Developing Nutrient Criteria for Streams: an Evaluation of the Frequency Distribution Method1

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ABSTRACT:

The U.S. Environmental Protection Agency recommends two statistical methods to States and Tribes for developing nutrient criteria. One establishes a criterion as the 75th percentile of a reference-population frequency distribution, the other uses the 25th percentile of a general-population distribution; the U.S. Environmental Protection Agency suggests either method results in similar criteria. To evaluate each method, the Montana Department of Environmental Quality (MT DEQ) assembled data from STORET and other sources to create a nutrient general population. MT DEQ's reference- stream project provided reference population data. Data were partitioned by ecoregions, and by seasons (winter, runoff, and growing) defined for the project. For each ecoregion and season, nutrient concentrations at the 75th percentile of the reference population were matched to their corresponding concentrations in the general population. Additionally, nutrient concentrations from five regional scientific studies were matched to their corresponding reference population concentrations; each study linked nutrients to impacts on water uses. Reference-to-general population matches were highly variable between ecoregions, as nutrients at the 75th percentile of reference corresponded to percentiles ranging from the 4th to the 97th of the general population. In contrast, case studies- to-reference matches were more consistent, matching on average to the 86th percentile of reference, with a coefficient of variation of 13%.

(KEY TERMS: algae; rivers/streams; environmental regulations; nutrients.)

INTRODUCTION

The over enrichment of rivers and streams by nitrogen and phosphorus (eutrophication) is a serious water quality problem. Eutrophication can, for example, impact recreational and water supply uses (Freeman, 1986; Dodds et al., 1997), result in diel oxygen swings that impact fisheries and aquatic life (Welch, 1992), and increase the levels of organochlorine compounds (PCBs) in localized trout populations (Berglund, 2003). Eutrophication has been recognized as a water quality problem for a long time, well illustrated by the fact that the U.S. Environmental Protection Agency (U.S. EPA) commenced a national eutrophication survey of streams (Omernik, 1977) shortly after the passage of the 1972 Clean Water Act. To address the national eutrophication problem, the U.S. EPA in 1998 announced that it expected all States and Tribes to adopt numeric nutrient standards by 2003. However, recognizing the complexity of developing and implementing such standards, the U.S. EPA subsequently provided a more flexible approach.

This approach allows States and Tribes to submit to the U.S. EPA plans outlining the process and schedule of how they intend to adopt numeric nutrient standards (memorandum to States and Tribes from U.S. EPA, Office of Science and Technology; November 14, 2001). The Montana Department of Environmental Quality (MT DEQ) developed and submitted such a plan in 2002.

It has been widely recognized that numeric nutrient standards would not be the same everywhere, due to natural influences on nitrogen (N) and phosphorus (P) concentrations by landscape-level characteristics such as climate, geology, soils, vegetation, watershed area, etc. (Johnson et al., 1997; U.S. EPA, 1998; Rohm et al, 2002; Snelder and Biggs, 2002; Snelder et al., 2004). Ecoregions integrate into a single mapping system a number of these nutrientinfluencing geographic factors (Omernik, 1987). Ecoregions have been used to partition the United States into zones expected to manifest relatively uniform nutrient concentrations (U.S. EPA, 1998, 2000a; Rohm et al., 2002). This partitioning process is a necessary first step towards establishing numeric nutrient standards. However, there remains the need to identify appropriate nutrient criteria for each ecoregional zone.

Two statistically based approaches have been recommended by the U.S. EPA to select a criterion for any particular nutrient (e.g., total N, total P), within any particular ecoregion (U.S. EPA, 20006). The first approach identifies the criterion as the 75th percentile of the frequency distribution of nutrient data from reference stream sites within an ecoregion. Reference stream sites are relatively undisturbed examples (i.e., they have minimal human impacts and support all beneficial water uses) that can represent the natural biological, physical, and chemical integrity of a region (Hughes et al, 1986; Barbour et al, 1996; Kershner et al, 2004). The second approach selects as the criterion the 5th to 25th percentile of the frequency distribution from the general-population of nutrient data (U.S. EPA, 2000b). In practice, however, the 25th percentile is more frequently discussed in the U.S. EPA's nutrient documents than the 5th percentile, and is the basis for the U.S. EPA's national nutrient criteria recommendations (see U.S. EPA, 2000a, 2001; and related Clean Water Act section 304(a) nutrient- criteria documents). The option to select as criterion either the 75th percentile of reference or the 25th percentile of the general population is presumptive, as it assumes that reference and general- population frequency distributions will have a particular relationship to one another (Figure 1), and so nutrient concentrations selected via either approach will be similar.

In accordance with its nutrient criteria plan, the MT DEQ has been examining in detail the two criteria-selection approaches outlined above. MT DEQ identified a number of stream reference sites in the early 1990s (Bahls et al., 1992), and has had a project in place since 2000 to identify and sample reference stream sites around the state (Suplee et al., 2005). The availability of reference stream nutrient data enabled us to examine the relative merits of the reference vs. the general-population approach to developing nutrient criteria. Our purpose in writing this paper was to describe our finding that nutrient concentrations at the 25th percentile of general-population frequency distributions may represent overly stringent - or insufficiently protective - criteria. This will be dependent upon the relationship between the nutrient distribution of the general population and that of the corresponding reference population. We also report that nutrient concentrations at the 86th percentile of reference-site frequency distributions appear to be reasonable for establishing criteria. This is because nutrient concentrations at the 86th percentile of reference generally matched nutrient concentrations that begin to cause impacts to beneficial water uses (e.g., recreation and aesthetics, aquatic life) that are published in regional scientific studies.

METHODS

Data Sources for the Development of a River and Stream Nutrient Database

The primary data source for the analyses was from the U.S. EPA's Storage and Retrieval (STORET) database. In March 2001, a request was placed with the then-functioning mainframe STORET database for all ambient surface water-quality data from Montana, excluding data from pipes, wells and springs. The delimited text file received was then transferred to a Microsoft Access relational database. The STORET data (also referred to as Legacy STORET) contained data collected by 33 agencies or entities (organizations), and held nutrient data from the early 1960s to 1998. A query was run in the "Type" field (a field indicating the waterbody type) to remove lake data. The database was supplemented with all river and stream nutrient data from MT DEQ found in modernized STORET, which were collected from 2000 to 2004. Also added to the relational database were Montana river and stream data collected by the University of Montana, Utah State University, the U.S. EPA's Environmental Monitoring and Assessment Program (EMAP; Lazorchak et al., 1998), and reference-stream nutrient data up through 2005 (reference streams will be discussed further on in Methods.) The database contained 5,300 sampling sites and over 140,000 total records. Readers should note that the data sources we used are comparable to those used by the U.S. EPA in developing its nutrient criteria recommendations. The U.S. EPA used data sources that included Legacy STORET, two United States Geological Survey projects (the National Stream Quality Accounting Network and the National WaterQuality Assessment [NAWQA] Program), and regional U.S. EPA data (see U.S. EPA, 2000a and related documents). Our database contained more records per level III ecoregion than the database the U.S. EPA used to develop its criteria recommendations, because the U.S. EPA restricted its dataset to information collected from 1990 to 1998 (U.S. EPA, 2000a).

Each analytical measurement in Legacy STORET was uniquely identified by a parameter code (e.g., 00665; total P). Other data that were incorporated into the relational database, including those from modernized STORET, did not use these codes. To assure consistency and to facilitate the grouping of data (discussed below), the appropriate parameter code was ass\igned to each observation lacking a code. The water quality data in the assembled database, which included latitude and longitude coordinates for each observation, were then spatially joined to Geographic Information System (GIS) layers containing information on level III and level IV ecoregions (Figure 2; Woods et al., 2002). Observations were also labeled with the stream order (Strahler, 1964) of the stream reach from which they were collected. Strahler stream orders were derived from the U.S. EPA's reach file 3 (RF3) GIS layer (1:100,000 scale; U.S. EPA, 1994).

The final database was transferred to Stata (version 7), which was more amenable to statistical analysis programming, and was referred to as the "allobservations" database to distinguish it from a "median" database. The median database was developed from the all- observations database and contained only the medians of the observed values for each nutrient, for each station, and for each season. (Seasonal data stratification will be detailed in a following Methods subsection.) The median database was developed because it was less likely than the all-observations database to be influenced by outliers, and was therefore more amenable to parametric statistical analyses.

Data Quality Control Methodology

Examination of the Legacy STORET dataset confirmed that it did not contain water quality data from pipes, wells or springs. Pipe, well, and spring sampling stations had been included in a Legacy STORET metadata (station-information) file. We linked this metadata file to the water quality database and verified that none of the pipe, well or spring sampling stations could be joined with any water quality data. To eliminate potentially erroneous or highly uncertain data from the analyses, data bearing certain comments codes were excluded (Table 1). Also, observations in the database bearing comment codes indicating the analytical result was below detection were replaced with values equal to 50% of the reported detection limits (DL/2; Table 1). For datasets skewed to the right, which were common in our nutrient database, the DL/2 method is reported to be sufficiently accurate for determining descriptive statistics like the mean and standard deviation (Hornung and Reed, 1990). Further, if less than 15% of the total dataset is below detection, the U.S. EPA (2006) indicated that the nondetect observations may be substituted, preferably with DL/2 values. Less than 15% of total observations in our database were below detection. Finally, nutrient observations with reported values of zero were excluded from use, since they probably represented data entry errors. Most analytical results in the database provided a result value, a detection limit and an indication when the measurement was below detection. True analytical result values of zero are very unlikely; for example, zeros are not reported for low-level organic pesticide analyses using HPLC methods even when no peak is detected (technical memorandum 94-12 from National Water Quality Laboratory to NAWQA study-unit chiefs, July 8, 1994, http://nwql.usgs.gov/ Public/tech_memos/ nwql.94-12.html).

Water quality data collected from streams and rivers are rarely normally distributed and are frequently skewed to the right (i.e., lognormally distributed), and the presence of high outlier values in such datasets is common (Helsel and Hirsch, 1992). We did not have knowledge of the flow conditions or other important factors prevalent at the time the data were collected, and it would have been inappropriate to eliminate outlier data simply because they inconvenienced the statistical analyses (Helsel and Hirsch, 1992). Therefore, beyond the quality control measures described above, we did not further eliminate any data from the database.

Nutrient Data Grouping Methodology

We identified thirty different nutrient analytical measurements of N and P in the database, each bearing its own parameter code. Many appeared to be closely related, and rather than select a single parameter code to represent a given nutrient type (e.g., total P, 00665), we opted to aggregate the analytical measurements into groups. This approach allowed us to retain many nutrient analytical measurements that would have otherwise not been used. The objective was to group nutrient analytical measurements together that were fundamentally equivalent, while at the same time avoiding double- counts in cases where an agency may have reported two or more grouped analytical measurements from the same sample. The approach was undertaken in a series of steps. First, the different analytical measurements were identified by their parameter codes and other identifying information, checked against records (U.S. EPA, 1979; Alexander et al., 1996; Clesceri et al., 1998) to determine what they measured, and then organized into groups. The thirty nutrient measurements in the database were thus aggregated into seven groups (Table 2). Although we developed this grouping methodology independently, it is nearly identical to that used by Mueller et al. (1995) to aggregate nutrient data for an analysis of surface and groundwater. Next, a series of exploratory queries were made in the database for each group and for each agency, to ascertain if any analytical measurements within the group were derived from the same sample. In cases where this occurred, only one of the analytical measurements was retained for that agency (generally the largest sample contributor). Stata programs were developed to create the nutrient groups, convert all reporting units to "as N" or "as P", and to prevent sample double counts.

Entire analytical measurements were eliminated (those in gray- shaded areas; Table 2) if a clear definition for the measurement could not be located. And although placed in the nitrate & nitrite group, nitrite-only measurements were completely excluded from use. In most ambient waters exposed to oxygen, nitrite is only present in trace quantities and most dissolved inorganic N is nitrate (Home and Goldman, 1994). A review of the database showed that most nitrite measurements were very low or below the reported detection limit. Therefore, by aggregating analytical measurements that jointly report nitrite + nitrate (e.g., parameter code 00630; Table 2) with measurements that only report nitrate (e.g., 00618), we assumed that the nitrite + nitrate samples were mostly nitrate.

Development of Seasonal Periods to Partition Nutrient Data

Nutrient concentrations in flowing waters can show distinct seasonal patterns (Lohman and Priscu, 1992; Home and Goldman, 1994). Our objective here was to define seasonal (time) periods for each level III ecoregion, which we assumed would reduce intraecoregional variability in nutrient concentrations. Hydrological, biological and climatic data were all used to derive starting and ending dates of each season. Data from United State Geological Survey (USGS) gauge stations were used to address the hydrologic aspect. Two conditions were established to select the USGS gauge stations used to define flow patterns. First, each gauge station had to have at least 5 years of continuous flow records, although the stations did not need to be sampled up to the present (e.g., a continuous record from 1942 to 1963 was acceptable). Second, gauge stations were selected from stream segments having no major hydrologic modifications such as dams. Every effort was made to ensure that the selected stations provided good spatial coverage of each ecoregion, while at the same time meeting the conditions listed above. All together, 63 USGS gauge stations were selected (Appendix A), with from 10 to 12 stations per ecoregion. Two ecoregions (Idaho Batholith and Wyoming Basin; Woods et al., 2002) have very limited geographic extents in Montana, however, and only six and three suitable gauge stations, respectively, could be located.

Flow duration hydrographs based on daily-mean flows were developed for each station in order to derive onset and termination dates for the runoff period. These hydrographs were developed using the complete period of record of gauge-station flow data extracted from the USGS's National Water Information System (NWIS) database. For each hydrograph, the average of all daily flow records was calculated separately for each day of the year. Each of the longterm average daily flows calculated in this manner was then plotted, and the hydrograph curve thus generated represented the average annual flow pattern at the station for the period of record (Figure 3). The two points of greatest inflection on the hydrographs were used to define the runoff onset and termination dates (e.g., day 101 and 205; Figure 3).

After the hydrologically based dates for the onset and termination of runoff were compiled, it became obvious that the runoff termination dates suggested by some of the flow-duration hydrographe located in the mountainous ecoregions (Northern, Middle, and Canadian Rockies) extended longer into the summer than the MT DEQ has generally found there to be discernable scouring effects on aquatic life. Therefore, we turned to biological data to further define the seasons. The MT DEQ uses June 21st as the start date for biological sampling in streams of mountainous regions of the state (MT DEQ, Standard Operating Procedures for Sample Collection, Sorting, and Taxonomic Identification of Benthic Macroinvertebrates, Water Quality Planning Bureau, WQPBWQM-009, April 2005), as runoff effects have typically subsided by that time. A number of hydrographs in the mountainous ecoregions showed that runoff was still occurring on June 21st. Therefore, for ecoregions in which this occurred, we selected the runoff termination date as the earliest day after June 21st on which all flow-duration hydrographs in the ecoregion were at least on the declining limb of the peak flow.

The selection of the start-of-winter dates could not be readily dete\rmined using hydrograph characteristics. After runoff ends, base flow begins and can be fairly uniform into November and December (day 235-365; Figure 3). However, regional climatic influences such as lowered temperatures and light intensity typically cause by the end of September major reductions in aquatic plant life growth, as well as reductions in aquatic macroinvertebrate productivity (Richards, 1996). In general, the MT DEQ uses September 21st as the termination date for biological sampling (Standard Operation Procedures, cited above), and only rarely collects biological samples after October 1st. After having examined the hydrological, biological and climatic factors discussed, the onset and termination dates of the seasons were finalized for each ecoregion. The onset and termination dates were then rounded to the nearest end-ofmonth or mid-month date (Table 3).

Nutrient data in the databases were associated with the appropriate season by their dates of collection. Significant differences (95% confidence) between seasons were tested using the Kruskal-Wallis test (Conover, 1999). Kruskal-Wallis tests were performed on the nutrient general population separately for each level III ecoregion; that is, the data were first stratified by ecoregion before the significance of seasonal groups was tested. (The tests for the reference population had very low power because of the reference population's small size, and the results are not presented here.) Kruskal-Wallis tests were performed for the general population in the all-observations database and the median database.

Selection of Reference Sites

The identification and assessment of Montana reference stream sites is discussed in detail in Suplee et al. (2005), and will be only briefly summarized here. A group of candidate reference stream sites was assembled and then assessed using a consistent set of criteria that included both quantitative and qualitative evaluations. Data were examined at two scales: site specific, and watershed (5th or 4th hydrologic unit codes; Seaber et al., 1987). The qualitative component was undertaken by using best professional judgment (BPJ) to assess criteria such as "presence of point sources,""grazing use,""aesthetics,""condition of stream bank vegetation," and "mining impacts." Quantitative analyses consisted of watershed-level assessments and a site-specific analysis. At the watershed level, the proportion of agricultural land use and the total density of roads (km/km^sup 2^) was determined for the watershed upstream of each candidate reference site using a GIS. Criteria were then located in the literature (Kershner et al., 2004; Sheeder and Evans, 2004) to estimate thresholds for impacts to aquatic life and other beneficial water uses. At the site-specific level, water quality data for each site were reviewed to determine if they exceeded state water quality standards (MT DEQ (Montana Department of Environmental Quality), 2006) for a suite of metals contaminants commonly released from mining areas (Cd, Cu, Pb, Zn, Hg, and dissolved Al).

Some candidate reference sites were in a reference condition for certain characteristics (e.g., riparian condition), but failed in another category, for example having high density of abandoned mines in the watershed or metals concentrations that exceeded the state standards. Sites of this nature were not retained as reference sites. That is, none of the reference sites that passed to the final list contained any "fatal" flaws, and only sites passing all criteria were included. The final reference site list contained streams ranging in stream-order size (Strahler, 1964) from 1st to 6th, which generally comprised wadeable streams and small rivers. All data associated with reference sites were flagged in the Stata database to distinguish them from nonreference population data. The locations of reference sites are shown in Figure 2.

Percentile Mapping: Reference-to-General Population

Percentile mapping is the identification of corresponding percentile values of equivalent nutrient concentrations in two different data distributions. Percentile mapping for the reference- to-general population was carried out in two major steps. In the first step, summary statistics were computed for nutrient groups in the reference, nonreference, and general (reference plus nonreference) populations by each alternative stratification methodology (i.e., combinations of ecoregions and seasons). Specific summary statistics included the total number of observations, minimum, maximum, mean, standard deviation, and skewness. The summary statistics also included concentrations at the 25th, 50th, and 75th percentiles for reference, nonreference and general population observations. Percentile mapping was only undertaken when four or more nutrient observations were available at nonreference and reference locations.

In the second step, the reference and general population frequency distributions were matched within each stratification combination. Stata programs were developed to compute the nutrient concentrations corresponding to the 75th and 90th percentiles of the reference population. Next, an empirical cumulative distribution function was generated to assign a percentile rank to each nutrient concentration observation in the general population. The percentiles in the general population corresponding to the nutrient concentrations at the 75th and 90th percentiles in the reference distribution were then determined using a linear interpolation method. A cubic interpolation method was also tested. However, in most cases, the cubic interpolation method did not differ from the linear method and it resulted in missing values in a few boundary cases. Therefore, the linear interpolation method was exclusively applied for this analysis.

Percentile Mapping: Case Studies-to-Reference Population

Four conditions were used to select stressor-response case studies that were used to make comparisons against the reference- population frequency distributions. These were: (1) the case study reported a scientifically defensible linkage between nutrient concentrations and an impact to a beneficial water use (e.g., recreation & aesthetics, aquatic life, fisheries); (2) each case study's geographic extent was within a level III ecoregion found in Montana; (3) the stream or river in the case study generally fell within the scope of the present work (i.e., similar Strahler stream order); and (4) the case study was documented in some kind of publication. The nutrient concentrations recommended in or derived from these case studies were then mapped to their corresponding concentrations in the reference-population frequency distributions from the same ecoregion and season. In cases where more than one percentile in the reference distribution had the same concentrations (e.g., both the 50th and 75th percentile were equal to 0.05 mg total P/L), the higher percentile was selected.

Five scientific case studies that met the conditions for use were located for four different level III ecoregions. Welch et al. (1989) modeled the influence of SRP concentrations on periphyton biomass in the Spokane River of Idaho and Washington. The Spokane River is a sixth-order river in the Northern Rockies ecoregion, which extends into Montana. Watson et al. (1990) used artificial stream channels utilizing water from the Clark Fork River in Montana (4th7th-order) and control nutrient inputs (N and P) to determine the peak biomass of diatom algae and the filamentous algae Cladophora. Dodds et al. (1997) used a river and stream database comprised of sites from North America, Europe, and New Zealand to develop regression equations between nutrients and algal standing crop, and then recommend criteria for Montana's reach of the Clark Fork River. Based on a 16-year study, Sosiak (2002) recommended P concentrations intended to maintain algae density below nuisance levels in the Bow River (5th order; Alberta, Canada). Lastly, Suplee (2004) presented a regression equation between standing crop of algae and nitrate concentrations in Montana prairie streams (3-4th order), and recommended maximum concentrations for total N, total P and algal standing crop.

Other Descriptive Statistics and Statistical Analyses

As described earlier, we generated summary statistics for nutrient concentrations in the all-observations database for each alternative stratification methodology (i.e., combinations of ecoregions and seasons). This was also carried out for the median database. In addition, we were concerned that nutrient data from large rivers (Strahler order 7 and 8), for example the Missouri and Yellowstone rivers, might bias comparisons between the general and reference-population frequency distributions. (Recall that the reference sites came from first through sixth-order streams and small rivers). Therefore, we also generated summary statistics from an all-observations dataset that excluded data from seventh and eighth-order rivers. Statistically significant differences (95% confidence level) between nutrient concentrations of the reference and general populations were determined using the Wilcoxson ranksum test (Conover, 1999).

RESULTS

Seasonal Differences in Nutrient Concentrations

The results of the Kruskal-Wallis tests for ecoregionally stratified seasonal differences in nutrient concentrations are presented in Tables 4a and b. For nutrient zones based on level III ecoregions, there were significant seasonal differences in median nutrient concentrations in the general population. This was true for the majority of cases in the all-observation database, and for many cases in the median database. In the all-observations database, the majority of the nutrient groups showed significant seasonal differences for each level III ecoregions, except for the Wyoming Basin (Table 4a). The Wyoming Basin has a very limited geographic extent in Montana, whi\ch resulted in low power of the tests. For other nutrient groupings for which the trends are not significant, mainly in the median database, the results may reflect the low power of the tests because of the relatively small sample sizes associated with those nutrients.

Percentile Mapping, Descriptive Statistics and Statistical Test Results

Based on the all-observations database, Tables 5a through 5d present the 75th and 90th reference percentile equivalents in the general population for all seven nutrient groups in each level III ecoregion, for all seasons combined (Table 5a) and for each season (Tables 5b through 5d). Reference-to-general population matches for specific nutrients were highly variable between ecoregions, as nutrient concentrations at the 75th percentile of reference corresponded to general-population percentiles ranging from the 4th percentile to the 97th percentile. In general, the mountainous ecoregions (Northern, Middle and Canadian Rockies) showed greater separation between reference and general-population data than did the two prairie ecoregions (Northwestern Glaciated and Great plains). That is, general population streams in mountainous ecoregions had elevated nutrient concentrations relative to their corresponding reference streams whereas, in the prairie ecoregions, nutrients in reference and general-population streams were much more similar. Furthermore, the cross-nutrient standard deviations (and coefficient of variation, CV) around the mean of the mapped percentiles were fairly low in the two prairie ecoregions (see bottoms of Tables 5a to 5d). It is also apparent from Tables 5a through 5d that seasonal trends were not very pronounced in the percentile mappings. The only exceptions to this finding were for the Middle Rockies and the Canadian Rockies, where general- population percentiles corresponding to the 75th and 90th percentiles in the reference population were lower in the winter season than for other seasons. In another analysis not presented here, cross-ecoregional percentile mapping (e.g., grouping all total P percentile matches together across ecoregions) showed that, for a given nutrient, the cross-nutrient standard deviation around the mean in a given ecoregion was generally lower than the cross- ecoregional standard deviation around the mean.

There were only a limited number of cases (11%) for which the 75th percentile of the reference population mapped closely (5 percentiles) to the 25th percentile of the general population (Tables 5a through 5d). Similarly, of 19 aggregate cross-nutrient results (see "Mean" rows, Tables 5a through 5d) there was only one case (Middle Rockies, winter season) where the 75th percentile of reference population closely mapped (5 percentiles) to the 25th percentile of the general population.

Tables 6a through 6c show nutrient concentrations (all seasons) at the 25th, 50th, and 75th percentiles of the reference and nonreference populations, for each ecoregion. Table 6a was generated from the allobservations database, Table 6b from the same but excluding stream order

7 & 8 data, and Table 6c was generated from the median database. Overall, all three databases produce very comparable results. One anomaly in the datasets is the fact that TKN concentrations are often higher than TN in equivalent ecoregions and seasons. This resulted because TN data have generally been collected more recently, and have relatively low detection limits, whereas TKN was part of many older datasets, and TKN detection limits where commonly higher in the past. Table 7 shows the results of significance comparisons between reference and nonreference populations (all seasons), by ecoregion, for the all-observations and median databases. (Significance tests were performed for the all- observations database excluding stream order 7 & 8 data, but the results were virtually identical to the all-observations database and are not shown.) Although there was 100% agreement in significance-test results between the all-observations and median databases for the Canadian Rockies, in the remaining ecoregions there was disagreement between database results in about 35% of cases. For the great majority of nutrients in the mountainous ecoregions (Northern, Middle, and Canadian Rockies), there were significant differences between the reference and non-reference nutrient concentrations (Table 7). However, in the two prairie ecoregions (Northwestern Glaciated and Great plains), nutrient concentrations in the reference and nonreference populations were significantly different in only half of the cases or less.

The results of the case studies-to-reference population mapping are shown in Table 8. As for the reference-to-general population mapping, these results are based on the all-observations database. Case studies were located for four of Montana's seven level III ecoregions (Northern Rockies, Middle Rockies, Canadian Rockies, and the Northwestern Glaciated Plains). Overall, nutrient concentrations from case studies mapped to nutrient concentrations in reference-population distributions across a much smaller range than was observed for the reference-to-general population mappings. The case studies-to-reference population mappings ranged from the 68th to the 99th percentiles (Table 8). Overall, nutrient concentrations from the case studies mapped to the 86th (mean) and 86th (median) percentile of the reference populations, with a CV of 13% (Table 8, bottom row).

DISCUSSION

The databases used in the present study comprised data from longitudinal samplings of the same streams, most data were not sampled probabilistically and therefore a number of samples are not truly independent. Our goal, however, was to create a nutrient database having the greatest possible spatial and temporal coverage of the state. To achieve this, we assembled data from as many organizations as possible, over the greatest possible period of time, knowing that each organization had its own sampling goals, objectives and timeframes. We assumed that compiling data from many organizations would minimize bias associated with any one organization's dataset. Even probabilistically collected datasets may contain some type of bias. For example, the one truly probabilistic dataset we incorporated (EMAP; 2000-2004) was entirely collected during a statewide dry cycle when moderate to extreme drought was common (hydrological drought index; Palmer, 1965; NCDC (National Climate Data Center), 2006). In contrast, our database contained data collected during numerous wet/dry climatic cycles, including several periods of extreme drought and extreme moisture (Palmer, 1965; NCDC (National Climate Data Center), 2006). Drought, and precipitation patterns in general, can influence water quality (Ojima et al., 1999; Little et al., 2003), and our database is capable of reflecting these influences because of its relatively long period of record.

In its guidance on the development of river and stream nutrient criteria, the U.S. EPA has recommended that for any given physiographic region the 75th percentile of a reference-site frequency distribution be selected or, alternatively, the 5th to 25th percentile of the general-population frequency distribution (U.S. EPA, 2000b). This recommendation assumes that either method "should approach a common reference condition along a continuum of data points" (page 95, U.S. EPA, 2000b). This presumption is based on three case studies - one in Tennessee, one in Minnesota, and one in New York - where it was found that the 75th percentiles of the reference site frequency distributions for nutrients closely matched to the 25th percentile of the general population frequency distributions (U.S. EPA, 2000a,b,c). However, two of these three case studies are from lakes (New York and Minnesota), waterbody types that are different from rivers and streams. Aside from the vast body of scientific literature on the topic of lotie and lentic waters, the fundamental difference between rivers/streams and lakes is illustrated by the fact that the U.S. EPA has developed its nutrient criteria recommendations separately for each of these two waterbody types (e.g., U.S. EPA, 2000a,d). Therefore, it is questionable whether the finding in lakes that nutrient concentrations at the 75th percentile of a reference population are similar to nutrient concentrations at the 25th percentile of the general population can be, unexamined, directly transferred to rivers and streams. The remaining case study (Tennessee) was undertaken in rivers and streams using an approach similar to ours. However, when the reference-to-general population nutrient relationship was examined for Tennessee's level III ecoregions, only three out of four of Tennessee's ecoregions showed a close match between the 75th percentile of reference and the 25th percentile of the general population (Appendix A; U.S. EPA, 2000b). Similarly, an analysis of reference and general-population nutrient data for small streams in parts of North Carolina and Tennessee shows that the 75th percentile of the reference distribution matches to about the 45th and 40th percentile of the general population for TN and TP, respectively (Rohm et al., 2002).

The use of the 5th to 25th percentile of a general population frequency distribution to identify nutrient criteria is a secondary approach, to be used when reference data are unavailable (U.S. EPA, 2000a). Our results and those of Rohm et al. (2002) demonstrate that caution should be taken when using this general-population approach to selecting criteria because, in effect, it creates a "moving target" because of its complete reliance upon the degree of eutrophication prevalent when the data were collected (Dodds and Oakes, 2004). If the ecoregion in question has not had a substantial degree of eutrophication, then the 25th percentile of the general population will result in overly restrictive criteria; Fi\gure 4 demonstrates this point. In Figure 4, the reference and general population distributions for TN in the Northwestern Glaciated Plains of Montana overlap a great deal. The 75th percentile of the reference population maps to about the 63rd percentile of the general population, and so the general population 25th percentile represents an unduly restrictive criterion. The corollary to this is that in highly eutrophied regions, the general-population 25th percentile is probably not sufficiently protective of water beneficial uses. How one would go about systematically selecting more restrictive criteria (e.g., the 5th percentile) in the absence of reference sites, at least using these statistically based approaches, is not entirely clear in the U.S. EPA's guidance (U.S. EPA, 2000b).

Results from the present study also illustrate that it is not always easy to predict upfront, for any particular ecoregion, what the reference-to-general population relationship for any given nutrient will be. Prior to the analysis of Montana's data, we would have predicted - based on our general understanding of land use in Montana - that the prairie region east of the Rocky Mountain Front would have demonstrated a greater degree of elevated nutrients than the western, mountainous region of the state. The two prairie ecoregions comprising most of eastern Montana's land area (Northwestern Glaciated Plans and Northwestern Great Plains) are almost entirely used for grazing, dry-land agriculture (cereal crops such as wheat and barley) and, to a lesser degree, irrigated agriculture, and we assumed that nutrients in those ecoregions' streams would be highly elevated relative to their corresponding reference streams. However, we found that in these two ecoregions the reference and general-population nutrient concentrations were significantly different in only about a third of the cases (Table 7), much less often than was observed in the mountainous ecoregions of the state. There are four likely explanations for this: (1) the reference sites were poorly selected and actually represent eutrophied conditions; (2) most of the nutrients were sequestered by heavy growth of algae and aquatic plants and nutrient concentrations were, consequently, low; (3) not all nutrients are good indicators of regional eutrophication, and special attention should be paid to certain nutrient groups; or (4) the region - as a whole - is not as heavily eutrophied as initially thought.

Of these four possibilities, the latter two are probably closest to the truth, and can be exemplified using the Northwestern Glaciated Plains ecoregion. To address the first possibility, two specific reference sites demonstrate the overall quality of the reference sites. The reference site "Rock Creek below Horse Creek, Near Int. Boundary" (USGS gauge station 06169500) is a USGS Hydrologie Benchmark Network (HBN) site located on the U.S.- Canadian border in the Northwestern Glaciated Plains ecoregion. The HBN network comprises stream sites located in relatively undeveloped basins which serve as controls for separating natural from human- caused changes in stream water quality (Alexander et al., 1996; Clark et al., 2000). Much of Rock Creek's watershed upstream of the site is contained within the Grasslands National Park of Canada (Parks Canada - Grasslands National Park of Canada, website, http:// www.pc.gc.ca/pn-np/sk/grasslands, accessed October 21, 2005), and only about 7% is used for crop agriculture (U.S. and Canada combined). The reference site "Bitter Creek" (same ecoregion) has as its immediate upstream drainage a land area that has been described by the Montana Natural Heritage Program (a branch of the Nature Conservancy) as the largest intact grassland in northern Montana, and one of the most extensive naturally functioning glaciated plains grasslands in North America (Cooper et al., 2001). Bitter Creek's drainage is not used for dry-land or irrigated agriculture, and grazing use of the area is highly compatible with natural ecological processes that maintain grasslands of this type (Cooper et al., 2001). These two stream sites are arguably as close to true reference as one is likely to find today in the Northern Great Plains. Available nutrient concentration data (all seasons) from these two sites were combined, and the 75th percentile of four nutrient groups - TN, TP, SRP and NO^sub 2^ + NO^sub 3^ - were matched to their corresponding general-population data in the Northwestern Glaciated Plains ecoregion. The four nutrient groups matched to the 84th, 78th, 58th, and 39th percentiles, respectively. As an aggregate, nutrient concentrations in Rock and Bitter creeks matched to the 65th percentile of the general population, lower than the percentile for the aggregate of all reference sites in the ecoregion (73rd; Table 5a) but clearly not at the 25th percentile. So even when nutrient data from the very best reference sites of the Northwestern Glaciated Plains ecoregion in Montana are examined, their frequency distributions overlap a great deal with the general population, which suggests that the general-population 25th percentile would represent too stringent criteria.

Regarding the second possibility, the winter season data do not support the assertion that nutrients were sequestered in dense growths of algae and aquatic plants. The winter season for the Northwestern Glaciated Plains (October 1st to March 14th; Table 3) occurs when aquatic plant growth has greatly slowed due to low light and freezing temperatures, and so the plant's ability to sequester nutrients and diminish water-column concentrations is negligible. Because soluble nutrients are most biologically available, they are probably the most sensitive measure of potential nutrient uptake by aquatic plants. In the winter season, the concentration at the 75th percentile of reference for ammonia, NO^sub 3^ + NO^sub 2^ and SRP matched to the 83rd, 75th, and 52nd percentiles of the general population (Table 5b). If general population streams were highly eutrophied and had heavy algal and aquatic plant growth taking up nutrients in the growing season, the plants' uptake would not be manifested in winter and one might expect soluble nutrient concentrations to become elevated in the winter season. The net result would be that reference site concentrations would match to much lower general-population percentiles (i.e., more like Figure 1) in winter than we observed.

Concerning the third possibility, note that NO^sub 3^ + NO^sub 2^ was significantly different between reference and nonreference sites in the Northwestern Glaciated Plains (Table 7). NO^sub 3^ + NO^sub 2^ is also, among the seven nutrient groups in Tables 5a and 5d, the nutrient showing the greatest separation from the 75th percentile of the reference sites. Suplee (2004) showed that NO^sub 3^ + NO^sub 2^ is significantly correlated to algae density in the region's streams, and another study in the ecoregion found that dryland crop- fallow practices elevate nitrate concentrations in soil pore-water and groundwater (Nimick and Thamke, 1998). These facts suggest that special attention should be paid to this particular nutrient, as it is the one most likely to be linked to eutrophication problems in the region.

Finally, the fourth possibility can best be gauged relative to other parts of the state. In the mountainous ecoregions, which have forestry activities and also comprise intermountain valleys that have substantial agricultural activity, reference and nonreference streams were significantly different for many more nutrient groups than was found to be the case for the Northwestern Glaciated Plains. Furthermore, the reference 75th percentiles of the mountainous ecoregions mapped to much lower percentiles in their corresponding general populations than was observed in the Northwestern Glaciated Plains. So, relative to the mountainous region of the state, Northwestern Glaciated Plains nutrients are not as elevated, and there are fewer nutrient groups that are elevated. One is left to conclude that, in this prairie ecoregion of Montana, eutrophication is not as severe and is more nutrient-specific than in the western, mountainous part of the state.

The idea that the water quality of reference sites should be acceptable and support all beneficial water uses is fairly intuitive. This idea is intrinsic in the U.S. EPA's recommendation that the 75th percentile of a nutrient-concentration reference distribution be used to set criteria, because the 75th percentile will assure that the majority of the nutrient data from reference sites will not exceed the criteria thresholds. Nevertheless, the 75th percentile is still a cautious (i.e., protective) approach, as 25% of nutrient data collected from reference sites could exceed the criteria. Our results indicated that a somewhat higher percentile (about the 86th) from nutrient-concentration reference distributions is more appropriate for Montana streams, as this percentile has been ground truthed to regional case studies that demonstrate nutrient impacts to beneficial water-uses.

Impact-to-use nutrient concentrations (i.e., those at or above the 86th percentile of reference in the present study) are altogether different from "pristine" nutrient concentrations. Estimates of pristine nutrients concentrations in streams are reported in the literature, however (Kemp and Dodds, 2001; Smith et al., 2003; Dodds and Oakes, 2004), and some of these concentrations can be compared with the present study. The best estimate of "pristine" from our study would be approximately the 50th percentile of reference, as it represents the central tendency for groups of reference sites. In the Central Cultivated Great Plains of the United States, pristine TN concentrations are estimated to range from 200 to 566 g/l (Kemp and Dodds, 2001; Smith et al., 2003; Dodds and Oakes, 2004), whereas this study suggests 7\10 g/l (Northwestern Glaciated Plains; 50th percentile; Table 6a). Pristine TP concentrations for the same region range from 23 to 58 g/l (Smith et al., 2003; Dodds and Oakes, 2004), while this study suggests 60 g/l (Table 6a). In the Western Forested Mountains of the United States, the results of this study are lower than other literature values. For example, in the Western Forested Mountains pristine concentrations range from 19 to 45 g/l (Smith et al., 2003; Dodds and Oakes, 2004), and this study suggests 3-10 g/l (Northern, Middle and Canadian Rockies; Table 6a).

We acknowledge that in some regions of the United States (like Montana) the possibility of locating reference sites is much greater than in areas having widespread intensive agriculture (e.g., the U.S. corn belt). The process of identifying appropriate nutrient criteria in areas of intensive agriculture is clearly challenging, and although difficult to accomplish it would be prudent in such regions to try to locate at least a few reference sites, so that some sense of the reference-to-general population relationship can be developed. If this cannot be done, another approach would be to model the factors controlling a region's water quality and then factor out the affects of land use (e.g., Robertson et al., 2001; Dodds and Oakes, 2004) or, alternatively, develop stressor-response models (e.g., Biggs, 2000b; Dodds et al., 2002) between nutrients and demonstrable impacts to beneficial water uses.

In conclusion, our findings indicated that the relationship between nutrient concentrations in reference populations and nutrient concentrations in their corresponding general populations can vary a great deal from ecoregion to ecoregion. We found in this study that the 75th percentile of reference corresponded to the 4th to 97th percentile of the general population. Further, an expected relationship between reference and general population nutrient data - based on an a priori understanding of land use in a region - may not always manifest itself as anticipated. As a result, if the 25th percentile of a general-population frequency distribution is used to establish nutrient criteria, then the resulting nutrient standards could be overly stringent or insufficiently protective, depending upon what the actual relationship between the reference and the general population looks like. On the other hand, nutrient concentrations derived from five regionally applicable scientific studies (nutrient as stressor, impact to a beneficial water use as response) fell within a relatively narrow band around the 86th percentile of the reference-site nutrient frequency distributions. The latter result indicated that nutrient concentrations at high percentiles of reference-site frequency distributions (this study suggests the 86th) represent, fairly consistently, the threshold where impacts to beneficial water uses begin to occur.

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