic boundary. This is consistent with data from Missouri lakes and reservoirs (Jones and Knowlton 1993, Knowlton and Jones 1993) where TP-chlorophyll relationships are similar to broader analyses but Secchi depths are shallower than expected. This occurred even though very turbid reservoirs were excluded from our analyses. Furthermore, the TP and TN concentrations considered as reference values for lakes and reservoirs are similar to those suggested for the rivers and streams draining ecoregions of Kansas (Table 4) and feeding the lakes and reservoirs of interest. The numbers for streams should be viewed with some caution because the Missouri ecoregions were aggregated somewhat differently than they were in this paper. Given that nutrient concentrations can be expected to be generally lower in lakes and reservoirs than in inflow streams (because denitrification and settling cause nutrient losses), it is encouraging that the levels in reference rivers and streams are generally similar to those in reference lakes and reservoirs. That is, it would appear possible to control the trophic state of Kansas lakes and reservoirs through the application of nutrient criteria.

The best currently attainable conditions in all ecoregions have levels of TP, TN and planktonic chl *a* near or well below those at which water quality problems are known to become more severe. Had reference nutrient levels in Kansas lakes and reservoirs been solidly in the eutrophic range, the eventual control of eutrophication problems in these systems would have appeared less probable.

Cyanobacteria are of particular interest because eutrophication can lead to toxic algal blooms (Anderson *et al.* 2002). Downing *et al.* (2001) demonstrated that the probability of cyanobacterial blooms increases dramatically when mean summer epilimnetic TP exceeds 30-70 $\mu g \cdot L^{-1}$. Similarly, research in New Zealand and Australia demonstrated a low risk of adverse biological effects when TP is <35 $\mu g \cdot L^{-1}$ (Hart *et al.* 2004). Finally, Cook *et al.* (1993) suggested planktonic communities in lakes shift to gas vacuolated cyanobacteria between 50-100 $\mu g \cdot L^{-1}$ TP. Our analysis of cyanobacterial biovolume as a function of TP agrees closely with these findings. When TP exceeds 50 $\mu g \cdot L^{-1}$, the probability that cyanobacterial biovolume will exceed 50% of total biovolume increases dramatically.

Total phytoplankton is usually expected to increase with increasing TP concentrations. The relationship between TP and cyanobacterial blooms is also consistent with observations elsewhere when considered concurrently with our reference nutrient values for Kansas lakes and reservoirs. It should be possible to attain nutrient conditions in Kansas lakes and reservoirs under which cyanobacterial blooms, or at least frequent and recurring blooms, are unlikely. However, based on mean values of chl *a* data used in our analysis of each water body, sporadic episodes of greater algal biomass are likely (Bachman *et al.* 2003). For example, it would be reasonable to expect a lake with a mean chl *a* of 8 $\mu g \cdot L^{-1}$ to experience maxima of 15-20 $\mu g \cdot L^{-1}$, although infrequently, during a typical summer.

In conclusion, available data suggest that oligotrophic lakes and reservoirs are not likely to occur in Kansas. A mesotrophic state for Kansas water bodies, however, is a reasonable management expectation. Previous attempts to calculate reference condition in Kansas lakes and reservoirs agree with this conclusion (Carney 2002). That the different methods for determining reference condition are in approximate agreement (*e.g.*, mean TP and TN values can range over several orders of magnitude among Kansas lakes and reservoirs, but reference levels generally vary by less than three fold) is reassuring, and having at least three potential approaches available for determining reference values offers some degree of certainty. Any one approach may yield an unexpectedly high or low reference value, but such a value can be checked using the other methods.

Although all three methods could be used simultaneously to provide different lines of evidence for comparison, some states or regions probably lack the large amount of data needed to satisfactorily implement all three approaches. Therefore, a hierarchical order of preference could be considered for applying these various techniques for assigning official threshold values.

Where suitable minimally impacted reference lakes and reservoirs exist in a region, the use of a selected reference subgroup should be given preference for determining the regional reference condition. In areas where the cohort of lakes and reservoirs reflects a wide range of anthropogenic influences and appropriate data exist to adequately quantify those influences, the extrapolation approach provides a statistically defensible method for evaluating the effect of land use. However, the method should be applied with caution given the difficulties inherently associated with statistical extrapolation. While the trisection method can act as a surrogate for identifying a reference subgroup, this method is not recommended for regions with widespread anthropogenic impacts because the subgroup represented by the best one-third of the data will not reflect a minimally impacted reference condition, and anthropogenic impacts may confound efforts to aggregate across ecoregions. In all instances, analysis of variance or analysis of covariance can be used to evaluate the ecoregional effect and may support the aggregation of data across ecoregions to make better use of data from a limited number of reference sites.

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