LIMNOLOGY
and
OCEANOGRAPHY: METHODS

Limnol. Oceanogr.: Methods 2, 2004, 333–341

A technique for establishing reference nutrient concentrations across watersheds affected by humans

Walter K. Dodds and Robert M. Oakes*
Division of Biology, Kansas State University, Manhattan, KS 66506, USA

Abstract

Establishing reference nutrient conditions for rivers and streams is necessary to assess human impact on aquatic ecosystems and protect water quality and biotic integrity. Several methods have been proposed: (1) percentiles from statistical distributions of all rivers and streams in a region or dataset, (2) reference stream approaches, and (3) modeling river networks from existing reference streams. We propose an additional statistical method to estimate the influence of anthropogenic land uses on lotic nutrient concentrations. First, we quantify regional variation by using analysis of covariance, where total nitrogen or total phosphorus is the dependent variable, region is the categorical predictor, and percentage of anthropogenic land use (e.g., cropland, urban land) is the covariate. This allows for the aggregation of regions if there is not a significant regional effect, or if there is a significant regional effect, identifies the need to analyze regions separately. Second, we develop multiple linear regression models with best-model techniques in which anthropogenic land-use classifications are the independent variables, and the logarithms of in-stream nutrient concentrations are the dependent variables. The intercept of these regression models (i.e., expected nutrient concentration in the absence of human activities assuming linear extrapolation to the origin) represents the reference nutrient concentrations. This analysis suggests that larger percentages of cropland and urban land have strong positive influences on in-stream nutrient concentrations, both in eastern Kansas and across the conterminous United States. The most appropriate method for regions may depend on the relative availability of reference sites and other data sources. The covariance/reference approach offers a potential method for regions with limited numbers of reference sites.

One of the central issues of pollution control is the establishment of reference conditions. This is particularly true of efforts to control anthropogenic eutrophication because some amount of nutrient enrichment of aquatic systems from land is a natural part of pristine ecosystems. Identifying reference conditions is an especially difficult issue when establishing nutrient criteria in rivers and streams. Water quality conditions downstream are affected by the entire watershed upstream, and few watersheds are minimally affected by humans (Lewis 2002). Reference conditions (i.e., background nutrient concentrations) provide an indication of the maximum obtainable water quality if human impacts are completely controlled and may approximate the natural trophic state of a lotic ecosystem. Reference conditions in aquatic ecosystems also provide insight into the abiotic habitat in which the biotic community evolved. In some instances, it may be necessary to establish pristine natural conditions to maintain biotic integrity (Dodds and Welch 2000).

Establishing nutrient criteria has recently assumed greater importance (Dodds and Welch 2000) as regulatory agencies in numerous developed countries have extended efforts to control eutrophication. Originally, efforts were aimed toward controlling eutrophication in lakes and reservoirs, but efforts have now expanded to streams and wetlands. As part of this strategy, several approaches have been used to establish reference conditions. Most approaches rely on the concept that nutrient concentrations in waters may be based upon site-specific characteristics that are in some way independent of anthropogenic impacts. One approach to delineating ecoregions for North America is based on soils, vegetation, and dominant land uses (Omernik 1995) and explicitly recognizes that reference conditions may vary spatially across landscapes. The United States Environmental Protection Agency (USEPA) has

*E-mail: wkdodds@ksu.edu

Acknowledgments

We thank Dolly Gudder for technical assistance and members of the LAB journal club and Ed Carney for helpful criticism. Jim Omernik provided useful insights, and the Kansas Department of Health and Environment, the Konza Prairie Long Term Ecological Research project, the USGS, and the USEPA (with special thanks to Jeffrey Comstock) provided data. Support for this project came from the USGS GAP program and from the NSF Long Term Ecological Research program. This is contribution 04-286-J from the Kansas Agricultural Experiment Station.

aggregated Omernik's ecoregions to suggest geographically delineated nutrient criteria (USEPA 1998).

The USEPA suggests using one of three strategies for determining reference conditions (Buck et al. 2000). The first approach characterizes reference reaches for each stream class within a region according to best professional judgment, and then uses these reference conditions to develop criteria. This method fails when reference reaches are not available, as is the case in some agriculturally dominated regions of the midwestern United States. The second approach identifies the 75th percentile of the frequency distribution of reference streams for a class of streams and uses this percentile to develop the criteria. The third approach calculates the fifth to 25th percentile of the frequency distribution of the general population of a class of streams and uses the selected percentile to develop the criteria. In regions that are extensively affected by human nutrient loading, the method of using the 25th percentile can lead to the establishment of artificially large values for nutrient criteria (Smith et al. 2003), perhaps leading to failure to protect water quality.

A biologically based approach would use the functional response between biotic indicators and stream nutrient concentrations. This approach could be based on community analyses of algae (e.g., ordination analyses of diatom assemblages; Pan et al. 1996, 2000). Alternatively, the natural breakpoint beyond which no additional benthic chlorophyll yield is expected (Dodds et al. 2002) could be used to set upper limits on in-stream nutrient concentrations. While some regions (e.g., England, northeastern United States) have well defined biotic indices, community responses of biota to elevated nutrients are not well developed for many specific regions. To our knowledge, biologically based index approaches have received little general application to nutrient control strategies employed by regulatory agencies. Such approaches may ultimately provide important data as biotic responses at the species level to nutrients are documented for each specific region of interest.

A promising approach to estimate reference conditions involves using data from small, moderately affected systems and applying statistical modeling techniques to obtain reference nutrient values for large rivers (Smith et al. 2003). This method uses available reference reaches, predicts in-channel nutrient removal rates from existing data, and attempts to model in-channel nutrient concentrations in the absence of human impact. This technique is useful in that it provides an alternative to the difficult (or impossible) task of identifying large reference watersheds, and it accounts for in-stream nutrient processing that may remove nutrients from the water column as water flows through drainage systems. This method can also account for atmospheric deposition. But the technique works only if small, moderately affected systems are available, and if modeling assumptions are met.

The considerations described in the previous paragraphs identify the need for a method of determining baseline nutrient concentrations in areas with different degrees of human impact. Such a method would be most useful in regions that have few or no minimally affected sites, such as heavily agricultural or urban areas in the United States. In the examples we present in this paper, digital land cover and population density data, which are available for the entire contiguous United States, are used to generate reference nutrient concentrations. We will discuss the potential applicability and limitations of this approach.

In this paper, we explore statistical techniques to distinguish human land-use effects from naturally variable nutrient concentrations. We use three data sets to explore this approach: a detailed water-chemistry data set from central and eastern Kansas, data from an extensively monitored pristine watershed in the Flint Hills region of northeast Kansas, and a national dataset generated from the United States Geological Survey (USGS) survey network (Alexander et al. 1998).

Materials and procedures

Kansas data—The mean of the nutrient concentration for all samples at each site was used as the response variable. Chemical data were taken from the ambient monitoring network maintained by the Kansas Department of Health and Environment (KDHE). Data for total phosphorus (TP) were collected from 1990 to 2002. Data for total nitrogen (TN) were collected from January 2000 to May 2003. Total nitrogen and phosphorus were analyzed by a colorimetric automated phenate method, after digestion by metal-catalyzed acid and persulfate techniques, respectively, according to USEPA standard methods. Nitrate concentrations were added to the amount of N determined by metal-catalyzed digestions to represent TN. Minimum detection limits were 0.1 and 0.01 mg L^{-1} for TN and TP, respectively. Samples lower than the minimum detection were encountered less than 1% of the time, and values were set to the detection limit if this occurred. Data were taken from watersheds in four ecoregions from central-eastern Kansas (Central Great Plains, Central Irregular Plains, Corn Belt, and Flint Hills). Only watersheds that were entirely within a USEPA level III ecoregion were selected (Oakes 2003). No watersheds were selected that had large numbers of confined animal feeding operations or permitted sewage outfalls close to the sampling location. We were unable to control for animal feeding operations holding fewer than 200 animals.

Additional TN and TP data were collected on Kings Creek, a pristine prairie stream that has been monitored for nutrient chemistry. We used stream site data from 1994 to 2001 (Dodds 2003). Within the Kings Creek site, there are 4 small pristine watersheds where samples were taken. Total N and TP samples were collected three times a week during the period when flow was occurring, leading to 1727 individual samples, which were analyzed as reported previously (Dodds 2003). The TN and TP values were never below the limit of detection (4 µg L^{-1} P and N).

USGS data for United States—Nutrient data were obtained from a large data set compiled by the USGS for sampling that occurred from 1970 to 1983 (Alexander et al. 1998). All sampling dates when both TN and TP were collected were included in these analyses. Samples were below the detection limits less than 0.3% of the time for TP, and these values were set to zero. There were no values below detection for TN. The dataset was further restricted to sites sampled more than 20 times, those that included land-cover data as of 1987, and sites that did not appear to be statistical outliers. The mean of the nutrient concentrations for all samples at each site was used as the response variable. Land-cover classifications were obtained from the U.S. Soil Conservation Service (now officially titled the Natural Resources Conservation Service of the U.S. Department of Agriculture). The classifications assumed to include anthropogenic impacts were cropland (areas used for production of crops for harvest), pasture land (land managed primarily for the production of introduced forage plants for livestock grazing), range land (plant cover that is principally grasses or small plants suitable for grazing), farm land (buildings and livestock-holding facilities), and urban land (residential, industrial, commercial, and institutional land). Population data from 1990 were also included in models. These areas were classified by hydrologic units in the USGS database. Monitoring stations that met the criteria listed above for both TN and TP were used to create a spatially explicit Geographic Information System (GIS) coverage of sampling site locations that was overlaid on a digital stream network (USEPA river reach file, RF1) and a GIS layer of the USEPA level III ecoregions (USEPA 1998). Hydrologic units are not defined solely by the watershed above a single point. For example, a hydrologic unit on the Mississippi river may only cover a portion of the watershed above that point (i.e., the tributaries entering for a relatively short distance upstream from that point). Thus, hydrologic units do not always encompass the entire watershed above a sampling station (Omernik and Bailey 1997). Therefore, as a final data refinement step, stations were only included where 2/3 or more of the entire watershed draining into the station was contained in one ecoregion. This last selection avoids problems with stations that may be influenced by multiple upstream ecoregions (Griffith et al. 1999). A total of 519 stations were used in analyses, with 5 to 113 stations present in each of the 13 USEPA level III aggregate National Ecoregions (USEPA 1998). The aggregate National Ecoregions are hereafter referred to simply as ecoregions.

Statistical methods—Statistical analyses were performed by using Statistica 6.1 (Statsoft). All nutrient data were $\log_{10}$ transformed for two reasons: (1) it led to normal data distributions (Kolmogorov-Smirnoff test, $P > 0.05$) and (2) data distributions did not yield intercept estimates that were less than zero once data were log transformed. This is particularly important when applying the regression approach described next, inasmuch as a negative nutrient concentration is not possible, but the method could potentially yield a negative intercept when using non–log-transformed nutrient concentrations as the dependent variable.

Statistical analyses were accomplished in two steps. Analysis of covariance (ANCOVA) was first used to test for significant differences among ecoregions while accounting for the effects of land use variables on water column nutrients (Sokal and Rolhf 1981). Second, within ecoregions, or across ecoregions that were not significantly different ($P > 0.05$) as determined by ANCOVA, multiple linear regression was used to establish relationships between land use and nutrient concentrations. All possible subsets regression was used to determine the best fit model with Mallow's Cp as an index to control for the effect of adding additional variables into the model.

The regression approach is reflective of that used by Omernik (1977). In that study, however, the percentage of urban land and cropland were simply summed, whereas the approach outlined herein finds the best regression model of all potential model subsets, assigns relative weights to each land-use category, and considers additional land-use categories that were not available to Omernik (1977). Results from the multiple linear regression analyses were examined in two ways. First, the intercept (β_o) and associated error were used to extrapolate nutrient concentrations in the absence of predominantly anthropogenic landscapes, and the variance in this prediction could be characterized by the 95% prediction band around the estimate for β_o. Second, the values for β, excluding the intercept, were condensed into a single axis to visualize the influence of multiple significant land uses. For example, if the regression model yielded a relationship of $\log_{10}\text{TP} = \beta_o + \beta_1 \times \%$ cropland $+ \beta_2 \times \%$ urban land, then β_o was taken as the expected reference level and its 95% prediction band was used to establish the uncertainty in the estimate for reference nutrient concentrations. In addition, a two-dimensional plot was created in which the x value for each site was calculated as $\beta_1 \times \%$ cropland $+ \beta_2 \times \%$ urban and was plotted against TP concentrations at sites on the y axis.

Methods developed by the USEPA for determining reference nutrient concentrations (Buck et al. 2000) were also used; the fifth to the 25th percentile of the frequency distribution of the general population of a class of streams were calculated for Flint Hills data (using data from the state of Kansas only) to allow comparison with reference and regression methods.

Assessment

Kansas data—When data taken from KDHE were used, the Flint Hills were characterized by relatively small percentages of cropland and urban land, the Corn Belt by large percentages of cropland, and the Central Irregular Plains by the largest proportion of urban lands (Fig. 1). The Flint Hills were characterized by the lowest TN and TP concentrations (Fig. 1).

Analyses related to TP are used first to exemplify the methodology developed in this article. Analysis of covariance demonstrated that there was not a significant ecoregion effect, or a significant interaction effect on TP between ecoregion

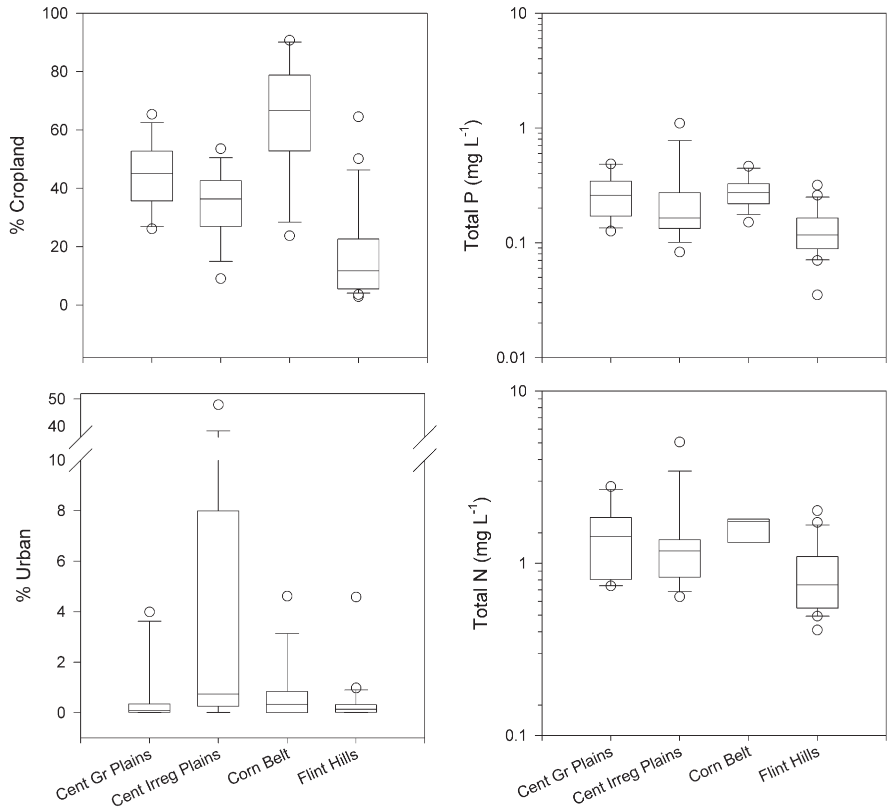

Fig. 1. Box plots of cropland, urban land, TP, and TN for water quality sites in eastern Kansas by level III ecoregion. Lines in center of boxes are the medians, tops and bottoms of boxes are 75th and 25th percentiles, respectively. Bars are 95% confidence intervals, and outliers are plotted as open points.

and percentages of cropland or urban land (Table 1). This lack of an ecoregion effect indicated that the ecoregions could be combined; therefore, data were pooled across ecoregions for the multiple regression analysis. Both the percentage of cropland and urban land, as well as the intercept, were significantly related to TP concentrations in the regression model (Table 2). The strength of this relationship can be visualized by creating an x-axis value from the results of the regression across all sites with varying land use (Fig. 2). In this example, the regression equation was Log_{10} TP = –0.724 + 0.00668 × % cropland + 0.1465 × % urban land (Table 2).

Therefore, for each sampling site, an x variable was created by using the equation: x = 0.00668 × % cropland + 0.1465 × % urban land. This plot also includes the individual ecoregion-specific regression lines to allow visual verification of the statistical result from ANCOVA that slopes did not vary significantly across ecoregion. Although the line for the Corn Belt has a slightly lower slope, this was not statistically significant.

The intercept (β_o = –0.724) is then used to establish reference criteria in the TP regressions. The intercept represents the point where percentages of cropland and urban land are zero. Given the regression, a 95% prediction band could be calculated around this predicted value such that the reference level, the lower confidence interval, and the upper confidence interval were 97, 38, and 251 µg P L^{-1}, respectively.

In contrast to the results from the analyses of TP, TN did vary significantly across Kansas ecoregions (Table 3, ANCOVA with significant ecoregion effect and ecoregion × % cropland interaction). Thus, it was necessary to perform the regressions separately by ecoregion in this example.

Table 1. Analysis of covariance of log transformed total P, with four Kansas ecoregions as categorical predictors and with percentages of urban and cropland as the covariates

	Sum of squares	d.f.	Mean square	F	P
Intercept	2.331	1	2.331	71.59	<0.0001
Ecoregion	0.175	3	0.059	1.80	0.1593
% crop	0.161	1	0.161	4.96	0.0303
% urban	0.112	1	0.112	3.43	0.0697
Ecoregion × % crop	0.175	3	0.058	1.80	0.1595
Ecoregion × % urban	0.223	3	0.074	2.29	0.0896
% crop × % urban	0.051	1	0.051	1.56	0.2169
Ecoregion × % crop × % urban	0.155	3	0.056	1.58	0.2043
Error	1.693	52	0.033		

Table 2. Results of regression analysis for relationship between percentages of urban land, cropland, and $\log_{10}$ transformed TP (mg L^{-1}), with aggregated data across four Kansas ecoregions (R^2 = 0.431).

	B	Standard error	t(65)	*P* level
Intercept	–0.72376	0.048511	–20.8900	<0.000001
% crop	0.00668	0.001097	6.0872	<0.000001
% urban	0.01465	0.003280	4.4652	0.000033

The regression method was compared with two other methods suggested by USEPA, the fifth and 25th quartile of a general population of streams sampled by KDHE and comparison with a known reference stream (Table 4). In this instance, the upper 75% of data taken from a known reference stream (Kings Creek on Konza Prairie) had substantially less TP than results arrived at by any other method. The quartile methods had slightly smaller reference values than did the TP regression method across the four ecoregions. The regression method for TN yielded very similar results to the percentile method, and results from both methods were slightly more than two times greater than those of the pristine reference site (Table 4).

United States Geological Survey (USGS) data—Analysis of covariance indicated significant differences in TN and TP across ecoregions when using percentages of cropland and urban land as the covariates ($F_{12,466}$ = 8.8693, P < 0.00001 for TN; $F_{12,466}$ = 8.1841, P < 0.00001 for TP), so each of the USEPA aggregate level III ecoregions were analyzed separately for both TN and TP. Ecoregion 6 (Corn Belt and Northern Great Plains) had, on average, substantially greater mean TN and TP values than the other ecoregions, and ecoregions 2 (Western forested mountains) and 8 (Nutrient poor glaciated upper midwest and northeast) had significantly smaller mean values (P < 0.05) than other ecoregions.

All possible subsets regression using Mallow's Cp for model selection of anthropogenic land use classifications (population, % urban land, % cropland, % pasture land, % range land, and % farm land) was used to predict TP (Table 5). Regression analysis for TP suggested that percentage of cropland was a strong predictor of TP within ecoregions (6 of 13 models), as were percentages of urban land and total population above the sampling site (6 of 13 cases for both predictor variables). In ecoregion 4 (Great Plains grass and shrublands), there was a weak relationship between land-use factors and TP concentrations, which contrasts with the results of the Kansas analyses (Table 2) that included smaller portions of this ecoregion. In general, the TP values predicted in the absence of human land use were less than 50 µg L^{-1} TP, except when the relationship between

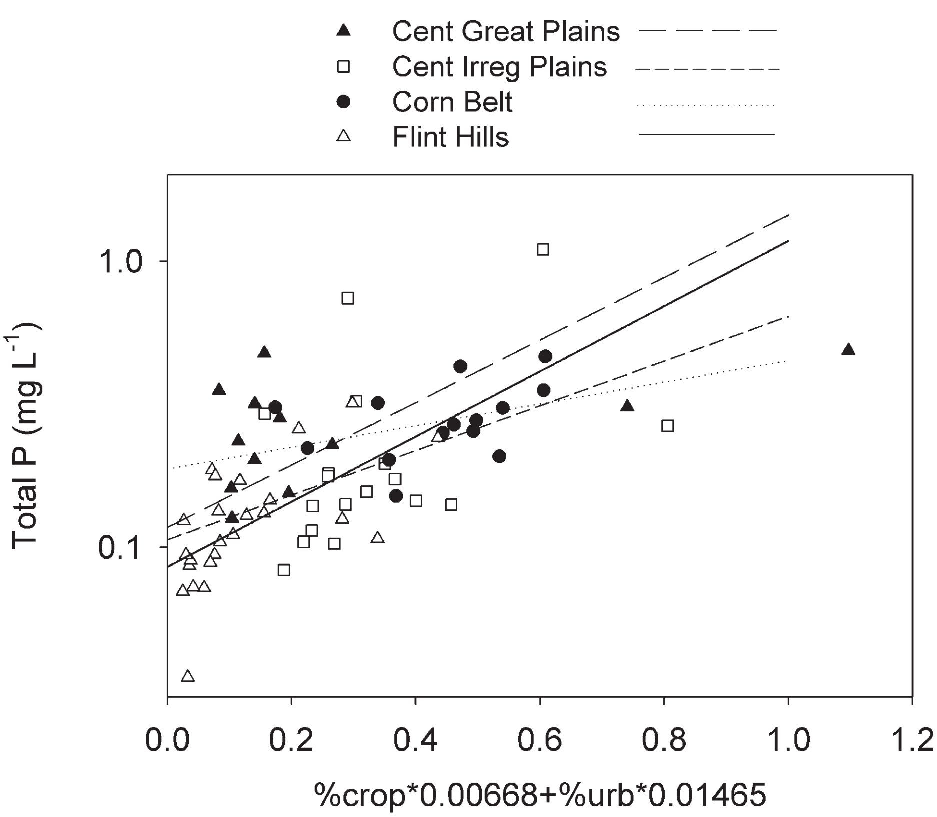

Fig. 2. Relationships between land use and TP by ecoregion from ecoregions of eastern Kansas. Data on x axis were constructed from results of regression analysis across ecoregions (*see Materials and procedures* for details). Individual lines represent regressions within ecoregions.

Table 3. Analysis of covariance of $\log_{10}$ TN, with percentage of cropland as the covariate and ecoregion as the categorical predictor

	Sum of squares	d.f.	Mean square	F	P
Intercept	0.013	1	0.013	0.402	0.5291
Ecoregion	0.632	3	0.210	6.457	0.0009
% crop	0.011	1	0.011	0.327	0.5701
Ecoregion × % crop	0.469	3	0.156	4.793	0.0053
Error	1.600	49	0.033		

land use and TP was weak (i.e., the Xeric west and the Great Plains grass and shrublands) and in the Texas-Louisiana coastal and Mississippi alluvial plains ecoregion.

All possible subsets regression of anthropogenic land-use factors was also used to predict TN (Table 6). As with the regression analyses for TP, the percentage of cropland was the variable most often included in the model that best predicted TN within ecoregions (9 of 13 cases), followed by watershed population (7 of 13 cases) and the percentage of urban land in the watersheds (5 of 13 cases). In ecoregion 12 (Southern coastal plains), there were no significant relationships between land-use factors and TN concentrations, and in ecoregion 4 (Great Plains grass and shrublands), the predictive ability of the model was very weak. Predicted background TN concentrations were less than 600 µg L^{-1} TN in 8 of 12 instances.

Discussion

In a broad sense, the regression method for determining reference nutrient concentrations in the absence of anthropogenic land uses provided numbers comparable to those generated with the USEPA 25% method and that of Smith et al. (2003). The data suggest that generally the reference values should be suspect if they fall above 60 µg L^{-1} TP or 600 µg L^{-1} TN (Table 7). There were some instances of serious divergence among methods, however, with each approach having its own limitations and benefits. Correlation analyses and paired-difference *t* tests failed to find significant correlations among the methods ($P > 0.05$) or significantly higher or lower values predicted by any of the methods ($P > 0.05$).

The regression model we present here has the primary limitation of not quantifying all sources of human impacts because such data were not readily available. Quantification of impacts such as atmospheric deposition of N, confined animal feeding units, effects of riparian buffer strips and other best-management practices, and point discharges from sewage treatment likely would have improved model accuracy. Specifically, accounting for other anthropogenic nutrient inputs would further lower the estimated reference nutrient concentrations. Accounting for additional factors such as drainage area and slope could improve the accuracy of the models. In-channel processing (i.e., the effect of stream size) cannot be assessed with the regression approach.

The regression approach relies upon the percentage of land use in each category to compare anthropogenic impacts across watersheds. This leads to potential problems with non-normal data distribution (Sokal and Rohlf 1981). Unfortunately, the transformations generally employed to correct non-normal proportional data are not defined at zero. Because the point of the approach is specifically to determine the intercept when the dependent variable (degree of anthropogenic impact) is zero, we used percentages in our analyses. Perhaps the greatest weakness of our regression approach is the need to extrapolate beyond known data points. However, where reference reaches are not available, this may be the only option for determination of reference conditions.

The regression approach does not require data from a large number of reference or low impact sites. If there are no low impact sites however, this method requires prediction of data far from the data points that anchor the relationship. Predictions made farther away from the observed data result in broader prediction bands. Thus, the accuracy of this approach should be greatest when sites include a relative continuum of land-use intensity.

The use of ANCOVA before application of the regression approach allowed for a statistically defensible aggregation of

Table 4. Mean and 95% confidence intervals for estimated reference-nutrient concentrations in a pristine Flint Hills site (Kings Creek), and intercepts and 95% prediction bands according to regression analysis of TP across four ecoregions in Kansas and TN across the Flint Hills (Kansas Department of Health and Environment data), and using the 25th and fifth percentile of all Flint Hills data (Kansas Department of Health and Environment data)

Method	Parameter	Concentration (µg L^{-1})	*n*	Low 95% (µg L^{-1})	High 95% (µg L^{-1})
Flint Hills reference site (Kings Creek)	TP	6.56	1146	6.28	6.87
Regression across ecoregions	TP	97	65	38	251
25th percentile Flint Hills data	TP	90	24	—	—
Fifth percentile Flint Hills data	TP	70	24	—	—
Flint Hills reference site (Kings Creek)	TN	223	1727	214	232
Regression across Flint Hills	TN	575	22	285	1148
25th percentile Flint Hills data	TN	569	24	—	—
Fifth percentile Flint Hills data	TN	493	24	—	—

Table 5. Best model regression results (Mallow's CP) for TP

Ecoregion number	Ecoregion name	n	R^2	β_0	Standard error	Best model land uses
1	Willamette and Central Valleys	5	0.90	–1.710	0.134	% crop
2	Western forested mountains	39	0.34	–1.348	0.137	pop, % urban, % past, % range
3	Xeric west	84	0.20	–0.820	0.099	pop, % urban
4	Great Plains grass and shrublands	36	0.19	–1.226	0.427	pop, % urban
5	Central cultivated Great Plains	35	0.30	–1.645	0.359	% urban, % range, % farm
6	Corn belt and Northern Great Plains	42	0.47	–1.640	0.314	pop, % urban, % crop, % range, % farm
7	Mostly glaciated dairy region	32	0.56	–1.630	0.101	pop, % crop
8	Nutrient poor glaciated upper Midwest and Northeast	41	0.32	–1.555	0.044	% crop, % range
9	Southeastern temperate forested plains and hills	113	0.48	–1.514	0.071	% urban, % crop, % range, % past
10	Texas-Louisiana coastal and Mississippi alluvial plains	30	0.64	–0.950	0.162	pop, % range
11	Central and Eastern forested uplands	27	0.32	–1.370	0.097	% urban, % crop
12	Southern coastal plain	15	0.54	–1.316	0.239	% range, % farm
14	Eastern coastal plain	18	0.19	–1.397	0.127	% farm

β_0 is calculated for mg L^{-1} phosphorus.

ecoregions. This is beneficial because it may be possible to aggregate ecoregions with few or no reference or low-impact sites with other ecoregions that do include reference sites, and subsequently decreases the prediction bands around estimates of reference concentrations.

Another benefit of the regression approach is that it identifies specific anthropogenic land use practices that contribute to nutrient pollution in an ecoregion and, thus, may guide management efforts. Finally, the graphical method used to represent the regression results can be used to visualize the data in two dimensions, even when more than one independent variable is significantly related to the dependent variable.

The modeling approach taken by Smith et al. (2003) used reference data to model the expected increase in N in the stream channel as stream order increased in order to estimate reference conditions. Smith et al. (2003) also accounted for atmospheric deposition of N into watersheds and for the loss of N and P from the water column of rivers as the water moved down the drainage network. However, their approach requires using reference reaches in which human impacts are relatively moderate as a starting point for modeling, requires extrapolation from small streams to larger rivers, and requires substantial modeling expertise to implement.

The establishment of reference watersheds to delineate nutrient criteria may be most desirable because it allows true

Table 6. Best model regression results (Mallow's CP) for total N*

Ecoregion number	Ecoregion name	n	R^2	β_0	Standard error	Best model land uses
1	Willamette and Central valleys	5	0.98	–0.583	0.048	% crop
2	Western forested mountains	39	0.41	–0.319	0.080	pop, % urban, % past, % range
3	Xeric west	84	0.32	–0.037	0.089	pop, % urban, % crop, % past, % range
4	Great Plains grass and shrublands	36	0.11	–0.181	0.291	pop, % urban
5	Central cultivated Great Plains	35	0.40	–0.247	0.179	pop, % range, % farm
6	Corn belt and Northern Great Plains	42	0.67	–0.668	0.205	% urban, % crop, % past, % range
7	Mostly glaciated dairy region	32	0.69	–0.248	0.069	pop, % crop
8	Nutrient poor glaciated upper midwest and northeast	41	0.62	–0.230	0.031	% crop
9	Southeastern temperate forested plains and hills	113	0.68	–0.432	0.040	% urban, % crop, % past, % range
10	Texas-Louisiana coastal and Mississippi alluvial plains	30	0.61	–0.128	0.069	pop, % crop, % range
11	Central and Eastern forested uplands	27	0.52	–0.091	0.042	% crop, % range
12	Southern coastal plain	15	NA†			
14	Eastern coastal plain	18	0.53	–0.445	0.114	pop, % crop, % farm

*β_0 is calculated for mg L^{-1} nitrogen.
†NA signifies that regression was not significant.

Table 7. Comparison of results from this paper and with 25% values suggested by the United States Environmental Protection Agency and values modeled by Smith et al. (2003) corrected for atmospheric N loading*

Ecoregion number	Ecoregion name	TP †	TP ‡	TP §	TN †	TN ‡	TN §
1	Willamette and Central valleys	20	47	16	261	310	156
2	Western forested mountains	45	10	19	479	120	157
3	Xeric west	151	22	24	918	380	44
4	Great Plains grass and shrublands	59	23	60	659	560	95
5	Central cultivated Great Plains	23	67	58	566	880	258
6	Corn belt and Northern Great Plains	23	76	54	215	2180	355
7	Mostly glaciated dairy region	23	33	22	565	540	147
8	Nutrient poor glaciated upper midwest and northeast	28	10	13	589	380	165
9	Southeastern temperate forested plains and hills	31	37	48	370	690	150
10	Texas-Louisiana coastal and Mississippi alluvial plains	112	128	48	745	760	439
11	Central and Eastern forested uplands	43	10	20	1102	310	156
12	Southern coastal plain	48	40	24	NA	900	548
14	Eastern coastal plain	40	31	15	359	710	561

*All values reported in μg L^{-1} N or P; NA = not applicable.
† This study.
‡ United States Enviromental Protection Agency.
§ Smith et al. (2003)

assessment of baseline, or reference, nutrient concentrations. But reference reaches simply are not available in many areas. As watershed size increases, the probability of identifying a suitable reference watershed decreases, as reference reaches tend to be in small headwater streams. Therefore, the reference reach method has limited applicability for medium-to-large rivers. Additionally, reference sites may be unaffected because of specific characteristics that make them undesirable for human uses (e.g., rocky terrain unsuitable for cropland), and may not be reflective of reference conditions across a region.

The USEPA 25% method is entirely dependent on the data that are incorporated, because it relies on the statistical distribution of all existing data. Thus, the USEPA method may present a moving target of criteria as nutrient pollution amounts increase with population growth and development (Dodds et al. 1998). The benefit of the 25% method is that it allows the maximum number of data points available to be used in the analysis, which may be important in regions without extensive sampling. Additionally, it requires the least technical expertise (i.e., no local determination of what is a reference was reached by experts, only moderate statistical training, and no modeling expertise) to implement.

The Corn Belt and Northern Great Plains ecoregions included few reference sites, and applying the USEPA 25% method to these sites yielded very large values. Most of the region is dominated by agricultural practices, and strong correlations existed between TN, TP, and row-crop agriculture when these relationships were analyzed using the USGS nationwide dataset. The USEPA 25% method resulted in reference values of 76 and 2180 μgL^{-1} for TP and TN, respectively, whereas the regression method with USGS data yielded substantially smaller numbers (3.3 and 10.1 times less for TP and TN, respectively). In this instance, the regression model was in better accord with the modeling approach of Smith et al. (2003).

Comments and recommendations

There is no optimal method for determining reference conditions for nutrient levels in rivers and streams. Fewer and fewer minimally affected streams are available to sample, particularly with regard to atmospheric contamination by N and agricultural influences. There is no better option than using detailed local data collected at pristine sites to establish baseline conditions, but many instances arise in which such data are not available. In these instances, it is important that multiple approaches are available to assess reference conditions. Individuals undertaking such analyses should be aware of the strengths and weaknesses of the datasets they have to work with and supplement their analyses with data from a variety of sources. Reference values provide regulators with a theoretical framework for best- and worst-case nutrient-control scenarios for a specific region.

Whereas this paper uses EPA ecoregions as a framework in which to use the regression approach, other spatial characteristics could be considered (e.g., geology, slope, drainage area). Accounting for any additional variance would allow for tightening of the confidence limits on the predicted reference concentrations. Thus, the regression method can be tailored for specific areas. It may also be possible to use the various techniques in concert with each other. For example, ANCOVA could indicate that it is reasonable to aggregate ecoregions, allowing for inclusion of more than a few reference sites.

This paper underscores the concept that there are multiple approaches to determining reference nutrient conditions. Each method has strengths and weaknesses; the regression method presented here provides yet another tool for estimating reference conditions. Perhaps the most useful aspect of the numbers generated with this analysis is to examine the variations between the data presented by Smith et al. (2003) and the methodology currently recommended by the USEPA. Unless a reasonable mechanism can be found to explain why certain values are high (e.g., TN and TP values in the Corn Belt using the USEPA approach, or high values in the Xeric west using the regression method), the two lower agreeing values should probably be considered. The availability of multiple techniques to establish reference nutrient conditions allows analyses to be tailored to the individual circumstances of each situation and gives scientists more options for determining suitable nutrient targets for surface waters.

References

Alexander, R. B., J. R. Slack, A. S. Ludtke, K. K. Fitzgerald, and T. L. Schertz. 1998. Data from selected U.S. Geological Survey national stream water quality monitoring networks. Water Resourc. Res. 34:2401-2405.

Buck, S., and others. 2000. United States Environmental Protection Agency. Nutrient criteria technical guidance manual, rivers and streams. EPA-822-B-00-002.

Dodds, W. K. 2003. Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. J. North Am. Benthol. Soc. 22:171-181.

———, J. R. Jones, and E. B. Welch. 1998. Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus. Water Res. 32:1455-1462.

——— and E. B. Welch. 2000. Establishing nutrient criteria in streams. J. North Am. Benthol. Soc. 19:186-196.

———, V. H. Smith, and K. Lohman. 2002. Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Can. J. Fish. Aquat. Sci. 59:865-874.

Griffith, G. E., J. M. Omernik, and A. J. Woods. 1999. Ecoregions, watersheds, basins, and HUCs: How state and federal agencies frame water quality. J. Soil Water Conserv. 54:667-676.

Lewis, W. M., Jr. 2002. Yield of nitrogen from minimally disturbed watersheds of the United States. Biogeochem. 57/58:375-385.

Oakes, R. M. 2003. Riparian impacts on water quality in eastern Kansas watersheds. M.S. thesis, Kansas State Univ.

Omernik, J. M. 1977. Nonpoint source-stream nutrient level relationships: a nationwide survey. U.S. Environmental Protection Agency EPA-600/3-77-105.

———. 1995. Ecoregions: A spatial framework for environmental management, p. 49-66. *In* W. S. Davis and T. P. Simon [eds.], Biological assessment and criteria. Tools for water resource planning and decision making. Lewis.

——— and R. G. Bailey. 1997. Distinguishing between watersheds and ecoregions. J. Am. Water Res. Assn. 33:935-949.

Pan, Y., R. J. Stevenson, B. H. Hill, A. T. Herlihy, and G. B. Collins. 1996. Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment. J. North Am. Benthol. Soc. 15:481-495.

———, R. J. Stevenson, B. H. Hill, and A. T. Herlihy. 2000. Ecoregions and benthic diatoms assemblages in Mid-Atlantic Highlands streams, USA. J. North Am. Benthol. Soc. 19:518-540.

Smith, R.A., R. B. Alexander, and G. E. Schwarz. 2003. Natural background concentrations of nutrients in streams and rivers of the conterminous United States. Environ. Sci. Tech. 37:2039-3047.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry 2nd ed. W. H. Freeman. New York.

U.S. EPA (U.S. Environmental Protection Agency). 1998. Level III ecoregions of the continental United States (revision of Omernik, 1987). U.S. Environmental Protection Agency.

Submitted 5 March 2004
Revised 24 June 2004
Accepted 5 July 2004