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Ecological Indicators 7 (2007) 371–386

ECOLOGICAL INDICATORS

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# A nutrient biotic index (NBI) for use with benthic macroinvertebrate communities

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Received 3 January 2006; received in revised form 22 March 2006; accepted 23 March 2006

**Abstract**

Aquatic macroinvertebrates have been among the principal biological communities used for freshwater monitoring and assessment for several decades, but macroinvertebrate biomonitoring has not incorporated nutrient measures into assessment strategies. Two nutrient biotic indices were developed for benthic macroinvertebrate communities, one for total phosphorus (NBI-P), and one for nitrate (NBI-N). Weighted averaging was used to assess the distributions of 164 macroinvertebrate taxa across TP and $NO_3^-$ gradients and to establish nutrient optima and subsequent nutrient tolerance values. Both the NBI-P and NBI-N were correlated with increasing mean TP and $NO_3^-$ values ($r = 0.68$ and $r = 0.57$, respectively, $p < 0.0001$). A three-tiered scale of eutrophication for TP and $NO_3^-$ (oligotrophic: $\leq$0.0175 mg/l TP, $\leq$0.24 mg/l $NO_3^-$, mesotrophic: >0.0175 to $\leq$0.065 mg/l TP, >0.24 to $\leq$0.98 mg/l $NO_3^-$, eutrophic: >0.065 mg/l TP, >0.98 mg/l $NO_3^-$) was also established through cluster analysis of invertebrate communities using Bray–Curtis (quantitative) similarity. Significant differences ($p < 0.0001$) were detected between median NBI-P and NBI-N scores among the three trophic states. Therefore, the nutrient biotic indices (NBIs) appear to accurately reflect changes in stream trophic state. Multimetric water quality assessments were also used to identify thresholds of impairment among the three trophic states. Hodges-Lehman estimation indicated that the greatest change in assessment results occurred between the mesotrophic and eutrophic states. The eutrophic state also represented the highest percentage of overall impairment. Therefore, the suggested threshold for nutrient impairment is the boundary between mesotrophic and eutrophic (0.065 mg/l TP and 0.98 mg/l $NO_3^-$). The corresponding NBI-P score (6.1) and NBI-N score (6.0) for this threshold incorporate predictive capabilities into the NBIs. The NBI and index score thresholds of impairment will provide monitoring programs with a robust measure of stream nutrient status and serve as a useful tool in enforcing regional nutrient criteria.


*Keywords:* Nutrient criteria; Macroinvertebrates; Biomonitoring; Biotic index; Optima

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1470-160X/$ – see front matter 
doi:10.1016/j.ecolind.2006.03.001

## 1. Introduction

Nonpoint source nutrient enrichment has become a dominant source of water quality impairment throughout the United States (USEPA, 2000a; Reckhow et al., 2005) with 20–40% of water quality impairment decisions arising from increased levels of nitrogen and phosphorus (USEPA, 1998a; Dodds and Welch, 2000; USEPA, 2000b). The current trend of increasing eutrophication of surface waters from various human induced sources such as fertilizer application and fossil fuel combustion (Vitousek et al., 1997; Smith et al., 2003; Turner et al., 2003) has caused the United States Environmental Protection Agency (USEPA) to develop a national strategy for the development of regional nutrient criteria (USEPA, 1998a,b). In a technical guidance manual published in 2000 by the EPA, response variables such as nutrients, chlorophyll *a*, total suspended solids, transparency, and turbidity are considered the primary targets for measuring the effects of nutrients on surface waters and developing criteria, because of their close relationship to the source of impairment (USEPA, 2000c). Listed as secondary response variables are macroinvertebrate multimetric indices (USEPA, 2000c).

As a result of nonpoint source pollution (NPSP) complexity, making determinations regarding the sources and extent of impairment due to any one component is inherently challenging. For this reason, it is important to develop and refine diagnostic measures that can address the complexity of water quality impairment as a result of nonpoint source pollution. The development of indices that incorporate taxon-specific tolerance values for specific sources of impairment may be one way to achieve this goal (Chessman and McEvoy, 1998; Yuan, 2004). Although heterotrophic organisms are often used in biological monitoring, the effects of increased levels of nutrients on such communities may vary (Deegan et al., 1997), creating difficulty in their use as robust measures of nutrient enrichment.

Aquatic macroinvertebrates have long been among the principal biological communities used for freshwater resource monitoring and assessment (Growns et al., 1997). Their diversity, ubiquitous occurrence, importance to ecosystem functioning, and sensitivity to environmental impact make them effective estimators of overall, integrated water quality (Rosenberg et al., 1986; Growns et al., 1997; Bode et al., 2002). The most frequently observed macroinvertebrate response to increased nutrient levels in streams is an alteration in abundance or density (Dudley et al., 1986; Hart and Robinson, 1990; Johnston et al., 1990; Perrin and Richardson, 1997; Stevenson, 1997; Robinson and Gessner, 2000), often in the form of increases in the abundance of certain feeding groups. Deegan et al. (1997) found higher densities of Trichoptera in fertilized reaches, when compared to unfertilized reaches, of the Kuparuk River, Alaska. Hart and Robinson (1990) reported grazing caddisflies developed faster, had higher body mass, and higher population densities in phosphorus-enriched study areas than in control areas. The primary catalyst behind such changes may be the result of bottom-up controls (Deegan et al., 1997; Perrin and Richardson, 1997; Miltner and Rankin, 1998) influencing food quality and quantity, such as increased periphyton abundance (Hart and Robinson, 1990). Aside from broader community changes, tolerance to different nutrient levels exhibits large among-species variation (Beketov, 2004). This suggests the establishment of biotic indices for nutrients based on species tolerances is possible.

This study addressed two major objectives. The first was to develop a biotic index to assess nutrient enrichment in wadeable streams using benthic macroinvertebrates. The second was to establish nutrient biotic index (NBI) score thresholds and corresponding nutrient concentrations, above which invertebrate communities show impairment due to increased stream water nutrient concentrations. The frequency of occurrences of taxa at varying nutrient concentrations would allow the identification of taxon-specific nutrient optima using a method of weighted averaging (Ter Braak and Juggins, 1993; Brandt, 2001; Black et al., 2004). The establishment of nutrient optima is possible based on the observation that most species exhibit unimodal response curves in relation to environmental variables (Jongman et al., 1987). The assignment of tolerance values to taxa based on nutrient optima would provide the ability to reduce community data to a linear scale of eutrophication from oligotrophic to eutrophic, similar to other biotic indices which have addressed other forms of pollution (Beck, 1954; Chutter, 1972; Cook, 1976; Hilsenhoff, 1987; Chessman and McEvoy, 1998). The

establishment of index score thresholds of impairment would serve as a useful tool in establishing and enforcing regional nutrient criteria.

## 2. Methods

The formation of the NBI consisted of development and application of two indices, one for total phosphorus (TP) (NBI-P) and another for nitrate ($NO_3^-$) (NBI-N). New York State Department of Environmental Conservation (NYSDEC) ambient water quality monitoring data were used for developing these indices. The data set contained benthic macroinvertebrate and water column chemistry samples collected between 1993 and 2002 from 129 locations on 116 different streams across New York State (Fig. 1). Benthic macroinvertebrate samples were collected once between July and September from each location, during the same year as water chemistries. A 5-min traveling kick sample along a 5-m diagonal transect through a riffle area was used. For each sample, a 100-specimen subsample was removed, and each organism was identified to the lowest taxonomic level possible (usually genus or species) (Bode et al., 2002). Water chemistry samples were collected 4–10 times a year at each location using a depth-integrated wading sampler, and composited from at least three points across the stream. Water samples were analyzed using automated colorimetry (USEPA method codes 353.2 and 365.2) by an environmental chemistry laboratory under contract to NYSDEC.

The results of water chemistry analysis from samples collected within 90 days prior to the invertebrate sampling event were averaged and used to establish the relationship between water nutrient concentrations and macroinvertebrate community structure. TP was the only measure of stream phosphorus included in the data set and therefore the only phosphorus measurement available for use in the present study. $NO_3^-$ is one of the most common measures of stream nitrogen (Simon et al., 2005), and is the typical form of soluble nitrogen in water (Paul and Clark, 1989). The inclusion of these nutrients in national nutrient criteria development and their presence in agricultural and urban runoff also made them primary targets for index development.

The TP and $NO_3^-$ values for the entire data set were split into 15 different ranges (bins) which contained

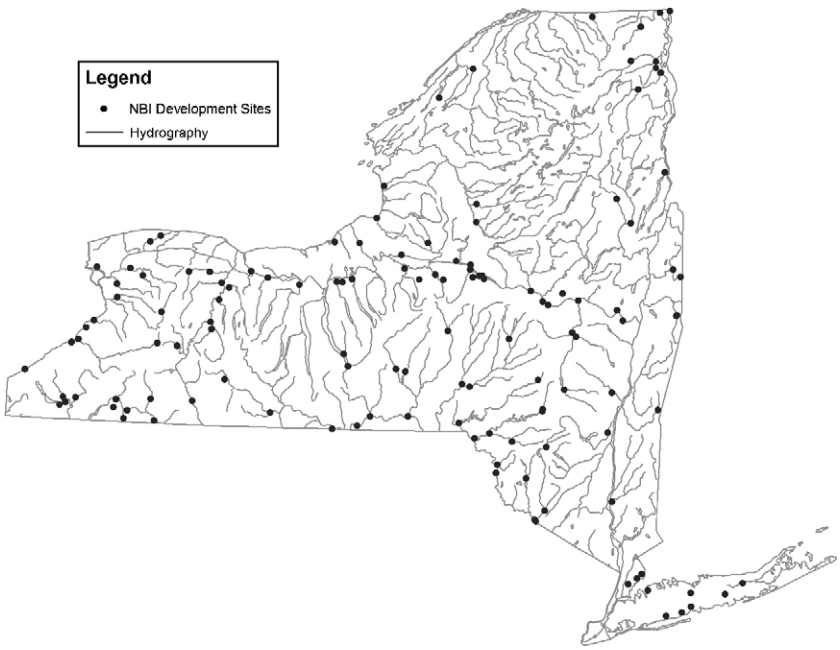


Fig. 1. Locations of sampling stations in New York State used in the development of the nutrient biotic indices (NBI-P and NBI-N). The data set provided an adequate distribution in the state, capturing a wide range of stream types and nutrient concentrations.

roughly equal numbers of samples in each. These bins provided the basis for observing invertebrate species response to varying nutrient concentrations. Many of the 367 taxa in the data set occurred too infrequently to be informative. Therefore, taxa that occurred in ≤2% of the samples were removed, leaving 164 taxa for which associated optima were developed.

A taxon's nutrient optimum for TP and $NO_3^-$ was calculated by dividing the sum of the weighted proportion of times a taxon occurred within the 15 nutrient bins, by the sum of the un-weighted proportion of times a taxon occurred within the bins (1):

$$\text{nutrient optima}_{(\text{TP or nitrate})} = \sum (W_{\text{prop}})_{\text{bin1+bin2+...+bin15}} / \sum (U_{\text{prop}})_{\text{bin1+bin2+...+bin15}} \quad (1)$$

where $W_{\text{prop}} = \text{Bin}\,\bar{x} \times U_{\text{prop}}$, and $U_{\text{prop}}$ = taxon frequency in Bin/Bin frequency.

The weighted proportion $W_{\text{prop}}$ was equal to the mean nutrient concentration of the bin Bin $\bar{x}$, multiplied by the proportion of times the taxon occurred within the bin $U_{\text{prop}}$. Therefore, nutrient optima are roughly equal to the mean nutrient concentration of the bin in which the taxon had the greatest number of occurrences. This method was adapted from a temperature index developed for 162 macroinvertebrate taxa by the Idaho Department of Environmental Quality (Brandt, 2001). Similar uses of weighted averaging methods have been developed for establishing environmental variable optima for various groups of organisms (Birks et al., 1990; Ter Braak and Juggins, 1993; Pan et al., 1996; Winter and Duthie, 2000; Black et al., 2004; Yuan, 2004).

Tolerance values were assigned on the basis of the nutrient optima. The ranges of nutrient optima were sorted from lowest to highest and split into 11 different groups. Each group consisted of 15 taxa except for group 11 which consisted of 14 taxa. Taxa within group one, which were associated with the lowest nutrient optima, were assigned a tolerance value of 0; taxa in group two were assigned a 1; taxa in group three were assigned a 2, and so on. Therefore a tolerance value of 0 represents nutrient intolerance and 10 represents tolerance.

Using the invertebrate data from the 185 different sampling events, NBI-P and NBI-N scores were calculated for each sample. Calculation of the indices (2) follows the approach of Hilsenhoff (1987):

$$\text{NBI score}_{(\text{TP or NO}_3^-)} = \sum (ab)/\text{c} \quad (2)$$

where $a$ is equal to the number of individuals for each taxon, $b$ the taxon's tolerance value, and $c$ is the total number of individuals in the sample for which tolerance values have been assigned. Using the calculated scores for each sample ordinary least squares (OLS) regression analysis was performed to assess the relation between mean $\log_{10}$ TP and $NO_3^-$ concentrations and corresponding NBI scores. This initial index assessment provided a basic understanding of the relation of the indices to increasing nutrient scores.

Site locations with furthest outlying NBI values were selected for calibration. Taxa at these sites driving index scores away from regression lines were selected for adjustment. Tolerance values for the dominant taxa within these outlying samples of the linear regression analysis were adjusted using the method described below. For samples in which the number of individuals of these taxa was ≥10 the NBI-P and NBI-N scores were recalculated after removing the taxa being adjusted. The mean NBI-P and NBI-N score for all sites where the taxon was ≥10 was rounded to the nearest whole number and designated as the new tolerance value for the taxon. Using the adjusted tolerance values a linear regression analysis was performed again with NBI scores plotted as functions of their respective nutrients.

After a satisfactory working pair of indices was established a three-tiered scale of eutrophication was developed that related the NBI-P and NBI-N scores to nutrient concentrations. Using the original series of nutrient bins as classifiers, a Bray–Curtis similarity analysis (Bray and Curtis, 1957) was performed using species abundance data. The similarity analysis used the mean of all pair-wise comparisons of samples within each nutrient bin, and between all pairs of nutrient bins, producing two correlation matrices. A cluster analysis was then performed on the inter-bin matrix. This cluster analysis assisted in the establishment of NBI score and nutrient concentration thresholds signifying the levels above which impairment occurs.

NBI-P and NBI-N scores were placed on the scale dictated by a site's nutrient concentration. Median NBI-P and NBI-N scores of the three trophic states

were analyzed using non-parametric analyses of variance (ANOVA). The Kruskal–Wallis statistic was used to test the null hypothesis, that the median index scores of the three trophic states were identical (Helsel and Hirsch, 1992). If the statistic resulted in rejection of the null hypothesis, Tukey's multiple comparison test was used to identify which median index scores were significantly different from others.

Overall water quality was determined for each of the sampling events in the data set using New York State's multimetric approach for determining water quality impairment (Bode et al., 2002; Riva-Murray et al., 2002). This method calculates species richness, Ephemeroptera–Plecoptera–Trichoptera richness (Lenat, 1987), Hilsenhoff's biotic index score (Hilsenhoff, 1987), and percent model affinity (Novak and Bode, 1992). The results of each of these indices are placed on a common 10 scale and the mean of these adjusted values is determined. The result, called the biological assessment profile (BAP) score, is a single value for which a four-tiered scale of water quality impact (non, slight, moderate, severe) has been established. New York State's water quality monitoring program has set the threshold between slight and moderate impact as the point above which remedial action for a waterbody typically occurs. Determining the concentrations for nutrients that cause impairment based on benthic macroinvertebrate community assessment should therefore relate in some way to the already established threshold of BAP scores between slight and moderate impact.

The clusters identified in the Bray–Curtis analysis provided a means for comparing water quality impact within different ranges of TP and $NO_3^-$ concentrations. Using a non-parametric ANOVA as was used during index reassessment, BAP scores were compared to determine if water quality impact changed significantly between clusters identified by Bray–Curtis analysis. Hodges-Lehmann estimation provided information on the magnitude of change in raw BAP scores between the different clusters. The percentage of sites determined to be non, slight, moderate or severely impacted within each of the clusters was also calculated.

## 3. Results

The data set provided a well-balanced distribution of nutrient concentrations for establishing taxon-specific nutrient optima, from very low to very high. For TP, bin means ranged from 0.0067 to 0.3596 mg/l and for $NO_3^-$ mean concentrations ranged from 0.0675 to 3.7642 mg/l. The upper and lower concentrations of each bin for both TP and $NO_3^-$ are provided in Table 1. A list of the taxa included in the

Table 1
Nutrient bins created for total phosphorus and nitrate used to derive taxa nutrient optima

| Bin number | TP (mg/l) upper limit | Mean TP (mg/l) | Std. dev. | *N* | $NO_3$ (mg/l) upper limit | Mean $NO_3$ (mg/l) | Std. dev. | *N* |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.0089 | 0.0067 | 0.003 | 14 | 0.0850 | 0.0675 | 0.025 | 12 |
| 2 | 0.0120 | 0.0105 | 0.002 | 13 | 0.1200 | 0.1025 | 0.025 | 13 |
| 3 | 0.0151 | 0.0136 | 0.002 | 13 | 0.1750 | 0.1475 | 0.039 | 13 |
| 4 | 0.0175 | 0.0163 | 0.002 | 14 | 0.2367 | 0.2059 | 0.044 | 13 |
| 5 | 0.0193 | 0.0184 | 0.001 | 12 | 0.2950 | 0.2659 | 0.041 | 13 |
| 6 | 0.0233 | 0.0213 | 0.003 | 12 | 0.3675 | 0.3313 | 0.051 | 13 |
| 7 | 0.0295 | 0.0264 | 0.004 | 13 | 0.4133 | 0.3904 | 0.032 | 12 |
| 8 | 0.0366 | 0.0331 | 0.005 | 12 | 0.5560 | 0.4847 | 0.101 | 12 |
| 9 | 0.0473 | 0.0419 | 0.008 | 12 | 0.6400 | 0.5980 | 0.059 | 12 |
| 10 | 0.0565 | 0.0519 | 0.006 | 12 | 0.7083 | 0.6742 | 0.048 | 12 |
| 11 | 0.0650 | 0.0608 | 0.006 | 12 | 0.9790 | 0.8437 | 0.191 | 12 |
| 12 | 0.0927 | 0.0789 | 0.020 | 12 | 1.1250 | 1.0520 | 0.103 | 13 |
| 13 | 0.1075 | 0.1001 | 0.010 | 12 | 1.4700 | 1.2975 | 0.244 | 12 |
| 14 | 0.1633 | 0.1354 | 0.039 | 11 | 1.8650 | 1.6675 | 0.279 | 12 |
| 15 | 0.5558 | 0.3596 | 0.278 | 11 | 5.6633 | 3.7642 | 2.686 | 11 |

The upper limit of each bin is given along with the mean concentration for each bin, standard deviation and the number of samples falling within each of the bins.

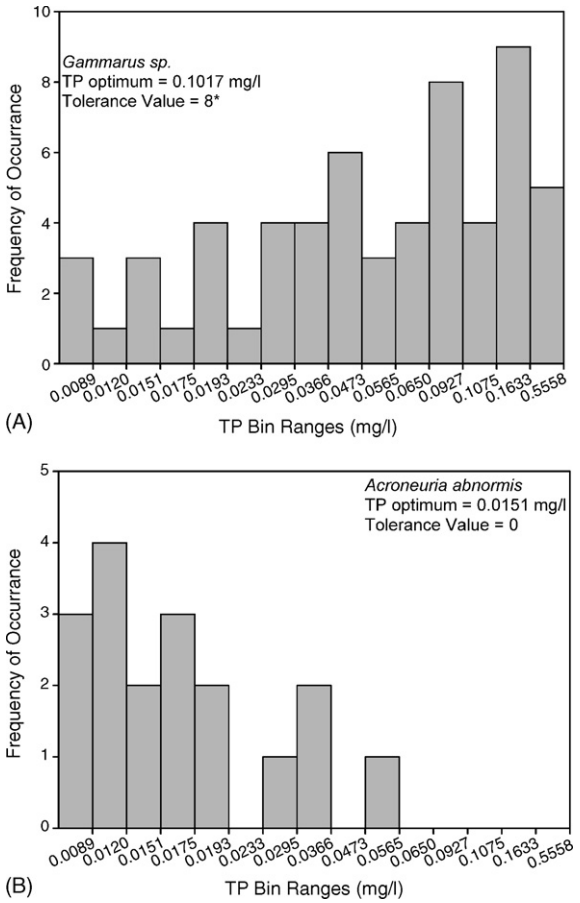


Fig. 2. Examples of taxon distribution over the TP gradient. *Gammarus* sp. (A) is considered TP tolerant. *Acroneuria abnormis* (B) is considered TP intolerant. * signifies the tolerance value was adjusted during calibration of the indices.

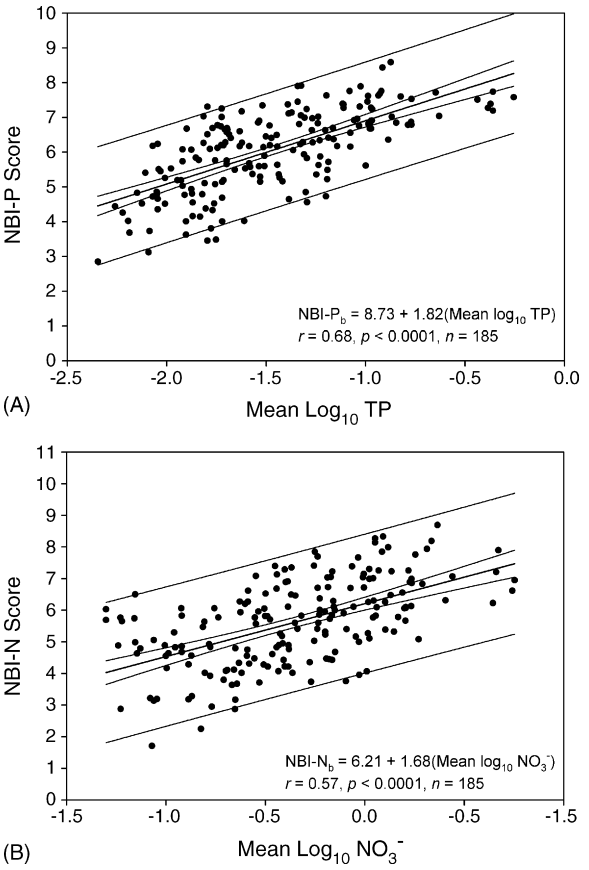


Fig. 3. NBI-P (A) and NBI-N (B) scores as functions of mean $\log_{10}$ TP and mean $\log_{10}$ $NO_3^-$. Confidence and predictions intervals set at 95%. Pre calibration results identified a wide array of scatter (NBI-P: $r = 0.65$, $p < 0.0001$, $n = 185$; NBI-N: $r = 0.56$, $p < 0.0001$, $n = 185$), but after calibration a significant amount of this was reduced (A and B above).

NBI-P and NBI-N, along with calculated nutrient optima, optimal nutrient ranges, and subsequent tolerance values is provided in Table 2. As hypothesized, many of the taxa had frequency distributions that displayed a distinct response to increasing nutrient concentrations. Two examples of these are given in Fig. 2.

Initial assessment of the NBI-P and NBI-N using OLS showed a positive linear relationship suggesting increasing NBI-P and NBI-N scores with increasing concentrations of the appropriate nutrient. An increase in the performance of the indices was noted after completion of the calibration phase (Fig. 3).

The Bray–Curtis analysis showed two distinct clusters of invertebrate communities for both TP and $NO_3^-$ (Fig. 4). There was a distinct grouping of bins 1, 2, 3, and 4 for both nutrients, and another consisting of bins 12, 13, 14, and 15. There was less of a distinct cluster of remaining bins for both nutrients, and clustering was more distinct among the $NO_3^-$ bins than among TP bins at the higher nutrient concentrations. The clustering of communities at the upper and lower ends of the nutrient ranges provided a logical designation of all samples that fell in the lower range cluster as oligotrophic, and the samples in the higher range cluster as eutrophic. The mesotrophic range was assigned to bins that did not cluster with either of the other two groups (Fig. 4). On the basis of bin clusters, the nutrient concentrations designating the boundaries between oligo-, meso-, and eutrophic were established.

Table 2
Taxa included in the NBI-P and NBI-N, optimal nutrient range, their nutrient optima (Opt.), and subsequent tolerance values (*T*-value)

| Genus species | TP optima range (mg/l) | TP optima (mg/l) | TP *T*-value | $NO_3^-$ optima Range (mg/l) | $NO_3^-$ optima (mg/l) | $NO_3^-$ *T*-value |
|---|---|---|---|---|---|---|
| Nemertea | | | | | | |
| *Prostoma graecense* | 0.0233–0.0295 | 0.0237 | 2 | 0.708–0.979 | 0.729 | 7 |
| Turbellaria | | | | | | |
| Undetermined Turbellaria | 0.0650–0.0927 | 0.0825 | 8 | 0.708–0.979 | 0.928 | 6* |
| Lumbricida | | | | | | |
| Undetermined Lumbricina | 0.0650–0.0927 | 0.0788 | 8 | 0.708–0.979 | 0.923 | 8 |
| Lumbriculida | | | | | | |
| Undetermined Lumbriculidae | 0.0175–0.0193 | 0.0181 | 5* | 0.413–0.556 | 0.452 | 6* |
| Tubificida | | | | | | |
| Undetermined Enchytraeidae | 0.0565–0.065 | 0.0572 | 7 | 0.708–0.979 | 0.940 | 8 |
| Undetermined Tubificidae w/ cap. setae | 0.1633–0.5558 | 0.2545 | 10 | 0.708–0.979 | 0.930 | 8 |
| Undetermined Tubificidae w/o cap. setae | 0.0565–0.065 | 0.0621 | 7 | 0.708–0.979 | 0.797 | 7 |
| *Nais variabilis* | 0.0151–0.0175 | 0.0162 | 5* | 0.175–0.236 | 0.217 | 0 |
| *Stylaria lacustris* | 0.0565–0.065 | 0.0619 | 5* | 0.295–0.367 | 0.351 | 2 |
| Hirudinea | | | | | | |
| Undetermined Hirudinea | 0.1075–0.1633 | 0.1102 | 9 | 1.125–1.47 | 1.288 | 10 |
| Gastropoda | | | | | | |
| *Physella* sp. | 0.0650–0.0927 | 0.0734 | 8 | 0.708–0.979 | 0.772 | 7 |
| *Ferrissia* sp. | 0.0927–0.1075 | 0.0995 | 9 | 0.556–0.64 | 0.565 | 5 |
| *Goniobasis livescens* | 0.1633–0.5558 | 0.2301 | 10 | 1.865–5.663 | 2.489 | 10 |
| Undetermined Hydrobiidae | 0.0473–0.0565 | 0.0494 | 6 | 0.708–0.979 | 0.763 | 7 |
| Pelecypoda | | | | | | |
| *Pisidium* sp. | 0.0650–0.0927 | 0.0681 | 8 | 1.125–1.47 | 1.407 | 10 |
| *Sphaerium* sp. | 0.0927–0.1075 | 0.1004 | 9 | 0.413–0.556 | 0.518 | 4 |
| Undetermined Sphaeriidae | 0.1075–0.1633 | 0.1264 | 10 | 0.708–0.979 | 0.952 | 8 |
| Isopoda | | | | | | |
| *Caecidotea racovitzai* | 0.0650–0.0927 | 0.0753 | 6* | 0.295–0.367 | 0.355 | 2 |
| *Caecidotea* sp. | 0.0650–0.0927 | 0.0869 | 7* | 1.125–1.47 | 1.198 | 9 |
| Amphipoda | | | | | | |
| *Gammarus* sp. | 0.0927–0.1075 | 0.1017 | 8* | 1.125–1.47 | 1.132 | 9 |
| Decapoda | | | | | | |
| Undetermined Cambaridae | 0.0473–0.0565 | 0.0553 | 6 | 0.556–0.64 | 0.585 | 5 |
| Ephemeroptera | | | | | | |
| *Isonychia bicolor* | 0.0233–0.0295 | 0.0259 | 5* | 0.295–0.367 | 0.315 | 2 |
| *Acentrella* sp. | 0.0295–0.0366 | 0.0300 | 5* | 0.413–0.556 | 0.507 | 5* |
| *Acerpenna pygmaea* | 0.0120–0.0151 | 0.0130 | 0 | 0.413–0.556 | 0.489 | 4 |
| *Baetis brunneicolor* | 0.0295–0.0366 | 0.0329 | 1* | 0.556–0.64 | 0.556 | 5 |
| *Baetis flavistriga* | 0.0565–0.065 | 0.0611 | 7 | 0.979–1.125 | 1.067 | 7* |
| *Baetis intercalaris* | 0.0473–0.0565 | 0.0506 | 6 | 0.413–0.556 | 0.550 | 5 |
| *Baetis tricaudatus* | 0.0650–0.0927 | 0.0765 | 8 | 1.125–1.47 | 1.145 | 9 |
| *Baetis* sp. | 0.0295–0.0366 | 0.0347 | 6* | 0.413–0.556 | 0.452 | 3 |
| *Plauditus* sp. | 0.0233–0.0295 | 0.0249 | 2 | 0.640–0.708 | 0.669 | 6 |
| *Epeorus* (*Iron*) sp. | 0.0120–0.0151 | 0.0136 | 0 | 0.175–0.236 | 0.234 | 0 |
| *Heptagenia* sp. | 0.0151–0.0175 | 0.0168 | 0 | 0.120–0.175 | 0.171 | 0 |
| *Leucrocuta* sp. | 0.0193–0.0233 | 0.0203 | 1 | 0.413–0.556 | 0.413 | 3 |
| *Nixe* (*Nixe*) sp. | 0.0175–0.0193 | 0.0181 | 1 | 0.640–0.708 | 0.641 | 5 |
| *Rhithrogena* sp. | 0.0151–0.0175 | 0.0151 | 0 | 0.236–0.295 | 0.270 | 1 |

Table 2 (*Continued*)

| Genus species | TP optima range (mg/l) | TP optima (mg/l) | TP *T*-value | $NO_3^-$ optima Range (mg/l) | $NO_3^-$ optima (mg/l) | $NO_3^-$ *T*-value |
|---|---|---|---|---|---|---|
| *Stenacron interpunctatum* | 0.0565–0.065 | 0.0627 | 7 | 0.708–0.979 | 0.808 | 7 |
| *Stenonema mediopunctatum* | 0.0295–0.0366 | 0.0296 | 3 | 0.413–0.556 | 0.448 | 3 |
| *Stenonema modestum* | 0.0233–0.0295 | 0.0237 | 2 | 0.556–0.64 | 0.565 | 5 |
| *Stenonema terminatum* | 0.0233–0.0295 | 0.0248 | 2 | 0.367–0.413 | 0.392 | 3 |
| *Stenonema vicarium* | 0.0473–0.0565 | 0.0519 | 6 | 0.708–0.979 | 0.787 | 7 |
| *Stenonema* sp. | 0.0295–0.0366 | 0.0352 | 5* | 0.367–0.413 | 0.381 | 5* |
| Undetermined Heptageniidae | 0.0366–0.0473 | 0.0430 | 5 | 0.295–0.367 | 0.300 | 2 |
| *Paraleptophlebia mollis* | 0.0233–0.0295 | 0.0236 | 2 | 0.236–0.295 | 0.281 | 1 |
| *Paraleptophlebia* sp. | 0.0193–0.0233 | 0.0216 | 2 | 0.367–0.413 | 0.380 | 3 |
| *Drunella cornutella* | 0.0193–0.0233 | 0.0222 | 4* | 0.413–0.556 | 0.542 | 4 |
| *Ephemerella subvaria* | 0.0295–0.0366 | 0.0304 | 4* | 0.236–0.295 | 0.273 | 1 |
| *Ephemerella* sp. | 0.0366–0.0473 | 0.0405 | 4 | 0.413–0.556 | 0.516 | 4 |
| *Serratella deficiens* | 0.0366–0.0473 | 0.0439 | 5 | 0.295–0.367 | 0.303 | 2 |
| *Serratella serrata* | 0.0193–0.0233 | 0.0194 | 1 | 0.175–0.236 | 0.198 | 0 |
| *Serratella serratoides* | 0.0175–0.0193 | 0.0178 | 0 | 0.236–0.295 | 0.279 | 1 |
| *Serratella* sp. | 0.0175–0.0193 | 0.0179 | 1 | 0.236–0.295 | 0.288 | 1 |
| Undetermined Ephemerellidae | 0.0233–0.0295 | 0.0286 | 3 | 0.640–0.708 | 0.641 | 6 |
| *Tricorythodes* sp. | 0.0295–0.0366 | 0.0351 | 4 | 0.979–1.125 | 1.092 | 9 |
| *Caenis* sp. | 0.0295–0.0366 | 0.0305 | 3 | 0.413–0.556 | 0.451 | 3 |
| *Anthopotamus* sp. | 0.0366–0.0473 | 0.0407 | 4 | 0.556–0.64 | 0.600 | 5 |
| *Ephoron leukon* | 0.0193–0.0233 | 0.0196 | 1 | 0.236–0.295 | 0.290 | 1 |
| Odonata | | | | | | |
| *Ophiogomphus* sp. | 0.0175–0.0193 | 0.0190 | 1 | 0.367–0.413 | 0.385 | 3 |
| Undetermined Gomphidae | 0.0193–0.0233 | 0.0210 | 2 | 0.175–0.236 | 0.235 | 0 |
| Plecoptera | | | | | | |
| *Acroneuria abnormis* | 0.0151–0.0175 | 0.0151 | 0 | 0.120–0.175 | 0.146 | 0 |
| *Acroneuria* sp. | 0.0089–0.012 | 0.0114 | 0 | 0.175–0.236 | 0.234 | 0 |
| *Agnetina capitata* | 0.0233–0.0295 | 0.0291 | 3 | 0.708–0.979 | 0.713 | 6 |
| *Neoperla* sp. | 0.0366–0.0473 | 0.0417 | 5 | 0.556–0.64 | 0.616 | 5 |
| *Paragnetina immarginata* | 0.0175–0.0193 | 0.0187 | 1 | 0.295–0.367 | 0.318 | 2 |
| *Paragnetina media* | 0.0473–0.0565 | 0.0562 | 6 | 0.367–0.413 | 0.411 | 3 |
| *Paragnetina* sp. | 0.0175–0.0193 | 0.0187 | 1 | 0.640–0.708 | 0.670 | 6 |
| Undetermined Perlidae | 0.0366–0.0473 | 0.0469 | 5 | 0.708–0.979 | 0.728 | 7 |
| Coleoptera | | | | | | |
| *Ectopria nervosa* | 0.1075–0.1633 | 0.1204 | 10 | 1.125–1.47 | 1.161 | 9 |
| *Psephenus herricki* | 0.1075–0.1633 | 0.1203 | 10 | 1.125–1.47 | 1.138 | 9 |
| *Psephenus* sp. | 0.0295–0.0366 | 0.0309 | 3 | 0.413–0.556 | 0.490 | 4 |
| *Optioservus fastiditus* | 0.0366–0.0473 | 0.0398 | 6* | 0.708–0.979 | 0.783 | 7 |
| *Optioservus ovalis* | 0.0650–0.0927 | 0.0868 | 9 | 0.413–0.556 | 0.468 | 4 |
| *Optioservus trivittatus* | 0.0565–0.065 | 0.0645 | 7 | 0.708–0.979 | 0.812 | 6* |
| *Optioservus* sp. | 0.0565–0.065 | 0.0614 | 7 | 0.979–1.125 | 0.995 | 8 |
| *Promoresia elegans* | 0.1633–0.5558 | 0.2129 | 10 | 1.865–5.663 | 1.916 | 10 |
| *Stenelmis concinna* | 0.0175–0.0193 | 0.0175 | 5* | 0.175–0.236 | 0.211 | 0 |
| *Stenelmis crenata* | 0.1075–0.1633 | 0.1199 | 7* | 0.708–0.979 | 0.897 | 7* |
| *Stenelmis* sp. | 0.0473–0.0565 | 0.0485 | 7* | 0.413–0.556 | 0.497 | 7* |
| Megaloptera | | | | | | |
| *Corydalus cornutus* | 0.0233–0.0295 | 0.0246 | 2 | 0.295–0.367 | 0.298 | 2 |
| *Nigronia serricornis* | 0.1075–0.1633 | 0.1473 | 10 | 0.979–1.125 | 1.017 | 8 |
| *Sialis* sp. | 0.0366–0.0473 | 0.0458 | 5 | 0.640–0.708 | 0.693 | 6 |
| Trichoptera | | | | | | |
| *Chimarra aterrima* | 0.0233–0.0295 | 0.0253 | 2 | 0.295–0.367 | 0.365 | 3 |

Table 2 (*Continued*)

| Genus species | TP optima range (mg/l) | TP optima (mg/l) | TP *T*-value | $NO_3^-$ optima Range (mg/l) | $NO_3^-$ optima (mg/l) | $NO_3^-$ *T*-value |
|---|---|---|---|---|---|---|
| *Chimarra obscura* | 0.0366–0.0473 | 0.0428 | 6* | 0.413–0.556 | 0.472 | 4 |
| *Chimarra socia* | 0.0151–0.0175 | 0.0165 | 4* | 0.236–0.295 | 0.273 | 1 |
| *Chimarra* sp. | 0.0233–0.0295 | 0.0246 | 2 | 0.175–0.236 | 0.229 | 0 |
| *Dolophilodes* sp. | 0.0175–0.0193 | 0.0186 | 4* | 0.413–0.556 | 0.450 | 3 |
| *Psychomyia flavida* | 0.0193–0.0233 | 0.0201 | 1 | 0.175–0.236 | 0.217 | 0 |
| *Neureclipsis* sp. | 0.0233–0.0295 | 0.0253 | 3 | 0.236–0.295 | 0.280 | 1 |
| *Polycentropus* sp. | 0.0366–0.0473 | 0.0395 | 4 | 0.295–0.367 | 0.352 | 2 |
| *Cheumatopsyche* sp. | 0.0565–0.065 | 0.0629 | 6* | 0.708–0.979 | 0.863 | 6* |
| *Hydropsyche betteni* | 0.0650–0.0927 | 0.0904 | 7* | 0.979–1.125 | 1.042 | 9 |
| *Hydropsyche bronta* | 0.0565–0.065 | 0.0617 | 7 | 0.708–0.979 | 0.930 | 6* |
| *Hydropsyche morosa* | 0.0295–0.0366 | 0.0350 | 5* | 0.236–0.295 | 0.273 | 1 |
| *Hydropsyche scalaris* | 0.0295–0.0366 | 0.0312 | 3 | 0.367–0.413 | 0.374 | 3 |
| *Hydropsyche slossonae* | 0.0473–0.0565 | 0.0534 | 6 | 1.125–1.47 | 1.419 | 10 |
| *Hydropsyche sparna* | 0.0650–0.0927 | 0.0785 | 6* | 1.125–1.47 | 1.264 | 7* |
| *Hydropsyche* sp. | 0.0366–0.0473 | 0.0380 | 5* | 0.413–0.556 | 0.496 | 4 |
| *Macrostemum carolina* | 0.0565–0.065 | 0.0575 | 7 | 0.295–0.367 | 0.324 | 2 |
| *Macrostemum* sp. | 0.0295–0.0366 | 0.0316 | 4 | 0.295–0.367 | 0.318 | 2 |
| *Rhyacophila fuscula* | 0.0233–0.0295 | 0.0243 | 2 | 0.556–0.64 | 0.618 | 5 |
| *Rhyacophila* sp. | 0.0151–0.0175 | 0.0166 | 0 | 0.236–0.295 | 0.275 | 1 |
| *Glossosoma* sp. | 0.0473–0.0565 | 0.0508 | 6 | 0.175–0.236 | 0.197 | 0 |
| *Hydroptila consimilis* | 0.0927–0.1075 | 0.0943 | 9 | 1.865–5.663 | 2.440 | 10 |
| *Hydroptila spatulata* | 0.1075–0.1633 | 0.1136 | 9 | 0.979–1.125 | 1.025 | 8 |
| *Hydroptila* sp. | 0.0565–0.065 | 0.0569 | 6 | 0.640–0.708 | 0.648 | 6 |
| *Leucotrichia* sp. | 0.0473–0.0565 | 0.0556 | 6 | 0.295–0.367 | 0.324 | 2 |
| Undetermined Hydroptilidae | 0.0366–0.0473 | 0.0436 | 5 | 0.295–0.367 | 0.355 | 2 |
| *Brachycentrus appalachia* | 0.0193–0.0233 | 0.0193 | 3* | 0.175–0.236 | 0.219 | 4* |
| *Micrasema* sp. | 0.0193–0.0233 | 0.0196 | 1 | 0.175–0.236 | 0.190 | 0 |
| *Apatania* sp. | 0.0151–0.0175 | 0.0168 | 3* | 0.236–0.295 | 0.294 | 4* |
| Undetermined Limnephilidae | 0.0233–0.0295 | 0.0271 | 3 | 0.413–0.556 | 0.530 | 4 |
| *Lepidostoma* sp. | 0.0233–0.0295 | 0.0250 | 2 | 0.175–0.236 | 0.216 | 0 |
| *Helicopsyche borealis* | 0.0175–0.0193 | 0.0186 | 1 | 0.295–0.367 | 0.327 | 2 |
| Lepidoptera | | | | | | |
| *Petrophila* sp. | 0.0366–0.0473 | 0.0470 | 5 | 0.413–0.556 | 0.466 | 3 |
| Diptera | | | | | | |
| *Antocha* sp. | 0.0650–0.0927 | 0.0705 | 8 | 0.640–0.708 | 0.657 | 6 |
| *Dicranota* sp. | 0.0366–0.0473 | 0.0444 | 5 | 1.125–1.47 | 1.327 | 10 |
| *Hexatoma* sp. | 0.0175–0.0193 | 0.0178 | 0 | 0.236–0.295 | 0.249 | 1 |
| *Tipula* sp. | 0.1633–0.5558 | 0.1661 | 10 | 1.125–1.47 | 1.290 | 10 |
| Undetermined Ceratopogonidae | 0.0650–0.0927 | 0.0744 | 8 | 0.979–1.125 | 1.098 | 9 |
| *Simulium jenningsi* | 0.0473–0.0565 | 0.0510 | 6 | 0.295–0.367 | 0.317 | 2 |
| *Simulium tuberosum* | 0.0193–0.0233 | 0.0204 | 1 | 0.120–0.175 | 0.159 | 0 |
| *Simulium vittatum* | 0.1075–0.1633 | 0.1565 | 7* | 1.470–1.865 | 1.672 | 10 |
| *Simulium* sp. | 0.0366–0.0473 | 0.0415 | 7* | 0.640–0.708 | 0.651 | 6 |
| *Atherix* sp. | 0.0650–0.0927 | 0.0751 | 8 | 0.556–0.64 | 0.610 | 5 |
| *Hemerodromia* sp. | 0.0366–0.0473 | 0.0412 | 5 | 0.708–0.979 | 0.894 | 6* |
| Chironomidae | | | | | | |
| *Pentaneura* sp. | 0.0151–0.0175 | 0.0154 | 0 | 0.236–0.295 | 0.275 | 1 |
| *Thienemannimyia* gr. spp. | 0.0650–0.0927 | 0.0682 | 8 | 0.708–0.979 | 0.912 | 8 |
| *Diamesa* sp. | 0.1633–0.5558 | 0.1685 | 10 | 1.470–1.865 | 1.616 | 10 |
| *Pagastia orthogonia* | 0.0366–0.0473 | 0.0366 | 4 | 0.979–1.125 | 1.041 | 8 |
| *Potthastia gaedii* gr. | 0.0650–0.0927 | 0.0842 | 9 | 1.470–1.865 | 1.477 | 10 |
| *Cardiocladius obscurus* | 0.0565–0.065 | 0.0587 | 8* | 0.413–0.556 | 0.476 | 6* |

Table 2 (*Continued*)

| Genus species | TP optima range (mg/l) | TP optima (mg/l) | TP *T*-value | $NO_3^-$ optima Range (mg/l) | $NO_3^-$ optima (mg/l) | $NO_3^-$ *T*-value |
|---|---|---|---|---|---|---|
| *Cricotopus bicinctus* | 0.0565–0.065 | 0.0589 | 7 | 0.640–0.708 | 0.687 | 6 |
| *Cricotopus tremulus* gr. | 0.0650–0.0927 | 0.0693 | 8 | 1.125–1.47 | 1.128 | 9 |
| *Cricotopus trifascia* gr. | 0.0650–0.0927 | 0.0886 | 9 | 0.979–1.125 | 1.068 | 9 |
| *Cricotopus vierriensis* | 0.0473–0.0565 | 0.0525 | 6 | 0.556–0.64 | 0.590 | 5 |
| *Eukiefferiella devonica* gr. | 0.0927–0.1075 | 0.0986 | 9 | 1.125–1.47 | 1.239 | 9 |
| *Orthocladius nr. dentifer* | 0.0295–0.0366 | 0.0314 | 3 | 0.708–0.979 | 0.758 | 7 |
| *Parametriocnemus lundbecki* | 0.0650–0.0927 | 0.0676 | 8 | 1.470–1.865 | 1.666 | 10 |
| *Rheocricotopus robacki* | 0.0295–0.0366 | 0.0356 | 4 | 0.413–0.556 | 0.476 | 4 |
| *Synorthocladius nr. semivirens* | 0.0473–0.0565 | 0.0498 | 6 | 0.979–1.125 | 1.065 | 9 |
| *Tvetenia bavarica* gr. | 0.0650–0.0927 | 0.0853 | 9 | 1.470–1.865 | 1.823 | 10 |
| *Tvetenia vitracies* | 0.0565–0.065 | 0.0631 | 7 | 0.640–0.708 | 0.688 | 6 |
| *Chironomus* sp. | 0.1075–0.1633 | 0.1077 | 9 | 0.640–0.708 | 0.664 | 6 |
| *Cryptochironomus fulvus* gr. | 0.0366–0.0473 | 0.0430 | 5 | 0.640–0.708 | 0.658 | 6 |
| *Dicrotendipes neomodestus* | 0.1075–0.1633 | 0.1360 | 10 | 0.413–0.556 | 0.494 | 4 |
| *Microtendipes pedellus* gr. | 0.0650–0.0927 | 0.0687 | 7* | 0.708–0.979 | 0.724 | 7 |
| *Microtendipes rydalensis* gr. | 0.0193–0.0233 | 0.0215 | 2 | 0.236–0.295 | 0.244 | 1 |
| *Phaenopsectra dyari* | 0.0366–0.0473 | 0.0392 | 4 | 0.413–0.556 | 0.550 | 5 |
| *Polypedilum aviceps* | 0.0193–0.0233 | 0.0231 | 5* | 0.708–0.979 | 0.754 | 7 |
| *Polypedilum flavum* | 0.0650–0.0927 | 0.0841 | 9* | 0.708–0.979 | 0.811 | 7 |
| *Polypedilum illinoense* | 0.1075–0.1633 | 0.1610 | 10 | 0.708–0.979 | 0.737 | 7 |
| *Polypedilum laetum* | 0.0565–0.065 | 0.0588 | 7 | 0.708–0.979 | 0.718 | 6 |
| *Polypedilum scalaenum* gr. | 0.1075–0.1633 | 0.1356 | 10 | 0.640–0.708 | 0.701 | 6 |
| *Stenochironomus* sp. | 0.0366–0.0473 | 0.0405 | 4 | 0.413–0.556 | 0.459 | 3 |
| *Cladotanytarsus* sp. | 0.0473–0.0565 | 0.0495 | 6 | 0.413–0.556 | 0.510 | 4 |
| *Micropsectra dives* gr. | 0.0473–0.0565 | 0.0497 | 6 | 0.979–1.125 | 1.077 | 9 |
| *Micropsectra polita* | 0.0120–0.0151 | 0.0133 | 0 | 0.708–0.979 | 0.753 | 7 |
| *Micropsectra* sp. | 0.0233–0.0295 | 0.0288 | 3 | 0.295–0.367 | 0.295 | 1 |
| *Paratanytarsus confusus* | 0.0366–0.0473 | 0.0410 | 5 | 0.979–1.125 | 1.015 | 8 |
| *Rheotanytarsus exiguus* gr. | 0.0565–0.065 | 0.0596 | 6* | 0.556–0.64 | 0.568 | 5 |
| *Rheotanytarsus pellucidus* | 0.0233–0.0295 | 0.0279 | 3 | 0.295–0.367 | 0.305 | 2 |
| *Sublettea coffmani* | 0.0233–0.0295 | 0.0284 | 3 | 0.556–0.64 | 0.591 | 5 |
| *Tanytarsus glabrescens* gr. | 0.0366–0.0473 | 0.0433 | 5 | 0.640–0.708 | 0.690 | 6 |
| *Tanytarsus guerlus* gr. | 0.0366–0.0473 | 0.0409 | 5 | 0.556–0.64 | 0.598 | 5 |
| *Zavrelia* sp. | 0.0650–0.0927 | 0.0900 | 9 | 1.125–1.47 | 1.148 | 9 |

Tolerance values accompanied by * indicates the value was adjusted from the original during the calibration phase. Tolerance values of 0 indicate high nutrient level intolerance, a 10 indicates high nutrient level tolerance.

The TP boundaries were set as follows: oligo–mesotrophic 0.0175 mg/l, meso–eutrophic 0.065 mg/l. The boundaries for $NO_3^-$ were set as oligo–mesotrophic 0.24 mg/l, meso–eutrophic 0.98 mg/l. Fig. 5 shows results of the index scores for samples when classified by the trophic state determined by these nutrient concentration boundaries.

Results of ANOVA (Kruskal–Wallis and Tukey's tests) suggested significant differences existed between median index scores among all possible comparisons of trophic states (Fig. 5). The Kruskal–Wallis test on both the NBI-P and NBI-N suggested the null hypothesis of the medians of the three trophic state populations being equal could be rejected and the alternative accepted (NBI-P: Kruskal–Wallis statistic = 87.9, d.f. = 2, $p < 0.0001$; NBI-N: Kruskal–Wallis statistic = 63.8, d.f. = 2, $p < 0.0001$). Tukey's test of association indicated that all comparisons between the different trophic states were significantly different (NBI-P: $F = 83.37$, d.f. = 2, $p < 0.0001$; NBI-N: $F = 48.37$, d.f. = 2, $p < 0.0001$).

Although none of the samples were identified as severely impacted using NYSDEC's multimetric approach, overall water quality impact increased with increased nutrient concentrations (Fig. 6). Kruskal–

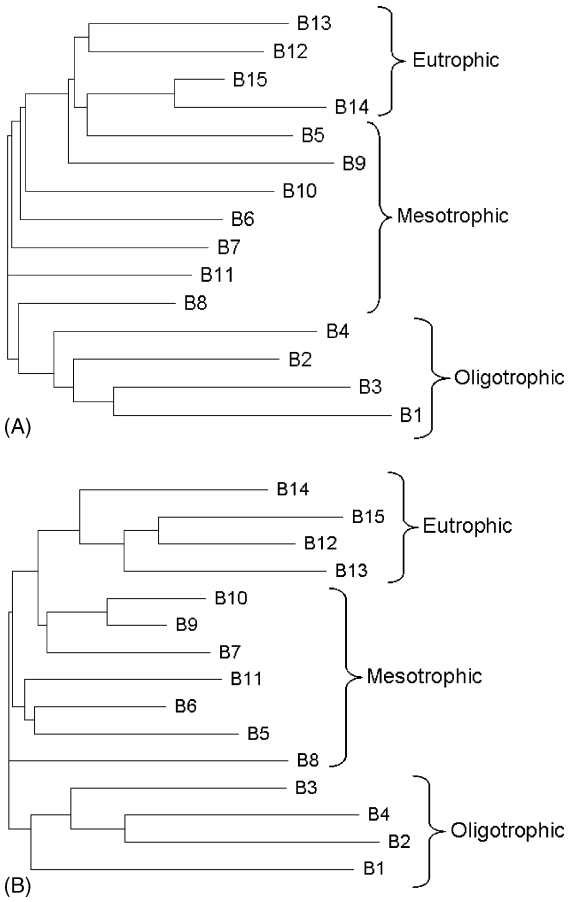


Fig. 4. Additive tree clusters based on mean pair-wise Bray-Curtis similarity between TP (A) and $NO_3^-$ (B) bins. Clusters of bins are designated with their appropriate trophic state. Bins with higher numbers signify greater nutrient concentration. On the basis of bin clusters trophic state boundaries were set as follows; TP oligo-mesotrophic 0.0175 mg/l, meso-eutrophic 0.065 mg/l: $NO_3^-$ oligo-mesotrophic 0.24 mg/l, meso-eutrophic 0.98 mg/l.

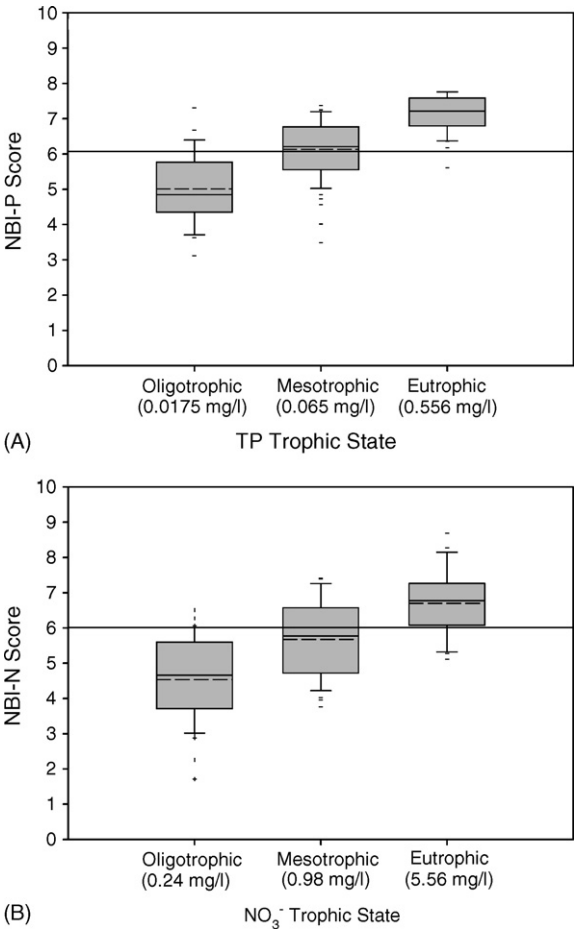


Fig. 5. Nutrient Biotic Index scores classified by trophic state determined by nutrient concentration boundaries developed using mean pair-wise Bray-Curtis similarity (Fig. 4, A and B). Dashed lines represent means, solid lines represent medians. Outliers are points falling outside of the 10th and 90th percentiles. Based on ANOVA all median NBI Scores were significantly different between trophic states. This suggests the ability of the NBI-P and NBI-N to accurately predict stream trophic state. Solid lines extending across plots represent NBI score thresholds. Above these points water quality impairment is likely to occur.

Wallis test results suggest median BAP scores were significantly different for both TP and $NO_3^-$ (BAP score, TP: Kruskal–Wallis statistic = 47.08, d.f. = 2, $p < 0.0001$; BAP score, $NO_3^-$: Kruskal–Wallis statistic = 29.28, d.f. = 2, $p < 0.0001$). Tukey's test identified median BAP scores were significantly different between all comparisons of trophic states for both TP and $NO_3^-$ (BAP score, TP: $F = 31.4$, d.f. = 2, $p < 0.0001$; BAP score, $NO_3^-$: $F = 17.22$, d.f. = 2, $p < 0.0001$). The greatest magnitude of change in BAP scores occurred between mesotrophic and eutrophic states for both TP and $NO_3^-$ (Table 3). The percentage of each of the four different levels of overall water quality impairment for sites within each of the three clusters identified in the Bray–Curtis analysis are summarized in Table 3 and Fig. 6. For both TP and $NO_3^-$ sites with mean nutrient concentrations within the oligotrophic ranges showed the least impact.

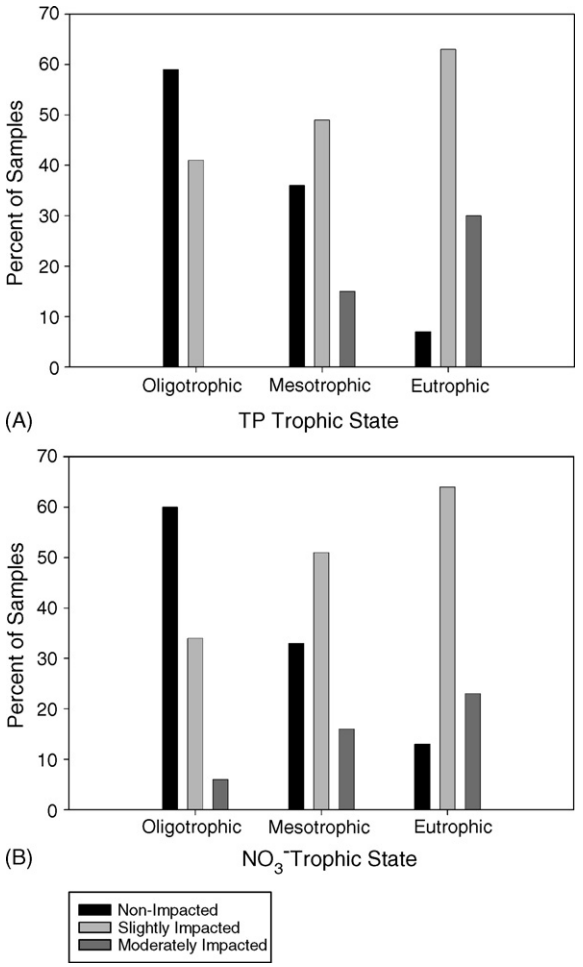


Fig. 6. Overall water quality assessment results derived using NYS's multimetric approach. Assessments are presented as percentages within the three different trophic states for both TP (A) and $NO_3^-$ (B). In general water quality assessments worsened with increased nutrient concentrations. The mesotrophic state shows a mixture of non, slight, and moderately impacted water quality. This may be a transitional zone of impairment.

## 4. Discussion

The biotic index has been an accepted method for determining water quality for several decades, arising out of the need for water quality assessments that were easy to understand and express (Chutter, 1972). Some of the best known indices, such as Chutter's (1972) empirical biotic index of water quality and Hilsenhoff's (1987) biotic index lacked a protocol for assigning tolerance values to the species encountered. In contrast the weighted averaging technique used in this investigation provided a protocol for assigning tolerance values based on species distributions in different concentration ranges of chemical variables. Weighted averaging methods have been widely used in recent years. Examples include the development of land cover optima for macroinvertebrates (Black et al., 2004), temperature optima for macroinvertebrates (Brandt, 2001), pH, turbidity, TN and TP optima for diatoms (Pan et al., 1996; Winter and Duthie, 2000), and for reconstructing environmental variables using biological community structure (Birks et al., 1990; Ter Braak and Juggins, 1993). These studies were based on the premise that a taxon's optimum for an environmental variable can be derived by weighting the mean of the variable by the taxon's relative abundance (Birks et al., 1990; Black et al., 2004). A taxon with an optimum closest to that of the level of an environmental variable of interest at a particular site is likely to be in the greatest abundance (Birks et al., 1990). Most studies utilizing weighted averaging used a method similar to Birks et al. (1990) known as "weighted averaging regression and calibration" in which taxon relative abundance is used for weighting the environmental variable. In developing the NBI, the method was that used for Brandt's (2001) temperature index, in which the environmental variable was weighted by the taxon's proportion of occurrence at a level of environmental variable, rather than its abundance. In addition the present study used ranges of environmental variables rather than single values.

A similar analysis of benthic macroinvertebrate taxa was performed by Yuan (2004), in which taxa were classified as sensitive, intermediately tolerant or tolerant to TP, $SO_4$, pH and habitat. A comparison of TP tolerance values with those applied by Yuan (2004) found some different results. For example in the present study, *Parametriocnemus* sp., *Nigronia* sp., and *Sialis* sp. have been assigned tolerances conflicting with those given by Yuan (2004). However, some taxa such as *Ephemerella* sp., *Epeorus* sp., and *Stenelmis* sp. were assigned similar tolerance values. Possible explanations for these discrepancies may stem from residual effects of differences in sampling protocols (i.e. riffles only in the present study and pool/riffle composites in Yuan, 2004), differences in methods used in analysis of taxa distributions,

Table 3
Hodges-Lehmann estimation results from comparing BAP scores of sites from the three trophic states and water quality assessments for each trophic state

| | Median BAP score | Median change | %Change | %Non | %Slightly | %Moderately | %Severely |
|---|---|---|---|---|---|---|---|
| TP | | | | | | | |
| Oligotrophic | 7.98 | 0.76 | 9.6 | 59 | 41 | 0 | 0 |
| Mesotrophic | 7.06 | | | 36 | 49 | 15 | 0 |
| Eutrophic | 5.86 | 1.22 | 17 | 7 | 63 | 30 | 0 |
| $NO_3^-$ | | | | | | | |
| Oligotrophic | 7.8 | 0.75 | 9 | 60 | 34 | 6 | 0 |
| Mesotrophic | 6.8 | | | 33 | 51 | 16 | 0 |
| Oligotrophic | 5.98 | 0.81 | 12 | 13 | 64 | 23 | 0 |

The median BAP score for each trophic state is given, along with the magnitude of change between trophic states and the overall % change between trophic states. The greatest change occurred for both TP and $NO_3^-$ between the mesotrophic and eutrophic states. The percent of non, slightly (Slt), moderately (Mod), and severely (Sev) impacted samples in each trophic state is also provided. Most impairment (moderately impacted) occurs after eutrophic status has been reached.

differences in study location (northeast as opposed to the Mid-Atlantic region). In addition, Yuan (2004) used genus-level identification, so species differences cannot be seen. Tolerance assignments for some genera will result in loss of information when species differences are apparent as in the present study. For example, the six species of the caddisfly genus *Hydropsyche* identified in the current study were assigned tolerance values between 3 and 7 (Table 2). Yuan (2004) assigned this entire genus as tolerant.

The results of this study demonstrate the advantages of weighted averaging for developing taxa optima around environmental variables (Table 2). Correlations observed between the nutrient biotic indices and their respective nutrients suggest that nutrient optima were accurately calculated. These optima now provide a starting place for further refinement of nutrient biotic indices. Plotting species frequency distributions along the nutrient gradient also indicates the desired response of organisms to increasing nutrients (Fig. 2). For many taxa, frequencies rise to some maximum at an optimal nutrient concentration and then decrease as nutrient concentrations become inhibiting. Some taxa displayed only a rise or fall in frequency as a result of increased nutrient concentrations, possibly because the entire nutrient gradient for that taxon was not measured.

The relationship observed between the indices and nutrients supports the conclusion of success in development of the NBI-P and NBI-N (Fig. 3). However, reduced performance was noted with regard to the NBI-N ($r = 0.57$, Fig. 3) in comparison with the NBI-P ($r = 0.68$, Fig. 3). Traditional views on stream limiting nutrients point towards phosphorus as the limiting nutrient, although this view has been contradicted in bioassay and correlation studies (Correll, 1998; Dodds and Welch, 2000). TP may be the limiting nutrient in these streams or at least less variable than $NO_3^-$ and easier to predict. This may explain why taxa responses to TP resulted in a more robust index.

The boundaries established between the three trophic states (Fig. 4) approximated those of Dodds et al. (1998) whose trophic state categories were derived using frequency distributions of TP, TN, and chlorophyll. The results of their investigation yielded upper boundaries of their oligotrophic category as 0.025 mg/l TP and 0.7 mg/l TN and mesotrophic category as 0.075 mg/l TP, 1.5 mg/l TN. These results are comparable to the boundaries established using invertebrates and nutrients in this study (oligotrophic–mesotrophic 0.0175 mg/l TP, 0.24 mg/l $NO_3^-$; mesotrophic–eutrophic 0.065 mg/l TP, 0.98 mg/l $NO_3^-$).

The three-tiered trophic state framework allows for the determination of trophic status based on nutrient concentrations in New York State streams and can likely be applied to other states in the region. The trophic state categories can be interpreted to have a direct connection with changes in invertebrate communities because their development is based on similarities between benthic invertebrate assemblages. The two distinct clusters of oligotrophic sites and eutrophic sites (Fig. 4) along with changes in water

quality assessment (Table 3 and Fig. 6) indicate that macroinvertebrate communities change significantly over the range of nutrient concentrations identified in this study.

Setting nutrient criteria at levels that correspond with invertebrate community impact is efficient since most states already utilize benthic macroinvertebrates in determining surface water quality. It would be cost effective to integrate the enforcement of regional nutrient criteria within the United States (USEPA, 1998a,b, 2000c) into already established monitoring programs. Surface waters that have nutrients in exceedance of criteria can be identified without having to use special sampling protocols or to enlist the use of other biological communities for assessment.

In an effort to conserve resources not yet affected by nutrient enrichment, nutrient criteria should be established at levels below which a majority of streams will not be assessed as impacted by traditional biological monitoring methods. Hodges-Lehmann estimation determined the greatest magnitude of change in median BAP scores to occur between the mesotrophic and eutrophic levels (Table 3). A substantial increase in the percentage of moderately impacted samples (TP: 15–30%; $NO_3^-$: 16–23%) and decrease in the percentage of non-impacted samples (TP: 36–7%; $NO_3^-$: 33–13%) was observed between these two trophic states (Fig. 6). If nutrient criteria are to be set at levels that will prevent impairment the nutrient boundary between mesotrophic and eutrophic for both TP (0.065 mg/l) and $NO_3^-$ (0.95 mg/l) is appropriate. These are the levels of nutrient concentration above which water quality impairment is likely to occur. Furthermore, Miltner and Rankin (1998) found that detrimental changes occurred to fish communities when phosphorus levels exceeded 0.06 mg/l. This concentration corresponds well with the mesotrophic/eutrophic boundary (0.065 mg/l TP) set by this study. Comparison with EPA guidance criteria finds these nutrient concentrations are roughly twice their stated values for the ecoregions found in New York State (USEPA, 2000c). This is similar to what others have found when evaluating proposed guidance criteria (Ice and Binkley, 2003). In addition not all of the samples within the eutrophic range were moderately impacted, suggesting this threshold is protective and conservative.

If the NBI is to become a tool for assessing nutrient enrichment and enforcing nutrient criteria, the corresponding NBI scores for these thresholds of impairment must be identified. This was done using the mean NBI-P and NBI-N scores from samples whose nutrient concentrations fell within 10% of the mesotrophic–eutrophic TP and $NO_3^-$ boundaries. This method sets the NBI scores above which a stream would be in exceedance of the nutrient criteria at an NBI-P score of 6.1 and an NBI-N score of 6.0. Some samples within the mesotrophic range did suggest impairment at the moderately impacted level for both TP and $NO_3^-$. The percentages of moderately impacted sites within the mesotrophic ranges were less than that of the eutrophic and the numbers of non-impacted samples within the mesotrophic ranges were still substantial (Fig. 6). Nutrient concentrations within the mesotrophic range are not enough to cause impairment alone, but coupled with other impacts impairment may occur. NBI scores that signify the beginning of the transitional zone of impairment can be established using the same method as previously used to identify NBI scores corresponding with the mesotrophic–eutrophic boundaries. Therefore the oligotrophic–mesotrophic boundaries for TP and $NO_3^-$ are 0.0175 and 0.24 mg/l and the NBI scores for samples with nutrient concentrations within 10% of these values are 5.5 and 4.8, respectively.

## 5. Conclusions

In general, the hypotheses of this investigation were supported. Nutrient optima were successfully developed from historical data (Table 2). Taxa responded to increasing nutrient concentrations in ways that provided the ability to locate an optimal nutrient concentration (Fig. 2). Subsequent tolerance values provided the information necessary to differentiate among stream sites of differing nutrient concentration and trophic state (Fig. 5). The performance of the NBI-P was better than the NBI-N (Fig. 3). This may be because phosphorus is the limiting nutrient in these streams yielding a more measurable biological response than $NO_3^-$.

To date, there has been no simple index that can be used in monitoring to link benthic macroinvertebrate community composition with stream nutrient concentrations. The complexity of nonpoint source pollution and its indirect effects on benthic macroinvertebrates

has made measuring the effects of nutrients on stream biota a difficult task. Establishment of the NBI will provide biological monitoring programs with a robust measure of stream nutrient status that is based on benthic macroinvertebrates. Furthermore, the use of the NBI should not be limited to New York State. The ability of invertebrate communities to show strong similarities at different levels along the nutrient gradients suggests the NBI's applicability in other areas at least in those places that share common ecoregions with New York. The development of nutrient optima for the 164 taxa allows managers to evaluate, through the indices themselves or direct evaluation of the taxa, the net effects of nutrients on wadeable streams. The NBIs provide easy-to-understand results for managers to convey and allows for spatial comparability along a stream or river. In addition, coupling the NBI-P and NBI-N with nutrient concentrations above which water quality impairment typically occurs allows the index to function as a useful tool in enforcing regional nutrient criteria.

Further study of the NBIs is needed before the NBI-P and NBI-N can be fully implemented in a water quality monitoring program. The testing of the NBIs should utilize a set of data that adequately represents both phosphorus and nitrogen gradients. Sites should be selected to minimize external variables that might contribute forms of impairment other than nutrients. Such a study was conducted on 32 stream sites in New York State in 2005, results of which will be published separately. In addition if testing of the NBI is used elsewhere than the Northeastern states a regional calibration may be necessary for tolerance values of some taxa.

## Acknowledgments

The authors would like to thank the Stream Biomonitoring Unit – Margaret Novak, Larry Abele and Diana Heitzman – for providing indispensable support in data collection and sample processing; Dr. George Robinson of SUNY Albany, Pat Phillips and Karen Riva-Murray of the USGS for providing statistical and theoretical advice; and Ken Markussen and the State of New York Department of Environmental Conservation for providing project funding.

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