Assessment of Chlorophyll-*a* as a Criterion for Establishing Nutrient Standards in the Streams and Rivers of Illinois

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Nutrient enrichment is a frequently cited cause for biotic impairment of streams and rivers in the USA. Efforts are underway to develop nutrient standards in many states, but defensible nutrient standards require an empirical relationship between nitrogen (N) or phosphorus (P) concentrations and some criterion that relates nutrient levels to the attainment of designated uses. Algal biomass, measured as chlorophyll-*a* (chl-*a*), is a commonly proposed criterion, yet nutrient–chl-*a* relationships have not been well documented in Illinois at a state-wide scale. We used state-wide surveys of >100 stream and river sites to assess the applicability of chl-*a* as a criterion for establishing nutrient standards for Illinois. Among all sites, the median total P and total N concentrations were 0.185 and 5.6 mg L^{-1}, respectively, during high-discharge conditions. During low-discharge conditions, median total P concentration was 0.168 mg L^{-1}, with 25% of sites having a total P of $\geq$0.326 mg L^{-1}. Across the state, 90% of the sites had sestonic chl-*a* values of $\leq$35 μg L^{-1}, and watershed area was the best predictor of sestonic chl-*a*. During low discharge there was a significant correlation between sestonic chl-*a* and total P for those sites that had canopy cover $\leq$25% and total P of $\leq$0.2 mg L^{-1}. Results suggest sestonic chl-*a* may be an appropriate criterion for the larger rivers in Illinois but is inappropriate for small rivers and streams. Coarse substrate to support benthic chl-*a* occurred in <50% of the sites we examined; a study using artificial substrates did not reveal a relationship between chl-*a* accrual and N or P concentrations. For many streams and rivers in Illinois, nutrients may not be the limiting factor for algal biomass due to the generally high nutrient concentrations and the effects of other factors, such as substrate conditions and turbidity.

The Federal Clean Water Act requires states to identify impaired water bodies and develop plans to reduce impairment. Nutrient enrichment, mainly with nitrogen (N) and phosphorus (P), is a frequently cited cause of impairment for streams and rivers (USEPA, 2000a). Nutrient loading can degrade the ecological integrity of streams and create human health concerns. For example, the U.S. Environmental Protection Agency (USEPA) has set a drinking water standard of 10 mg NO_3–N L^{-1} to prevent methemoglobenemia. No drinking water standard exists for P; however, P enrichment can affect drinking water supplies by stimulating blooms of toxin-producing organisms, such as cyanobacteria. Nutrient enrichment in streams stimulates algal growth with resulting impacts on habitat quality, trophic relations, community structure, dissolved O_2 concentrations, pH, and aesthetic qualities (e.g., Miltner and Rankin, 1998).

The goal in developing nutrient standards for streams and rivers is to prevent a particular ecological condition (e.g., excessive algal biomass) by controlling the presumably limiting factor for algal growth. The rationale behind nutrient standards is that ecological impairment in nutrient-enriched streams is due, at least in part, to excess algal biomass and the effect of the excess biomass on dissolved O_2 deficits via respiration and decomposition. However, the cause-and-effect relationship among nutrients, algal biomass, and O_2 deficits is complicated by other environmental factors that can maintain low algal biomass despite abundant nutrients (Dodds and Welch, 2000). For example, scouring, shading, grazing, and temperature can affect algal biomass independently of nutrient enrichment. Further complicating the development of nutrient standards is the fact that algae in streams and rivers occur in multiple forms, such as sestonic cells, epilithic biofilms, and filamentous mats. These various forms may differ in their response to nutrient enrichment and the degree to which they are affected by other environmental factors.

Chlorophyll-*a* (chl-*a*) is a commonly used proxy for algal biomass and has been proposed as a criterion for identifying streams that fail to

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Abbreviations: chl-*a*, chlorophyll-*a*; DRP, dissolved reactive phosphorus; Q, discharge.

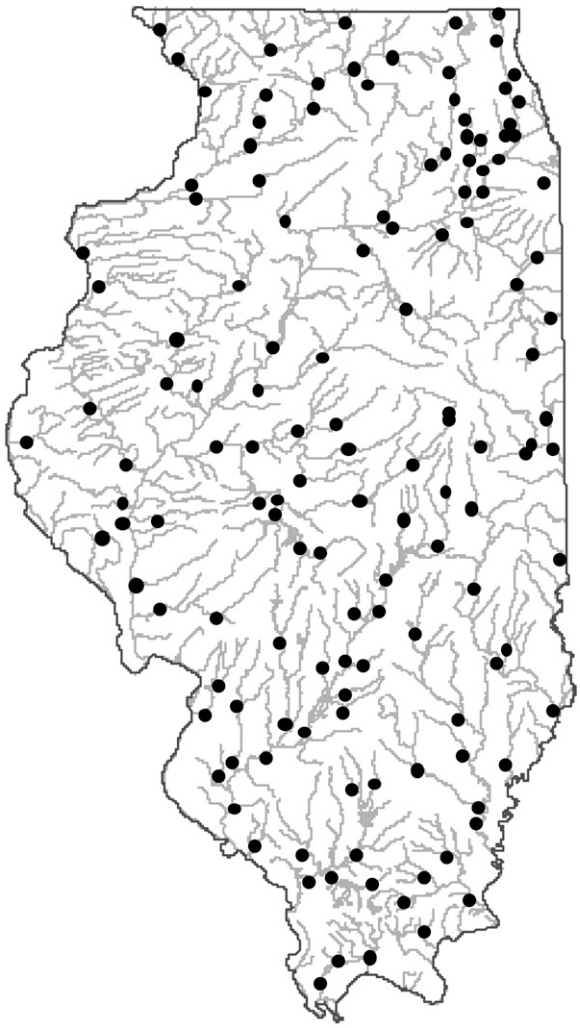

Fig. 1. Map of Illinois showing the major river networks and the distribution of the 138 sites used for the study.

attain their designated use(s) due to nutrient loading (e.g., USEPA, 2000b). Because of the large number of streams and rivers that will likely require management intervention to reduce nutrient loading, it is critical that the selected criterion be strongly associated with numerical water quality standards and attainment of designated uses (Reckhow et al., 2005). In Illinois, the strength of the relationship between nutrients and chl-*a* has not been assessed at a state-wide scale, and the appropriateness of sestonic or benthic chl-*a* as a state-wide criterion for establishing nutrient standards is unknown. Previous work in Illinois suggested that in wadeable agricultural streams sestonic chl-*a* was a poor indicator of eutrophic conditions (Figueroa-Nieves et al., 2006; Morgan et al., 2006), but its applicability in larger rivers has not been examined.

Across large geographic regions, such as states or level III ecoregions (e.g., Woods et al., 2006), it is often difficult to statistically relate nutrient concentrations to algal biomass (as chl-*a*) due to spatial and temporal variations in hydrology, light, temperature, and land use factors that influence algal abundance (Dodds et al., 2002). Nevertheless, the development of nutrient standards for streams will likely occur independently within each state, potentially with the goal of producing single, state-wide standards for N and P. To assist the state of Illinois with development of nutrient standards, we examined nutrient–chlorophyll relationships throughout the streams and rivers of the state. Because the state anticipates single, state-wide standards, our approach was at the state-wide scale. Our goals included examination of state-wide relationships between nutrients and chl-*a* as well as more mechanistically focused studies aimed at identifying environmental factors other than nutrients that may affect algal biomass in the streams and rivers of Illinois.

Materials and Methods

State-wide Surveys

Quantifying the ecological response to nutrient enrichment across Illinois presents a challenge due to the diversity of stream types and land uses within Illinois. Illinois covers slightly more than 150,000 km^2 and has a latitudinal gradient of 627 km, from 36°58′ N at the south to 42°30′ N at the northern boundary. Much of Illinois is rural and dominated by intensive row-crop agriculture with large inputs of N and P fertilizer (David and Gentry, 2000). The southern region of Illinois has more extensive tracks of hardwood forests and includes the Shawnee National Forest. Streams in the northeastern portion of the state are influenced strongly by urbanization, with the Illinois River system receiving the wastewater effluent from the approximately 8 million people living in the greater Chicago region. Statewide, land cover is 76% agricultural, 12% forest, 6% urban, 4% wetland, and 2% other uses (Illinois Department of Agriculture, 2001).

We conducted two state-wide surveys in 2004 designed to document conditions during distinct seasonal and hydrological conditions. A smaller state-wide survey was conducted in 2005 in conjunction with the artificial substrate study described below. Sites selected for the survey ranged in size from small, wadeable streams to the large rivers of the state but did not include the Mississippi, Ohio, or Wabash Rivers because these rivers were not wadeable even at low discharge and could not be safely sampled from bridges. Most sites corresponded to locations used by Illinois EPA in their ambient water quality monitoring network. The goal in site selection was to identify a representative group of sites that would allow for generalization to the streams and rivers of the state as a whole. A complete listing of all sites is presented in Appendix A.

The first survey examined 138 sites distributed across the state (Fig. 1) and was conducted from May to early July when most of the streams were at higher than baseflow discharge (Q) but not flooded. An analysis of 103 of the sites that were gauged by the US Geological Survey indicated that, at the time of sampling, average discharge across the sites was 81% of the long-term mean discharge for the month of May. For the second survey we revisited 109 of the sites during September when the streams were at baseflow. In this paper we refer to the first survey as the high-Q survey and the second survey as the low-Q survey. During all surveys, we sampled sestonic chl-*a* and benthic chl-*a* (if present), estimated canopy cover, and collected water samples for the determination of total P, dissolved reactive P (DRP), organic P, total N, NO_3–N, NH_4–N, organic N, and dissolved silica. We used

portable probes and meters to make on-site measurements of water temperature, pH, specific conductivity, and turbidity. Turbidity was measured at three locations across the width of each stream and averaged. Samples for dissolved constituents were filtered through a 0.45-µm membrane. Sample processing and preservation followed standard procedures (APHA, 1998).

At each site we established three cross-sectional transects separated by approximately 50 m. At each transect that had gravel or cobble substrate, we collected a representative rock for determination of benthic chl-*a* density. We did not attempt to sample benthic chl-*a* from soft sediments or sand. At the most up-stream transect, we collected three 500-mL samples from the left, right, and center of the channel for determination of sestonic chl-*a* concentration. Samples for chl-*a* were stored in the dark on ice until they were processed at the end of each day. For each sestonic chl-*a* sample, a known volume of water was filtered through a Whatman GF/F filter (0.7 µm), and the filters were immediately placed in individual plastic Petri dishes, wrapped in aluminum foil, and placed on ice. Rocks were individually wrapped and placed on ice for transport to the laboratory where they were processed within 30 d of collection (see below).

Nutrient and Chlorophyll Analyses

Nitrate concentrations were measured using ion chromatography (DX-120; Dionex, Sunnyvale, CA) with a detection limit of 0.1 mg L^{-1} of NO_3–N. Ammonium, DRP, and silica concentrations were analyzed colorimetrically by flow injection analysis with a QuikChem 8000 (Lachat, Loveland, CO) using the automated sodium salicylate, the automated ascorbic acid, and the automated heteropoly blue methods, respectively. Method detection limits were 0.01 mg NH_4–N L^{-1}, 0.005 mg P L^{-1}, and 0.2 mg SiO_2 L^{-1}. Water samples for total P were digested with sulfuric acid (11.2 N) and ammonium persulfate (0.4 g per 50 mL of sample), which converted all forms of P into DRP, and then analyzed as described previously. Samples for total N were digested with sulfuric acid, copper sulfate, and potassium sulfate in an aluminum block digester (BD-46; Lachat) that converted organic N compounds to ammonia, which was then analyzed as described previously. Organic N and P were determined as the difference between the total and the dissolved inorganic forms of each nutrient.

In general, we followed the procedures for chl-*a* analysis as described in detail by Morgan et al. (2006), with some modifications. Samples for sestonic chl-*a* were extracted in the dark with 90% acetone for 24 h at 4°C. For benthic chl-*a*, rocks were thoroughly scraped of material using a wire brush, and the dislodged material was collected onto a Whatman GF/F filter. Chl-*a* was extracted in the dark at 4°C for 24 h with 90% ethanol. For sestonic and benthic samples, 30 s of sonication was used to promote extraction. Chlorophyll-*a* was determined using a UV-Vis spectrophotometer (Aquamate; ThermoElectron, Waltham, MA). To correct for pheophytin, absorbance was determined before and after acidification as described in Morgan et al. (2006). The areal surface on the rocks from which the benthic chl-*a* was collected was determined using the aluminum foil method (Steinman and Lamberti, 1996).

Embarras River and Kaskaskia River Surveys

The 2004 surveys revealed unexpectedly high concentrations of sestonic chl-*a* in some of the larger rivers of the state. We examined this phenomenon further in 2005 by conducting synoptic surveys on the Embarras and Kaskaskia rivers. The surveys included sampling sestonic chl-*a* at multiple sites on the mainstem rivers and in several tributaries of each river. An 82-km study reach was used on the Embarras River, and a 36-km reach was used on the Kaskaskia. The goal was to determine if the high mainstem concentrations were the result of tributary loading or in-channel production. All methods were as described previously.

Artificial Substrate Study

Substrate condition is a critical factor for the development of periphyton in streams and can vary substantially among sites. We attempted to reduce the effects of this confounding variable by placing unglazed ceramic tiles at 35 sites across the state and measuring benthic chl-*a* accrual. The goal was to examine nutrient–chl-*a* relationships when provided a common and suitable benthic substrate. At nine sites, the water was too deep to place tiles, but sestonic chl-*a* and nutrient samples were collected. At the remaining 26 sites, a 20 cm × 20 cm tile was placed near the center of the channel at each of three transects and anchored in place with reinforcing bars. This method may not have accounted for the accrual of floating filamentous algal mats, but floating mats of algae were not observed at the time of placement or retrieval of the tiles at any of the sites. Tiles were placed in July 2005 and retrieved after 5 wk of incubation. Sestonic chl-*a* and nutrient samples were collected in conjunction with placement and retrieval of the tiles and analyzed as described previously. Benthic chl-*a* on the tiles was determined by thoroughly scraping a known area and processing the sample as described previously.

Data Analysis

Relationships between chl-*a* and environmental variables (including nutrients) were examined with Pearson correlation analysis or simple linear regression. Differences between the high-Q and low-Q surveys were examined with a two-sample *t* test if the data were normally distributed or with a nonparametric test if data could not be normalized. Normality of all data sets was examined with the Kolmogorov-Smirnov test ($\alpha = 0.05$). Water temperature was normally distributed, and the benthic chl-*a* data were normalized with a $\log_{10}(X + 1)$ transformation (Zar, 1999). Sestonic chl-*a*, nutrient, and turbidity data could not be normalized and therefore were examined with the Kruskal–Wallis nonparametric test. All statistical analyses were conducted with MINITAB release 14.2.

Results

Water Chemistry

Water temperatures were not different between the high-Q and low-Q surveys ($p = 0.143$) and averaged 21°C during both time periods. There was a large range in specific conductivity among the streams, from approximately 100 to >2000 µS cm^{-1}, but there was little difference between the high-Q and low-Q pe-

Table 1. Distribution of water chemistry values from the 2004 state-wide surveys.

	Minimum	25th Percentile	Median	75th Percentile	Maximum
High-Q† survey (May–July, *n* = 138)					
DRP‡ (mg L^{-1})	<0.005	0.038	0.069	0.156	1.9
Total P (mg L^{-1})	0.013	0.123	0.185	0.326	2.0
NH_4–N (mg L^{-1})	0.008	0.040	0.058	0.089	0.387
NO_3–N (mg L^{-1})	0.10	1.0	4.3	10.2	20.2
Total N (mg L^{-1})	0.37	2.2	5.6	11.0	20.9
Silica (mg L^{-1})	1.5	6.7	9.6	11.8	16.6
pH	7.0	7.7	7.9	8.1	8.7
Specific conductivity (μS cm^{-1} @ 25°C)	106	586	658	751	2240
Turbidity (NTU§)	<1	21	36	61	614
Low-Q survey (Sept., *n* = 109)					
DRP (mg L^{-1})	0.001	0.029	0.081	0.345	2.8
Total P (mg L^{-1})	0.007	0.112	0.168	0.456	2.8
NH_4–N (mg L^{-1})	0.002	0.011	0.022	0.042	0.696
NO_3–N (mg L^{-1})	<0.05	0.18	1.5	3.9	18.0
Total N (mg L^{-1})	0.21	1.0	2.5	5.0	18.7
Silica (mg L^{-1})	1.3	6.4	8.6	11.2	29.2
pH	6.8	7.6	7.9	8.2	8.9
Specific conductivity (μS cm^{-1} @ 25°C)	132	556	664	814	3246
Turbidity (NTU)	<1	10	18	29	159

† Low-Q, low discharge; high-Q, high discharge.

‡ Dissolved reactive phosphorus.

§ Nephelometric turbidity units

riods (Table 1). Turbidity declined significantly between the two surveys ($p < 0.001$) from a median of 36 nephelometric turbidity units during the high-Q period to a median of 18 nephelometric turbidity units during the low-Q period. The distribution of nutrient concentrations during the high-Q and low-Q periods is shown in Table 1. Across the state, the median total P concentration was 0.185 mg L^{-1} during the high-Q survey and 0.168 mg L^{-1} during the low-Q survey. There was no statistical difference between the two surveys in total P or DRP. The maximum DRP and total P values of $\geq$2 mg L^{-1} were recorded from streams in which the discharge was dominated by wastewater effluent.

Total N, nitrate, and ammonium concentrations were significantly lower during the low-Q survey than during the high-Q survey, but even during the low-Q period 75% of the streams had a total N concentration of 1.0 mg L^{-1} or greater (Table 1). The high nitrate concentrations reflect the heavily fertilized, agricultural landscape that typifies much of Illinois. As total N concentrations increased, nitrate comprised a greater fraction of the total N, particularly during the high-Q survey. Ammonium concentrations were generally 1 to 2 orders of magnitude lower than nitrate concentrations, and only 25% of the sites had ammonium N concentrations >0.089 mg L^{-1} during the high-Q survey or >0.042 mg L^{-1} during the low-Q survey.

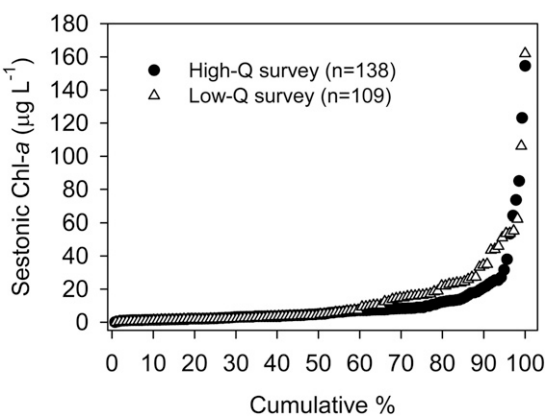

Fig. 2. Distribution of sestonic chlorophyll-*a* (chl-*a*) concentrations across the state of Illinois during the 2004 high-discharge (high-Q) and low-Q surveys (see text for explanation). See Appendix A for the list of sites used in each survey.

Sestonic chl-*a*

The median sestonic chl-*a* value was 5 μg L^{-1} during the high-Q and low-Q surveys, and statistically there was no difference in sestonic chl-*a* concentrations during the two time periods ($p = 0.642$) (Fig. 2). Across the state, 90% of the sites had sestonic chl-*a* values of $\leq$35 μg L^{-1}. There was no correlation between benthic chl-*a* (see below) and sestonic chl-*a*, suggesting that sloughing of periphyton was not the major source of algal cells to the water column. During all surveys, watershed area was the best predictor of sestonic chl-*a* (Fig. 3).

Large streams and rivers are capable of supporting planktonic algal communities and can accumulate sestonic cells from tributary inputs. The synoptic surveys on the Embarras and Kaskaskia Rivers suggested that the direct relationship between watershed area and sestonic chl-*a* was due mainly to in-channel production rather than to tributary inputs. Across the 82-km study reach on the Embarras River, mainstem sestonic chl-*a* values increased from 52 to 97 μg L^{-1}. In the Kaskaskia, mainstem sestonic chl-*a* increased from 30 to 86 μg L^{-1} along the 36-km study reach. The mainstem Embarras had a mean sestonic chl-*a* concentration of 69 μg L^{-1} (SD = 19; $n = 5$), whereas the tributaries had a mean concentration of 11 μg L^{-1} (SD = 12; $n = 5$). In the Kaskaskia, the mainstem had a mean of 63 μg L^{-1} (SD = 28; $n = 4$), whereas the tributaries had a mean of 8 μg L^{-1} (SD = 7; $n = 9$). We were unable to calculate sestonic chl-*a* loads because discharge data were not available for the tributaries. However, the tributaries were significantly smaller than the mainstem rivers, indicating that tributary loading could not account for the downstream increase in sestonic chl-*a* observed in both mainstem rivers.

There was no relationship between sestonic chl-*a* and any nutrient measure or other environmental factor during the high-Q survey. During the low-Q survey, there was no correlation between sestonic chl-*a* and total P for the data set as a whole. However, for those sites that had both canopy cover $\leq$25% and total P of $\leq$0.2 mg L^{-1} there was a correlation

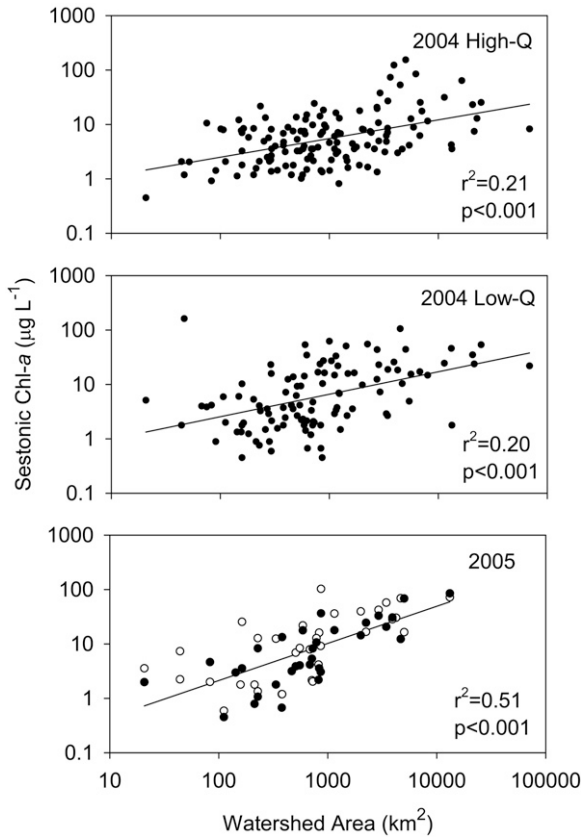

Fig. 3. Relationship between watershed area and sestonic chlorophyll-*a* (chl-*a*) concentrations across the state of Illinois during the 2004 surveys (see text for explanation) and during 2005. Samples for 2005 were collected in July (open symbols, *n* = 35) and 5 wk later in August or September (filled symbols, *n* = 35). See Appendix A for the list of sites used in each survey.

between total P and sestonic chl-*a* (Pearson correlation = 0.62; $p < 0.001$) (Fig. 4). Based on the 38 sites that met these criteria, there appeared to be a threshold value for total P of about 0.07 mg L^{-1}. Below that threshold, sestonic chl-*a* was $\leq$5 µg L^{-1}, whereas sestonic chl-*a* ranged from 1 to 55 µg L^{-1} among sites with $\geq$0.07 mg L^{-1} of total P and an open canopy.

Benthic chl-*a*

Less than 50% of the sites sampled during both the high-Q and low-Q surveys contained gravel or cobble substrate for analysis of benthic chl-*a*. Among the sites that contained suitable substrate, the median benthic chl-*a* value was 3 mg m^{-2} during the high-Q survey (n = 31) and 14 mg m^{-2} during the low-Q survey (n = 46). There was a significant increase in benthic chl-*a* between the two time periods (t = 7.04; $p < 0.001$; df = 74) (Fig. 5). During the low-Q survey, there was no relationship between benthic chl-*a* and any nutrient measure among the 46 sites that contained coarse substrate. During the high-Q survey, however, there was a weak correlation between total N and log-transformed benthic chl-*a* (Pearson correlation = 0.33; p = 0.07).

Of the 26 sites at which tiles were placed, 20 accrued benthic chl-*a* during the 5-wk incubation. At the other six sites, the tiles were buried by shifting sand and fine sediments. No significant flooding occurred at the sites during the incubation period (late

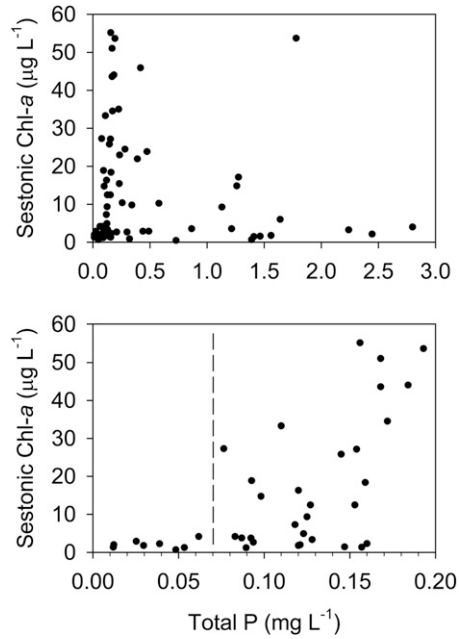

Fig. 4. Relationship between total P and sestonic chlorophyll-*a* (chl-*a*) concentrations during the 2004 low-discharge survey using all sites (upper panel), and only sites with an open canopy (<25%) and total P concentrations of <0.2 mg L^{-1} (lower panel; *n* = 38). The dashed vertical line indicates an apparent threshold value of 0.07 mg L^{-1} total P.

July to September), and discharge declined in 14 of the 15 sites that were gauged (Table 2). Among the 20 sites that accrued benthic chl-*a*, the density of chl-*a* on the tiles ranged from 3 to 67 mg m^{-2}, with a median value of 11 mg m^{-2}. Although the sites spanned a range in N and P concentrations (Table 2), there was no relationship between chl-*a* accrual on the tiles and any nutrient measure or other environmental factor.

Discussion

The establishment of defensible nutrient standards for streams and rivers requires a strong linkage between attainment of designated uses and the criterion used to measure the effect of nutrient enrichment. A successful criterion should allow resource managers to accurately predict attainment status based on the measured value of the criterion (Reckhow et al., 2005). Additionally, it is desirable from a management standpoint to have a single standard that can be applied to a large geographic region, such as a state or ecoregion, meaning the criterion must be broadly applicable to a potentially large range of stream types. Our goal was to examine patterns and relationships between algal biomass (as chl-*a*) and nutrient concentrations or other environmental factors and to do so at a state-wide scale. We focused on algal biomass as a potential criterion for nutrient standards because algae often respond directly to nutrient loading and because excess algal biomass can negatively affect O_2 concentrations, habitat quality, biotic community structure, and the aesthetic value of streams—all of which can affect attainment of designated uses.

Sestonic chl-*a* can occur in streams as a result of sloughing of periphyton or in-channel production if conditions are favorable (Swanson and Bachmann, 1976; Lohman and Jones, 1999).

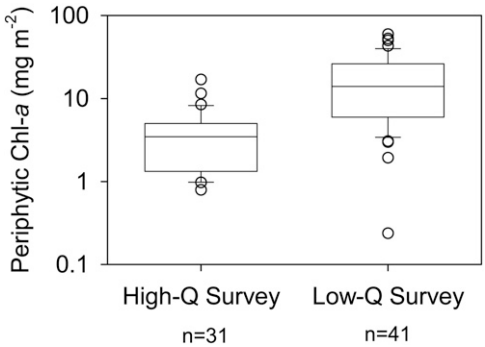

Fig. 5. Box-and-whisker plots of benthic chlorophyll-*a* (chl-*a*) density during the 2004 high- discharge (high-Q) and low-Q surveys (see text for explanation). Horizontal lines indicate the 25th, 50th, and 75th percentiles; whiskers indicate the 10th and 90th percentiles. Circles represent sites outside the 10th or 90th percentiles.

Similar to Lohman and Jones (1999), we found no correlation between benthic chl-*a* density and sestonic chl-*a* concentration, suggesting that in-channel production was the main source of sestonic chl-*a*. This conclusion is supported by the synoptic surveys on the Embarras and Kaskaskia Rivers in which we documented downstream increases in mainstem sestonic chl-*a* that were not attributable to tributary inputs. Across Illinois, sestonic chl-*a* was directly related to watershed area, and this pattern has been observed in other geographic regions (Van Nieuwenhuyse and Jones, 1996; Lohman and Jones, 1999). We did not find the expected relationship between watershed area and total P because several relatively small streams (drainage area <800 km^2) had total P concentrations >1.0 mg L^{-1} during low discharge conditions. Point-source discharges can elevate P concentrations in small streams, but the high rate of algal washout may keep sestonic chl-*a* concentrations lower than the P concentration would predict. In a study of agricultural streams in central Illinois, sestonic chl-*a* was correlated with total P only if a site that received wastewater effluent was excluded from the analysis (Morgan et al., 2006). In the present study, the positive relationship between sestonic chl-*a* and watershed area indicated that rivers and large streams supported conditions that favored the development of sestonic algal communities. As channel size increases, rivers tend to be less influenced by riparian shading and have lower flushing rates than do smaller streams. Van Nieuwenhuyse and Jones (1996) suggested that physical conditions and nutrients co-regulate sestonic chl-*a* concentrations in rivers, and our results support this notion.

We observed a correlation between total P and sestonic chl-*a* but only by limiting the analysis to sites with an open canopy and <0.2 mg L^{-1} total P (Fig. 4). There seemed to be a threshold value of approximately 0.07 mg L^{-1} total P that would be protective of excessive water column chl-*a*, but only eight sites out of 109 had total P concentrations below the apparent threshold, which limits our ability to generalize to the state as a whole. This apparent threshold for Illinois agrees closely with the concentration of 0.075 mg L^{-1} total P suggested by Dodds et al. (1998) as a boundary between mesotrophic and eutrophic conditions in streams of the temperate zone. We suggest that sestonic chl-*a* is not a useful criterion for streams and small rivers (drainage areas <2000 km^2), but it may have some applicability for larger rivers. Among sites with drainage areas >2000 km^2, there was a trend for increasing sestonic chl-*a* with increasing total P concentration (both on a $\log_{10}$ scale), but there was considerable variability, and the relationship was not statistically significant.

Table 2. Periphyton accrual on artificial substrate and the change in environmental variables from the start to the end of the 5-wk incubation. Description of the sites is given in Appendix A.

		Discharge		Total P		Total N		NO_3–N		Turbidity (NTU†)	
Site	Chl-*a*‡	Start	End	Start	End	Start	End	Start	End	Start	End
	mg m^{-2}	m^3 s^{-1}		mg L^{-1}							
1	67	0.04	0.03	1.92	2.50	9.8	14.4	9.4	14.2	1	2
2	43	4.05	3.42	0.22	0.13	0.6	0.7	0.4	0.3	6	7
3	33	0.17	0.05	0.20	0.18	1.2	2.1	0.2	1.6	7	7
4	30	13.41	9.91	2.94	4.25	1.4	4.8	0.7	3.5	8	16
5	27			0.62	0.60	1.2	1.4	0.1	0.4	14	16
6	19			0.74	0.92	4.5	9.1	3.8	8.7	5	19
7	18	0.15	1.56	0.11	0.08	3.5	3.5	3.2	3.2	6	2
8	17	3.45	1.53	0.03	0.04	0.6	0.4	0.1	0.2	2	12
9	14			0.10	0.10	2.8	1.4	2.0	0.9	17	17
10	12	1.08	0.65	0.07	0.05	5.8	1.7	5.5	0.1	7	5
11	11			0.11	0.09	0.7	1.3	0.2	0.6	11	15
12	10	1.73	1.25	0.24	0.19	1.5	0.5	0.1	0.2	10	15
13	9	0.34	0.05	0.15	0.15	0.9	0.6	0.1	0.1	9	14
14	9	0.31	0.17	0.23	0.20	1.1	0.6	0.5	0.3	25	9
15	7	0.42	0.18	0.07	0.07	0.5	0.6	0.1	0.1	4	6
16	7	0.57	0.37	0.09	0.07	0.5	0.8	0.1	0.4	10	12
17	6	0.37	0.27	1.43	1.26	12.7	11.8	11.3	11.6	42	14
18	5			0.09	0.05	0.6	0.2	0.2	0.1	3	3
19	4	0.09	0.05	0.50	0.22	0.9	1.7	0.7	1.3	28	69
20	3			0.17	0.12	0.7	0.4	0.1	0.1	4	11

† Nephelometric turbidity units.

‡ Chlorophyll-*a*.

The relationship between nutrients and the density of benthic chl-*a* is often confounded by factors such as flooding, grazing, and shading, which can make it difficult to separate the effects of human disturbance from natural variation (Dodds et al., 2002). After scouring of periphyton during high discharge, algae begin to accrue in relation to factors such as nutrient availability, light, and grazing, which makes the time since last disturbance a critical co-factor in explaining nutrient–periphyton relationships (Biggs, 2000). This presents practicable problems for state-wide monitoring programs, particularly for sites that are not continuously gauged for discharge. It often is filamentous macro-algae, rather than epilithic biofilms, that reach nuisance levels in streams (Welch et al., 1988). Filamentous mats can have a very patchy distribution within

a stream reach, which makes representative sampling difficult. In the current study, we encountered significant mats of filamentous algae only during the low-Q survey. Although nutrient loading is necessary for nuisance algal blooms, factors such as scouring, turbidity, and riparian shading can create situations of high nutrient concentrations and low algal biomass, as occurred during the high-Q survey.

We could not establish a nutrient–benthic chl-*a* relationship, even when a common and suitable substrate for algal growth was used, suggesting among-site variation in other (unmeasured) environmental factors influenced chl-*a* accrual more strongly than did nutrient availability. During both the high-Q and low-Q survey, the 25th percentiles for total N and total P were greater than the breakpoints reported by Dodds et al. (2006) for the nutrient–benthic chl-*a* relationships identified for temperate zone streams. This suggests that, for the state as a whole, nutrients occurred in excess of algal demands and that a factor(s) other than nutrients limited algal biomass. Additionally, this implies that for many streams and rivers in Illinois nutrients would have to be lowered below some threshold concentration before a response in algal biomass would occur (Dodds et al., 2002).

An ecoregion approach to using benthic chl-*a* as an indicator of nutrient enrichment has shown promise in streams of the Mid-Atlantic USA (Pan et al., 1999) and to some extent for streams in North America and New Zealand (Dodds et al., 2002). Whether an ecoregion approach would improve the nutrient–benthic chl-*a* relationship in Illinois streams is unknown, and the paucity of sites with measurable benthic chl-*a* in the current study precludes an ecoregion analysis. However, the Central Corn Belt Plains Ecoregion represents approximately 50% of the state of Illinois (Woods et al., 2006), and estimates have been made for baseline nutrient concentrations for various corn belt ecoregions. The total N and total P concentrations reported here and elsewhere (e.g., Royer et al., 2004; Gentry et al., 2007) for Illinois streams are generally 1 to 2 orders of magnitude greater than the estimated background total N and total P concentrations for corn belt ecoregions (Smith et al., 2003; Dodds and Oakes, 2004). Although historic data on nutrient concentrations for Illinois streams are sparse, it is clear that present-day nutrient concentrations are likely greatly elevated from background conditions, and this may obscure differences between ecoregions in algal–nutrient relationships.

Fewer than 50% of the sites we examined had coarse substrate from which to sample benthic chl-*a*. Because we did not attempt to sample benthic chl-*a* from sand and soft sediments, we cannot assess the role of these substrates in supporting benthic chl-*a*. However, our observations indicate that streams and rivers in Illinois that contain soft sediments are consistently turbid even during periods of low discharge (see Table 1) due to the recurring suspension of fine sediments. A previous study of 14 agricultural streams in central Illinois found that water column turbidity was a strong predictor of benthic chl-*a* (Figueroa-Nieves et al., 2006). Morgan et al. (2006) examined benthic chl-*a* in two open canopy agricultural streams in Illinois and found a significant inverse relationship between chl-*a* and water depth, suggesting that water column turbidity was limiting light penetration to the streambed. Based on the accumulated evidence, we suggest that benthic chl-*a* in Illinois streams and rivers is strongly influenced by substrate conditions and water column turbidity (which are themselves related) and that this may preclude isolating the direct effect of nutrients on benthic chl-*a* at a state-wide scale.

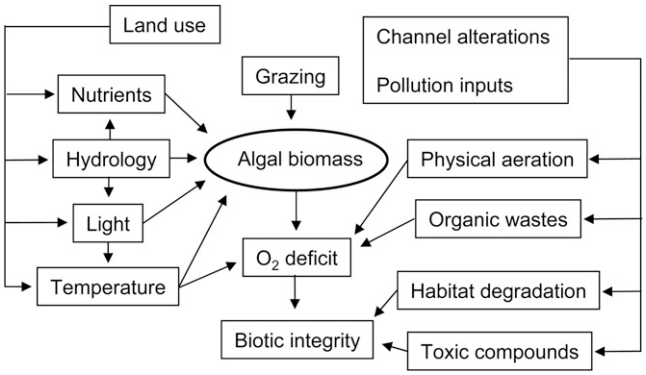

Fig. 6. Factors that influence the biotic integrity of Illinois streams. Land use, channel alterations, and pollution inputs can directly and indirectly decouple the expected cause-and-effect relationship between nutrient loading, algal biomass, O_2 deficit, and biotic integrity. Not all factors and interactions are shown.

Conclusions

Several challenges exist in the process of developing nutrient standards for streams and rivers. For example, unlike drinking water standards developed via toxicity testing to prevent a physiological outcome in individuals, nutrient standards are designed to prevent an ecological outcome (i.e., biotic impairment) across large geographic regions in the face of multiple stressors that influence biotic integrity. Furthermore, the ultimate goal of improving and protecting the biotic integrity of streams may not be accomplished solely with the implementation of nutrient standards. Biotic integrity is an outcome of many interacting factors (Fig. 6), and it may often be the case that nutrient concentration plays a relatively minor role in causing biological impairment of a particular site. Physical habitat quality played a strong role in controlling stream macroinvertebrate communities across Illinois, and habitat quality and nutrient concentrations were related, confounding efforts to isolate the influence of each (Heatherly et al., 2007).

Although nutrients are not the sole determinant of stream health, factors such as habitat quality, hydrology, light, temperature, and grazing are less amenable to management practices, leaving nutrients as the focus for attempts to control algal biomass and protect biotic integrity (Dodds and Welch, 2000). Currently in Illinois, it seems that for many streams and rivers nutrients may not be the limiting factor for algal biomass, due to the generally high nutrient concentrations and the effects of other factors, such as substrate conditions and turbidity. Nevertheless, management of nutrient concentrations in Illinois is important because ecological processes other than algal growth, including heterotrophic respiration, are influenced by nutrient loading (Dodds, 2006). Illinois is also a major contributor of N and P to the Mississippi River and Gulf of Mexico (Goolsby et al., 1999; David and Gentry, 2000), and efforts within the state to reduce nutrient loading may have far-reaching benefits.

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Appendix A. The 138 sites used in the study, watershed area for each site, and the corresponding Ambient Water Quality Monitoring Network code used by the Illinois Environmental Protection Agency (IEPA). Sites are organized by major drainage basins, and all location references are to towns or cities in Illinois. All of the listed sites were used in the 2004 high-Q survey, those sites used in other aspects of the study also are indicated.

IEPA code	Site name and location	Watershed area	Sites used in 2004 low-Q survey	Sites used in 2005 survey	Site number for artificial substrate study
		km^2			
	Ohio River Basin				
AD-02	Cache River at Forman	632	x		
AK-02	Lusk CR near Eddyville	111	x	x	8
AT-06	Saline River near Gibsonia,	2751	x		
ATF-04	North Fork Saline River near Texas City	448	x		
ATGC-01	Bankston Creek near Harrisburg	202			
ATH-05	South Fork Saline River near Carrier Mills	381	x	x	
	Little Wabash & Wabash River Basin				
BC-02	Bonpas Creek at Browns	591	x	x	
BE-01	Embarras River near Billet	6224			
BE-07	Embarras River at Ste. Marie	3926	x	x	13
BE-09	Embarras River near Diona	2380			
BE-14	Embarras River at Camargo	466	x	x	14
BEF-05	North Fork Embarras River near Oblong	824	x	x	20
BM-02	Sugar Creek near Elbridge	158	x		
BP-01	Vermilion River near Danville	3341	x		
BPG-09	North Fork Vermilion River near Bismarck	679	x		
BPJ-03	Salt Fork Vermilion River near Oakwood	1267	x		
BPJC-06	Saline Branch near Mayview	212	x	x	1
BPK-07	Middle Fork Vermilion River near Oakwood	1119	x		
C-21	Little Wabash River near Effingham	622	x		
C-22	Little Wabash River near Clay City	2929	x	x	
C-23	Little Wabash River at Carmi	7998	x		
CA-03	Skillet Fork near Carmi	2740	x		
CA-05	Skillet Fork at Wayne City	1202	x		
CD-01	Elm River near Toms Prairie	686	x	x	12
	Illinois River Basin				
D-23	Illinois River at Marseilles	21,391	x		
D-32	Illinois River at Valley City	68,801	x		
DA-04	Macoupin Creek near Macoupin	787	x	x	
DA-06	Macoupin Creek near Kane	2248	x	x	
DB-01	Apple Creek near Eldred	1046	x		
DD-04	Mauvaise Terre Creek near Merritt	378	x	x	
DE-01	McKee Creek at Chambersburg	883	x		
DG-01	La Moine River at Ripley	3349			
DG-04	La Moine River at Colmar	1696	x		
DJ-08	Spoon River near Seville	4237	x	x	
DJ-09	Spoon River near London Mills	2751			
DJB-18	Big Creek near Bryant	106	x		
DJL-01	Indian Creek near Wyoming	163	x	x	2
DK-12	Mackinaw River near Green Valley	2828			
DK-13	Mackinaw River near Congerville	2010	x	x	16
DQ-03	Big Bureau Creek at Princeton	508	x	x	4
DS-06	Vermilion River at McDowell	1427	x		
DS-07	Vermilion River near Leonore	3240			
DT-06	Fox River at Algonquin	3634			
DT-38	Fox River at Montgomery	4486	x		
DT-46	Fox River at Dayton	6843			
DTD-02	Blackberry Creek near Yorkville	181	x		
DTG-02	Poplar Creek at Elgin	91	x		
DTK-04	Nippersink Creek near Spring Grove	497	x		
DV-04	Mazon River near Coal City	1178	x		
DZZP-03	Farm Creek at East Peoria	158	x		
	Sangamon River Basin				

(continued)

Appendix A. Cont'd.

IEPA code	Site name and location	Watershed area	Sites used in 2004 low-Q survey	Sites used in 2005 survey	Site number for artificial substrate study
		km^2			
E-09	Sangamon River at Decatur	2429			
E-25	Sangamon River near Oakford	13,191	x	x	5
E-26	Sangamon River at Riverton	6781	x		
E-28	Sangamon River near Monticello	1484	x		
E-29	Sangamon River at Fisher	622	x		
EI-02	Salt Creek near Greenview	4672	x	x	3
EI-06	Salt Creek near Rowell	868	x	x	
EID-04	Sugar Creek near Hartsburg	862	x	x	6
EIE-04	Kickapoo Creek at Waynesville	588	x		
EIG-01	Lake Fork near Cornland	554	x	x	15
EL-01	Spring Creek at Springfield	282	x		
EO-01	South Fork Sangamon River near Rochester	2253			
EO-02	South Fork Sangamon River at Kincaid	1456	x		
EOH-01	Flat Branch near Taylorville	715	x	x	19
EZU-01a	Big Ditch near Dewey	142	x	x	10
	Kankakee River Basin				
F-01	Kankakee River near Wilmington	13,339	x		
F-02	Kankakee River at Momence	5941			
FL-02	Iroquois River near Chebanse	5416	x		
FL-04	Iroquois River at Iroquois	1777			
FLI-02	Sugar Creek at Milford	1155	x		
	Des Plaines River & Lake Michigan Basins				
G-07	Des Plaines River near Gurnee	601	x		
G-08	Des Plaines River at Russell	319			
G-22	Des Plaines River near Des Plaines	932			
G-39	Des Plaines River near Riverside	1632	x		
GB-10	Du Page River near Naperville	570			
GB-11	Du Page River at Shorewood	839	x		
GBK-05	West Branch Du Page River near Warrenville	233	x		
GBK-09	West Branch Du Page River near West Chicago	75	x		
GBL-10	East Branch Du Page River at Lisle	148	x		
GG-02	Hickory Creek at Joliet	277	x		
GI-01	Sanitary & Ship Canal at Romeoville				
GL-09	Salt Creek at Western Springs	295	x		
GLA-02	Addison Creek at Bellwood	47	x		
H-01	Calumet Sag Channel at Sag Bridge	1008			
HBD-04	Thorn Creek at Thornton	269	x		
HCC-07	North Branch Chicago River at Niles	259	x		
HCCC-02	North Branch Chicago River at Deerfield	52			
	Mississippi River Tributaries				
II-03	Marys River at Welge	293	x		
IX-04	Cache River at Sandusky	606	x		
IXJ-02	Big Creek near Balcom	21	x	x	
JN-02	Cahokia Canal near Collinsville	155	x		
JNA-01	Canteen Creek near Collinsville	67	x		
JQ-05	Cahokia Creek at Edwardsville	549	x		
KCA-01	Bay Creek at Nebo	417	x		
KI-02	Bear Creek near Marcelline	904	x		
LD-02	Henderson Creek near Oquawkao	1119	x		
LF-01	Edwards River near New Boston	1153	x	x	11
MJ-01	Plum River at Savanna	707	x		
MN-03	Apple River near Elizabeth	536	x		
MQ-01	Galena River at Galena	508	x		
	Big Muddy River Basin				
N-11	Big Muddy River at Plumfield	2056			
N-12	Big Muddy River at Murphysboro	5618	x		
NC-07	Beaucoup Creek near Vergennes	1238	x		
ND-01	Crab Orchard Creek near Carbondale	704	x		
ND-04	Crab Orchard Creek near Marion	83	x	x	
NJ-07	Casey Fork near Mount Vernon	228	x	x	
NK-01	Rayse Creek near Waltonville	228	x	x	

(continued)

Appendix A. Cont'd.

IEPA code	Site name and location	Watershed area	Sites used in 2004 low-Q survey	Sites used in 2005 survey	Site number for artificial substrate study
		km²			
	Kaskaskia River Basin				
O-02	Kaskaskia River at Cooks Mills	1225	x		
O-07	Kaskaskia River near Carlyle	7081			
O-08	Kaskaskia River at Vandalia	5025	x	x	
O-10	Kaskaskia River near Cowden	3445	x	x	9
O-20	Kaskaskia River near Venedy Station	11,378	x		
O-31	Kaskaskia River near Tuscola	293			
OC-04	Richland Creek near Hecker	334	x	x	17
OD-06	Silver Creek near Troy	399	x		
OD-07	Silver Creek near Freeburg	1202			
OI-08	Shoal Creek near Breese	1904			
OI-09	Shoal Creek near Walshville	728	x	x	
OK-01	East Fork Kaskaskia River near Sandoval	293	x		
OKA-01	North Fork Kaskaskia River near Patoka	101			
OL-02	Hurricane Creek near Mulberry Grove	394	x		
ON-01	Hickory Creek near Bluff City	202	x		
OQ-01	Beck Creek at Herrick	251			
OT-02	West Okaw River near Lovington	290	x		
OZC-01	Plum Creek near Baldwin	158	x	x	
	Rock River Basin				
P-04	Rock River near Joslin	24,732	x		
P-06	Rock River at Como	22,670			
P-14	Rock River at Byron	20,694	x		
P-15	Rock River at Rockton	16,480			
PB-02	Green River near Deer Grove	834	x	x	18
PB-04	Green River near Geneseo	2598			
PH-16	Elkhorn Creek near Penrose	378	x		
PHI-01	Five Mile Creek near Brookville	44	x	x	7
PL-03	Kyte River at Daysville	464	x		
PQ-10	Kishwaukee River at Garden Prairie	575	x		
PQ-12	Kishwaukee River near Perryville	2846	x		
PQC-06	South Branch Kishwaukee River near Fairdale	1002	x		
PW-08	Pecatonica River at Freeport	3434	x		

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