

A Method and Rationale for Deriving Nutrient Criteria for Small Rivers and Streams in Ohio

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Abstract A mechanistic understanding of the effects of nutrient enrichment in lotic systems has been advanced over the last two decades such that identification of management thresholds for the prevention of eutrophication is now possible. This study describes relationships among primary nutrients (phosphorus and nitrogen), benthic chlorophyll *a* concentrations, daily dissolved oxygen (DO) concentrations, and the condition of macroinvertebrate and fish communities in small rivers and streams in Ohio, USA. Clear associations between nutrients, secondary response indicators (i.e., benthic chlorophyll and DO), and biological condition were found, and change points between the various indicators were identified for use in water quality criteria for nutrients in small rivers and streams (<1300 km²). A change point in benthic chlorophyll *a* density was detected at an inorganic nitrogen concentration of 0.435 mg/l (± 0.599 SD), and a total phosphorus (TP) concentration of 0.038 mg/l (± 0.085 SD). Daily variation in DO concentration was significantly related to benthic chlorophyll concentration and canopy cover, and a change point in 24-h DO concentration range was detected at a benthic chlorophyll level of 182 mg/m². The condition of macroinvertebrate communities was related to benthic chlorophyll concentration and both minimum and 24-h range of DO concentration. The condition of fish communities was best explained by habitat quality. The thresholds found in relationships between the stressor and the response variables, when interpreted in light of the uncertainty surrounding individual change points, may now serve as a framework for nutrient criteria in water quality standards.

Keywords Nutrients · Phosphorus · Nitrogen · Benthic Chlorophyll · Water quality standards · Macroinvertebrates

Nutrients, sediment, and habitat alteration have consistently been identified as leading causes of impairment to rivers and streams in the United States for the past two decades (U.S. EPA 1996, 2007). In contrast, organic enrichment and other forms of pollution (e.g., metals) associated with municipal and industrial point sources have been largely controlled, often with dramatic results, under the aegis of the Federal Water Pollution Control Amendments of 1972, commonly known as the Clean Water Act (CWA). For example, organic enrichment and metals were the first and fourth leading causes of impairment to streams sampled prior to 1992 in Ohio (Ohio EPA 1995), a populous and heavily industrialized state. In 2006, fewer waters were considered impaired; however, of those, sediment, habitat alteration, flow alteration, and nutrients were the four leading causes of impairment (Ohio EPA 2006).

To address the issue of nutrient enrichment, the U.S. EPA (2001a) published nutrient criteria using a reference range approach and authorized states to develop regionally specific, scientifically defensible criteria (U.S. EPA 2001b). Similarly, the Water Framework Directive (2000) issued by the European Union has tasked member nations to develop strategies to control cultural eutrophication of shared waters. However, unlike toxicants and putrescible materials, the effects of nutrient enrichment on fish or macroinvertebrates are not predictable through dose-response curves or models. Furthermore, although relationships between nutrients and stream eutrophication have been well documented (Dodds and others 1997; Smith and others 1999;

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Biggs 2000), the nutrient–eutrophication relationship is complex, and the impact of eutrophication on higher trophic levels is difficult to quantify because fish and macroinvertebrate communities are strongly influenced by physical habitat (Miltner and Rankin 1998; Wang and others 2007), flow regime (Poff and Allan 1995), geomorphic condition (Walters and others 2003; Mazeika and others 2006), and land use (Passy and others 2004). These complexities have led many states and European Union (EU) members to begin deriving nutrient criteria empirically based on field studies (e.g., Camargo and others 2004; Skoulikidis and others 2004; Donohuea and others 2006; Ponader and others 2007; Smith and others 2007; Wang and others 2007; Heiskary and Markus 2003; Soranno and others 2008).

Nutrient enrichment has been shown to affect macroinvertebrate communities through direct pathways. For example, nutrient amendments to an arctic stream stimulated production of algae and macroinvertebrates and increased fish growth rates (Deegan and Peterson 1992; Peterson and others 1993). Nutrient addition to a shaded first-order stream in North Carolina increased abundance and production of both macroinvertebrate primary and secondary consumers via a heterotrophic path (Cross and others 2006). Niyogi and others (2007) demonstrated higher epilithic chlorophyll *a* levels, and both increased macroinvertebrate abundance and changes in community composition along a nutrient gradient driven by increasing pastoral land cover in New Zealand streams. Camargo and others (2005) similarly showed that stream reaches enriched with nutrients from deep release impoundments had increased benthic chlorophyll *a* concentrations and higher macroinvertebrate densities relative to upstream reaches. And Bowman and others (2007) demonstrated increased abundance of benthic algae and macroinvertebrates, especially those classed as scrapers, in oligotrophic streams receiving treated municipal wastewater.

Other studies have inferentially demonstrated negative effects of nutrient enrichment on macroinvertebrates or fish through direct gradient analysis (Carlisle and others 2007; Smith and others 2007; Meador and Carlisle 2007; Haase and Nolte 2008), associations with biotic indices (Miltner and Rankin 1998; Hering and others 2006; Wang and others 2007), or multivariate approaches including discriminant analysis (Norton and others 2000) and canonical correspondence analysis (Riva-Murray and others 2002). Although cause and effect was not directly demonstrated by these studies, eight of the studies partitioned the variance in biological response over several or more environmental gradients (i.e., land use, physical stream habitat quality, sediment, and water chemistry) in addition to a nutrient gradient, thereby building a circumstantial case for a causal link between nutrients and the biological response.

Apart from a direct trophic response, enrichment also affects fish and macroinvertebrates indirectly by influencing dissolved oxygen (DO) concentrations. Sabater and others (2000) observed a 10 mg/l difference between daytime and nighttime DO concentrations at an enriched site where benthic chlorophyll *a* levels exceeded 500 mg/m² and reported that short episodes of hypoxia associated with wide DO swings were responsible for fish kills in the study area. In a study of large Minnesota rivers (i.e., drainage area >2600 km²) the daily ranges in DO concentration were positively correlated with total phosphorus (TP) and sestonic chlorophyll *a* (Heiskary and Markus 2003), and in turn, the number of EPT taxa (i.e., macroinvertebrates in the orders Ephemeroptera, Plecoptera, and Trichoptera) in macroinvertebrate samples were negatively associated with increasing range of daily DO. In the same study, fish Index of Biotic Integrity (IBI) scores were negatively correlated with maximum daily DO and daily DO range but showed no significant relationship with minimum DO.

The objectives of this study are twofold. The first is to measure whether concentrations of primary nutrients (phosphorus and nitrogen) are positively associated with benthic chlorophyll *a* levels and, in turn, increasing daily variation in DO concentrations. If those relationships hold, then determine if the increasing expression of nutrient enrichment given by either benthic chlorophyll or DO concentrations (hereafter referred to collectively as enrichment indicators) corresponds to decreasing condition in fish or macroinvertebrate communities (i.e., biological condition indicators). Where clear associations between stressor and response variables are found, the second objective becomes identifying concentrations or levels in the stressors over which the respective response variables change appreciably. The change points then form the basis of defensible water quality standards for nutrients in small rivers and streams [i.e., watershed area less than ~1300 km² (500 mi²)].

Methods

Study Area

One hundred nine survey sites were selected to establish a gradient of anthropogenic enrichment and habitat quality based on a combination of historic water quality and stream habitat data, proximity to municipal wastewater plants, and land use from satellite imagery. Land use for each sampling location was derived from 30-m-resolution Landsat Thematic Mapper satellite imagery (September–October 1994) of land cover provided by the Ohio Department of Natural Resources. The percentage of land area in the satellite data classed as urban or agricultural for the

drainage upstream from each sampling point was used as an indicator of potential enrichment. For 19 sites that were situated on large rivers (i.e., an administrative demarcation for streams with drainage areas $>1300 \text{ km}^2$), the delineation of drainage land use upstream from a sampling point included all the area of principal tributaries up to a maximum area of 775 km^2 (i.e., one-half the drainage area of the smallest large river site). Drainage area and local stream gradient were calculated for each site. Figure 1 shows the location of sites in Ohio, a frequency distribution of site drainage areas, and quantile plots of the percentage of urban and agricultural land in the upstream drainage for the sampling points.

Chemical, Biological, and Physical Sampling

Sites were sampled in batches between 2004 and 2007 such that roughly one-quarter of the sites were sampled each year. For each site sampled in any given year, samples for nutrient water chemistry analysis were collected three to six times between June 15 and October 15, and the results expressed as the geometric mean for each measured parameter. Geometric means were used in lieu of arithmetic means given that nutrient concentrations had a log-normal distribution (i.e., an arithmetic mean taken from log transformed values yields a geometric mean in original units). Nutrients included in the analyses were nitrate–nitrite nitrogen ($\text{NO}_x\text{-N}$), ammonia nitrogen ($\text{NH}_3\text{-N}$), TP, and total Kjeldahl nitrogen (TKN). The method detection limit for TP was 0.01 mg/l . Values below method detection limits (14% for TP, 61% for $\text{NH}_3\text{-N}$) were halved. Other water chemistry parameters included total suspended solids. Laboratory methodology followed procedures in the APHA (1992). Hourly DO concentrations were recorded for a 24- to 48-h period at 86 sites with automatic data loggers (probe accuracies for DO are within $\pm 0.3 \text{ mg/l}$). Data loggers were deployed a week prior to or several days following a chlorophyll sample to coincide with stable, low-flow conditions.

Benthic chlorophyll *a* concentrations were measured from epilithic periphyton communities by scraping a known area (3.35 cm^2) from each of 10–20 (usually 15) large gravel to cobble-size rocks from a glide-riffle-run complex. Methodology followed that discussed in detail by Moulton and others (2002), Scrimgeour and Chambers (2000), Cattaneo and others (1997), and Lohman and others (1992). Only rocks that were undisturbed, as determined by a distinct, bicolored appearance between the exposed surface and the side facing the stream bed, were collected. Rocks were collected once per site from late July to early September at a minimum of 10 days following any significant rainfall to minimize effects from scouring (Biggs

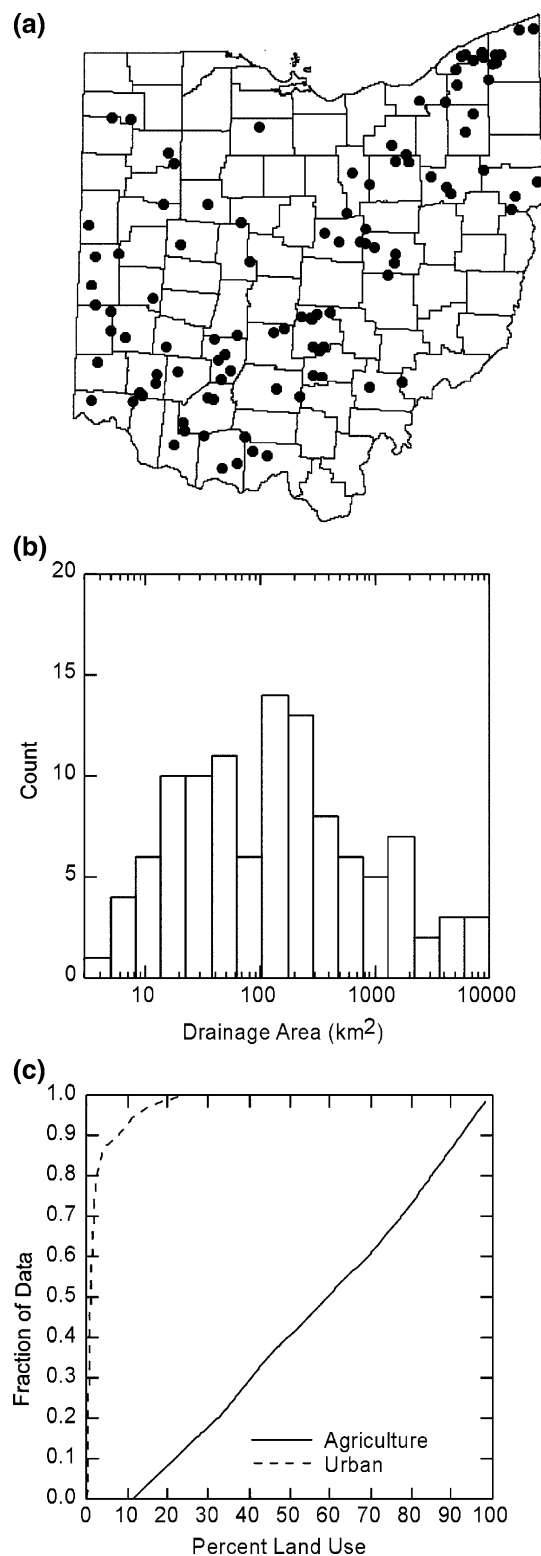


Fig. 1 a Sampling locations, b a frequency distribution of drainage areas, and c quantile plots of percentage urban and percentage agricultural land use for the drainage upstream from sampling locations

2000; Lohman and others 1992). Large gravel (>7.5 cm in diameter) to cobble-sized substrates were chosen to minimize potential spatial variation within the stream reach (Cattaneo and others 1997). The rock scrapings were combined and blended with a rechargeable Cuisinart (East Windsor, NJ, USA) model CSB-77 hand blender. Three 5-ml aliquots were drawn from the slurry and each filtered on Whatman GF/C 1.2- μm glass-fiber filters in the field and either placed on ice for daytrips or frozen on dry ice for overnight trips. The chlorophyll on the filters was extracted using a known quantity (10–15 ml) of 90% acetone. The amount of chlorophyll *a* and pheophytin *a* in a sample was determined using EPA Method 445 (U.S. EPA 1997). Calibration of the fluorometer was against a known standard. Results from each of the three filters were averaged, and the concentration of corrected benthic chlorophyll *a* or pheophytin *a* (hereafter referred to as benthic chlorophyll and pheophytin, respectively) at a given site was expressed as milligrams per square meter as extrapolated from the slurry volume and total rock area scraped.

Stream physical habitat quality was assessed using the Qualitative Habitat Evaluation Index (QHEI; Rankin 1995) at least once in a given year. The QHEI is a qualitative visual assessment of functional aspects of stream macrohabitats (e.g., amount and type of cover, riparian width, siltation, channel morphology). An estimate of light availability at a site was given by the degree of open arc between the canopy tops of either bank. A clinometer was used by an observer standing in the middle of the stream channel to measure the angle to the canopy top of opposite banks at three locations within the sampling reach. The sum of the two measured angles were subtracted from 180 and averaged for the three observation points to yield what is hereafter referred to as canopy cover.

Fish communities were sampled once at 100 of the 109 sites using generator-powered, pulsed DC electrofishing units and a standardized methodology (Ohio EPA 1987, 1989a, b; Yoder and Smith 1999). Samples were typically collected within 2 weeks following chlorophyll samples, under the same flow conditions. Fish community attributes were collectively expressed by the IBI (Karr 1981; Karr and others 1986), as modified for Ohio streams and rivers (Yoder and Smith 1999; Ohio EPA 1989a). Macroinvertebrates were sampled quantitatively at 56 sites using modified, multiple-plate artificial substrate samplers (fashioned after Hester and Dendy 1962) and sampled qualitatively for presence/absence at 102 sites (that included the previous 56). The artificial substrates were deployed 2–3 weeks prior to, and 3–4 weeks after, chlorophyll sampling. Qualitative samples were collected when the artificial substrate was retrieved (i.e., not all artificial substrates are retrieved, hence the disparity in sample types). Macroinvertebrate community structure for quantitative samples was expressed as the

Invertebrate Community Index (ICI; DeShon 1995). The ICI is a multimetric measure of the invertebrate community composed of ten metrics scoring functional, compositional and taxonomic attributes. ICI scores were binned into eight ranks based on narrative ranges (e.g., excellent, very good, good). For the 46 samples with only presence/absence data, staff biologists assigned one of the eight ranks to the samples based on both the relative composition of macroinvertebrates in the sample and the weighted tolerance values for individual taxa (DeShon 1995). The weighted tolerance values for individual taxon were derived from weighted average ICI scores; thus, the narrative assignments correspond to ranges of ICI scores (DeShon 1995). Hereafter, the rankings are referred to as the invertebrate community (IC) ranks. The number of taxa in the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) from qualitative samples was also used as a measure of community quality.

Statistical Analyses

Values for benthic chlorophyll, pheophytin, water chemistry parameters, stream gradient, drainage area, percentage urban land use, and canopy were \log_{10} transformed to normalize distributions prior to statistical analyses. Percentage agriculture was normally distributed. Relationships between benthic chlorophyll concentrations and the aforementioned variables were initially described by simple Pearson correlations and inspection of scatter plots. One site collected from the Mahoning River was identified as an outlier (by the Hadi 1994 algorithm of SYSTAT) and excluded from all subsequent analysis. Of the environmental variables identified as having a marginal ($P < 0.1$) or better ($P < 0.05$) association with benthic chlorophyll, all-subsets regression (Neter and others 1990) was used to suggest linear combinations that explain variation in benthic chlorophyll given dissolved inorganic nitrogen (DIN; the sum of ammonia nitrogen and nitrate-nitrite nitrogen) and canopy cover. This exercise was not done to find a predictive equation; rather it was to inform change point analysis by identifying which environmental variables account for variation in benthic chlorophyll beyond that explained by nutrients and light. DIN and canopy were forced a priori given that nutrients and light are well-established predictors of benthic chlorophyll in small streams. DIN was used in lieu of NO_x, as the two are statistically equivalent (see Table 1), and DIN would likely be used in management. DIN was forced in favor of TP because it showed a stronger association with benthic chlorophyll (Table 1). However, residuals from the regression of TP on DIN were used as a free predictor to assess the explanatory contribution of TP uncompromised by multicollinearity (Graham 2003). The model with the lowest value for Mallows' Cp, the smallest number of predictor variables, and the highest adjusted coefficient of

Table 1 Simple Pearson correlations between enrichment measures and selected physical and land use variables

	Chl <i>a</i>	Pheo <i>a</i>	DIN	NO _x -N	NH ₃	TKN	TP	TSS	Grade	DA	% Urban	% Agric.	Canopy
Chl <i>a</i>	1.00												
Pheo <i>a</i>	0.92	1.00											
DIN	0.48**	0.43**	1.00										
NO _x -N	0.49**	0.44**	0.99**	1.00									
NH ₃	0.20	0.24	0.25	0.19	1.00								
TKN	0.27	0.26	0.40**	0.39**	0.30	1.00							
TP	0.37**	0.37**	0.68**	0.66**	0.30	0.70**	1.00						
TSS	0.17	0.12	0.37*	0.37*	0.14	0.35*	0.43**	1.00					
Gradient	-0.32 ^{ns}	-0.31 ^{ns}	-0.26	-0.26	-0.28	-0.17	-0.21	-0.63**	1.00				
DA	0.18	0.26	0.30 ^{ns}	0.31 ^{ns}	0.00	0.19	0.30 ^{ns}	0.59**	-0.68**	1.00			
% Urban	0.32*	0.31 ^{ns}	0.51**	0.51**	0.06	0.51**	0.56**	0.29	-0.15	0.33*	1.00		
% Agric.	0.30 ^{ns}	0.39**	0.19	0.19	0.22	0.29	0.32*	0.02	-0.20	-0.09	-0.09	1.00	
Canopy	0.38**	0.36**	0.13	0.13	0.13	0.06	0.06	0.42**	-0.43**	0.43**	0.09	0.02	1.00

DA drainage area. Asterisks denote significant linear associations at the Bonferonni adjusted $P < 0.05$ and $P < 0.01$ levels

determination was subsequently run excluding data from the 19 large river sites to gauge changes in the explanatory power and slopes of the various parameter estimates, given that the 19 large river sites were potentially transitional between periphyton- and phytoplankton-dominated systems.

Change points in benthic chlorophyll concentrations in relation to TP or DIN were identified by first obtaining the residuals from the regression of benthic chlorophyll concentrations on canopy cover and percentage agricultural land use, given that the latter was consistently identified as a predictor in all subsets regression. The residuals then served as a dependent variable in a regression tree where either TP or DIN was an independent variable. The trees were constrained to a single split and a minimum of 10 cases (i.e., ~10% of the sample) in a terminal node. A change point in benthic chlorophyll in relation to canopy cover was similarly obtained using the residuals from the regression of benthic chlorophyll on DIN and percentage agricultural land. The reduction in variance afforded by the change point identified by each regression tree was gauged using an F -test (Qian and others 2003). Note that the F -tests were not formally testing respective null hypotheses of similar variances, as the change points were not chosen beforehand.

An estimate of uncertainty in the cut point for each regression tree model was evaluated with a 1000-count bootstrap sample (Qian and others 2003). To help interpret results, frequency histograms of cut values from the bootstrap samples were overlain onto scatter plots of benthic chlorophyll residuals and each of the three independent variables. Also, for each scatter plot, a locally weighted line was fitted to the data using the LOWESS ($\alpha = 0.5$) function in SYSTAT (San Jose, CA, USA).

Information from automated monitoring of DO at a sampling location was summarized as the maximum range in concentration and the minimum value recorded over a 24-h period for a given location. Linear models explaining variation in DO range were suggested by all-subsets regression that included benthic chlorophyll in all models and pheophytin, stream gradient, and QHEI scores as free predictors. Pheophytin was introduced as an independent variable because it serves as the primary electron receptor in photosystem II (Marshall and others 2000) and is, thus, an important accessory pigment in the living fraction. Also, as a measure of the senesced fraction, pheophytin may represent the potential for oxygen demand and, thus, serve as a proxy for daily swings in DO. However, because pheophytin was strongly correlated with benthic chlorophyll, residuals from the regression of pheophytin on benthic chlorophyll were used in lieu of the measured values (Graham 2003). Stream gradient was included as a rough proxy for re-aeration, and QHEI scores were included to account for variation due to overall physical habitat quality. Results from the all subsets regression indicated that pheophytin residuals and QHEI scores formed a parsimonious set of predictor variables; therefore, residuals from the regression of DO range on QHEI scores and pheophytin residuals were used in change point analysis. A change point in DO range over benthic chlorophyll was given by a regression tree constrained to a single split and a minimum of nine cases (i.e., 10% of the sample) in a terminal node. Uncertainty was evaluated by overlaying a frequency histogram of cut values from a 1000-count bootstrap sample on a scatter plot of DO range (residuals) over benthic chlorophyll concentrations in concert with a LOWESS ($\alpha = 0.5$) fitted line. Minimum DO concentrations were assumed a priori to be largely a function of DO

range and, therefore regressed, against the DO range, stream gradient, and drainage area.

Indicators of macroinvertebrate and fish community quality were regressed against indicators of nutrient enrichment, either benthic chlorophyll concentration, range of daily DO concentration, or daily minimum DO concentration, to test whether a linear relationship existed between any of the biological and enrichment indicators. For the macroinvertebrate community, community rankings and the number of EPT taxa were used as indicators of quality, and for fish, IBI scores and the number of sensitive fish species were used. Because habitat quality is a known predictor of the fish and macroinvertebrate indicators, QHEI scores were included as an independent variable in each of the regressions to ascertain if the enrichment indicators explained significant additional variation in the biological indicators. Similarly, drainage area was included as an independent variable in regressions between the number of sensitive fish species and the enrichment indicators. If an enrichment indicator explained variation in a biological indicator, change points between the two were identified with regression trees that followed the methods previously described for DO and benthic chlorophyll wherein residuals following regression on QHEI scores were the dependent variable.

Results

Summary statistics listing median concentrations and ranges for benthic chlorophyll, DIN, TP, and canopy cover are listed in Table 2. Benthic chlorophyll concentrations were associated with DIN, TP, canopy cover, and percentage urban land use in simple correlation analysis (Table 1). Significant correlations (Bonferonni adjusted $P < 0.05$) existed between several of the candidate explanatory variables, especially between TP and DIN, between NOx-N and TKN, and between percentage urban land use and TP, DIN, NOx-N , and TKN. All subsets regression suggested that, given DIN (partial $r^2 = 0.25$) and canopy (partial $r^2 = 0.10$), percentage agriculture accounted for an extra 5% of the variation in benthic chlorophyll (Tables 3, 4), whereas percentage urban land use and residuals from

the regression of TP on DIN provided little extra information. DIN and canopy cover explained an additional 10% (partial $r^2 = 0.28$ and 0.17, respectively) of the variation in benthic chlorophyll when large river sites were excluded from the linear model (Table 4).

Cut values obtained from regression trees ran against bootstrapped samples of the data consistently found a change point in residual benthic chlorophyll variation (i.e., following regression on canopy cover and percentage agriculture) over either DIN or TP. The median and modal value was 0.435 mg/l for DIN and 0.038 mg/l for TP (Table 5; Fig. 2a, b; values were transformed back to original units). F -tests indicated that the respective change points for DIN and TP partitioned a significant amount of variance in benthic chlorophyll levels. Uncertainty about the change point relative to DIN was manifest in a secondary mode at 1.095 mg/l that coincided with an inflexion in the LOWESS trend line. For TP, the distribution of cut values in Fig. 2b suggests 0.078 mg/l (i.e., the 90th percentile) approximates an upper limit for the change point. The change point for benthic chlorophyll against canopy cover (Table 5) given by the regression tree was 40° of open canopy. Cut points from bootstrap samples occurred most frequently at 40° and were infrequent beyond 50°.

All-subsets regression suggested that, given benthic chlorophyll, pheophytin and QHEI scores formed a parsimonious set variables explaining variation in the 24-h range of DO concentrations (Table 6). Benthic chlorophyll alone accounted for 7% of the variation in 24-h DO range. Residual variation in 24-h DO range (from the regression on QHEI scores and pheophytin residuals) was partitioned by a regression tree at a benthic chlorophyll concentration of 182 mg/m², and most frequently partitioned in bootstrap samples at benthic chlorophyll concentration of 190 mg/m² (Fig. 3a; Table 5). Minimum DO concentrations were linearly related to the 24-h DO range (Fig. 3b), stream gradient, and drainage area (Table 7).

Benthic chlorophyll, DO range, and DO minimum accounted for a significant, but minor, fraction of the variation in both IC ranks and the number of EPT taxa in regression models that included QHEI scores (Table 8). However, those indicators did not account for additional variation in either fish IBI scores or the number of sensitive fish species beyond that explained by QHEI scores (and drainage area for sensitive fish). Change points for residual variation in IC ranks (following regression on QHEI scores) in relation to benthic chlorophyll were most frequently detected at 320 mg/m² in bootstrap samples (Fig. 4a; Table 5); however, the change points were skewed toward lower concentrations. The F -test shows that the point at 320 mg/m² partitions a relatively small amount of variance in IC ranks. Change points from bootstrap samples for EPT residuals in relation to benthic chlorophyll

Table 2 Summary statistics for DIN, TP, benthic chlorophyll *a* (Chl *a*) concentrations, degree of open canopy, and drainage area measured at 109 sites in Ohio, 2004–2007

	DIN (mg/l)	TP (mg/l)	Chl <i>a</i> (mg/m ²)	Canopy
Maximum	8.244	1.715	856	161
Minimum	0.088	<0.010	32	9
Median	0.770	0.051	190	67

Table 3 Results of all-subsets regression for environmental variables associated with benthic chlorophyll given DIN and canopy as fixed predictors

Variables	R^2	Adj. R^2	Cp	S	Agricultural	Grade	Urban	TP ^a
2	42.7	40.5	3.6	0.21058	X		X	
1	41.1	39.4	4.4	0.21237	X			
3	43	40.2	5.1	0.21106	X		X	X
3	42.7	39.9	5.5	0.21149	X	X	X	
2	41.2	38.9	6.2	0.21324	X	X		
4	43	39.7	7.0	0.21198	X	X	X	X
1	36.5	34.7	12.5	0.22052			X	

^a Residuals from regression of TP on DIN

Table 4 Parameter estimates from linear regressions of benthic chlorophyll on explanatory variables selected by all subset regression (all sites) and for sites of <1300 km²

	All sites ($N = 108$; model $R^2 = 0.3943$)			Sites <1300 km ² ($N = 88$; model $R^2 = 0.4629$)		
	Coefficient	SE	Partial r^2	Coefficient	SE	Partial r^2
Constant	0.7939	0.1817		0.6149	0.1958	
DIN	0.2485	0.0472	0.2574	0.2785	0.0502	0.2820
Canopy	0.3333	0.0789	0.1028	0.4167	0.0856	0.1706
Agricultural	0.0024	0.0008	0.0511	0.0019	0.0009	0.0288

DIN dissolved inorganic nitrogen

Table 5 Estimates of uncertainty surrounding change points suggested by regression trees

Y	X	All data			Bootstrap samples			
		N	Change point	F	Median	Mode	75th%	90th%
Chl <i>a</i>	DIN (mg/l)	108	0.435	11.125	0.435	0.435	1.095	1.556
Chl <i>a</i>	TP (mg/l)	108	0.038	8.585	0.038	0.038	0.048	0.078
Chl <i>a</i>	Canopy (°)	108	40.0	10.151	41.0	40.0	50.0	84.0
DO range	Chl <i>a</i> (mg/m ²)	85	182.0	6.874	194.0	190.0	196.0	231.0
No. of EPTs	Chl <i>a</i> (mg/m ²)	102	107.0	5.722	111.0	96.0	122.0	214.0
IC rank	Chl <i>a</i> (mg/m ²)	102	320.0	2.484	261.0	320.0	320.0	365.0
No. of EPTs	Min. DO (mg/l)	83	5.86	5.459	5.86	5.86	5.86	6.14
IC rank	Min. DO (mg/l)	83	5.25	4.534	5.31	5.20	5.86	7.52
No. of EPTs	DO range (mg/l)	83	7.04	3.347	2.87	7.04	7.04	7.85
IC rank	DO range (mg/l)	83	9.36	6.389	8.69	9.85	9.85	9.85

Note: The change point is the point in the X variable that divides the corresponding Y variable into two groups. Medians, 75th percentiles, and 90th percentiles are from a 1000-count bootstrap sample. The F-test is for the difference between the variance in Y and the variance in Y when partitioned by the change point

occurred most frequently at 96 mg/m² (Fig. 4b; Table 5); however, the points were skewed toward higher concentrations, such that cuts were frequently detected up to 150 mg/m². In relation to minimum DO concentrations, tree splits for residual variation in EPT counts and IC ranks occurred most frequently at 5.9 and 5.2 mg/l, respectively (Fig. 5a, c). For the DO range, most tree splits for IC ranks occurred at a daily range of 9.9 mg/l, and for EPT counts, the mode was at 7.0 mg/l; however, the median occurred at 2.9 mg/l (Fig. 5b, d). The F-test indicated that the change

point of 7.0 mg/l partitioned comparatively little variance in the number of EPT taxa.

Discussion

Translating the Results into Criteria

The results of this study demonstrated clear links between increasing nutrient concentrations and stream eutrophication,

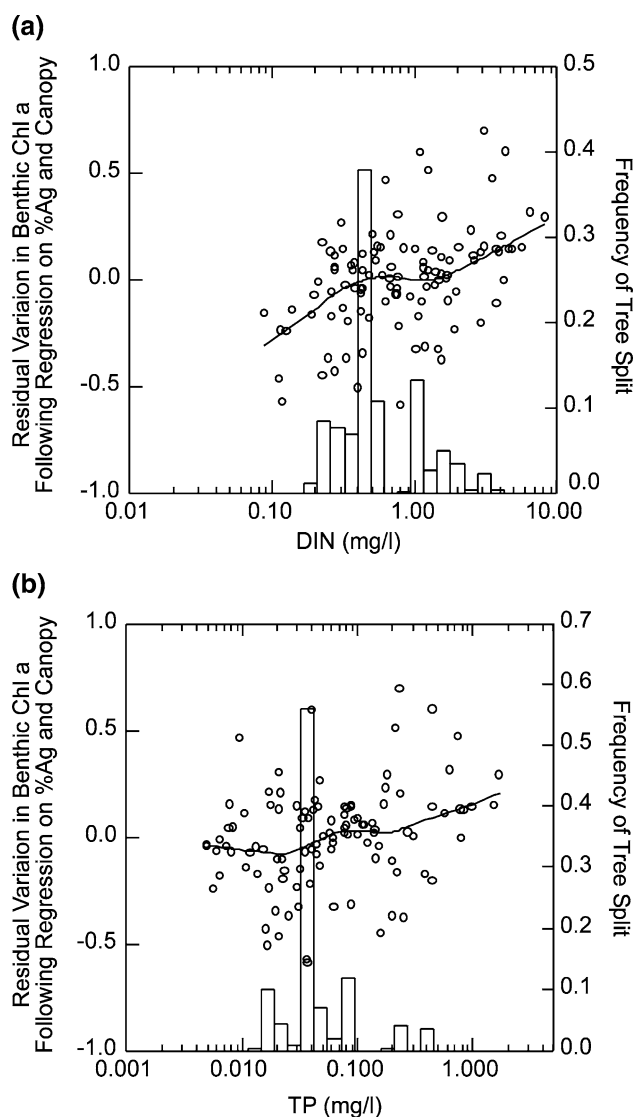


Fig. 2 Scatter plots of residuals from the regression of benthic chlorophyll on canopy cover and percentage agricultural land use plotted against **a** DIN and **b** TP. The *fitted lines* are from LOWESS smoothing ($\alpha = 0.5$). Histograms superimposed on the plots show the frequency distribution of cut values from regression trees performed on bootstrapped samples of the data shown on respective plots

Table 6 Results of all-subsets regression of DO range on stream gradient (grade), pheophytin (pheo), and stream habitat quality scores (QHEI) given benthic chlorophyll

Variables	R^2	Adj. R^2	Cp	S	Grade	Pheo ^a	QHEI
2	28	25.4	3.5	0.29617		X	X
3	28.4	24.9	5.0	0.29715	X	X	X
2	21.9	19.1	10.3	0.30840	X	X	
1	20.1	18.2	10.4	0.31005		X	
1	12.7	10.6	18.7	0.32412			X

^a Residuals from regression of pheo on benthic chlorophyll

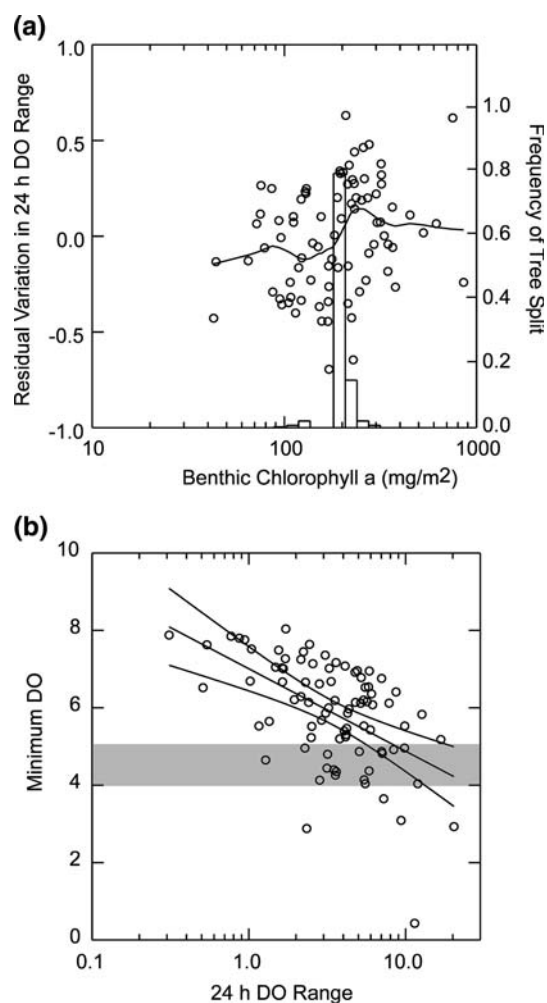


Fig. 3 **a** Residuals from the regression of daily DO range on canopy cover and pheophytin plotted against benthic chlorophyll concentrations. The *fitted line* is from LOWESS smoothing ($\alpha = 0.5$), and the superimposed histogram shows the relative frequencies of cut values from regression trees performed on bootstrap samples. **b** Scatter plot showing the relationship between the minimum DO concentration recorded within a 24-h period and the corresponding 24-h range in concentration. Fitted line is from ordinary least-squares regression. The *shaded region* depicts the lower range of DO concentrations required to support aquatic life consistent with beneficial uses

Table 7 Parameter estimates and coefficients of partial determination for the regression of minimum DO concentration on 24-h DO range, drainage area, and stream gradient

Parameter	Coefficient	SE	t	Partial r^2
Constant	4.5970	0.7513	6.12	
Gradient	1.3024	0.3936	3.31	9.86
Drainage area	0.5817	0.2100	2.77	9.00
24-h DO range	-1.8673	0.3668	-5.09	20.44

Table 8 Coefficients of partial determination for nutrient indicators accounting for significant variation in the listed dependent variable given QHEI scores already present in a first-order multiple regression (i.e., QHEI and either benthic chlorophyll or DO range or DO minimum)

Dependent variable	QHEI	Independent variable		
		Benthic chlorophyll	DO range	DO minimum
EPT taxa	0.23***	0.05**	0.05*	0.09**
Invertebrate rank	0.22***	0.04*	0.06*	0.13***
Sensitive fish ^a	0.51***	<0.01	<0.01	<0.01
IBI ^b	0.25***	<0.01	<0.01	0.03 ^{ns}

^a The variance explained given the QHEI. Note that for the number of sensitive fish, drainage area was included in the model

^b If coldwater sites are excluded, minimum DO explained significant ($P < 0.05$) variation in IBI scores, with a coefficient of partial determination, given QHEI scores, of 0.08

Significance levels are noted as follows: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ^{ns} $0.1 > P > 0.05$

and documented measurable stress to biological communities as a consequence. Although the amount of variance in either the fish or the macroinvertebrate indicator explained by any one of the enrichment indicators was generally $<10\%$ and, at best, 13% for minimum DO (Table 8), in the context of the multiple factors affecting biological integrity (Karr and Chu 1999), a narrow partitioning along a single gradient should be expected. Therefore, it is the strength of the circumstantial case between nutrients and biological condition that should be judged, rather than a partial r^2 value. Clearly, benthic chlorophyll levels in this study were largely a function of nutrient concentration and light, DO regimes were clearly related to benthic chlorophyll levels, and macroinvertebrate condition was unequivocally influenced by DO. Accepting that a compelling case has been made, identifying meaningful change points between the stressor and the response variables may now serve as a framework for criteria in water quality standards (Table 9). Meaningful change points, in this context, are those that ultimately result in protecting and maintaining beneficial aquatic life uses.

The change points for benthic chlorophyll relative to DIN and TP for this study were estimated as 0.435 and 0.038 mg/l, respectively. These values generally comport with thresholds identified using benthic chlorophyll as a response variable reported by Dodds and others (1997, 2002, 2006) and approach thresholds identified by Stevenson and others (2008) for TP but are an order of magnitude higher than those suggested by Biggs (2000). The Biggs (2000) targets were derived from regression equations using data from a relatively homogeneous set of streams, are designed to prevent benthic chlorophyll from exceeding 200 mg/m² based on a 50-day accrual period, and approximate values known to saturate algal growth

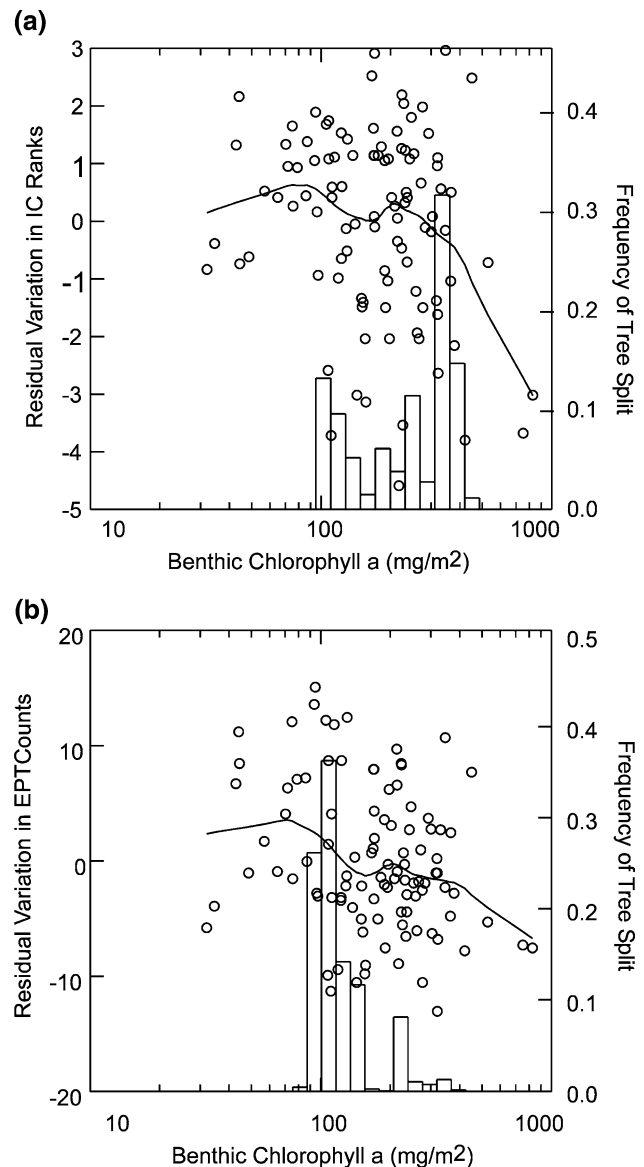


Fig. 4 **a** Residuals from the regression of invertebrate community ranks on QHEI scores and **b** residuals from the regression of EPT taxon counts on QHEI scores plotted against benthic chlorophyll. Fitted lines are from LOWESS smoothing ($\alpha = 0.5$), and the superimposed histograms show the frequency distributions of cut values from regression trees performed on bootstrapped samples of the data shown in respective plots

(e.g., Bothwell 1989; Chambers and others 2000). Stevenson and others (2008) derived targets using change point analysis from data that covered a broad range of stream sizes in the Mid-Atlantic Highlands region. The Dodds and others (2002, 2006) values were also derived using change point analysis based on data representing a broad range of stream sizes and geographic areas. Regardless of the differences between studies, all of the identified thresholds occur at comparatively low concentrations relative to the range typical of working landscapes.

Fig. 5 Residual variation in invertebrate community ranks following regression on QHEI scores in relation to **a** minimum DO concentrations and **b** daily range in DO concentrations. **c** Residual variation in EPT taxon counts in relation to minimum DO concentration and **d** daily range in DO concentrations. The fitted lines in each plot are from LOWESS smoothing ($\alpha = 0.5$); histograms show the relative frequencies of cut values from regression trees run against bootstrapped samples of the data shown in respective plots

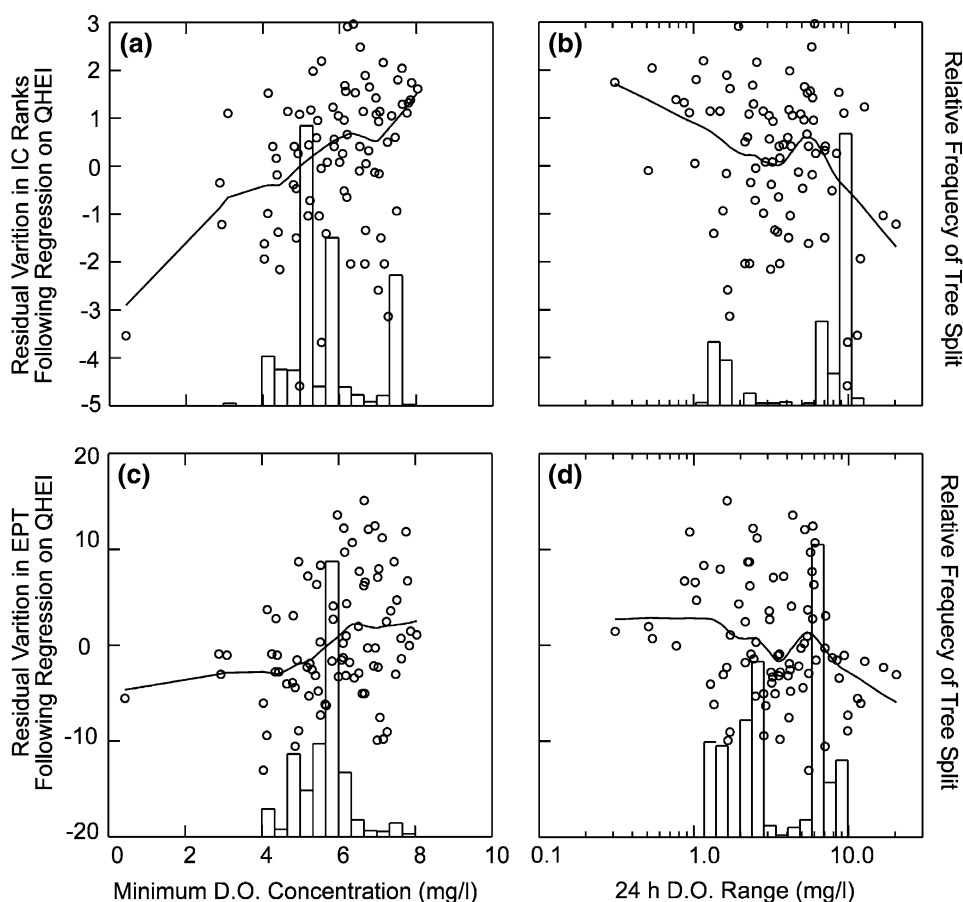


Table 9 Criterion values for enrichment indicators suggested to abate and prevent eutrophication of rivers and streams <1300 km² (500 mi²) in drainage area

Indicator	Protection	Management	Rationale (protection; management)
DIN (mg/l)	0.44	1.1	Change point for benthic chlorophyll; secondary mode in bootstrap samples
TP (mg/l)	0.04	0.1	Change point for benthic chlorophyll; achievable through current technology
Chl <i>a</i> (mg/m ²)	107	182	Protection of existing high-quality waters; maintain min. DO concentrations >4.0 mg/l
DO range (mg/l)	6.0	7.0	Maintain min. DO concentrations >4.0 mg/l; change point for number of EPT taxa
DO min. (mg/l)	6.0	5.0	Existing water quality standard for high-quality waters; change point for macroinvertebrate indicators
Canopy (deg open)	<40°	<40°	Change point for benthic chlorophyll

Where nutrient thresholds have been identified using fish or macroinvertebrates as response variables (Smith and others 2007; Wang and others 2007; Sheeder and Evans 2004; Miltner and Rankin 1998), the threshold concentrations tend to be higher, but of similar magnitude, compared to those obtained from benthic chlorophyll. This suggests that nutrient thresholds identified directly from fish or macroinvertebrate indicators can be used to inform development of numeric criteria. However, the tendency toward higher threshold concentrations may reflect cumulative indirect effects of nutrient enrichment, suggesting that information gathered from intervening steps (i.e., benthic

or sestonic chlorophyll and DO) will make application of numeric criteria more straightforward, especially with respect to diagnosing impairment. Macroinvertebrate community quality in this study was clearly related to DO concentrations, which were, in turn, mediated by periphytic biomass. Similarly, in a study of medium to large rivers in Minnesota, variation in DO concentrations forced by algal respiration was an important causal pathway between increasing nutrients and decreasing biological quality (Heiskary and Markus 2003). In that study, measures of fish and macroinvertebrate community quality showed stronger negative correlations with daily DO variation than

absolute minimum DO concentrations and noted that fluctuations exceeding 4.0 mg/l were particularly detrimental to the biological communities. Thus, Heiskary and Markus (2003) established daily DO range as a diagnostic measure of nutrient enrichment that is tied to impairment of beneficial aquatic life use.

The change point given by the regression tree for 24-h DO range relative to benthic chlorophyll for this study occurred at 182 mg/m². Benthic chlorophyll at sites with nutrient concentrations less than the change points averaged ~130 mg/m² (i.e., varies slightly between regression trees for DIN or TP), whereas those at sites exceeding the change points averaged ~213 mg/m². Note that Dodds and others (1998) proposed 200 mg/m² as an upper boundary for the prevention of nuisance conditions in streams. Of the 15 cases in the data set where the 24-h DO range was ≥7.0 mg/l (the change point for EPT relative to DO range), 13 occurred where the benthic chlorophyll concentrations were >190 mg/l (i.e., the modal change point in bootstrapped samples; Table 5). A daily DO range >6.0 mg/l carries a significant risk of minimum concentrations falling below the established water quality standard of 4.0 mg/l (Fig. 4). Conversely, ranges <6.0 mg/l tend to maintain minima >5.0 mg/l (the water quality standard for average daily minimum DO) and, therefore, should be protective of aquatic life based on both water quality standards, and the change points for macroinvertebrate indicators identified in this study, especially in light of the change points identified relative to IC ranks.

Although IBI scores and the number of sensitive fish species did not relate significantly with any of the nutrient indicators, one important caveat should be noted. Nine sites classified post hoc as coldwater (Mike Bolton, Ohio EPA, personal communication) had atypically low IBI scores and numbers of sensitive fish species. The IBI for Ohio rewards species richness because it is calibrated for warmwater streams and necessarily penalizes coldwater streams that lack diversity (Lyons and others 1996). If those data are culled, IBI scores show a significant relationship with minimum DO concentrations (partial $r^2 = 0.067$ given QHEI scores; $t = 2.52$, $P = 0.014$).

Applying Nutrient Criteria in Management

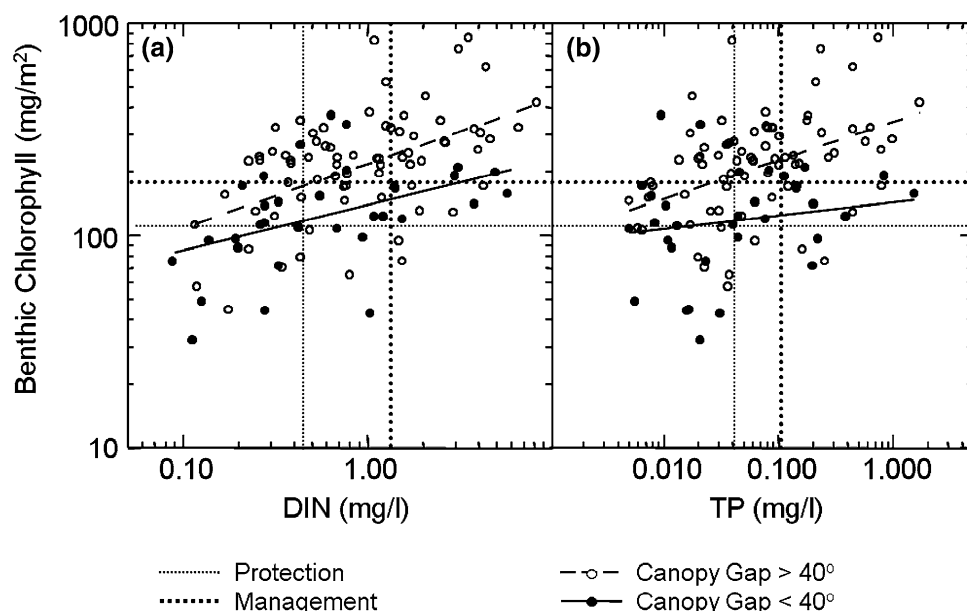
The change points listed in Table 9, when interpreted in light of these results, can help set management goals for small streams and rivers (i.e., <1300 km²). A practical upper limit of 1300-km² drainage area is suggested for the applicability of these results, given that most of the data were collected from streams less than that size, and because DIN and canopy cover had more explanatory power when the 19 large river sites were excluded from the regression predicting benthic chlorophyll (Table 4). That

said, wide DO swings and high levels of benthic or sestonic chlorophyll in large rivers are likely to similarly provide clear signals of overenrichment, once empirically defined.

If biological impairment (as judged by fish or macroinvertebrate indicators) is observed concurrently with DO swings or benthic chlorophyll levels that exceed the thresholds in Table 9, nutrient enrichment is very likely a contributing factor, and management should be directed toward reducing nutrient loads. However, application of the nitrogen and phosphorus targets must proceed with an appreciation for the uncertainty underlying the thresholds. Achieving respective seasonal average concentrations for DIN and TP of <0.44 and 0.04 mg/l would give a high probability of restoring beneficial uses to an erstwhile enriched stream; however, for effluent-dominated streams, meeting those targets off-the-shelf would require ultrafiltration techniques that may be prohibitively expensive if not spread over a large customer base (Jiang and others 2004). Effluent concentrations of TP between 0.1 and 0.5 mg/l can be achieved through chemical or biological removal (Clark and others 2005; Jiang and others 2004; Kim and others 2009), suggesting that, given some dilution to work with, seasonal average concentrations approaching 0.1 mg/l TP in the downstream receiving waters are feasible and, therefore, should be a management target for presently overenriched streams. Although nitrogen limitation is rare in Ohio streams, it was observed in 12 of the 109 cases in this study, based on molar ratios. Given the cost of nitrogen removal, removing phosphorus to force phosphorus limitation might be a cost-effective strategy. A nitrogen management goal of 1.1 mg/l is therefore proposed as a soft target because it coincides with the secondary mode of cut values in regression trees ran against bootstrapped samples taken from the chlorophyll and DIN data (Fig. 2).

Plotting the nutrient and chlorophyll results from this study against a backdrop of the criteria proposed in Table 9 helps to visualize potential management outcomes (Fig. 6). In Fig. 6, ordinary least-square regression lines are fitted to the data points for DIN (Fig. 6a) and TP (Fig. 6b) stratified by the threshold for canopy cover (i.e., 40°; Table 5). The suggested protection and management criteria for benthic chlorophyll and DIN or TP are identified by superimposing stippled lines on the plots. These plots suggest that for cases where point sources discharge to physically intact streams with closed canopies, meeting the management targets for TP or DIN will likely control benthic chlorophyll to levels <182 mg/m². Managing to this level offers a good chance for restoring biological condition in an impaired stream, as it will maintain benthic chlorophyll and 24-h DO range below threshold levels identified for the macroinvertebrate community as a whole (i.e., the change points for IC rank in Table 5).

Fig. 6 Benthic chlorophyll plotted against **a** DIN and **b** TP. Ordinary least-squares regression lines are fitted to the points coded by the canopy cover threshold of 40°. The *stippled lines* intersecting the axes correspond to the suggested nutrient criteria from Table 9



For open-canopied streams, the more restrictive limits may be necessary, perhaps in concert with riparian restoration. Similarly, in agricultural settings, where many streams are managed as open ditches to expedite drainage, creating wooded buffers would offer an immediate palliative benefit and may be necessary in areas where manure and fertilizers are applied at high agronomic rates. Optimally, riparian and channel restoration to improve assimilative capacity and habitat quality should co-occur with agricultural management practices aimed at reducing nutrient yield to surface waters. Keep in mind that habitat quality accounted for more variation in biological scores than did the nutrient indicators.

The plots also suggest that the more restrictive targets for nitrogen, phosphorus, and benthic chlorophyll in Table 9 should be applied as protective caps for presently un-enriched systems, such that new or additional loads, regardless of the source, will not result in those levels being exceeded. This is justified because nutrient concentrations exceeding background levels carry measurable risk of eutrophication, as was evidenced by a detectable change point for the number of EPT taxa occurring at a benthic of chlorophyll level of ~ 107 mg/m² (Table 5). The more restrictive targets may also serve as fall-back criteria in cases where achieving the management targets fails to effect restoration. In this sense, the numbers in Table 9 define risk management thresholds (*sensu* Mainstone and Parr 2002), as opposed to guarantees of protection, and should be applied judiciously and iteratively in management.

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