In cooperation with the Texas Commission on Environmental Quality

Nutrient and Biological Conditions of Selected Small Streams in the Edwards Plateau, Central Texas, 2005–06, and Implications for Development of Nutrient Criteria

Scientific Investigations Report 2007–5195

U.S. Department of the Interior
U.S. Geological Survey

Front cover:

Left: Cow Creek above FM 1431, March 2005.

Top right: South Rocky Creek at U.S. Highway 183, March 2005.

Bottom right: Cypress Creek at FM 962, March 2005.

Back cover:

Top: Flathead catfish at the Blanco River, August 2006.

Bottom: Largemouth bass at the Blanco River, August 2006.

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Conversion Factors

Inch/Pound to SI

Multiply	By	To obtain
	Length	
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
	Area	
square inch (in^2)	6.452	square centimeter (cm^2)
	Flow rate	
inch per year (in/yr)	25.4	millimeter per year (mm/yr)

SI to Inch/Pound

Multiply	By	To obtain
	Length	
kilometer (km)	0.6214	mile (mi)
micrometer (µm)	3.937×10^{-5}	inch (in.)
millimeter (mm)	0.03937	inch (in.)
	Volume	
liter (L)	33.82	fluid ounce (fl oz)
milliliter (mL)	0.034	fluid ounce (fl oz)

Abbreviations

grams per square meter, g/m^2

milligrams per liter, mg/L

milligrams per square meter, mg/m^2

Nutrient and Biological Conditions of Selected Small Streams in the Edwards Plateau, Central Texas, 2005–06, and Implications for Development of Nutrient Criteria

By Jeffrey A. Mabe

Abstract

During the summers of 2005 and 2006 the U.S. Geological Survey, in cooperation with the Texas Commission on Environmental Quality, evaluated nutrient and biological conditions in small streams in parts of the Edwards Plateau of Central Texas. Land-cover analysis was used to select 15 small streams that represented a gradient of conditions with the potential to affect nutrient concentrations across the study area, which comprises two of four subregions of the Edwards Plateau ecoregion. All 15 streams were sampled for water properties, nutrients, algae, benthic invertebrates, and fish in summer 2005, and eight streams were resampled in summer 2006. Streams that did not receive wastewater effluent had relatively low nutrient concentrations and were classified as oligotrophic; streams receiving wastewater effluent had relatively high nutrient concentrations and were classified as eutrophic. Nutrient concentrations measured in the least-disturbed streams closely matched the U.S. Environmental Protection Agency nutrient criteria recommendations based on estimated reference concentrations. Nitrogen/phosphorus ratios indicated streams not affected by wastewater effluent might be limited by phosphorus concentrations. Algal indicators of nutrient condition were closely related to dissolved nitrogen concentrations and streamflow conditions. Ambient dissolved nitrogen concentrations (nitrite plus nitrate) were positively correlated with benthic algal chlorophyll-*a* concentrations. The correlation of benthic algal chlorophyll-*a* with instantaneous nitrite plus nitrate load was stronger than correlations with ambient nutrients. Increased nutrient concentrations were associated with increased macroalgae cover, wider diel dissolved oxygen ranges, and reduced diel dissolved oxygen minimums. Benthic invertebrate aquatic life use scores generally were classified as High to Exceptional in study streams despite the influence of urbanization or wastewater effluent. Reductions in aquatic life use scores appeared to be related to extremely low flow conditions and the loss of riffle habitats. Benthic invertebrate aquatic life use scores and several of the metrics used to compute composite aquatic life use scores tended to increase with increasing total nitrogen concentrations. Fish community aquatic life use scores generally were classified as High or Exceptional with the exception of a few samples collected from streams receiving wastewater effluent that were classified as Intermediate. Fish community aquatic life use scores and several fish community metrics were positively correlated with nutrient concentrations and macroalgae cover. The majority of the positive correlations among nutrient concentrations, macroalgae cover, and fish metrics were strongly influenced by relatively high nutrient concentrations. Both benthic and planktonic chlorophyll-*a* measures were related to nutrients, but this study indicates that benthic chlorophyll-*a* was the better choice for monitoring nutrient enrichment because (1) the relation between benthic chlorophyll-*a* and nutrients was stronger, and (2) a strong relation between benthic chlorophyll-*a* and nutrients persisted after removal of the sites influenced by wastewater effluent, which indicates superior ability of benthic chlorophyll-*a* to discriminate between conditions at lower nutrient concentrations. The transect-based algal abundance estimate technique is a useful tool for identifying eutrophic conditions, assessing nuisance algal growth, and making broad comparisons among sites, but it appears to lack the fine resolution to identify lesser degrees of nutrient enrichment. Several individual benthic invertebrate and fish metrics were correlated with nutrient conditions, but correlations were generally positive and the reverse of what would be expected when nutrient enrichment causes a proliferation of algal growth and stream degradation. However, the benthic invertebrate functional feeding group metrics showed some promise as measures of nutrient condition.

Introduction

Nutrients, broadly defined, are chemical elements essential to the growth, reproduction, and metabolic processes of living organisms. Aquatic ecosystems require nutrients to support the biological communities they contain. However, overabundant nutrients can contribute to various water-quality problems. Excessive amounts of nitrogen or phosphorus, or both, can promote the growth of aquatic vegetation and result in problems ranging from degraded water quality and altered aquatic habitats to a loss of recreational and aesthetic value. Recent water-quality inventories compiled by the U.S. Environmental Protection Agency (USEPA) identify nutrient enrichment as one of the leading causes of water-resource impairment in the Nation (U.S. Environmental Protection Agency, 1996, 1998a, 2000). Historically, State efforts to control nutrients generally have taken the form of narrative criteria aimed at avoiding nuisance algal growth. For example, the present standard for Texas states, "Nutrients from permitted discharges or other controlled sources shall not cause excessive growth of aquatic vegetation which impairs an existing, attainable, or designated use" (Office of the Texas Secretary of State, 2007).

To effectively address issues related to nutrient enrichment, the USEPA has directed States to develop numeric nutrient criteria for their surface waters. To assist States in the development process, the USEPA created a strategy for developing ecoregion-based numeric nutrient criteria focused on specific water bodies—that is, streams and rivers, lakes and reservoirs, estuaries and coastal marine waters, and wetlands (U.S. Environmental Protection Agency, 1998b). In December 2001 the USEPA published nutrient-criteria recommendations for rivers and streams (U.S. Environmental Protection Agency, 2001) in Level III Ecoregion 30, the Edwards Plateau (Griffith and others, 2004) (fig. 1A). Recommendations were based on an estimate of reference conditions (25th percentile for all data) and focused on two nutrient constituents, total nitrogen and total phosphorus, and two biological variables known to respond to nutrient enrichment, water-column chlorophyll-*a* and turbidity. However, evidence indicates that water-column chlorophyll-*a*, a measure of the biomass of suspended algae (phytoplankton), is a poor indicator of nutrient enrichment in small, often fast-flowing, Texas streams, and that benthic (attached) algal chlorophyll-*a* might be a better indicator (Texas Commission on Environmental Quality, 2006).

Benthic algae are sessile organisms that colonize the surfaces of submerged rocks and other stable substrate. As primary producers, benthic algae in the clear streams of Central Texas take up nutrients from the environment and make them available to higher trophic levels. Benthic algae require nutrients to maintain a healthy community, but an overabundance of nutrients can promote excessive algae growth and result in wide-ranging ecological effects. The increased metabolic activity associated with high algal biomass can alter diel (24-hour) dissolved oxygen (DO) and pH concentrations (Allen, 1995). Reductions in DO concentrations coupled with high temperatures and low flows during the summer can affect the distribution, survival, and reproductive success of sensitive fish (Lowe and others, 1967; Matthews and Maness, 1979) and benthic invertebrate species (Allen, 1995; Rosenberg and Resh, 1996). The proliferation of benthic algae can lead directly to changes in community structure and function by altering the food base and cover habitat (Quinn and Hickey, 1990; Feminella and Hawkins, 1995). Additionally, high algal biomass often is viewed as objectionable and can degrade the aesthetic and recreational uses of a stream (Biggs, 1985; Welch and others, 1988).

The Texas Commission on Environmental Quality (TCEQ), the agency charged with developing nutrient criteria in Texas, does not routinely collect information on benthic algae, and data useful for developing nutrient criteria are lacking in the Edwards Plateau and other Texas ecoregions. Accordingly, the U.S. Geological Survey (USGS), in cooperation with TCEQ, did a study during 2005–06 to characterize nutrient and biological conditions and to identify relations between nutrient conditions and biological conditions in selected small streams of Central Texas.

Purpose and Scope

This report presents the results of data collection and analysis of nutrient and biological conditions in small streams in the Edwards Plateau of Central Texas during the summers of 2005 and 2006. More specifically this report (1) describes the range of nutrient and biological conditions in selected small streams in the eastern part of the plateau; and (2) identifies and examines relations between nutrient concentrations and biological-response variables, including the effects of streamflow on relations between nutrients and biological conditions. In addition, the report discusses the findings in light of USEPA-recommended nutrient criteria for the Edwards Plateau ecoregion. Data to characterize water properties, nutrients, algae, benthic invertebrates, and fish were collected from 15 streams in 2005 and from a subset of eight streams in 2006 in two of four subregions (the study area) of the Edwards Plateau ecoregion.

Description of Study Area

The Edwards Plateau of Central Texas is a dissected limestone uplift bounded on the south and east by the Balcones escarpment and grading into the Chihuahuan Desert to the west and the Great Plains to the north. The area generally is typified by thin soils underlain with Cretaceous-age limestone formed from marine deposits. The plateau can be divided into four subregions (fig. 1A) with distinct characteristics (Griffith and others, 2004), two of which contain the watersheds of streams sampled for this report. The Edwards Plateau Woodland subregion in the central part of the plateau contains broad, moderately dissected uplands typified by juniper-oak and mesquite-oak savannas. In contrast, the southeastern part of the plateau, the Balcones Canyonlands subregion, encompasses rugged terrain heavily dissected by stream systems with steep-sided canyons and a higher percentage of deciduous woodland. Although some farming occurs in the broader stream valleys, the plateau is better known as a grazing region

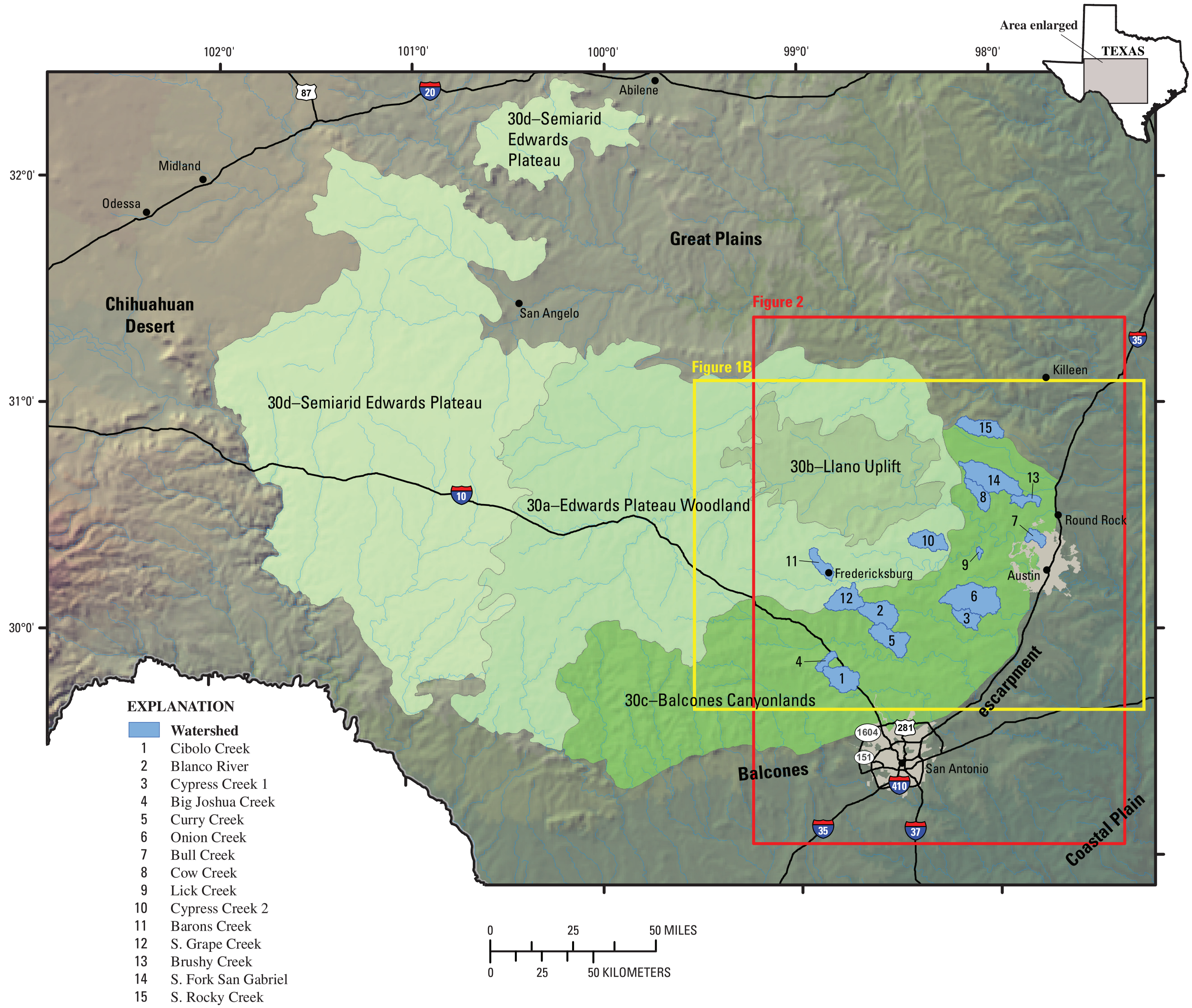


Figure 1A. Location of Level III Ecoregion 30, Edwards Plateau, and subregions and study watersheds, Central Texas.

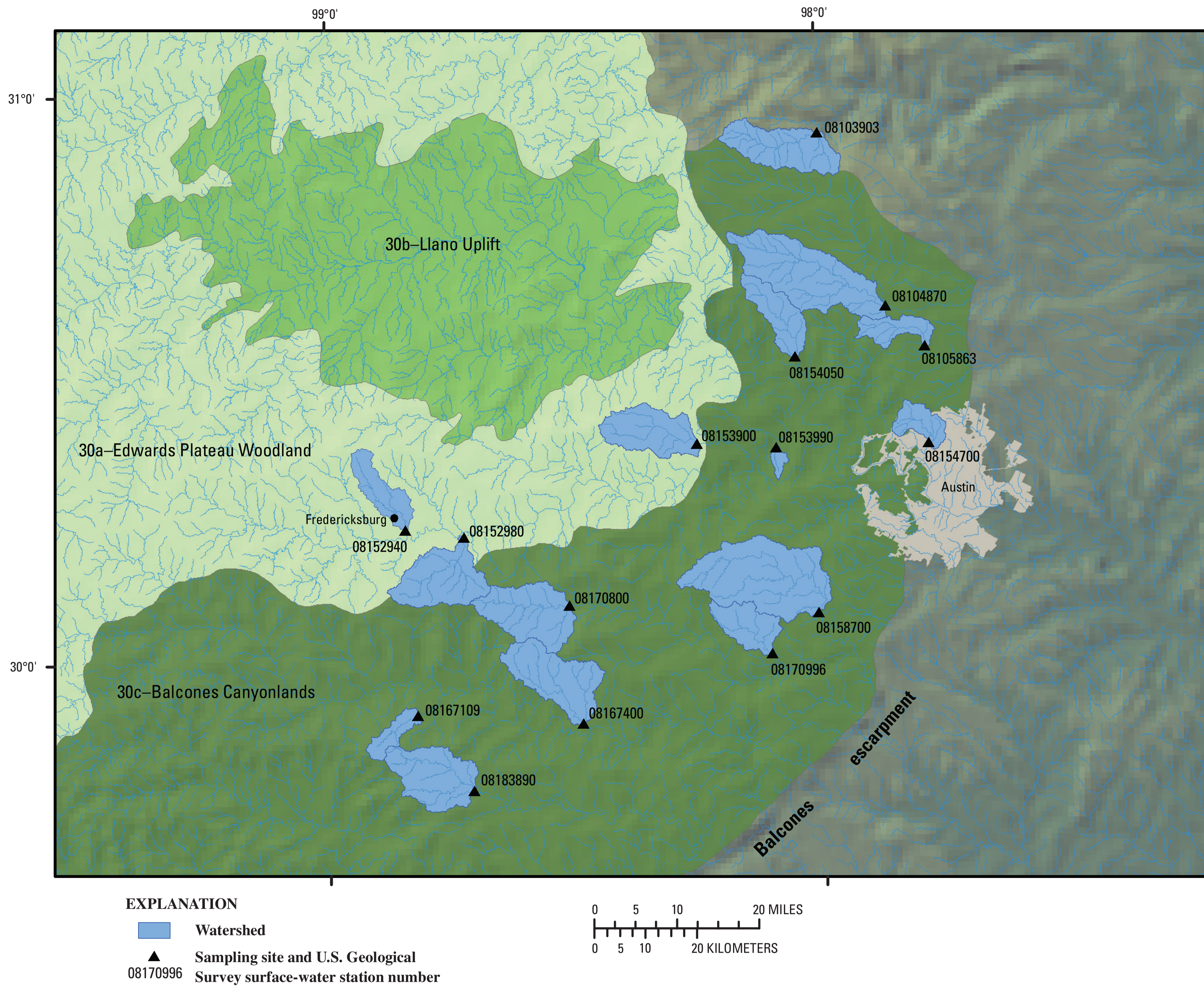


Figure 1B. Locations of sampling sites and stream networks in study watersheds, Central Texas.

for cattle, sheep, and goats. In recent years exotic game ranching has replaced traditional ranching in some areas.

Climatic conditions follow a gradient from semiarid to arid in the western parts of the plateau to more humid conditions in the eastern part. Average annual rainfall across the study area watersheds ranges from about 34 inches per year near Austin (fig. 1A) to about 28 inches per year near Fredericksburg (U.S. Department of Agriculture, Natural Resources Conservation Service, 1999). Streams in the region are primarily two types: spring-fed and perennial or intermittent and only flowing after rainstorms (Ashworth, 1983). Dry summer conditions and sporadic, intense rainstorms combined with steep, primarily bedrock slopes make the Balcones Canyonlands prone to extreme variation in streamflow (Caran and Baker, 1986).

Methods of Study

Water and biological samples were collected from small streams in study-area watersheds in September 2005 and August 2006. Samples were collected in the late summer to assess conditions during the period of the year when low streamflow and high water-temperature conditions stress biota and threaten the maintenance of aquatic life use (ALU) standards (Texas Commission on Environmental Quality, 2005). Streams were selected to represent a gradient of conditions with the potential to influence nutrient concentrations. Sites sampled twice were used to assess the year-to-year variation in nutrient concentrations and associated biological conditions.

Site Selection

The initial selection of candidate streams was done in consultation with TCEQ. Topographic maps were used to identify potential sampling sites, which then were plotted with 2001 National Land Cover Data (MRLC Consortium, 2007) to evaluate the presence of watershed characteristics and land use practices that could affect nutrient concentrations. Potential sampling sites were visited once in spring 2005 to evaluate habitat conditions and to screen for potential differences in channel form, substrate, riparian vegetation, and the availability of microhabitat or instream cover that could affect the biological sampling. Fifteen small wadeable streams were selected for this study (table 1). Watersheds of 12 streams are entirely within the Balcones Canyonlands, and watersheds of three streams are entirely or partly within the Edwards Plateau Woodland (fig. 1B). A study reach was established at each of the selected stream sites according to TCEQ protocols. Stream sites were evaluated to identify the best biological sampling locations, the number and extent of geomorphic channel units (riffles, runs, and pools), and the average stream width. Study reaches encompass the chosen biological sampling locations and the maximum variety of geomorphic channel units. Reach lengths were equal to 40 times the average stream width. Most stream watersheds are characterized by relatively low levels of urban and agricultural land cover (fig. 2), however, three streams (Barons Creek, Brushy Creek, and Cibolo Creek) receive wastewater discharges upstream from the study reach. The Bull Creek watershed in Austin has the largest percentage of urban land cover, and the South Grape Creek watershed has the largest percentage of agricultural land cover. Five streams (Big Joshua Creek, Cow Creek, Curry Creek, Cypress Creek 2, and South Rocky Creek) were designated for comparison as least-disturbed. The initial selection of least-disturbed streams was based on the lowest levels of urban and agricultural land cover in the watershed. The final designation of least-disturbed streams accounted for various land use factors identified during site reconnaissance. For example, the Blanco River watershed has low percentages of both urban and agricultural land cover, but site inspection revealed residential housing close to the study reach. Land cover in the least-disturbed watersheds was dominated by forest and shrubland with lesser percentages of grassland.

Data Collection and Analysis

Water Sampling

Water samples were collected once at each site (fig. 1B) at the time of biological sampling in accordance with TCEQ protocols (Texas Commission on Environmental Quality, 2003). Whole-water nutrient samples (total nitrogen, total phosphorus, and Kjeldahl nitrogen) were collected directly with a grab sample from the centroid of streamflow with a 125-milliliter translucent polyethylene bottle and preserved with 1 milliliter 1:7 sulfuric acid. Dissolved nutrients (ammonia, nitrite plus nitrate, and orthophosphate) were collected in a 1-liter polyethylene container and processed through a 45-micrometer (μm) glass-fiber filter into a 125-milliliter brown polyethylene bottle. All nutrient samples were placed on ice for shipping to the laboratory except for orthophosphate samples, which were frozen for preservation and shipped on dry ice. Nutrient samples were shipped overnight and processed by the USGS National Water Quality Laboratory (NWQL) in Lakewood, Colo.

A Hydrolab MiniSonde 4a multiprobe was used to measure DO, pH, specific conductance, and temperature continuously at 15-minute intervals for 2 to 3 days before sampling. Each multiprobe was calibrated with traceable standards in controlled conditions before placement in flowing water at approximately one-third the stream depth. After recovery, calibration was rechecked to evaluate instrument performance and screen for drift in any of the probes.

A single water sample cannot fully characterize the long-term water quality of a stream. The composition of stream water varies with time and can fluctuate with seasons and patterns of rainfall and runoff. Water composition of

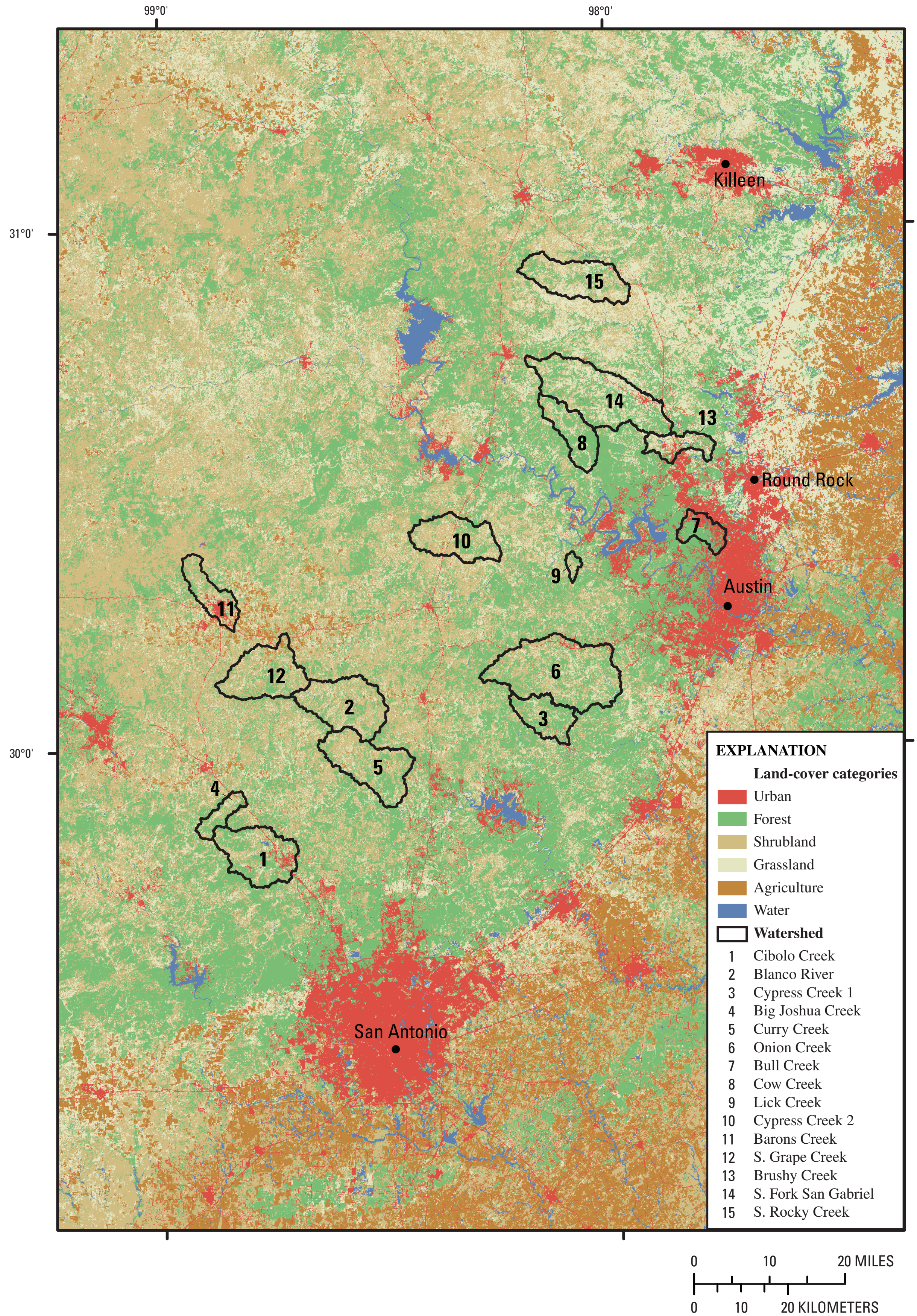


Figure 2. Land cover in study watersheds, Edwards Plateau, Central Texas, 2001.

Table 1. Data-collection sites, group designations, and percentage land cover for nutrient study sites in the Edwards Plateau, Central Texas, 2005–06.

[TCEQ, Texas Commission on Environmental Quality; USGS, U.S. Geological Survey; ddmm.sss, degrees minutes.seconds; WW, wastewater effluent; NWW, no wastewater effluent; LD, least disturbed]

Watershed (fig. 1A)	TCEQ station number	USGS station number (fig. 2)	USGS site name	Site short name	Stream group	Location of sampling site		Land cover (percent)				
						Latitude (ddmm.sss)	Longitude (ddmm.sss)	Urban	Forest	Shrubland	Grassland	Agriculture
1	16702	08183890	Cibolo Creek at Nature Center near Boerne	Cib	WW	N2947.048	W9842.839	6.31	45.7	33.6	13.4	0.19
2	18664	08170800	Blanco River at Crabapple Rd. near Blanco	Bla	NWW	N3006.106	W9830.714	.04	26.6	56.6	16.0	.50
3	12676	08170996	Cypress Creek near Ranch Road 12 in Wimberley	Cy1	NWW	N3000.723	W9806.321	1.42	56.3	30.0	11.9	0
4	18665	08167109	Big Joshua Creek at Interstate Highway 10 near Comfort	BJo	LD	N2954.601	W9849.391	1.57	41.9	40.3	16.2	.03
5	18666	08167400	Curry Creek at Acker Rd. near Spring Branch	Cur	LD	N2953.609	W9829.230	.08	36.3	48.7	13.9	.89
6	12451	08158700	Onion Creek near Driftwood	Oni	NWW	N3008.620	W9802.918	1.89	41.7	38.8	17.3	.06
7	12216	08154700	Bull Creek at Loop 360 near Austin	Bul	NWW	N3022.381	W9747.068	42.8	53.1	1.19	.93	0
8	18660	08154050	Cow Creek near Cow Creek Rd. near Lago Vista	Cow	LD	N3032.069	W9802.826	0	60.0	22.4	17.5	0
9	18661	08153990	Lick Creek near Pedernales Canyon Rd. near Bee Cave	Lic	NWW	N3022.522	W985.299	.40	42.5	38.8	18.2	0
10	12258	08153900	Cypress Creek at Ranch Road 962 near Cypress Mill	Cy2	LD	N3022.946	W9815.001	1.04	26.2	54.9	17.4	.38
11	12269	08152940	Barons Creek at Old San Antonio Rd. in Fredericksburg	Bar	WW	N3014.080	W9850.468	15.3	23.8	44.4	12.7	3.56
12	12267	08152980	South Grape Creek at U.S. Highway 290 near Fredericksburg	SGr	NWW	N3013.406	W9843.526	.52	27.3	54.6	13.8	3.68
13	18659	08105863	Brushy Creek near County Road 272 near Cedar Park	Bru	WW	N3032.912	W9747.006	14.0	31.8	17.0	34.7	.45
14	12116	08104870	South Fork San Gabriel River at U.S. Highway 183 near Leander	SSG	NWW	N3037.157	W9751.630	2.36	42.4	22.0	32.7	.07
15	18657	08103903	South Rocky Creek at U.S. Highway 183 near Briggs	SRo	LD	N3055.631	W9759.659	.71	26.5	37.9	34.6	.17

streams affected by point-source discharges such as wastewater releases can vary daily or even hourly. However, a single sample collected in an area of the stream where the water is well mixed and the chemical composition is homogeneous can adequately characterize conditions at the time of sampling (Hem, 1992). The purpose of this study was not to characterize the suite of nutrient conditions in any one stream, but rather to compare nutrient and biological conditions among sites during critical summer low-flow periods. Climatic conditions in the study area during the month before sampling were stable with high temperatures and little rainfall (National Climatic Data Center, 2007). Streamflow patterns in gaged streams within the study area generally were stable or slowly declining (U.S. Geological Survey, 2007). Therefore, single samples collected at the same time as the biological sampling were considered adequate to represent the overall water-quality conditions influencing the biological assemblage at summer low flow.

Biological Sampling

Two methods were used to sample benthic algal biomass at each site. The first followed standard USGS protocols developed for the National Water Quality Assessment (NAWQA) program (Moulton and others, 2002) and used the top-rock scrape method. Five large cobbles were collected from five locations in each study reach. Cobbles were collected in riffles when present and runs when riffles were not present. Benthic algae were removed from the cobble surfaces and combined to form a composite sample. Subsamples (5 milliliters each) were collected from the composite sample for the analysis of chlorophyll-*a* and ash-free dry weight (AFDW) and filtered onsite through a 45-μm glass-fiber filter. Filters were wrapped in foil, placed in a sealed petri dish, and frozen with dry ice for shipment to the NWQL for analysis. The remainder of the composite sample was preserved with a sufficient volume of buffered formaldehyde to obtain a final concentration of 5-percent buffered formalin and retained for possible future taxonomic identification.

The second method involved a transect-based technique, modified from Hawkins and others (2001), for sampling and estimating stream-algal abundance. Transects originated on the left bank at the downstream boundary of each reach and ran diagonally upstream across the channel to the right bank. When stream bends were encountered, transects were anchored at the bend and run diagonally back across the channel. This technique results in a single transect laid in a zigzag pattern down the length of the reach and facilitates the assessment of algal abundance in both mid-channel and near-bank environments. Transects were divided into 100 equally spaced survey points and walked; at each survey point the amount of stream bottom covered by macroalgae (filamentous algae) was estimated for a 1-foot-square area centered on the survey point. Coverage was estimated using six cover categories: none, less than 5 percent, 5–25 percent, 25–50 percent, 50–75 percent, and more than 75 percent. A composite macroalgae cover score was computed by assigning each cover category a numeric value (0 for no cover to 5 for more than 75-percent cover), multiplying the number of points in each category by the category value, and summing the total. Additionally, at each survey point the closest piece of loose substrate (rock or woody debris) was selected and evaluated for percentage cover of macroalgae using the same categorical approach. The thickness of microalgae (microscopic algae such as diatoms) growing on the loose substrate also was evaluated using categorized estimates: rough (no cover), slimy (microalgae present but not visible), visible, 0.5 to 1 millimeter (0.02 to 0.04 inch), and 1 to 5 millimeters (0.04 to 0.20 inch).

Phytoplankton biomass was assessed by collecting 1 liter of water from the centroid of flow and filtering it onsite through a 45-μm glass-fiber filter. Filters then were treated in the same manner as benthic algae samples. Phytoplankton biomass analysis consisted of chlorophyll-*a* only.

Benthic invertebrate samples and fish assemblage surveys were done at each site according to TCEQ protocols (Texas Commission on Environmental Quality, 2005). Benthic invertebrates were collected throughout the reach using a 500-μm D-frame kick net for 5 minutes in loose (gravel to cobble) substrate. The sampled material was placed in a shallow pan and randomly subsampled with a 4-inch-square frame. Subsamples were sifted, and all visible benthic invertebrates were collected until a minimum count was reached. The minimum count in 2005 was 100 individuals, but a change in TCEQ protocols increased the minimum to 200 individuals in 2006. However, TCEQ protocols require complete picking of the last subsample after the minimum count is obtained. As a consequence, total benthic invertebrate sample sizes were similar for sites sampled in 2005 and 2006. Samples were preserved with 80-percent ethanol and shipped to a contract laboratory (Twin Oaks Biological in Dripping Springs, Tex.) for taxonomic identification and enumeration.

The fish community at each site was sampled by making a single electrofishing pass through the entire reach for a minimum of 900 seconds. Most streams were sampled with a backpack electrofishing unit, but two sites, Cypress Creek 2 and Cibolo Creek, were too deep for the backpack unit and were sampled with a barge electrofishing unit. Six effective seine hauls per reach were done to supplement the electrofishing. Seine hauls were done using a 15- by 6-foot minnow seine with a 0.25-square-inch mesh and distributed among stream geomorphic units (riffles, runs, and pools) according to their relative abundance in a reach. All fish that could be identified in the field were identified. Problematic species were preserved in 10-percent buffered formalin and delivered to Dr. Dean Hendrickson, Memorial Museum, University of Texas at Austin, for expert identification. Voucher specimens (specimens retained for reference) were collected for all species at each site. Small species were vouchered by preserving a representative specimen in 10-percent buffered formalin, and large species were vouchered by photographing a representative specimen.

Data Analysis

The NWQL uses two statistically determined values to reduce the possibility of reporting erroneous results when analyzing very low concentrations of water constituents. The first and smallest value is the long-term method detection level (LT-MDL), which designates the smallest concentration that can be reported reliably with only a 1-percent chance of reporting a false positive (Oblinger Childress and others, 1999). The second, larger value is the laboratory reporting level (LRL), which is calculated on the basis of the LT-MDL and designates the value that can be reported with only a 1-percent chance of reporting a false negative. When a NWQL analysis results in a value that falls between the LT-MDL and the LRL, the laboratory reports the analysis value, but qualifies it as estimated. Analysis values less than the LT-MDL are reported as less than the LRL and are considered censored values. Statistical analyses of nutrient concentrations in this report included estimated values as they were reported, but one-half the value of the LT-MDL was used when results were reported as less than the LRL. The majority of censored concentrations were related to phosphorus and ammonia nitrogen data in streams with low nutrient concentrations.

Before sampling, the 15 streams of the study were grouped on the basis of their potential for nutrient enrichment. Designated groups were (1) streams receiving wastewater effluent (WW), (2) streams classified as least-disturbed on the basis of low percentages of urban and agricultural land cover (LD), and (3) streams not receiving wastewater effluent but that were excluded from the least-disturbed category because site reconnaissance indicated a potential nutrient source—for example, a home septic system close to the stream (NWW).

All data were reviewed for errors and imported into a statistical software package (STATISTICA, 1999) for summary and analysis. Summary statistics such as means and medians were computed from the raw data.

Study variables were compared among stream groups with a nonparametric Kruskal-Wallis multiple comparison (KWMC) test on ranked data. The nonparametric KWMC test does pairwise comparisons between all possible pairs of groups to indicate whether there are differences among groups, and if so, which differ from others—that is, whether pairs of group mean ranks differ at a particular significance level (Helsel and Hirsch, 1992). Because this is a nonparametric test, the overall shapes of the individual variable distributions do not affect the power of the KWMC test to detect differences among groups.

Variables thought to be indirectly related, such as nutrient concentrations and benthic invertebrate taxa richness, were assessed using correlation, which is considered appropriate for variables that are not functionally dependant (Zar, 1998). Spearman's rank correlation was used to indicate the significance of relations because it is sensitive to all monotonic relations (y changes as x changes) regardless of whether they are linear or not.

Relations between variables thought to be directly related, such as nutrient concentrations and algal chlorophyll-*a*, were assessed using simple linear regression. Regression is useful in assessing the relations between variables when the magnitude of one variable is assumed to be determined by the magnitude of one or more other variables (Zar, 1998). In some cases multiple regression was used to more fully explore variable relations. The individual variables were checked for normality using a Shapiro-Wilk test (Zar, 1998) before regression, and any non-normal variables were transformed to better approximate a normal distribution. Some proportional variables were arcsine transformed by taking the arcsine of the square root of the proportion. All other variable transformations were $\log_{10}$ transformations.

Two levels of significance were used to classify and discuss the statistical results: one for the KWMC test to indicate whether there were differences among groups and another with Spearman's rank to indicate whether variables were related. Grouping of streams on the basis of their potential for nutrient enrichment produced small sample sizes in group WW and LD streams. In general, small sample sizes reduce the power of a statistical test to indicate a difference, if one exists; and in general, sample size is the most important component affecting statistical power (Park, 2004). Increasing the significance level of a statistical test increases the power of the test. Thus, to offset potential loss of statistical power of the KWMC tests because of small sample sizes, the significance level for those tests was set to a relatively lenient .10 (p-value thus less than or equal to .10). The downside of increasing the power by increasing the significance level is that the probability that the test will indicate that a group is different, when in fact it is not, is increased. For the Spearman's rank correlations, which used the full dataset (with a few exceptions) and therefore involved larger sample sizes, the significance level was set to the more common .05 (p-value thus less than or equal to .05).

Nutrient Conditions

Nutrient conditions in the small streams of the Edwards Plateau can be broadly understood in terms of their trophic state. Streams have been classified nationally into trophic states on the basis of generally accepted limits (boundaries) for total nitrogen and total phosphorus (U.S. Environmental Protection Agency, 2001) (table 2). In this study trophic-state classifications were dependent on the presence of wastewater. Group NWW and LD streams generally were classified as oligotrophic (low nutrient concentrations) on the basis of USEPA criteria. Group WW streams had larger nutrient concentrations and were classified as eutrophic (high nutrient concentrations) on the basis of USEPA criteria.

Trophic states also were reflective of the type of influence in the watersheds. Group WW streams were associated with more urbanized watersheds, but the most urbanized watershed, Bull Creek, does not receive wastewater effluent and had some

Table 2. U.S. Environmental Protection Agency recommended boundaries for trophic classification of streams (U.S. Environmental Protection Agency, 2001).

[mg/m^2, milligrams per square meter; mg/L, milligrams per liter]

Response variable (units)	Oligotrophic-mesotrophic boundary	Mesotrophic-eutrophic boundary
Mean benthic chlorophyll-*a* (mg/m^2)	20	70
Maximum benthic chlorophyll-*a* (mg/m^2)	60	200
Total nitrogen (mg/L)	.70	1.50
Total phosphorous (mg/L)	.025	.075

of the lowest nutrient concentrations. In addition, the one stream (South Grape Creek) that was not receiving wastewater effluent and that could be classified as eutrophic on the basis of a 2005 total nitrogen concentration of 2.55 milligrams per liter [mg/L]) has the highest percentage of agricultural land cover in its watershed. When the three group WW streams were removed from the dataset, total nitrogen concentrations were significantly correlated with the percentage of agricultural land cover in the watershed ($p = .0004$) (fig. 3).

Constituent Concentrations

Nitrogen

Total nitrogen concentrations for all streams ranged from 0.12 to 4.81 mg/L (table 3) with a median concentration of 0.35 mg/L. Total nitrogen concentrations for group WW streams ranged from 0.57 to 4.81 mg/L with a median of

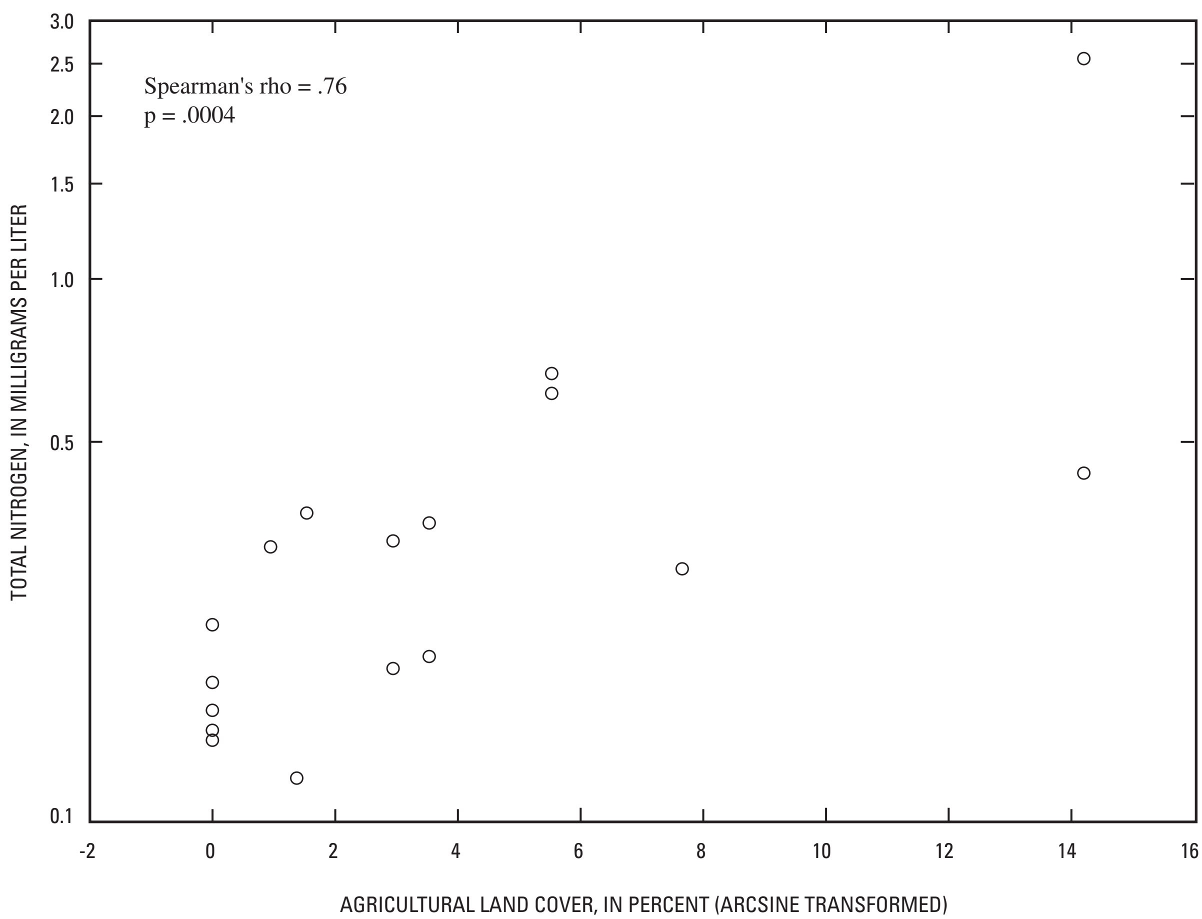


Figure 3. Relation between land cover and total nitrogen concentrations for all streams in the study, excluding those receiving wastewater effluent (group WW streams), Edwards Plateau, Central Texas, 2005–06.

Table 3. Summary of nutrient concentrations for selected small streams, Edwards Plateau, Central Texas, 2005–06.

[Total nitrogen and total phosphorus are measured values and might not equal sum of constituents. Values from single sample collected at summer low flow before biological sampling. Amm, ammonia; mg/L, milligrams per liter; KN, Kjeldahl nitrogen; NO_2+NO_3, nitrite plus nitrate; TN, total nitrogen; TP, total phosphorus; Ortho, orthophosphate; WW, wastewater effluent; NWW, no wastewater effluent; E, estimated; --, not sampled; LD, least disturbed]

Site short name (table 1)	Stream group	2005						2006					
		Amm (mg/L)	KN (mg/L)	NO_2+NO_3 (mg/L)	TN (mg/L)	TP (mg/L)	Ortho (mg/L)	Amm (mg/L)	KN (mg/L)	NO_2+NO_3 (mg/L)	TN (mg/L)	TP (mg/L)	Ortho (mg/L)
Bar	WW	0.024	0.41	4.67	4.81	1.09	1.06	0.032	0.47	0.172	0.57	1.37	1.359
Bru	WW	.032	.57	1.40	1.98	.057	.031	.018	.64	.423	1.05	.993	.914
Cib	WW	.039	.32	.420	.72	.340	.320	.041	.78	3.506	3.93	3.52	3.391
Bla	NWW	.016	.17	.532	.67	.004	[1].002	.046	.34	.300	.61	.006	[1].002
Bul	NWW	E.005	.17	.049	.16	.005	[1].002	[1].003	.12	.017	.15	[1].001	[1].002
Cy1	NWW	E.008	.11	.058	.14	.004	[1].002	--	--	--	--	--	--
Lic	NWW	.014	.28	[1].004	.23	.010	[1].002	--	--	--	--	--	--
Oni	NWW	E.009	.11	.022	.12	.004	.008	--	--	--	--	--	--
SGr	NWW	.022	.18	2.41	2.55	.007	[1].002	.051	.41	.068	.44	.0192	[1].002
SSG	NWW	.022	.34	.027	.37	.006	[1].002	--	--	--	--	--	--
BJo	LD	E.008	.11	.210	.32	E.002	[1].002	--	--	--	--	--	--
Cow	LD	[1].003	.23	[1].004	.18	E.003	.008	--	--	--	--	--	--
Cur	LD	.033	.14	.141	.29	.006	[1].002	--	--	--	--	--	--
Cy2	LD	E.006	.17	.068	.20	E.003	[1].002	.012	.23	E.015	.35	E.002	[1].002
SRo	LD	E.009	.15	.035	.19	E.002	.017	.018	.20	[1].004	.33	E.004	[1].002

[1] Value is one-half long-term method detection level, substituted for reported value when reported value less than laboratory reporting level.

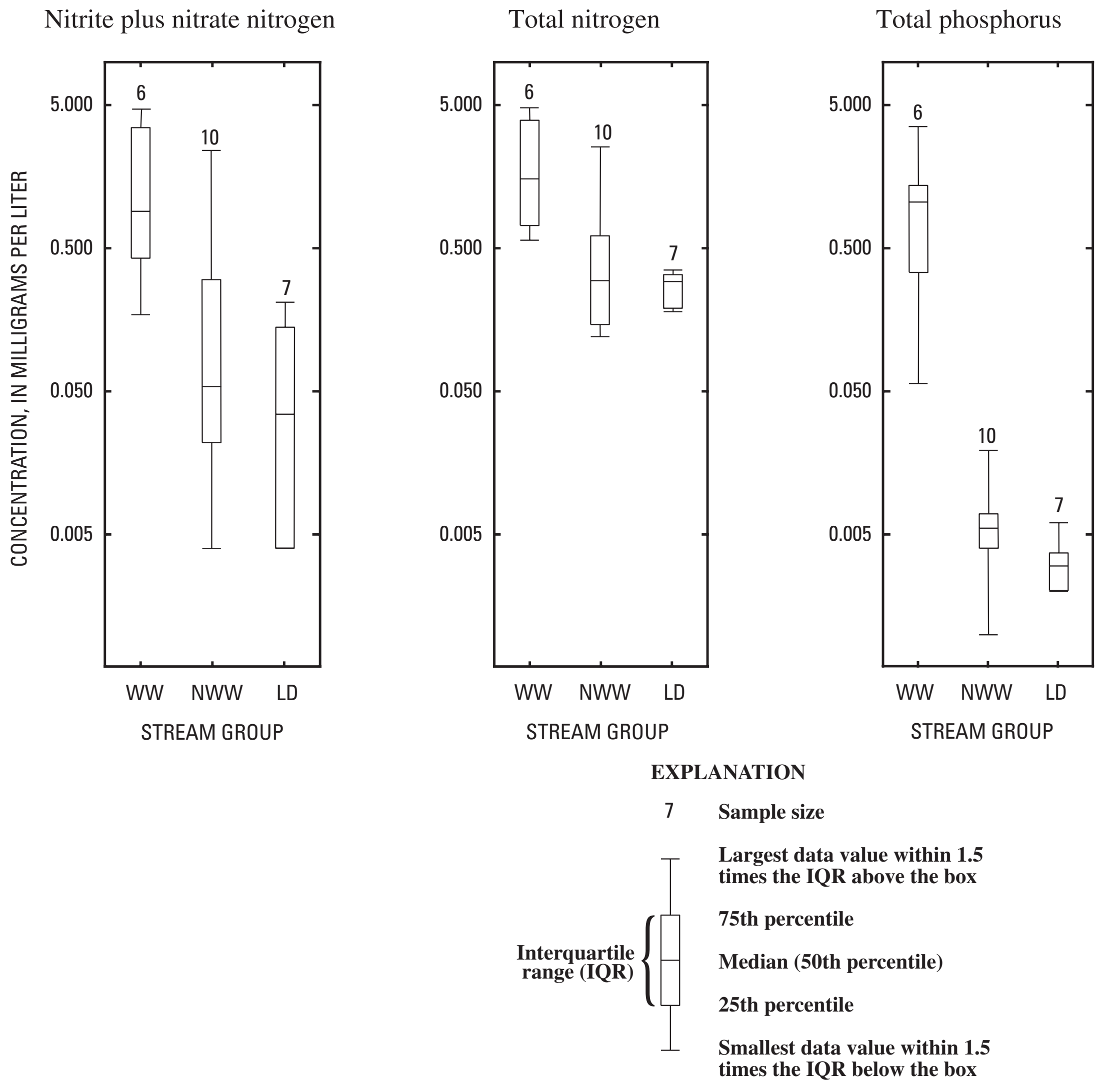


Figure 4. Distribution of nutrient concentrations by stream group, Edwards Plateau, Central Texas, 2005–06.

1.51 mg/L (fig. 4). Total nitrogen for group NWW streams ranged from 0.12 to 2.55 mg/L with a median of 0.30 mg/L. The largest total nitrogen concentration in group NWW streams (2.55 mg/L) was measured in the agriculturally influenced stream, South Grape Creek, in 2005 and was 3.8 times the next highest concentration. Total nitrogen for group LD streams ranged from 0.18 to 0.35 mg/L with a median of 0.29 mg/L.

The KWMC test comparing total nitrogen concentrations by group (table 4) indicated that group LD and NWW streams were significantly different from group WW streams ($p =$ .0113 and .0184, respectively). There was no significant difference between group LD and NWW streams ($p = 1.0$). The presence of wastewater effluent clearly elevates concentrations of total nitrogen in the small streams of the study.

Nitrogen occurs in many chemical forms in water but only the dissolved inorganic forms (nitrite, nitrate, and ammonium) are available for assimilation by most algae (Barsanti and Gualtieri, 2006). In contrast, organic nitrogen forms must first undergo mineralization (conversion to ammonium) before they are available to most algae. In aerobic waters the dominate form of inorganic nitrogen is nitrate (Stumm and Morgan, 1996) and nitrite plus nitrate can be considered a measure of the nitrogen directly available to algae.

Nitrite plus nitrate concentrations for all streams ranged from 0.004 to 4.67 mg/L (table 3) with a median concentration of 0.068 mg/L. Nitrite plus nitrate concentrations for group WW streams ranged from 0.172 to 4.67 mg/L with a median of 0.912 mg/L (fig. 4). Nitrite plus nitrate concentration in group NWW streams ranged from 0.004 to 2.41 mg/L with a median of 0.054 mg/L. Nitrite plus nitrate concentrations in group LD streams ranged from 0.004 to 0.21 mg/L with a median of 0.035 mg/L.

The KWMC test comparing nitrite plus nitrate concentrations by group indicated that group LD and NWW streams were significantly different from group WW streams

Table 4. Results for Kruskal-Wallis multiple comparison tests for differences among stream groups, Edwards Plateau, Central Texas, 2005–06.

[For each variable, groups classified with same letter are not significantly different at .10 level. WW, wastewater effluent; NWW, no wastewater effluent; LD, least disturbed; mg/L, milligrams per liter; mg/m², milligrams per square meter; g/m², grams per square meter; DO, dissolved oxygen]

Response variable (units)	Stream group		
	WW	NWW	LD
Nutrient measures			
Nitrite plus nitrate (mg/L)	(A)	(B)	(B)
Total nitrogen (mg/L)	(A)	(B)	(B)
Total phosphorus (mg/L)	(A)	(B)	(B)
Algal biomass measures			
Benthic algal chlorophyll-*a* (mg/m²)	(A)	(B)	(A, B)
Ash free dry weight (g/m²)	(A)	(B)	(B)
Phytoplankton chlorophyll-*a* (mg/L)	(A)	(A, B)	(B)
Composite score for macroalgae by area	(A)	(B)	(B)
Composite score for macroalgae by substrate	(A)	(A)	(A)
Composite score for macroalgae thickness	(A)	(A, B)	(B)
Diel dissolved oxygen and pH			
Diel DO mean (mg/L)	(A)	(A)	(A)
Diel DO minimum (mg/L)	(A)	(A)	(A)
Diel DO range (mg/L)	(A)	(A)	(A)
Diel pH minimum (standard units)	(A)	(A)	(A)
Diel pH maximum (standard units)	(A)	(B)	(A, B)
Diel pH range (standard units)	(A)	(B)	(A, B)
Benthic invertebrates			
Aquatic life use scores	(A)	(A)	(A)
Fish			
Aquatic life use scores	(A)	(B)	(A, B)

(p = .0100 and .0514, respectively). There was no difference between group LD and NWW streams (p = 1.0). The larger concentrations of nitrite plus nitrate in group WW streams reflect the effects of point-source inputs of wastewater effluent. Secondary treatment of wastewater promotes the conversion of ammonia and organic nitrogen to nitrate (U.S. Environmental Protection Agency, 2004).

Phosphorus

Phosphorus concentrations were measured as total phosphorus and as dissolved orthophosphate. Several total phosphorus samples were reported by the NWQL as estimated and one was reported as less than the LRL. Concentrations of total phosphorus ranged from 0.001 to 3.52 mg/L (table 3) with an overall median of 0.006 mg/L. Similar to nitrogen, total phosphorus concentrations were largest in streams receiving wastewater (group WW). Median total phosphorus concentrations by group were 1.04 mg/L for group WW streams, 0.006 mg/L for group NWW streams, and 0.003 mg/L for group LD streams (fig. 4). Unlike total nitrogen, total phosphorus was only slightly elevated in the agriculturally influenced South Grape Creek.

The KWMC test comparing total phosphorus concentrations by group (table 4) yielded results similar to those for total nitrogen; group LD and NWW streams were significantly different from group WW streams (p = .0002 and .0270, respectively) but not from each other (p = .2632).

In freshwater, phosphorus can exist in various dissolved ionic forms, both organic and inorganic, and can be sorbed to suspended sediment. However, dissolved inorganic phosphorus, primarily in the form of orthophosphate, is the principal form used by algae and aquatic plants. Orthophosphate concentrations in streams not influenced by wastewater (groups NWW and LD) were quite low; concentrations of orthophosphate in 82 percent of samples were below the LT-MDL of 0.004 mg/L. Medians for orthophosphate were not computed for these two groups of streams. Detectable concentrations of orthophosphate were in only two samples in the group LD streams. The median orthophosphate concentration for group WW streams was 0.987 mg/L and the median orthophosphate contribution to total phosphorus was about 95 percent.

Wastewater discharges are well known contributors of phosphorus to receiving waters (U.S. Environmental Protection Agency, 2004); thus the increased concentrations of total phosphorous and orthophosphate in the streams receiving wastewater effluent are not surprising.

U.S. Environmental Protection Agency Recommended Criteria

Historical datasets compiled by the USEPA and the USGS were used to develop estimates of reference-condition nutrient concentrations in the Edwards Plateau (U.S. Environmental Protection Agency, 2001). Reference-condition estimates for nutrient forms were the 25th percentile of all data (across all sites and seasons) reported for the Edwards Plateau between 1990 and 2000.

Nutrient concentrations measured in the group LD streams were very similar to the USEPA reference-condition estimates for nutrient concentrations in the Edwards Plateau (U.S. Environmental Protection Agency, 2001) (table 5). Mean concentrations of Kjeldahl nitrogen and total nitrogen were consistent with the USEPA estimates, whereas the mean for nitrite plus nitrate was slightly less than the USEPA estimate. The mean concentration of total phosphorus measured in the group LD streams was about 40 percent of the USEPA estimate. However, both estimated (0.008 mg/L) and measured

(0.003 mg/L) total phosphorus concentrations were quite small, and mean total phosphorus for the group LD streams was within the range of error for the NWQL measurement method at the time of this study (LRL = 0.004 mg/L and LT-MDL = 0.002 mg/L). In addition, the USEPA estimates were made using all available data from the entire Edwards Plateau ecoregion and data from all seasons of the year, whereas the group LD streams were concentrated in the Balcones Canyonlands subregion and were only sampled in the summer. Inclusion of least-disturbed streams from the entire ecoregion and data from all seasons of the year might increase mean concentrations of total phosphorus.

Nutrient Limitation

The concept of single nutrient limitation of algal growth is based on the theory that the rate of production is constrained by the nutrient that is in shortest supply. Understanding which nutrient is limiting in a system could be beneficial to the development of nutrient criteria and help focus nutrient reduction efforts. In general, phosphorus is considered limiting to benthic algae when the atomic ratio of nitrogen to phosphorus (N:P ratio) is greater than 20:1, and nitrogen is considered limiting at N:P ratios less than 10:1 (Borchardt, 1996). Limitation is difficult to discern for ratios in the 10–20:1 range, and nutrients might be co-limiting.

Nutrient limitation in the 15 study streams could be divided between streams receiving wastewater effluent and streams not receiving wastewater effluent. Streams that did not receive wastewater effluent (groups NWW and LD) had N:P ratios that ranged from 35:1 to 558:1, which indicates phosphorus limitation. One caution, however—nutrient ratios can only provide a general indication of nutrient limitation, and algal assays are needed to clearly identify nutrient limitation (S.D. Porter, Texas State University, written commun., 2007). In contrast, group WW streams generally had large phosphorus concentrations and low N:P ratios that ranged from 0.6:1 to 6.7:1. Low N:P ratios are common in streams that receive wastewater effluent because of the high phosphorus content of effluent (Hem, 1992). However, the results noted do not necessarily indicate nitrogen limitation in these streams. In general when nutrients are in excess, the supply ratio is irrelevant, and nutrient limitation is not a factor in algal production (Borchardt, 1996).

Biological Conditions

Algae

The issues most often associated with excessive nutrient concentrations in streams generally are related to the growth of algae and other aquatic plants. Algal growth commonly is assessed by measurements of biomass (mass of algal organic matter per unit area of substratum or volume of water). Two methods were used to estimate the biomass of benthic algae at each site: chlorophyll-*a* and AFDW. A transect-based method was used to estimate benthic algal abundance. The water-column chlorophyll-*a* concentration also was measured to estimate phytoplankton biomass for comparison and to evaluate its potential as an indicator of nutrient enrichment.

Table 5. U.S. Environmental Protection Agency nutrient concentration estimates for reference streams in the Edwards Plateau (U.S. Environmental Protection Agency, 2001) and mean measured concentrations from selected least-disturbed streams, Edwards Plateau, Central Texas, 2005–06.

[In milligrams per liter]

Constituent	U.S. Environmental Protection Agency estimated reference concentration	Mean measured concentration from least-disturbed sites
Total Kjeldahl nitrogen	0.18	0.18
Nitrite + nitrate	.090	.068
Total nitrogen	.270	.265
Total phosphorous	.008	.003

Chlorophyll-*a* and Ash-Free Dry Weight

Median benthic algal chlorophyll-*a* across all sites was 40.8 milligrams per square meter (mg/m^2) and ranged from 11.2 to 148 mg/m^2 (table 6). Medians for benthic algal chlorophyll-*a* were lowest in group NWW streams (29.5 mg/m^2), intermediate in group LD streams (40.8 mg/m^2), and highest in group WW streams (77.9 mg/m^2) (fig. 5). The KWMC tests indicated group WW and NWW streams were significantly different (p = .0759) (table 4). However, group LD streams were not significantly different from either group NWW streams (p = 1.0) or group WW streams (p = .6415). The lack of significant difference for group LD streams is the result of the wide distribution in rank values for benthic algal chlorophyll-*a* in the group LD streams, which might be related to streamflow. In general, benthic algal chlorophyll-*a* samples with low ranks were associated with relatively low streamflow, but the two group LD streams with benthic chlorophyll-*a* values ranked relatively high, Cy2 and Cur, were characterized by relatively high streamflow similar to flow in the streams receiving wastewater effluent.

Trophic-state boundaries for temperate streams based on mean benthic algal chlorophyll-*a* values have been developed by Dodds and others (1998) and recommended for use in nutrient criteria development (U.S. Environmental Protection Agency, 2001) (table 2). Values used to develop benthic algal chlorophyll-*a* criteria generally were seasonal means obtained over a 2–3 month period in a single year (Dodds and others, 1998). Although algal chlorophyll-*a* samples in this study are

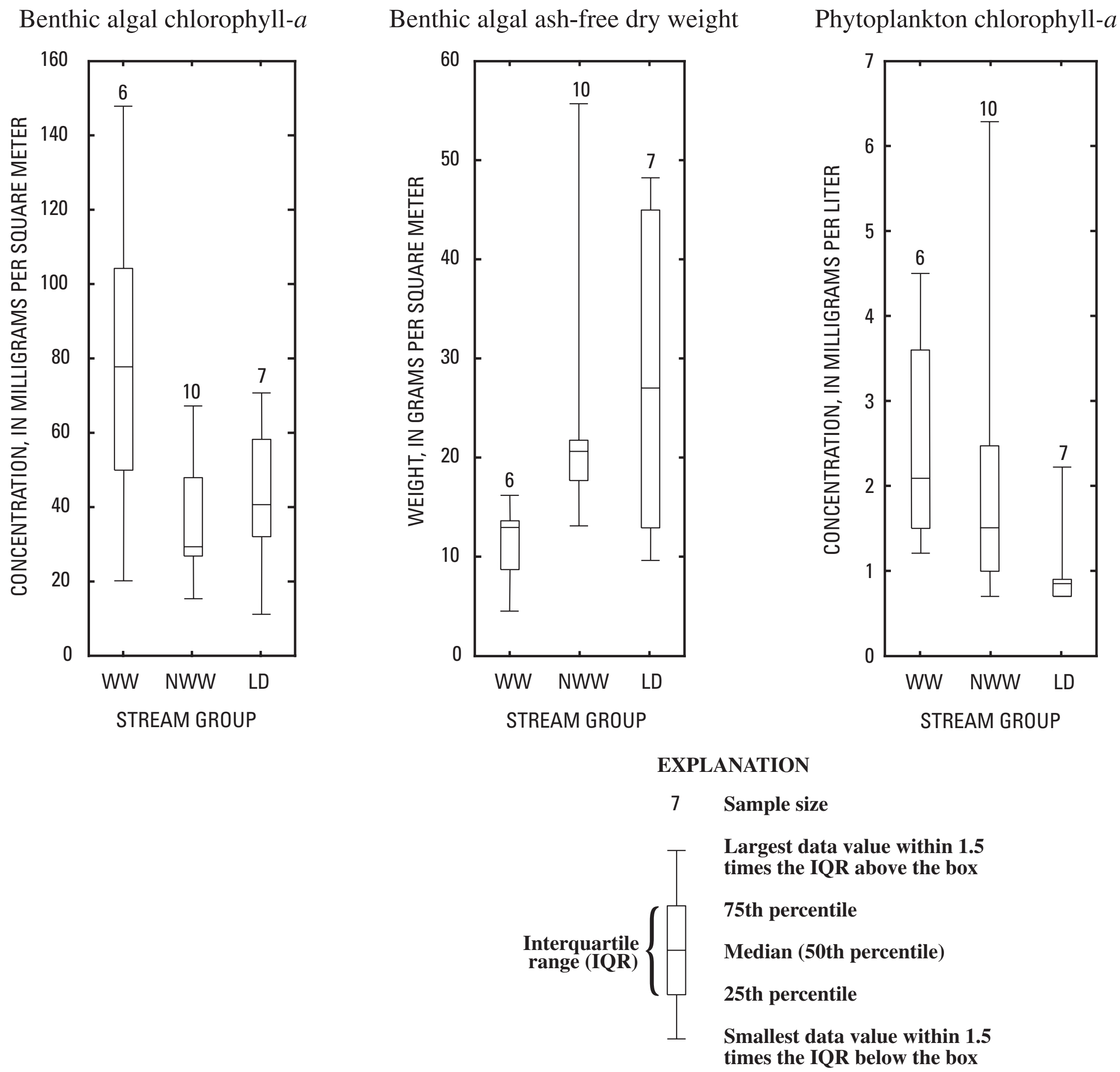


Figure 5. Distribution of benthic algal biomass by stream group, Edwards Plateau, Central Texas, 2005–06.

one-time late-summer samples and might not be strictly comparable to criteria for mean values, a comparison to published benthic algal chlorophyll-*a* criteria might still be informative.

Trophic states for streams not receiving wastewater (groups NWW and LD) evaluated using criteria based on mean benthic chlorophyll-*a* classifications generally were higher than those indicated by nutrient concentrations. The majority of samples from group NWW and LD streams (76.5 percent) were classified as mesotrophic. Three samples, two from group NWW streams and one from a group LD stream, were classified as oligotrophic, and one sample from a group LD stream was classified as eutrophic. In contrast, trophic classifications based on benthic chlorophyll-*a* were reduced (mesotrophic) for three group WW streams in comparison to those indicated by nutrient concentrations. Group WW streams generally had relatively high concentrations of chlorophyll-*a*, but trophic-state classifications based on benthic chlorophyll-*a* were not as clearly defined by wastewater as those based on measured nutrient concentrations (see “Nutrient Conditions” section).

AFDW commonly is used in conjunction with chlorophyll-*a* to assess benthic algal biomass. In this study the AFDW results were not consistent with those for chlorophyll-*a*. Correlation between AFDW and benthic algal chlorophyll-*a* across all samples was poor (p = .5055). However, if sites were categorized by the presence of wastewater effluent, AFDW was strongly correlated with benthic algal chlorophyll-*a* in streams receiving wastewater (group WW) (p = .0083) and streams not receiving wastewater (groups NWW and LD combined) (p = .0178) (fig. 6). The mean ratio of benthic chlorophyll-*a* to AFDW in group WW streams (6.6 mg/m^2:1 gram per square meter [g/m^2]) was more than four times the ratio in streams not affected by wastewater (1.6 mg/m^2:1 g/m^2).

Table 6. Summary of chlorophyll-*a* and ash-free dry weight results for selected small streams, Edwards Plateau, Central Texas, 2005–06.

[Chl-*a*, chlorophyll-*a*; mg/m^2, milligrams per square meter; AFDW, ash-free dry weight; g/m^2, grams per square meter; mg/L, milligrams per liter; WW, wastewater effluent; NWW, no wastewater effluent; --, not sampled; LD, least disturbed]

Site short name (table 1)	Stream group	2005			2006		
		Benthic Chl-*a* (mg/m^2)	AFDW (g/m^2)	Phytoplankton Chl-*a* (mg/L)	Benthic Chl-*a* (mg/m^2)	AFDW (g/m^2)	Phytoplankton Chl-*a* (mg/L
Bar	WW	52.8	12.7	4.5	20.1	4.50	3.6
Bru	WW	148	13.6	1.5	50.0	8.70	1.2
Cib	WW	103	13.0	1.9	104	16.2	2.3
Bla	NWW	67.2	55.7	1.1	30.5	21.2	6.3
Bul	NWW	47.9	18.0	1.9	26.9	21.3	2.5
Cy1	NWW	33.6	17.7	.70	--	--	--
Lic	NWW	15.4	13.1	.90	--	--	--
Oni	NWW	26.9	21.7	1.1	--	--	--
SGr	NWW	53.6	25.9	1.9	28.4	20.1	2.5
SSG	NWW	18.4	15.4	1.0	--	--	--
BJo	LD	40.8	41.9	.80	--	--	--
Cow	LD	32.0	27.1	.70	--	--	--
Cur	LD	58.2	12.9	.90	--	--	--
Cy2	LD	70.8	48.2	.90	49.8	45.0	.90
SRo	LD	35.9	17.6	.70	11.2	9.60	2.2

Measured AFDW as an estimate of algal biomass is subject to bias when non-algal organic material such as detritus and heterotrophic organisms compose a substantial part of the sample (Stevenson, 1996). However, the chlorophyll-*a*/AFDW ratios in this study are the reverse of what might be expected. Wastewater discharges are a substantial source of organic carbon (U.S. Environmental Protection Agency, 2004), and heterotrophic organisms, which are dependant on organic carbon, would be expected to proliferate in wastewater-influenced streams and thus decrease chlorophyll-*a*/AFDW ratios. The larger chlorophyll-*a*/AFDW ratios in group WW streams in this study might be related to streamflow. Substrates in the streams not receiving wastewater, where water velocities were relatively slow, were commonly covered by a thick layer of calcium carbonate precipitate. In contrast, substrates in streams where flow velocities were maintained by wastewater effluent generally were clear. The layer of calcium carbonate precipitate might have affected the AFDW results by (1) entraining non-algal organic material or (2) providing a habitat matrix for the growth of heterotrophic microfauna such as bacteria, fungi, and microinvertebrates. A significant positive correlation between instantaneous discharge and chlorophyll-*a*/AFDW ratios ($p = .0141$) supports this hypothesis.

Measured AFDW ranged from 4.50 to 55.7 g/m^2 (table 6) with an overall median of 17.7 g/m^2. Group medians for AFDW were reversed from those of chlorophyll-*a*; group WW streams were lowest (12.9 g/m^2), followed by group NWW streams (20.7 g/m^2), and then group LD streams (27.1 g/m^2) (fig. 5). The KWMC test comparing AFDW by group indicated that group LD and NWW streams were significantly different from group WW streams ($p = .0423$ and .0251, respectively) but not significantly different from each other (table 4).

Phytoplankton chlorophyll-*a* concentrations ranged from 0.70 to 6.3 mg/L (table 6) with an overall median of 1.2 mg/L. Group medians were smallest in group LD streams (0.85 mg/L), intermediate in group NWW streams (1.5 mg/L), and largest in group WW streams (2.1 mg/L) (fig. 5). The KWMC test results for phytoplankton chlorophyll-*a* showed a significant difference (table 4) between group LD and WW streams ($p = .0156$). Group NWW streams were not significantly different from either group LD or WW streams ($p = .8593$).

Algal Abundance Estimates

Scores for estimates of composite macroalgae cover by area (MacA) ranged from 64 to 417 (appendix 1) with an

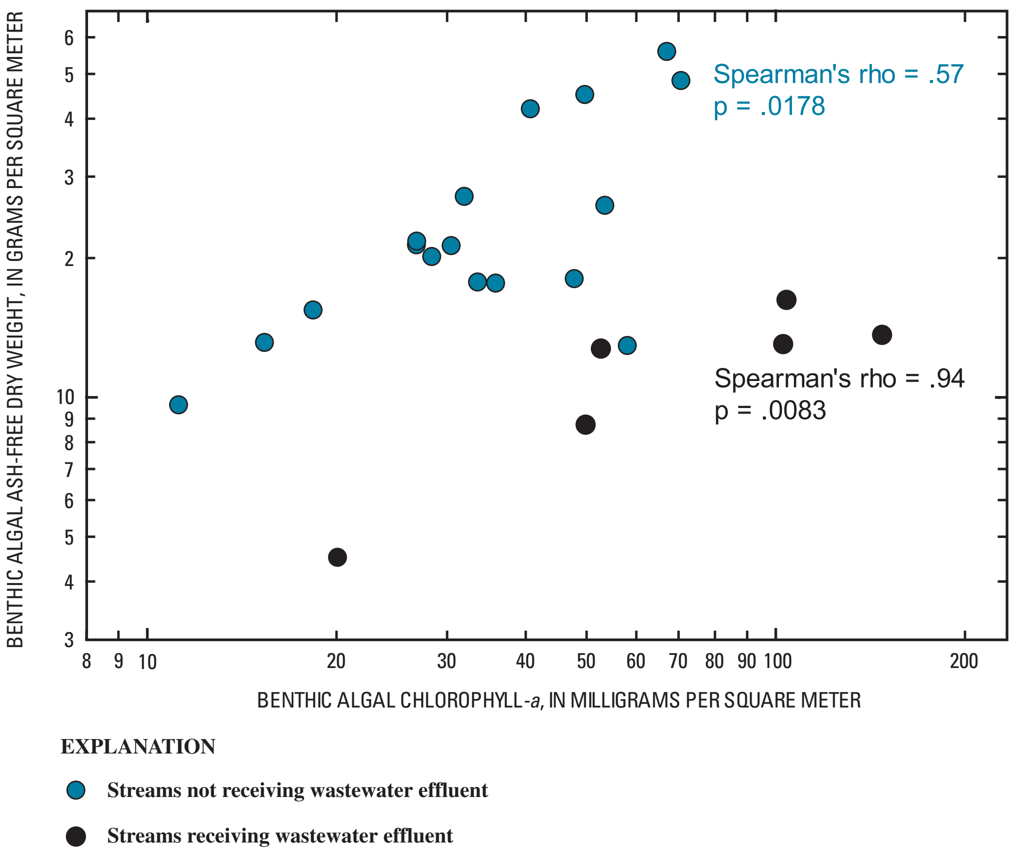


Figure 6. Relations between benthic algal chlorophyll-*a* and benthic algal ash-free dry weight in streams receiving wastewater effluent and streams not receiving wastewater effluent, Edwards Plateau, Central Texas, 2005–06.

overall median of 224. Median scores for MacA were highest in group WW streams (320) followed by group LD streams (224) and group NWW streams (156) (fig. 7). The KWMC test on MacA scores indicated that group LD and NWW streams were significantly different from group WW streams ($p = .0539$ and .0390, respectively), but not from each other ($p = 1.0$) (table 4).

Scores for estimates of composite macroalgae cover by loose substrate (MacS) ranged from 52 to 294 (appendix 2) with an overall median of 174. The distribution of median scores for MacS were similar to those for MacA and were highest in group WW streams (200), followed by group LD streams (158), and group NWW streams (118) (fig. 7). However, there were no significant differences in MacS among stream groups (table 4).

MacA and MacS were strongly correlated ($p < .0000$) and tended to have the largest scores in streams with high nutrient concentrations (high-nutrient streams) with cobble substrates and the smallest scores in streams with low nutrient concentrations (low-nutrient streams) with primarily bedrock bottoms, although these distinctions were not absolute. For example, Cow Creek, a low-nutrient stream with low water velocities and an open canopy had a relatively high MacA score (311); and Brushy Creek, a wastewater-influenced stream with relatively high water velocities, high nutrients concentrations, and a closed canopy, had relatively low MacA scores (131 in 2005 and 271 in 2006).

Composite estimates of the thickness of microalgae cover on loose substrate (MicT) showed a pattern opposite that of macroalgae cover and generally were larger in low-nutrient streams. Values for MicT ranged from 19 to 151 (appendix 3) with an overall median of 93. Medians for MicT by group were smallest for group WW streams (60), intermediate for group NWW streams (95), and largest for group LD streams (101) (fig. 7). KWMC tests for MicT indicated group WW and LD streams were significantly different ($p = .0558$) (table 4). Group NWW streams were not significantly different from either group LD streams ($p = 1.0$) or group WW streams ($p = .3170$).

Dense macroalgae cover in nutrient-enriched conditions might have shaded benthic substrate and reduced microalgae growth. In addition, lower flow velocities in streams not receiving wastewater effluent tended to favor the buildup of the calcium carbonate precipitate common in the limestone-dominated streams of the Edwards Plateau. Distinguishing algal thickness from calcium carbonate precipitate often

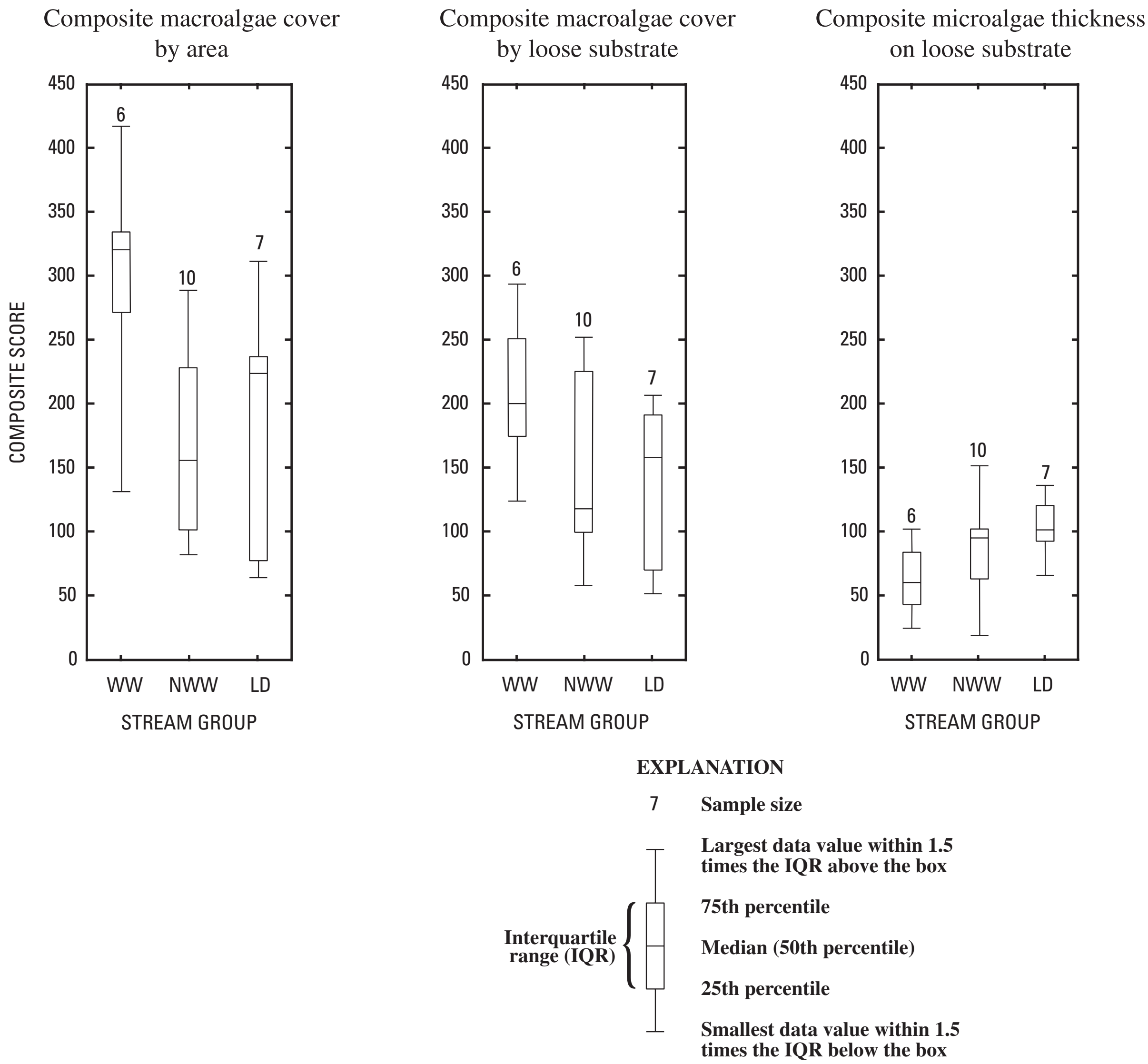


Figure 7. Distribution of composite scores for algal cover estimates by stream group, Edwards Plateau, Central Texas, 2005–06.

was difficult and might have influenced the microalgae results by confounding microalgae thickness estimates.

Duplicate algal abundance estimates were done by a second person at Bull Creek and Barons Creek in 2005 to assess the repeatability of the algae estimates. The largest mean difference in duplicate composite scores, 19 points, was for MacA (appendix 1). Mean differences between composite scores for MacS (appendix 2) and MicT (appendix 3) were 7.3 and 4.6 points, respectively. The largest single point difference (28) was between composite scores for MacA at Bull Creek, which had a base score of 289 and a duplicate score of 317; this was a 10-percent difference. The largest percentage differences between duplicate composite scores for MacS and MicT were 5.4 and 7.8 percent, respectively. Differences between estimates are minimal and likely do not influence the findings of the report.

Algal Biomass Estimates and Nutrients

Benthic Chlorophyll-*a*

Simple regression indicated relations between log transformed benthic chlorophyll-*a* (logChl) and total nutrients were relatively weak; regression of logChl on log transformed total nitrogen (logTN) yielded a significant coefficient of determination (R^2) of .26 ($p = .0132$), whereas regression of logChl on log transformed total phosphorus (logTP) was not significant ($R^2 = .11$, $p = .1141$) (table 7). In contrast, log transformed nitrite plus nitrate (logN+N), was strongly related to logChl ($R^2 = .50$, $p = .0002$) (fig. 8A). Multiple regression using logTP and logTN or logN+N did not account for any more variation in logChl than logN+N alone.

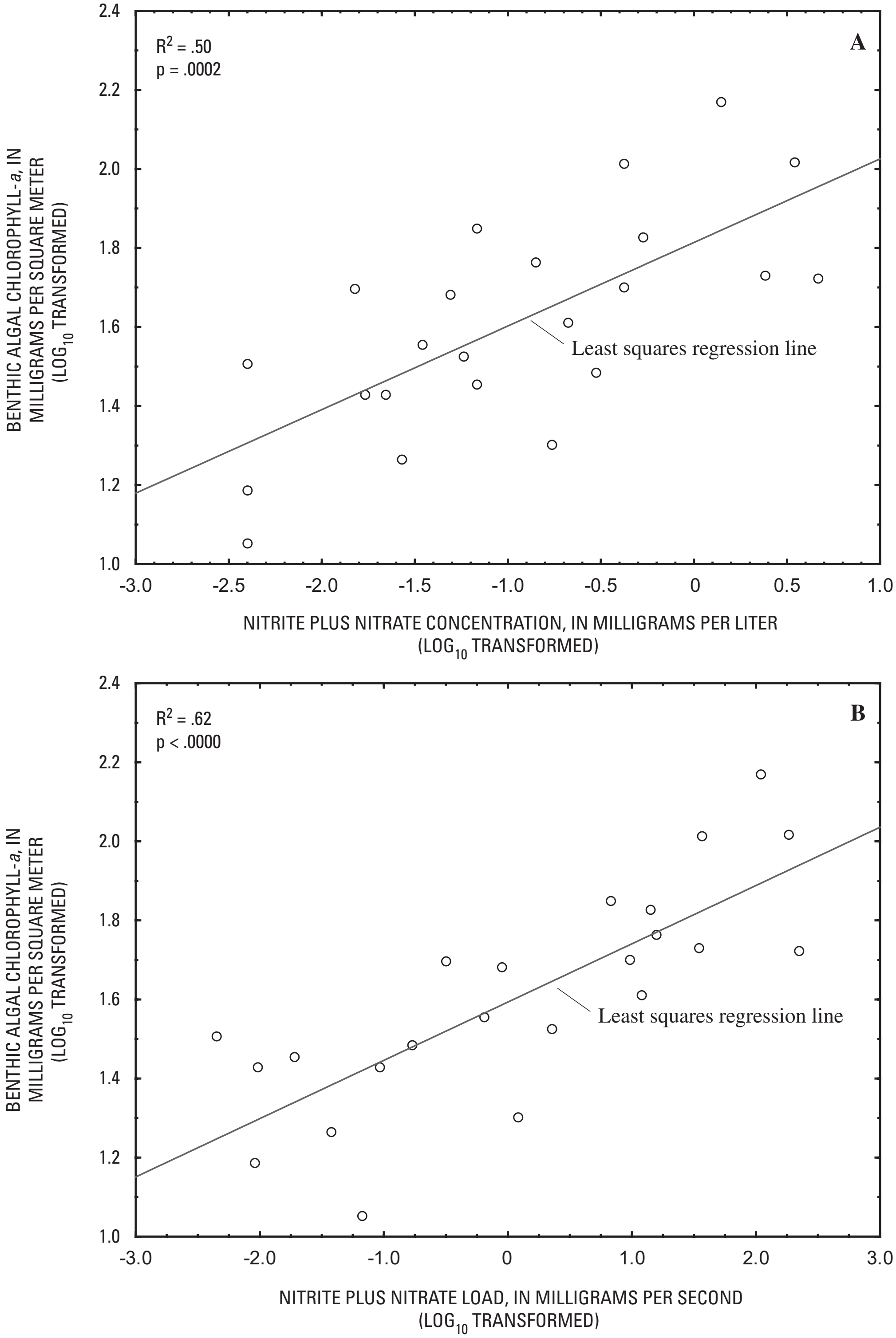


Figure 8. Regression relation between log transformed benthic algal chlorophyll-*a* and (A) log transformed nitrite plus nitrate concentrations and (B) log transformed nitrite plus nitrate instantaneous loads in selected small streams, Edwards Plateau, Central Texas, 2005–06.

Some evidence indicates that water movement can enhance the uptake of nutrients by benthic algae (Borchardt, 1996). Thus nutrient load of a stream, the product of nutrient concentration (mass/volume) and discharge (volume/time), might better describe the relation between benthic algae and nutrients (Borchardt, 1996). Total nitrogen, total phosphorus, and nitrite plus nitrate concentrations (in milligrams per liter) were multiplied by the instantaneous discharge (in cubic

Table 7. Regression analyses for variables considered directly related to nutrient concentrations in selected small streams, Edwards Plateau, Central Texas, 2005–06.

[Results presented for log transformed ambient nutrient concentrations. Results significant at 5-percent level ($p < .05$) in bold. R^2, coefficient of determination for regression model; p, probability of Type I error in statistical results; >, greater than; %, percent]

Response variable	R^2	p
Log transformed total nitrogen		
Log transformed benthic chlorophyll-*a*	**.26**	**.0132**
Log transformed ash-free dry weight	.04	.3331
Log transformed phytoplankton chlorophyll-*a*	**.26**	**.0137**
Composite score for macroalgae by area	.11	.1163
Macroalgae by area >75%[1]	**.28**	**.0108**
Composite score for macroalgae by substrate	.07	.2282
Macroalgae by substrate >75%[1]	**.23**	**.0217**
Composite score for microalgae thickness	.14	.0801
Log transformed total phosphorus		
Log transformed benthic chlorophyll-*a*	.11	.1141
Log transformed ash- free dry weight	**.38**	**.0019**
Log transformed phytoplankton chlorophyll-*a*	**.23**	**.0222**
Composite score for macroalgae by area	**.36**	**.0025**
Macroalgae by area >75%[1]	**.59**	**<.0000**
Composite score for macroalgae by substrate	.17	.0516
Macroalgae by substrate >75%[1]	**.37**	**.0022**
Composite score for microalgae thickness	**.28**	**.0088**
Log transformed nitrite+nitrate		
Log transformed benthic chlorophyll-*a*	**.50**	**.0002**
Log transformed ash-free dry weight	.00	.7649
Log transformed phytoplankton chlorophyll-*a*	**.18**	**.0408**
Composite score for macroalgae by area	.09	.1711
Macroalgae by area >75%[1]	**.20**	**.0337**
Composite score for macroalgae by substrate	.05	.3195
Macroalgae by substrate >75%[1]	.13	.0978
Composite score for microalgae thickness	**.22**	**.0258**

[1]Arcsine transformed data.

Table 8. Regression analyses for variables considered directly related to nutrient loads in selected small streams, Edwards Plateau, Central Texas, 2005–06.

[Results presented for log transformed nutrient instantaneous load estimates. Results significant at 5-percent level ($p < .05$) in bold. R^2, coefficient of determination for regression model; p, probability of Type I error in statistical results; >, greater than; %, percent]

Response variable	R^2	p
Log transformed total nitrogen instantaneous load		
Log transformed benthic chlorophyll-*a*	**.58**	**<.0000**
Log transformed ash-free dry weight	.00	.8455
Log transformed phytoplankton chlorophyll-*a*	.00	.7987
Composite score for macroalgae by area	.05	.2900
Macroalgae by area >75%[1]	**.20**	**.0333**
Composite score for macroalgae by substrate	.00	.7879
Macroalgae by substrate >75%[1]	.05	.2879
Composite score for microalgae thickness	**.22**	**.0245**
Log transformed total phosphorus instantaneous load		
Log transformed benthic chlorophyll-*a*	**.39**	**.0013**
Log transformed ash-free dry weight	.15	.0660
Log transformed phytoplankton chlorophyll-*a*	.05	.3046
Composite score for macroalgae by area	**.24**	**.0188**
Macroalgae by area >75%[1]	**.48**	**.0003**
Composite score for macroalgae by substrate	.07	.2328
Macroalgae by substrate >75%[1]	**.19**	**.0360**
Composite score for microalgae thickness	**.35**	**.0032**
Log transformed nitrite+nitrate instantaneous load		
Log transformed benthic chlorophyll-*a*	**.62**	**<.0000**
Log transformed ash-free dry weight	.00	.9044
Log transformed phytoplankton chlorophyll-*a*	.02	.5697
Composite score for macroalgae by area	.05	.2946
Macroalgae by area >75%[1]	**.19**	**.0402**
Composite score for macroalgae by substrate	.01	.7350
Macroalgae by substrate >75%[1]	.06	.2756
Composite score for microalgae thickness	**.26**	**.0121**

[1]Arcsine transformed data.

feet per second) measured at the time of sampling and by a conversion factor of 28.32, for consistent units, to estimate instantaneous loads (in milligrams per second) for these constituents. These estimates are instantaneous loads computed for the time of sampling only and do not reflect long-term load estimates. However, given the stable condition of these streams during and before the sampling period (see discussion in “Water Sampling” section) these estimates were considered adequate to characterize the nutrient and flow conditions affecting the benthic algae before sampling.

Regression of logChl on nutrient load estimates yielded strong statistical relations for all of the individual constituents. LogChl was significantly related to log transformed total nitrogen load (logTNL) ($R^2 = .58$, $p < .0000$) and log transformed total phosphorus load (logTPL) ($R^2 = .39$, $p = .0013$) (table 8). However, the relation between logChl and log transformed nitrite plus nitrate load (logN+NL) was again the strongest ($R^2 = .62$, $p < .0000$) (fig. 8B). Multiple regression using logTPL and logTNL or logN+NL again did not account for any more variation in logChl than logN+N alone.

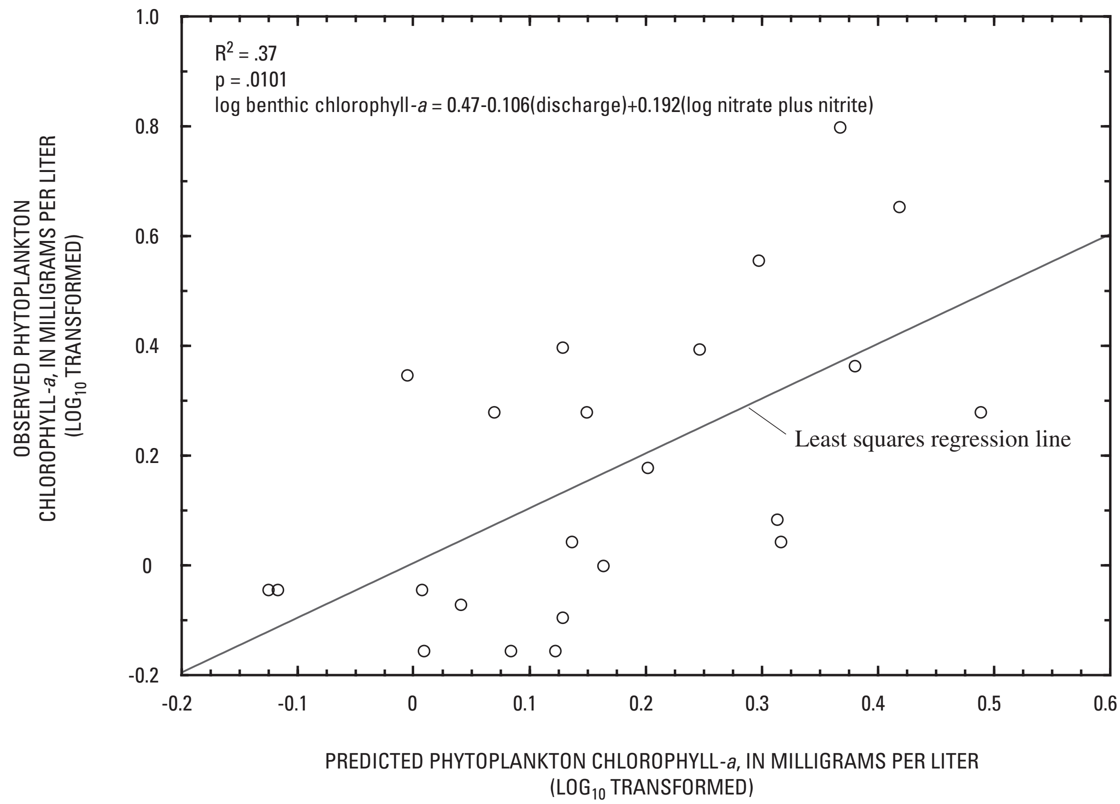


Figure 9. Regression relation between log transformed phytoplankton chlorophyll-*a* observed and predicted values in selected small streams, Edwards Plateau, Central Texas, 2005–06.

A regression was done without group WW streams to evaluate the relation between nutrient load estimates and benthic chlorophyll-*a* at the lowest nutrient concentrations. Relations between the nitrogen measures, logTNL and logN+NL, and logChl were weakened but still relatively robust (R^2 = .46, p = .0026 and R^2 = .55, p = .0006, respectively), whereas the relation between logTPL and logChl was strengthened (R^2 = .41, p = .0059).

Analysis of nutrient ratios indicated phosphorus limitation in the streams not receiving wastewater effluent, but statistical analysis indicated that benthic chlorophyll-*a* is more closely related to dissolved nitrogen concentrations and streamflow. These results reflect the effects of water movement as well as an important distinction in nutrient limitation with regard to measures of biomass such as chlorophyll-*a*: The supply rate of a limiting nutrient, along with light, controls the rate of algal growth, but the total amount of biomass production, or standing crop, is more closely linked to the total quantity of nutrients available (Borchardt, 1996) and the time since the last disturbance event (Biggs, 2000). Downstream transport of nutrients represents a virtually endless quantity for biomass production. Thus it might be possible for a flowing system to be growth-rate limited but still develop a large standing crop prior to a disturbance event. In addition, some algae have the ability to store phosphorus in the cell, and they require nitrogen in greater concentrations than phosphorus. As a consequence they are capable of reaching growth-saturation concentrations at relatively low ambient concentrations of phosphorus (Bothwell, 1988; Horner and others, 1990). The ability to store phosphorus coupled with continuous delivery, provided streams are flowing, might make natural phosphorus concentrations in the small streams of the Edwards Plateau sufficient, and long-term biomass accrual might be more closely tied to nitrogen concentrations.

Benthic Ash-Free Dry Weight

Regression indicated that log transformed AFDW (logAFDW) was significantly related to logTP (R^2 = .38, p = .0019), but the relation was negative, indicating that as phosphorus concentrations increased algal biomass decreased (table 7). These results appear to be related to the disparity in the ratio of chlorophyll-*a* to AFDW discussed in the “Chlorophyll-*a* and Ash-Free Dry Weight” section. No significant relation was indicated between logAFDW and logTN or logN+N. In addition, nonsignificant relations were indicated when regressions were done without group WW streams.

Phytoplankton Chlorophyll-*a*

Total nutrient measures include organic components that might be affected by suspended algae concentrations; therefore phytoplankton chlorophyll-*a* was assessed only against the

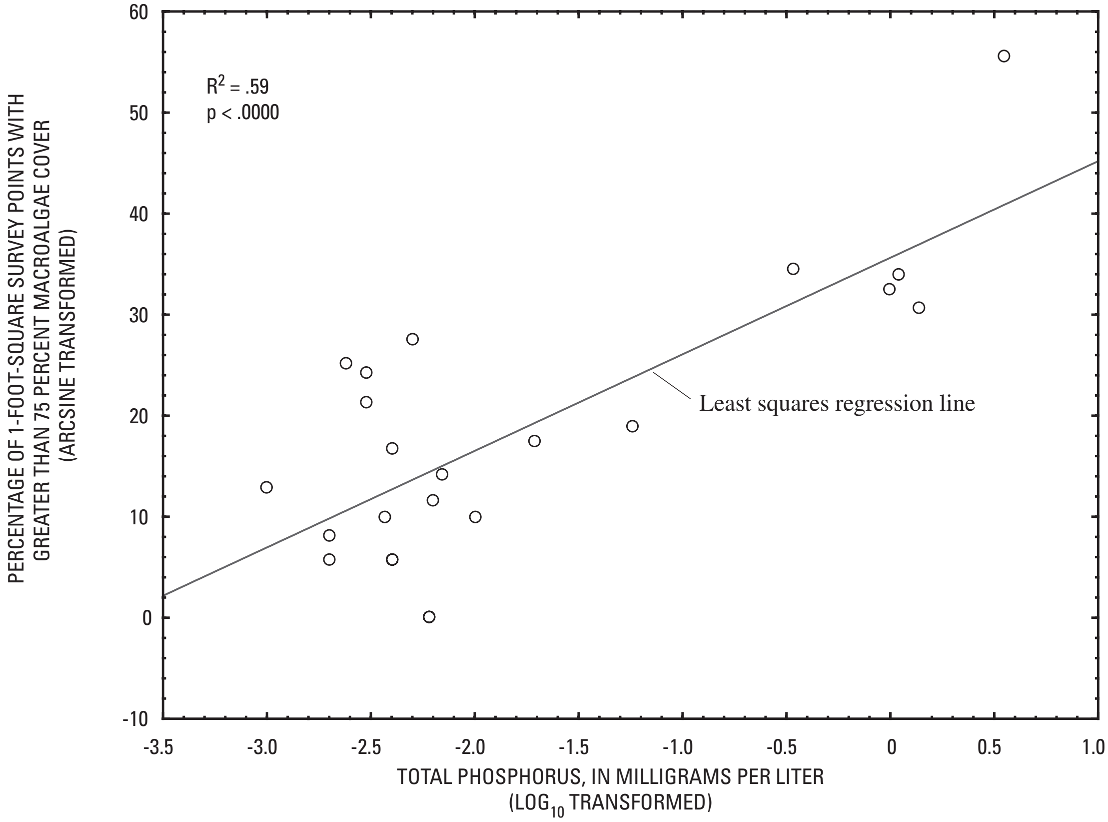


Figure 10. Regression relation between log transformed total phosphorus concentration and percentage of survey points with greater than 75 percent macroalgae cover (arcsine transformed) in selected streams, Edwards Plateau, Central Texas, 2005–06.

dissolved form of nitrogen (nitrite plus nitrate). Log transformed phytoplankton chlorophyll-*a* (logChl_P) was significantly related to logN+N, but the relation was again fairly weak ($R^2 = .18$, $p = .0408$) (table 7). However, multiple regression using logN+N and discharge as a separate variable yielded a relation that was stronger than regression with logN+N alone ($R^2 = .37$, $p = .0101$) (fig. 9). The equation produced by multiple regression explains 37 percent of variation in phytoplankton across streams and indicates that logChl_P increased as a function of increased nitrite plus nitrate concentration and decreased streamflow:

$$\text{logChl\_P} = 0.47 - 0.106(\text{discharge}) + 0.192(\text{logN+N}).$$

The regression equation was applied without group WW streams to assess the ability of the predictor variables to estimate logChl_P at the lowest nutrient concentrations. Without group WW streams the relation was weakened and not significant at the .05 level ($R^2 = .31$, $p = .0749$).

Streamflow in the small streams of the Edwards Plateau commonly is low in the summer months, even in the streams receiving wastewater effluent. Low flow in streams in which nutrient concentrations are elevated likely results in increased phytoplankton production in pools and in relatively slow-moving runs. The subsequent downstream movement of phytoplankton would account for relatively high water-column chlorophyll-*a* concentrations.

Algal Abundance Estimates

Regression indicated the composite scores for MacA were significantly related to logTP ($R^2 = .36$, $p = .0025$) (table 7). However, regression using only the highest MacA cover category (MacA greater than 75 percent [>75%]) produced a stronger relation with logTP ($R^2 = .59$, $p < .0000$). Graphical analysis of the relation between MacA >75% and logTP indicated that the statistical relation was heavily influenced by the substantially higher total phosphorus concentrations in many of the group WW streams (fig. 10). Regression of MacA >75% on logTP using only streams that do not receive wastewater effluent (groups LD and NWW) resulted in a weak statistical relation ($R^2 = .12$, $p = .1758$). These results indicate (1) high levels of macroalgae are associated with increased total phosphorus concentrations in streams receiving wastewater effluent, (2) the macroalgae survey by area is effective for identifying nuisance macroalgae growth associated with conditions of high nutrient enrichment, and (3) the macroalgae survey by area cannot, in its present form, discriminate between nutrient concentrations under low-nutrient conditions.

Table 9. Texas Commission on Environmental Quality criteria for diel (24-hour) dissolved oxygen aquatic life use categories in freshwater.

[mg/L, milligrams per liter]

Aquatic life use category	Dissolved oxygen criteria, mean/minimum (mg/L)
Exceptional	6.0/4.0
High	5.0/3.0
Intermediate	4.0/3.0
Limited	3.0/2.0

Scores for MicT were significantly related to logTP (R^2 = .28, p = .0088; table 7) and logTPL, (R^2 = .35, p = .0032; table 8), but the relations were negative, which indicates that as total phosphorus increased benthic microalgae decreased. These results reflect the influence of shading and flow velocities discussed in the “Algal Abundance Estimates” section (under “Algae” section). Additionally, the relations between MicT and the measures of total phosphorus are the inverse of the relation between logChl and nutrient concentrations. Benthic chlorophyll-*a* samples tended to be collected in shallow, fast-moving environments, such as riffles, where macroalgal growth was limited and calcium carbonate precipitate was not an issue. In contrast, microalgae thickness was estimated across the entire reach and included relatively slow water velocities where calcium carbonate precipitate was thick and relatively deep environments where macroalgal growth was abundant. When the issues associated with microalgae thickness estimates are taken into account, benthic algal cholorphyll-*a* more accurately reflects nutrient conditions in these small streams.

Diel Dissolved Oxygen and pH, and Relations Between Diel Dissolved Oxygen, pH, Nutrients, and Algae

TCEQ classifies water bodies into ALU categories partially on the basis of criteria for mean and minimum DO concentrations over a 24-hour (diel) period (table 9). DO measurements from the Hydrolab Minisondes were used to compute mean and minimum diel DO values for each stream. Mean diel DO concentrations ranged from 4.88 to 7.62 mg/L (table 10) with an overall median of 6.28 mg/L. Minimum diel DO concentrations ranged from 2.35 to 6.86 mg/L with an overall median of 4.61 mg/L.

Table 10. Summary of mean, minimum, maximum, and range of diel dissolved oxygen concentrations in selected small streams, Edwards Plateau, Central Texas, 2005–06.

[mg/L, milligrams per liter; WW, wastewater effluent; NWW, no wastewater effluent; --, not sampled; LD, least disturbed]

Site short name (table 1)	Stream group	Diel dissolved oxygen, 2005 (mg/L)				Diel dissolved oxygen, 2006 (mg/L)			
		Mean	Minimum	Maximum	Range	Mean	Minimum	Maximum	Range
Bar	WW	6.98	4.54	11.88	7.34	5.10	2.35	10.85	8.49
Bru	WW	6.41	5.42	7.91	2.49	6.00	4.13	8.21	4.08
Cib	WW	6.90	4.12	11.80	7.68	4.91	3.15	8.03	4.87
Bla	NWW	5.36	4.57	6.34	1.77	6.90	5.02	10.65	5.63
Bul	NWW	5.06	3.74	8.05	4.31	6.72	5.52	7.70	2.18
Cy1	NWW	6.81	6.35	7.37	1.02	--	--	--	--
Lic	NWW	5.89	3.95	9.28	5.33	--	--	--	--
Oni	NWW	6.05	4.64	7.47	2.83	--	--	--	--
SGr	NWW	7.62	6.86	8.18	1.32	6.94	3.54	11.38	7.84
SSG	NWW	4.88	3.94	6.40	2.46	--	--	--	--
BJo	LD	7.38	5.63	9.86	4.23	--	--	--	--
Cow	LD	6.15	2.87	10.81	7.94	--	--	--	--
Cur	LD	--	--	--	--	--	--	--	--
Cy2	LD	7.07	6.36	8.19	1.83	6.69	5.42	8.50	3.08
SRo	LD	5.76	4.89	8.04	3.15	5.81	5.03	7.86	2.82

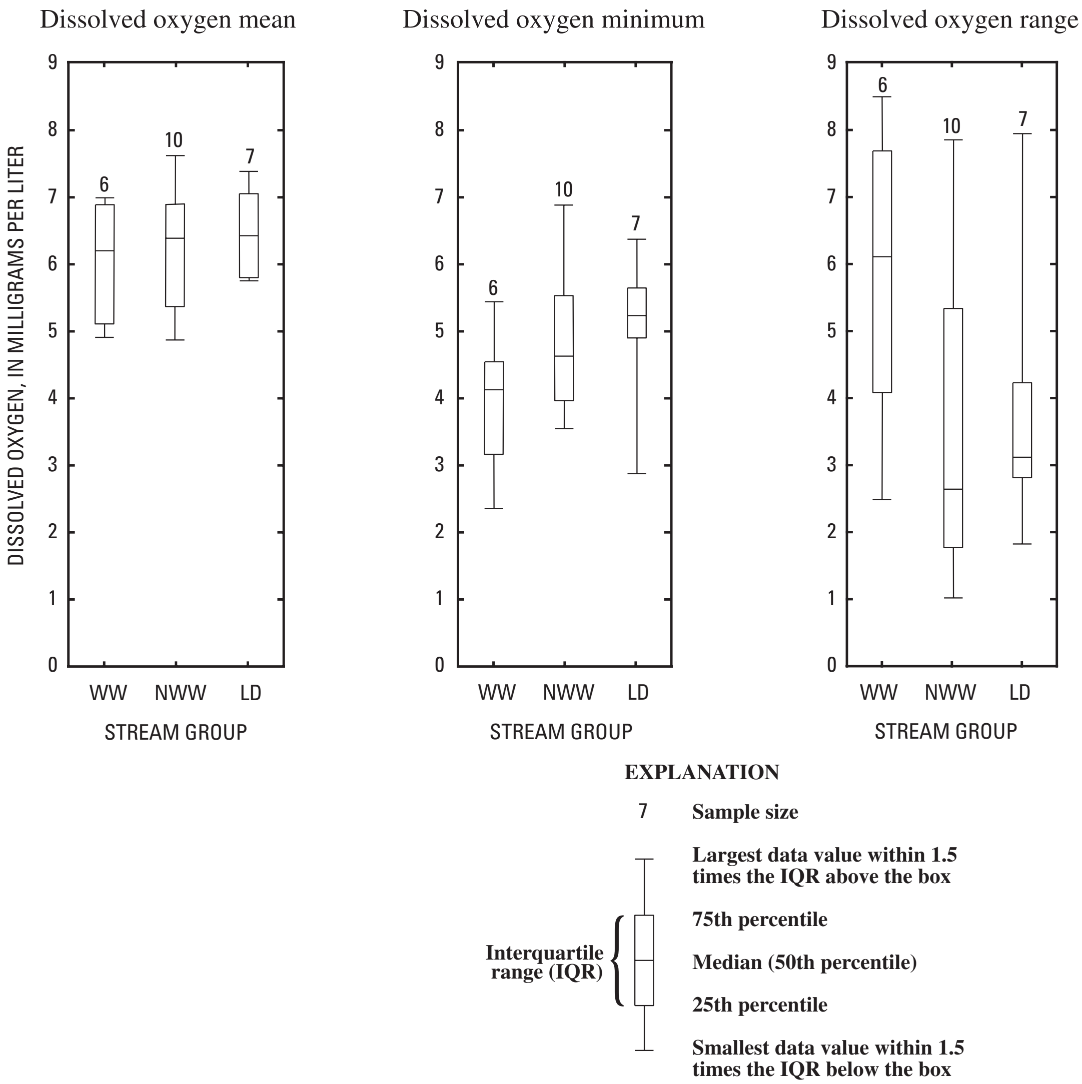


Figure 11. Distribution of mean, minimum, and range of diel dissolved oxygen concentrations by stream group, Edwards Plateau, Central Texas, 2005–06.

Median values for mean and minimum diel DO by stream group were largest for group LD streams (6.42 and 5.23 mg/L respectively), followed by group NWW streams (6.39 and 4.61 mg/L), and group WW streams (6.20 and 4.13 mg/L) (fig. 11). The KWMC tests for mean and minimum diel DO indicated no significant differences among groups (table 4).

When mean and minimum diel DO concentrations for each stream were compared to TCEQ criteria, all group WW streams scored as Exceptional in 2005. However, two group WW streams (Barons Creek and Cibolo Creek) had reduced scores in 2006 (Limited and Intermediate, respectively) because of low minimum diel DO concentrations. Group NWW streams generally scored as Exceptional or High. The single exception was South Fork San Gabriel River, which scored as Intermediate in 2005 based on low mean and minimum diel DO concentrations. All of the group LD streams scored as Exceptional or High except for Cow Creek, which scored as Limited because of a low minimum diel DO concentration. The low diel DO scores in Cow Creek and South Fork San Gabriel River were associated with very low flows in which mixing and aeration were reduced. Similarly, the reductions in diel DO scores in group WW streams between 2005 and 2006 are associated with reduced flows.

TCEQ does not have general pH criteria for classifying waters into ALU categories, but they do have site-specific criteria for minimum and maximum diel pH (6.5 and 9.0, respectively) in some classified waters. Hydrolab Minisonde pH measurements were used to compute minimum and maximum diel pH for all streams. Minimum diel pH ranged from 7.24 to 8.07 (table 11) with a median across all sites of 7.73. Maximum diel pH ranged from 7.49 to 8.99 with a median across all sites of 8.06. Medians for minimum and maximum diel pH by stream group were largest for group WW streams (7.87 and 8.38, respectively), followed by group LD streams (7.75 and 8.17), and group NWW streams (7.64 and 7.91) (fig. 12). No streams exceeded the minimum or maximum criteria for diel

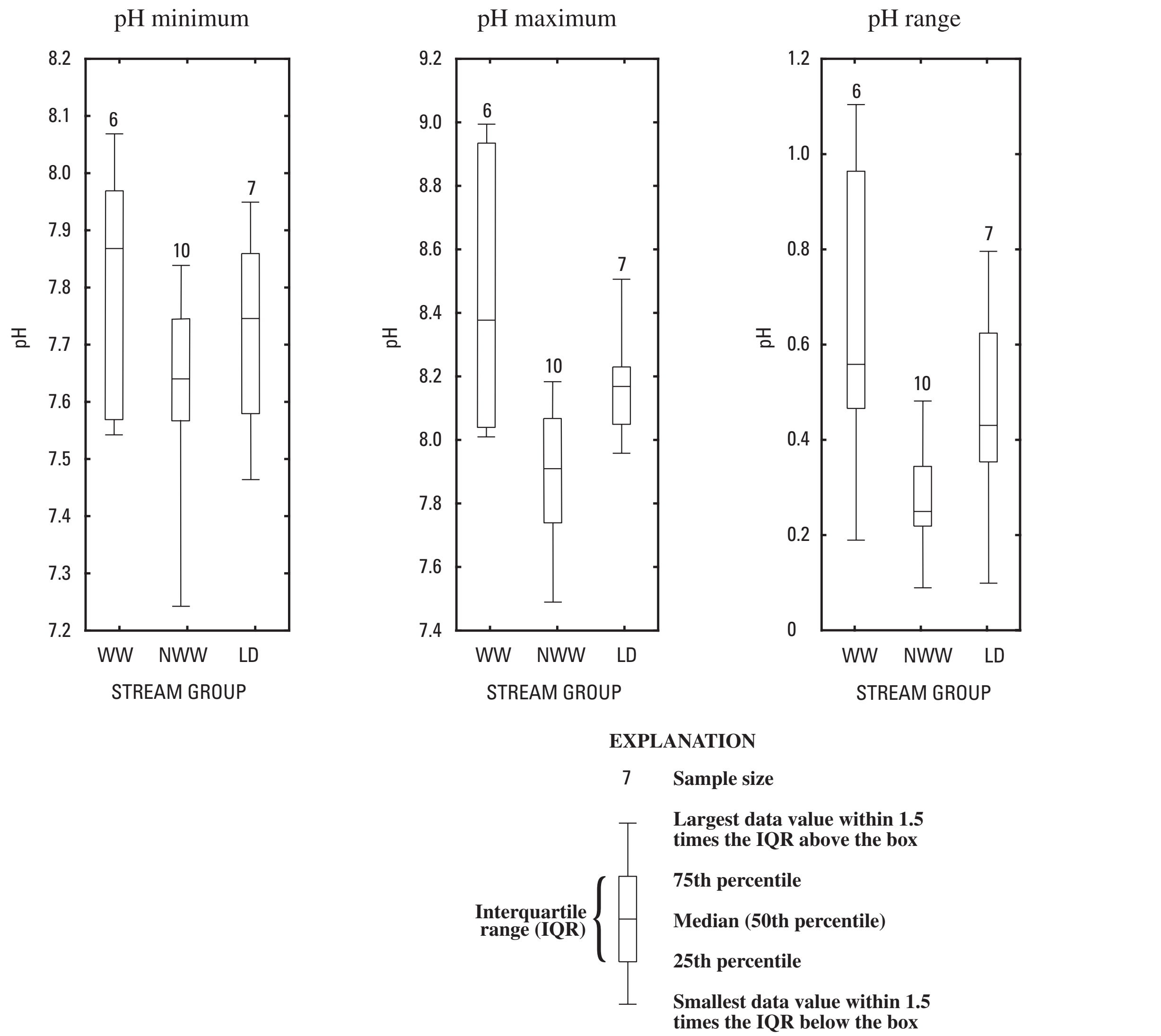


Figure 12. Distribution of minimum, maximum, and range of diel pH by stream group, Edwards Plateau, Central Texas, 2005–06.

pH, although Barons Creek was borderline with a maximum of 8.94 in 2005 and 8.99 in 2006. The KWMC tests for minimum and maximum diel pH (table 4) indicated no significant differences among groups for minimum diel pH. Maximum diel pH for group NWW streams was significantly different from that for group WW streams (p = .0491). Maximum diel pH was not significantly different between group WW and LD streams.

Dissolved oxygen and pH concentrations typically followed a diel pattern that increased during the day and decreased at night. The magnitude of this pattern, which generally is attributed to the dominance of photosynthetic processes during the day and respiration at night, can be an indicator of aquatic plant productivity (Allen, 1995). Diel DO range varied from 1.02 to 8.49 mg/L (table 10) with an overall median of 3.62 mg/L. Medians for diel DO range by stream group were largest for group WW streams (6.12 mg/L), followed by group LD streams (3.12 mg/L), and group NWW streams (2.65 mg/L) (fig. 11). The KWMC test for diel DO range indicated no significant differences among groups (table 4). Diel pH range varied from 0.09 to 1.11 (table 11) with an overall median of 0.35. Medians for diel pH range by stream group were largest for group WW streams (0.56), followed by group LD streams (0.43), and group NWW streams (0.25) (fig. 12). The KWMC test for diel pH range indicated that group WW streams were significantly different from group NWW streams (p = .0323). Diel pH range was not significantly different between group WW and LD streams.

Spearman's rank correlation indicated no strong relations between diel DO means and any of the measures of nutrient concentration (appendix 4), algal biomass (appendix 5), or algal abundance (appendix 6). However, total phosphorus was negatively correlated with diel DO minimums (p = .0134) and positively correlated with diel DO ranges (p = .0446) (appendix 4). Diel DO relations with the composite algal abundance estimate MacA were similar to those with total

Table 11. Summary of mean, minimum, maximum, and range of diel pH in selected small streams, Edwards Plateau, Central Texas, 2005–06.

[WW, wastewater effluent; NWW, no wastewater effluent; --, site not sampled; LD, least disturbed]

Site short name (table 1)	Stream group	Diel pH, 2005				Diel pH, 2006			
		Mean	Minimum	Maximum	Range	Mean	Minimum	Maximum	Range
Bar	WW	8.38	7.97	8.94	0.97	8.35	7.89	8.99	1.11
Bru	WW	7.93	7.85	8.04	.19	8.37	8.07	8.68	.61
Cib	WW	7.75	7.57	8.08	.51	7.73	7.54	8.01	.47
Bla	NWW	7.44	7.39	7.49	.10	7.86	7.75	8.09	.35
Bul	NWW	7.81	7.74	7.97	.23	7.98	7.81	8.07	.26
Cy1	NWW	7.7	7.65	7.74	.09	--	--	--	--
Lic	NWW	7.32	7.24	7.57	.33	--	--	--	--
Oni	NWW	7.7	7.59	7.83	.24	--	--	--	--
SGr	NWW	7.8	7.63	7.85	.22	7.77	7.57	8.05	.48
SSG	NWW	7.95	7.84	8.19	.35	--	--	--	--
BJo	LD	8.02	7.86	8.23	.37	--	--	--	--
Cow	LD	8.03	7.71	8.51	.80	--	--	--	--
Cur	LD	--	--	--	--	--	--	--	--
Cy2	LD	7.99	7.95	8.05	.10	7.94	7.79	8.14	.36
SRo	LD	7.57	7.47	7.96	.50	7.83	7.58	8.21	.63

phosphorus—that is, a negative correlation with diel DO minimums (p = .0259) and a positive correlation with diel DO range (p = .0459) (appendix 6). The correlation between diel pH range and MacA (p = .0402) was the only significant relation indicated for the diel pH measures.

Results of statistical analyses indicate that relatively low diel DO minimums and relatively high diel DO ranges observed in some of the study streams are associated with increased total phosphorus concentrations and increased macroalgae abundance. The influence of algae and other aquatic plants on DO and pH concentrations is a well-known phenomenon (Allen, 1995). Photosynthesis occurring during daylight hours consumes carbon dioxide, which raises pH concentrations, and photosynthesis generates oxygen, which increases DO concentrations. At night, when respiration and decomposition processes dominate, the effect is reversed. In addition, algal abundance can affect the magnitude of diel changes in DO and pH (Odum, 1956; Allen, 1995).

Benthic Invertebrates, and Relations Between Benthic Invertebrates, Nutrients, and Algae

The State of Texas evaluates benthic invertebrate kick-net samples with a set of metrics that describes structural and functional aspects of the invertebrate community (Texas Commission on Environmental Quality, 2005). Individual metrics are scored and summed to determine a composite benthic invertebrate ALU score for a stream. A stream benthic invertebrate ALU score is described by ranking scores as Limited (less than 22), Intermediate (22–28), High (29–36), and Exceptional (greater than 36). The majority of the benthic invertebrate samples scored as High with four samples scoring as Exceptional (table 12). Exceptional scores were recorded in streams in group LD (one stream twice) and group WW (two streams once). An ALU score of Intermediate was recorded in one group LD stream and in one group NWW stream. At the time of sampling, flow in the streams with Intermediate scores was extremely low and suitable riffle habitat was lacking. All group WW streams scored as High or Exceptional, which indicates no degradation of the benthic invertebrate community associated with wastewater effluent. Furthermore, the median ALU score for group WW streams (34) was larger than the medians for group LD streams (33) and group NWW streams (32) (fig. 13). However, stream composite benthic invertebrate ALU scores were not strong indicators of nutrient enrichment; the KWMC test indicated benthic invertebrate ALU scores were not significantly different among the three stream groups (table 4).

Spearman's rank correlation indicated that total nitrogen was the only nutrient measure showing a significant correlation with benthic invertebrate ALU scores (p = .0155)

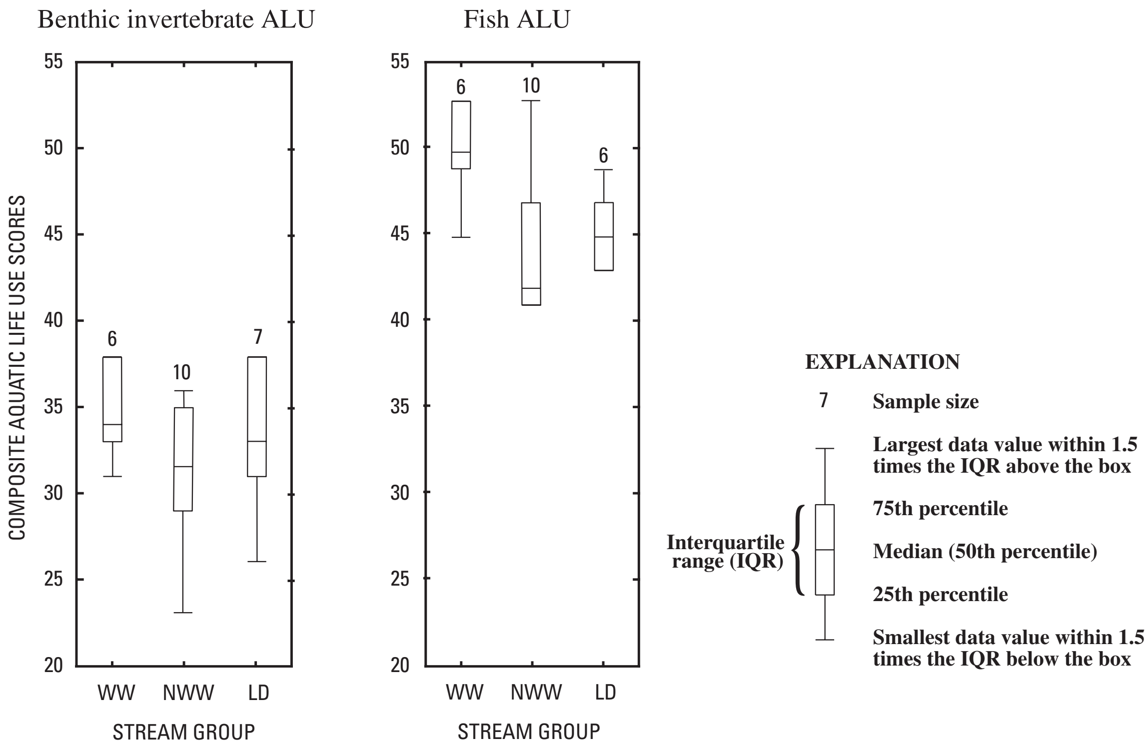


Figure 13. Distribution of aquatic life use (ALU) scores for benthic invertebrates and fish by stream group, Edwards Plateau, Central Texas, 2005–06.

(appendix 4). Several individual metrics that contribute to the composite benthic invertebrate ALU scores were correlated with both total nitrogen and dissolved nitrogen, but total nitrogen showed stronger relations. Three metrics generally thought to increase with improving water quality were positively correlated with total nitrogen: taxa richness ($p = .0420$), Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa richness ($p = .0268$), and the ratio of intolerant to tolerant taxa ($p = .0096$) (appendix 4). Two metrics that contribute to benthic invertebrate ALU scores and generally thought to decrease with improving water quality were negatively correlated with total nitrogen: the Hilsenhoff Biotic Index ($p = .0141$) and the percentage of Tricoptera as Hydropsychidae ($p = .0054$) (appendix 4).

Graphical analysis of the biological data indicated that two samples (Barons Creek in 2005 and Cibolo Creek in 2006) heavily influenced some of the statistical results between benthic invertebrate metrics and nutrient concentrations (fig. 14). When these samples were removed from the dataset, correlations between taxa richness and total nitrogen and between EPT taxa richness and total nitrogen were strengthened ($p = .0010$ and $p < .0000$, respectively).

Nutrient enrichment commonly is associated with increased benthic invertebrate biomass (Hart and Robinson, 1990; Mundie and others, 1991), but documented increases in benthic invertebrate species richness in response to nutrient enrichment are few. Local richness of benthic invertebrate species has been positively correlated with total nitrogen concentrations in oligotrophic headwater streams at high latitudes (Heino and others, 2003). However, investigations in community ecology indicate unimodal (increasing to a peak and then decreasing) relations between ecosystem productivity and species richness are common (Rosenzweig and Abransky, 1993; Mittelbach and others, 2001). Positive relations between nutrient concentrations and measures of algal biomass in this study indicate increasing productivity in these streams in response to nutrient enrichment. Positive relations between benthic invertebrate taxa richness, EPT taxa richness, and total nitrogen observed in this study might represent the ascending limb of a unimodal relation between productivity and species richness. More research is required to determine whether the two suspected outliers (Barons Creek in 2005 and Cibolo Creek in 2006) (fig. 14) represent natural variation, sampling error, or the descending limb of a unimodal relation between benthic invertebrate species richness and nitrogen concentrations.

Changes in nutrient concentrations also were correlated with changes in benthic invertebrate functional feeding group percentages. The percentage of grazing invertebrates (scrapers) was strongly positively correlated with both total nitrogen ($p = .0008$) and total phosphorus ($p = .0002$) (appendix 4). A significant multiple regression result using logTN and logTP

Table 12. Summary of benthic invertebrate and fish aquatic life use scores and rankings for selected small streams, Edwards Plateau, Central Texas, 2005–06.

[ALU, aquatic life use score; WW, wastewater effluent; H, high; E, exceptional; NWW, no wastewater effluent; I, intermediate; --, not sampled; LD, least disturbed; >, greater than]

Site short name (table 1)	Stream group	2005				2006			
		Benthic invertebrates[1]		Fish[2]		Benthic invertebrates[1]		Fish[2]	
		ALU	Rank	ALU	Rank	ALU	Rank	ALU	Rank
Bar	WW	35	H	49	H	38	E	51	H
Bru	WW	38	E	53	E	33	H	49	H
Cib	WW	33	H	53	E	31	H	45	H
Bla	NWW	36	H	41	I	34	H	41	I
Bul	NWW	29	H	41	I	23	I	41	I
Cy1	NWW	29	H	47	H	--	--	--	--
Lic	NWW	35	H	41	I	--	--	--	--
Oni	NWW	30	H	43	H	--	--	--	--
SGr	NWW	35	H	53	E	31	H	49	H
SSG	NWW	32	H	43	H	--	--	--	--
Bjo	LD	35	H	43	H	--	--	--	--
Cow	LD	26	I	43	H	--	--	--	--
Cur	LD	33	H	--	--	--	--	--	--
Cy2	LD	31	H	47	H	33	H	47	H
SRo	LD	38	E	43	H	38	E	49	H

[1] I (22–28), H (29–36), E (>36).

[2] I (30–41), H (42–51), E (>51).

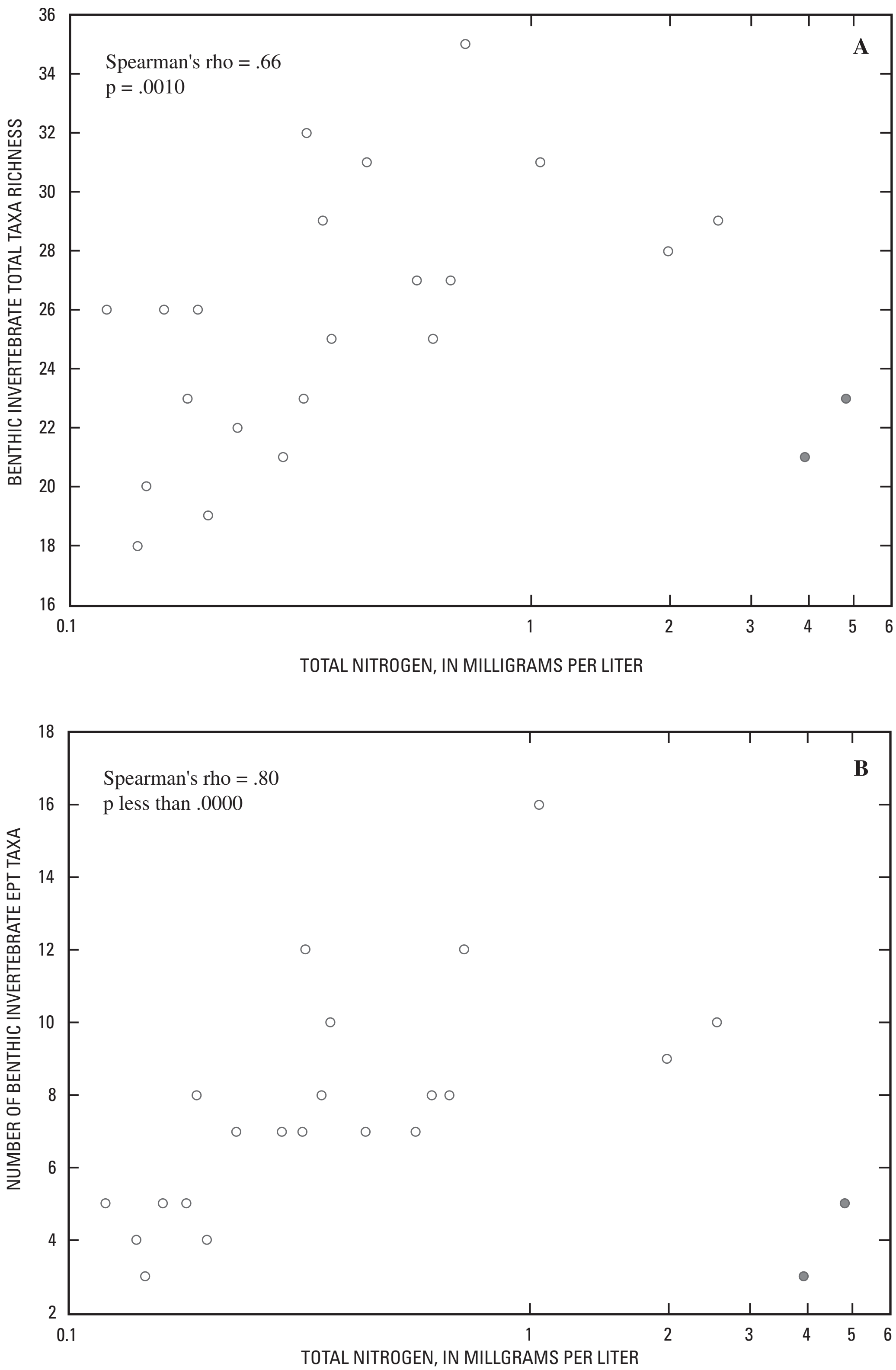
explained 53 percent of the variation in this metric across sites ($R^2 = .53$, $p = .0005$), (fig. 15). The multiple regression equation describing the community scraper percentage is

$$\text{Percent scrapers} = 32.82 + 5.69(\log TN) + 4.45(\log TP).$$

Benthic invertebrate metrics describing functional feeding groups also were correlated with measures of algal abundance (appendix 6). Composite scores for MacA and MacS were positively correlated with the percentage of collector-gatherers (p = .0235 and .0017, respectively) and negatively correlated with the percentage of filter-collectors (p = .0401 and .0136, respectively). The percentage of the benthic invertebrate community classified as scrapers was not correlated with the composite macroalgae scores MacA and MacS, but it was positively correlated with the percentage of survey points classified in the highest macroalgae cover categories: MacA >75% (p = .0041) and MacS >75% (p = .0041).

Benthic invertebrates classified as scrapers use periphyton (attached algae and associated microfauna) as a food source (Cummins and Merritt, 1996), and increased algal biomass frequently has been associated with increased scraper densities (Dudley and others, 1986; Feminella and Hawkins, 1995). In contrast, benthic invertebrates classified as collector-gatherers utilize fine particulate organic matter (FPOM) as a primary food resource (Cummins and Merritt, 1996). The reason for the relation between benthic invertebrate collector-gatherers and algal abundance is not clear, but algae might constitute an important source of detritus and FPOM in these oligotrophic streams.

Functional feeding group classifications for benthic invertebrates are based on morphological and behavioral adaptations for food acquisition (Cummins and Merritt, 1996), and changes in the relative composition of benthic invertebrate functional feeding groups can indicate a change in the food resource base (Texas Commission on Environmental Quality, 2005). The numerous strong relations among benthic invertebrate scrapers, collector-gatherers, nutrient concentrations, and measures of algal abundance in this study indicate nutrient enrichment can alter food sources in these generally low-nutrient streams.



EXPLANATION

- ○ **Data included in correlation analyses**
- ● **Data excluded from correlation analyses**

Figure 14. Correlations between total nitrogen concentration and (A) benthic invertebrate taxa richness and (B) Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa richness in selected small streams, Edwards Plateau, Central Texas, 2005–06.

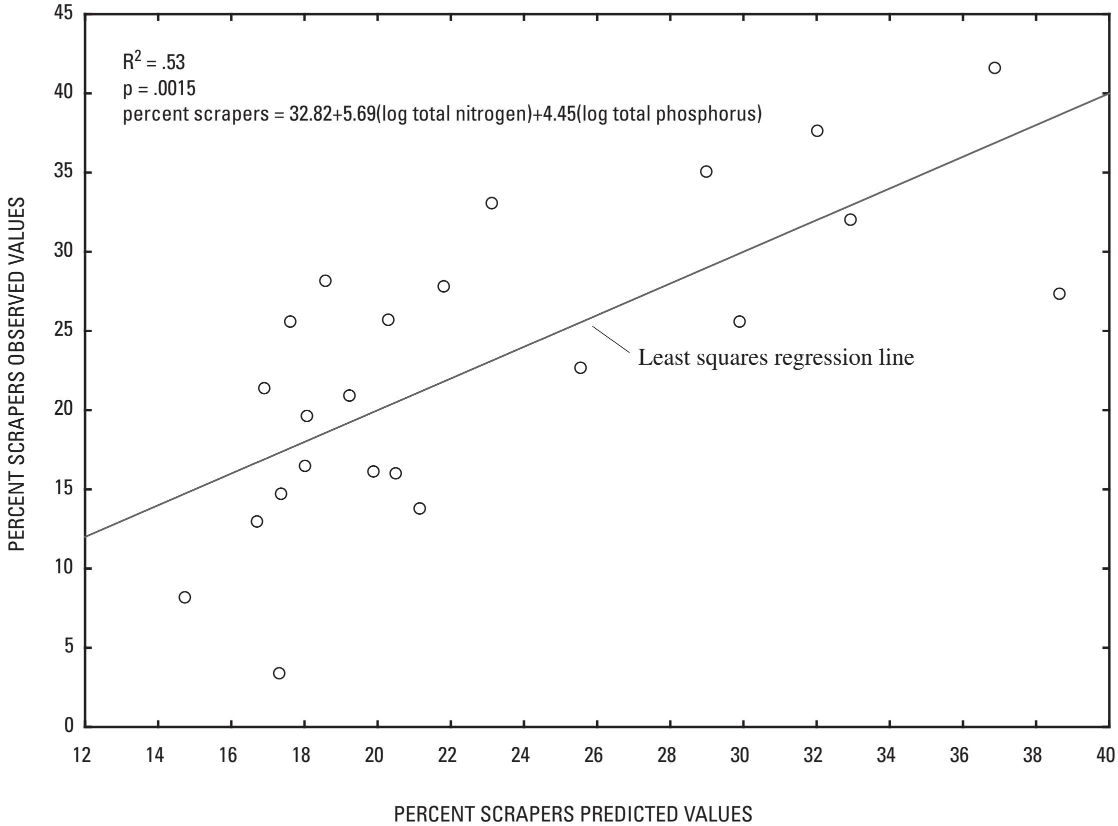


Figure 15. Regression relation between relative abundance of grazing benthic invertebrates (scrapers) observed and predicted values in selected small streams, Edwards Plateau, Central Texas, 2005–06.

Fish, and Relations Between Fish, Nutrients, and Algae

The State of Texas uses an ecoregion-focused set of metrics to compute ALU scores for fish community data (Texas Commission on Environmental Quality, 2005). Individual fish metrics are scored according to ecoregion-specific criteria and then summed to determine the composite fish ALU score. Fish ALU scores are categorized as Limited (less than 30), Intermediate (30–41), High (42–51), and Exceptional (greater than 51). Fish ALU scores for the Edwards Plateau streams in this study were similar to the invertebrate scores; the majority of ALU scores were categorized as High with a few exceptions (table 12). Five samples, all in group NWW streams, were categorized as Intermediate. Bull Creek, the site with the largest percentage of urban land cover in its watershed, was classified as Intermediate in both 2005 and 2006. Three samples, two in group WW streams and one in a group NWW stream, were categorized as Exceptional in 2005, but were reduced to High in 2006. The reason for these reductions is not clear, but streamflows in these streams in 2006 were lower relative to 2005.

Median fish ALU scores were largest for group WW streams (50), followed by group LD streams (45), and group NWW streams (42) (fig. 13). The KWMC test for fish ALU scores indicated group WW and NWW streams were significantly different (p = .0159) (table 4). Group LD streams were not statistically different from group WW or NWW streams.

Spearman's rank correlation indicated a significant positive correlation between fish ALU scores and measures of total nutrients (total nitrogen p = .0105 and total phosphorus p = .0217) and a relatively weaker but nearly significant positive correlation with the dissolved nitrogen measure (nitrite plus nitrate [p = .0542]) (appendix 4). ALU scores also were positively correlated with the percentage of survey points classified in the highest macroalgae cover categories, MacA >75% (p = .0188) and MacS >75% (p = .0156) (appendix 6).

Results from correlation using the individual fish metrics indicated that total fish species had a strong influence on ALU scores. Total fish species, like the ALU scores, was significantly positively correlated with total nitrogen (p = .0147), total phosphorus (p = .0066), nitrite plus nitrate (p = .0091) (appendix 4), and MacA >75% (p = .0074) (appendix 6). However, graphical analyses indicated that the correlation between total fish species and total phosphorus primarily was related to the larger total phosphorus concentrations found in the group WW streams (fig. 16A) and that the correlation between total

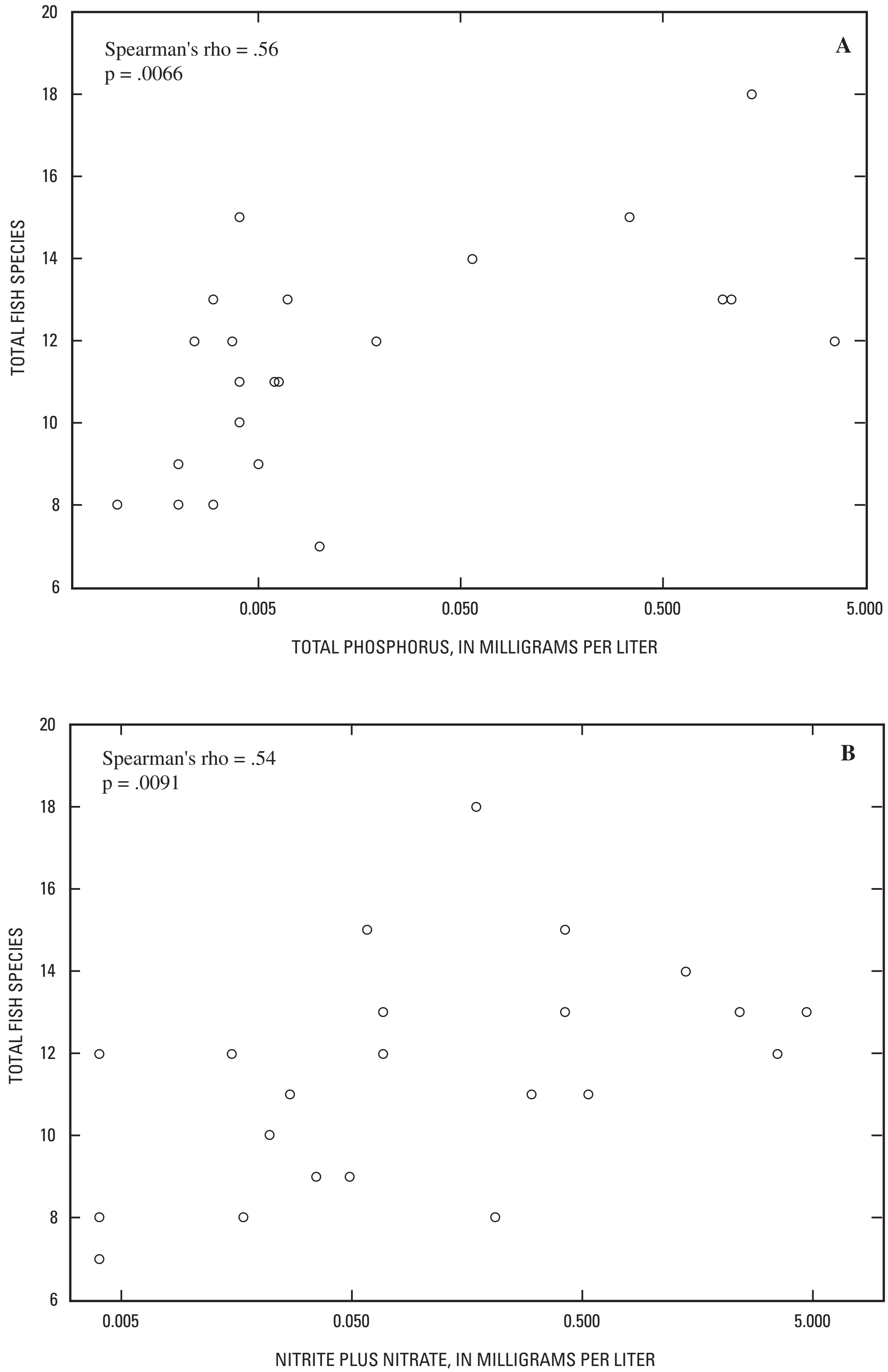


Figure 16. Correlations between total fish species and (A) total phosphorus concentrations and (B) nitrite plus nitrate concentrations in selected small streams, Edwards Plateau, Central Texas, 2005–06.

fish species and nitrite plus nitrate was more continuous across the suite of study streams (fig. 16B).

Graphical analysis of another species-related metric, the number of native cyprinid species, indicated that the Cibolo Creek 2006 sample again was influencing the statistical relations. Spearman rank correlations between the number of native cyprinid species and all three nutrient concentration measures (total nitrogen, nitrite plus nitrate, and total phosphorus) were increased and became statistically significant with the removal of the Cibolo Creek 2006 sample from the dataset. For example, the correlation between native cyprinid species and total phosphorus had a rho of .33 ($p = .1279$) before removal of the Cibolo Creek 2006 sample and a rho of .55 ($p = .0199$) after removal of the Cibolo Creek 2006 sample. Native cyprinid species are considered sensitive to habitat and water-quality degradation (Linam and others, 2002), and the positive correlations with nutrient concentration obtained in this study are the opposite of what generally might be expected.

The number of intolerant fish species showed positive correlations with nutrients (total nitrogen [$p = .0032$] and total phosphorus [$p = .0017$]) (appendix 4), as well as estimates of algal abundance (MacA >75% [$p = .0153$] and MacS >75% [$p = .0167$]) (appendix 6). This fish metric is assumed to increase with increasing stream quality (Linam and others, 2002), and again positive correlations with nutrient concentrations are the opposite of what generally might be expected.

The percentage of the fish community composed of non-native individuals was not correlated with measures of nutrient concentration but was positively correlated with MacA ($p = .0213$), MacA >75% ($p = .0076$), and MacS ($p = .0189$) (appendix 6). An increase in non-native species is not necessarily correlated with habitat or water-quality conditions, but instead represents a general disruption to the original fish assemblage and a deviation from natural conditions (Linam and others, 2002).

Numbers of fish species are known to increase with increasing drainage area (Linam and others, 2002), but such was not the case in this study. The metric total fish species was not strongly correlated with drainage area ($p = .5322$). Graphical analysis of the relations among nutrient concentrations, estimates of algal abundance, and fish metrics indicated that the majority of the positive correlations were strongly influenced by relatively high nutrient concentrations (group WW streams) (for example, fig. 16A). In general, strong correlations in the complete dataset were substantially weakened when Spearman's rank correlation was done without group WW streams. Increased productivity in group WW streams, resulting from nutrient enrichment in what are naturally low-nutrient streams, might lead to increased fish species richness. Similar to the benthic invertebrates, these results might describe the ascending limb of a unimodal relation between species richness and productivity.

The positive correlation between total fish species and estimates of macroalgae abundance indicates an alternative explanation is possible. Stream fish species richness tends to increase with increasing habitat heterogeneity (Gorman and Karr, 1978; Schlosser, 1991). Much of the streambed in the small streams of the Edwards Plateau is bare bedrock, and fish habitat commonly is scarce. Increased algal abundance stemming from nutrient enrichment might increase structural complexity and habitat heterogeneity in these streams.

A related factor that might affect fish species richness in this study is the consistently stable streamflow in the wastewater-influenced streams. Fish species richness has been shown to be lowest in headwater streams with high flow variability (Horwitz, 1978). The smaller streams in the Edwards Plateau that do not receive wastewater effluent are prone to drying and have greater flow variability in the summer months compared to the wastewater-influenced streams. Further evidence supporting this hypothesis is the fish species richness for the group LD stream Cypress Creek 2. This stream has a very consistent spring-fed flow (Brune, 1981) and a fish species richness comparable to the wastewater-influenced streams. In addition, most of the smaller streams that had low flow and relatively diverse fish communities also contained large pools that could act as refugia during periods of dewatering.

Implications for Development of Nutrient Criteria

The USEPA nutrient concentration recommendations for the Edwards Plateau (Level III Ecoregion 30) were computed to reflect the mean conditions for reference streams. Mean nutrient concentrations in the group LD streams were consistent with the USEPA recommendations (table 5). The group LD streams were sampled seven times during this study, and the USEPA-recommended total nitrogen concentration of 0.270 mg/L was exceeded in 57 percent of samples. No clear pattern was associated with these exceedances, but the two largest total nitrogen concentrations for group LD streams were associated with increased Kjeldahl nitrogen and were observed in 2006 when streamflows were reduced. The highest exceedance concentration (0.35) was 76 percent higher than the USEPA-recommended concentration but still relatively low when compared to total nitrogen concentrations from group WW streams. The mean total phosphorus concentration for the group LD streams (0.003 mg/L) was well below the USEPA-recommended mean of 0.008 mg/L, and no individual total phosphorus concentrations exceeded the USEPA recommendation. The USEPA nutrient criteria recommendations for the Edwards Plateau ecoregion took into account data from the entire ecoregion and all seasons, whereas this study involved data from only two subregions in summer. The results of this study indicate that total phosphorus concentrations in least-disturbed streams in the Balcones Canyonlands and the Edwards Plateau Woodland in the low-flow critical summer period might be smaller in comparison to those of the ecoregion as a whole.

Measured total nitrogen/total phosphorus ratios indicate that the small streams of the Edwards Plateau might be naturally phosphorus-limited, but biological responses were mixed: Benthic algal chlorophyll-*a* tended to be related more closely to measures of nitrogen whereas macroalgae cover was clearly related to phosphorus. Nutrient ratios are not definitive, and research using algal assays might be needed to determine what constituents limit algal production in these streams.

A primary focus of this study was the assessment of benthic algal chlorophyll-*a* as an indicator of eutrophication in the small streams of the Edwards Plateau. Results were promising: median benthic algal chlorophyll-*a* concentrations were higher in the nutrient-enriched group WW streams, and dissolved nitrogen concentrations were strongly associated with benthic algal chlorophyll-*a*. However, the KWMC test was not able to separate group WW streams from group LD streams on the basis of benthic chlorophyll-*a*. The failure of the KWMC test to indicate a significant difference might be related to a lack of statistical power because of the small sample sizes associated with group WW and LD streams.

When nutrient concentrations were evaluated using an instantaneous load approach, the relations to chlorophyll-*a* were improved. This implies that benthic algal productivity might be related not only to nutrient concentrations but water movement as well. Similar findings have been published by other authors (see comprehensive summary in Borchardt, 1996). These results might be particularly important because the low-flow critical period conditions typical of summer are most relevant for development of criteria protective of ALUs.

Although benthic chlorophyll-*a* was positively correlated with increased streamflow, this relation in all likelihood would not be continuous over a larger range of streamflow than that of this study. Reductions in benthic algae commonly are associated with floods and the accompanying high water velocities that disturb and abrade the streambed (Biggs, 1996). Streamflows during this study were low and stable, and no large flows occurred.

Phytoplankton chlorophyll-*a* also was evaluated as an indicator of nutrient enrichment. The relations between nutrients and phytoplankton chlorophyll-*a* were similar to those between nutrients and benthic chlorophyll-*a*—that is, significant positive but relatively weak relations with ambient nutrients and an increase in the strength of relations when streamflow also was considered. However, the relation between phytoplankton chlorophyll-*a* and streamflow was negative, which indicates that reduced streamflow allows for the development of phytoplankton in these streams.

Both benthic and planktonic chlorophyll-*a* measures were related to nutrients, but this study indicates that benthic chlorophyll-*a* was the better choice for monitoring nutrient enrichment because (1) the strength of the relation between nutrients and benthic chlorophyll-*a* was stronger, and (2) a strong relation between benthic chlorophyll-*a* and nutrients persisted after removal of the wastewater sites, which indicates superior ability to discriminate between conditions at lower nutrient concentrations. Regardless of the response variable, a measure of streamflow might be an important component with chlorophyll-*a* as an indicator of nutrient enrichment in the small streams of the Edwards Plateau.

An alternative approach to assessing algal biomass in streams using a transect-based estimate of algal abundance also was evaluated in this study. Results using estimates of algal abundance were promising: (1) The KWMC test using the composite score for macroalgae cover by area (MacA) clearly separated the nutrient-enriched wastewater streams from the other streams, and (2) both MacA and the highest macroalgae cover by area category (MacA >75%) were significantly related to total phosphorus concentrations. The strong relations between total phosphorus and macroalgae cover are of particular importance to nutrient criteria development because an overabundance of algal growth is the underlying cause of many problems associated with eutrophication. Furthermore, public perceptions of stream health often are associated with the amount of observable algal biomass in streams (Biggs, 1985; Welch and others, 1988). An encouraging result of this approach is the significant relation between MacA and dissolved oxygen minimum values. Failure to meet dissolved oxygen criteria was cited as a cause of impairment for 1,669 of 22,776 stream miles surveyed in Texas in 2006 (Texas Commission on Environmental Quality, 2007). A better understanding of the variables capable of influencing dissolved oxygen concentrations will aid in developing mitigation measures for streams that do not attain dissolved oxygen criteria.

However, the algal abundance estimate technique did have some drawbacks. When the nutrient-enriched group WW streams were removed from the dataset, no relations were identified between nutrients and algal abundance estimates. In addition, microalgae thickness estimates were negatively associated with nutrient concentrations and appeared to be influenced by macroalgae shading and the calcium precipitate commonly observed in the streams with slower water velocities.

The algal abundance estimate technique is a useful tool for identifying eutrophic conditions, assessing nuisance algal growth, and making broad comparisons among sites, but it appears to lack the fine resolution to identify lesser degrees of nutrient enrichment. Some of the variation in algal abundance among similar sites was because of the variation in algal cover among geomorphic channel units (riffles, runs and pools) and, in turn, the extent of various geomorphic channel units in the reach. For example, two of the wastewater-influenced sites, Brushy Creek and Cibolo Creek, had relatively high algal cover in the runs and less in the riffles; but the riffle section at the Brushy Creek reach was very long, and the Cibolo Creek reach had only one short riffle. As a result Cibolo Creek appears to have a higher degree of algal cover. Better resolution with the algal abundance estimate technique might be possible by stratifying estimates of algal cover by geomorphic channel units.

The composite ALU scores were marginally successful in identifying eutrophic conditions, but Spearman's rank correlations were relatively weak. More importantly, the correlation between increased nutrient concentrations and ALU scores, which was positive, was the reverse of what would be expected when nutrient enrichment causes a proliferation of algal growth and stream degradation. Fish and benthic ALU scores in the stream with the largest composite algal cover score (Cibolo Creek in 2006) were reduced relative to other group WW streams. More research might be needed to determine if increased ALU scores are the result of increased productivity and habitat heterogeneity in what are naturally low-nutrient streams, and if the levels of algal biomass in Cibolo Creek in 2006 represent the levels at which fish and benthic invertebrate communities begin to degrade.

Several individual fish and benthic invertebrate metrics showed relatively strong correlations with nutrient concentrations. These correlations were similar to those with ALU scores in that they generally indicated improving conditions associated with increasing nutrient enrichment and likely are the result of increased productivity in what are naturally low-nutrient streams. The utility of such metrics in other streams with relatively larger natural nutrient concentrations is uncertain. However, the benthic invertebrate functional feeding group metrics showed some promise as measures of nutrient condition. Changes in benthic invertebrate functional feeding group percentages, especially the percentage of scrapers, were clearly related to both nutrient concentrations and algal conditions in the study streams.

Summary

Excessive amounts of nutrients in aquatic ecosystems can promote the growth of aquatic vegetation and result in problems ranging from degraded water quality and altered aquatic habitats to a loss of recreational and aesthetic value. To effectively address issues related to nutrient enrichment, the U.S. Environmental Protection Agency (USEPA) has directed States to develop numeric nutrient criteria for their surface waters. In December 2001 the USEPA published nutrient-criteria recommendations for rivers and streams in the Edwards Plateau of Central Texas. USEPA recommendations were based on an estimate of reference conditions (25th percentile for all data) and focused on two nutrient constituents, total nitrogen and total phosphorus, and on two biological variables known to respond to nutrient enrichment, water-column chlorophyll-*a* and turbidity. Evidence indicates, however, that water-column chlorophyll-*a*, which is essentially a measure of the biomass of suspended algae (phytoplankton), is a poor indicator of nutrient enrichment in small, often fast-flowing, Texas streams, and that benthic (attached) algal chlorophyll-*a* might be a better indicator.

The U.S. Geological Survey (USGS), in cooperation with Texas Commission on Environmental Quality (TCEQ) (the agency charged with developing nutrient criteria for Texas), did a study during 2005–06 to characterize nutrient and biological conditions and identify relations between nutrient conditions and biological conditions in selected small streams of Central Texas. Water and biological samples were collected from small streams in parts of the Edwards Plateau of Central Texas in September 2005 and August 2006. Samples were collected in late summer to assess conditions during the period of the year when low streamflow and high water-temperature conditions stress biota and threaten the maintenance of aquatic life use (ALU) standards. Streams were selected to represent a gradient of conditions with the potential to influence nutrient concentrations. All streams were sampled in 2005, and a subset of eight streams was resampled in 2006.

Water, benthic invertebrate, and fish samples were collected once per sampling event at each site and in accordance with TCEQ protocols. Two methods were used at each stream to sample benthic algae: (1) the top-rock scrape method which is used to calculate estimates of benthic algal chlorophyll-*a* and ash-free dry weight (AFDW) in the USGS National Water Quality Assessment (NAWQA) program and (2) a transect-based technique for sampling/estimating stream-algal abundance. Phytoplankton biomass (water column chlorophyll-*a*) also was sampled for comparison to nutrient concentrations and benthic algal biomass.

The 15 streams of the study were grouped before sampling on the basis of their potential for nutrient enrichment. Designated groups were (1) streams receiving wastewater effluent (WW), (2) streams classified as least disturbed on the basis of low percentages of urban and agricultural land cover (LD), and (3) streams not receiving wastewater effluent but excluded from the least-disturbed category because site reconnaissance indicated a potential nutrient source (NWW). Study variables were compared among stream groups with a nonparametric Kruskal-Wallis multiple comparison (KWMC) test on ranked data. Individual variables thought to be indirectly related, such as nutrient concentrations and benthic invertebrate taxa richness, were assessed using Spearman's rank correlation, which is considered appropriate for variables that are not functionally dependant. Relations between variables thought to be directly related, such as nutrient concentrations and algal chlorophyll-*a*, were assessed using simple linear regression.

In this study trophic-state classifications based on nutrient concentrations were dependent on the presence of wastewater. Group NWW and LD streams generally were classified as oligotrophic on the basis of USEPA criteria. Group WW streams had larger nutrient concentrations and were classified as eutrophic. When the three group WW streams were removed from the dataset, total nitrogen concentrations were significantly correlated with the percentage of agricultural land cover in the watershed. Nutrient concentrations measured in group LD streams were very similar to the USEPA reference-condition estimates for nutrient concentrations in the Edwards Plateau. Nitrogen/phosphorus ratios indicated streams not affected by wastewater effluent might be limited by phosphorus concentrations.

Stream-group medians for benthic algal chlorophyll-*a* were lowest in group NWW streams, intermediate in group LD streams, and highest in group WW streams. Group WW streams generally had relatively high concentrations of chlorophyll-*a*, but trophic-state classifications based on benthic chlorophyll-*a* were not as clearly defined by wastewater as those based on measured nutrient concentrations.

Results for AFDW were not consistent with those for chlorophyll-*a*. The mean ratio of benthic chlorophyll-*a* to AFDW in group WW streams was more than four times the ratio in streams not affected by wastewater. The reason for this difference might be related to the relatively low water velocities common in the streams without wastewater input. A significant positive correlation between instantaneous discharge and benthic chlorophyll-*a*/AFDW ratios supports this hypothesis. Streams with low water velocities tended to develop an accumulation of calcium carbonate precipitate on bottom substrates that could have affected the AFDW results by (1) entraining non-algal organic material or (2) providing a habitat matrix for the growth of heterotrophic microfauna such as bacteria, fungi, and microinvertebrates.

Median values for phytoplankton chlorophyll-*a* were largest in group WW streams, and the KWMC tests indicated phytoplankton chlorophyll-*a* was significantly different between group LD and WW streams. However, group NWW streams were not significantly different from either group LD or WW streams.

Median scores for estimates of composite macroalgae cover by area (MacA) were highest in group WW streams. The KWMC test on MacA scores indicated that group LD and NWW streams were significantly different from group WW streams, but not from each other.

Simple regression indicated log transformed benthic algal chlorophyll-*a* (logChl) was weakly related to log transformed total nitrogen (logTN) and log transformed total phosphorus (logTP), but relatively strongly related to dissolved nitrogen (log transformed nitrite plus nitrate [logN+N]). Regression of logChl on nutrient load estimates (nutrient concentration multiplied by instantaneous discharge) yielded stronger statistical relations for all of the individual constituents. However, the relation between logChl and log transformed nitrite plus nitrate load (logN+NL) was again the strongest. Regressions done without group WW streams to evaluate relations at the lowest nutrient concentrations indicated relations between the nitrogen load measures and logChl were weakened but still relatively robust, whereas the relation between log transformed total phosphorus load (logTPL) and logChl was strengthened.

A multiple regression to estimate log transformed phytoplankton chlorophyll-*a* (logChl_P) using logN+N and discharge as a separate variable yielded a relation that explains 37 percent of the variation in phytoplankton across streams. The multiple regression equation indicated that logChl_P increased as a function of increased nitrite plus nitrate concentration and decreased streamflow. When the multiple regression equation was applied without group WW streams, the relation was weakened and not significant at the .05 level.

The regression of the highest MacA cover category (MacA greater than 75 percent [>75%]) on logTP produced a strong positive relation. However, regression of MacA >75% on logTP using only streams that do not receive wastewater effluent (groups LD and NWW) resulted in a weak statistical relation. These results indicate (1) high levels of macroalgae are associated with increased total phosphorus concentrations in streams receiving wastewater effluent, (2) the macroalgae survey by area is effective for identifying nuisance macroalgae growth associated with conditions of high nutrient enrichment, and (3) the macroalgae survey by area cannot, in its present form, discriminate between nutrient concentrations under low-nutrient conditions.

TCEQ classifies water bodies into ALU categories partly on the basis of criteria for mean and minimum dissolved oxygen (DO) concentrations over a 24-hour (diel) period. DO ALU categories in study streams generally were classified as High to Exceptional but might be reduced when streamflows are reduced. Results of statistical analyses indicate that relatively low diel DO minimums and relatively high diel DO ranges observed in some of the study streams are associated with increased total phosphorus concentrations and increased macroalgae abundance.

Benthic invertebrate ALU scores generally were High to Exceptional in study streams despite the influence of urbanization or wastewater. Reductions in ALU scores appeared to be related to extremely low flow conditions and the loss of riffle habitats. Benthic invertebrate ALU scores and several of the metrics used to compute composite ALU scores tended to increase with increasing total nitrogen concentrations. These positive relations likely are caused by nutrient enrichment increasing productivity in what are naturally low-nutrient streams. Increases in nutrient concentrations were correlated with increases in benthic invertebrate functional feeding group percentages. A multiple regression using logTN and logTP explained 53 percent of the variation in grazing invertebrates (scrapers) across streams.

The relative amount of invertebrates classified as scrapers and collector-gatherers also increased with increasing estimates of algal abundance (MacA and MacA >75%, respectively). The numerous strong relations among benthic invertebrate scrapers, collector-gatherers, nutrient concentrations, and measures of algal abundance in this study indicate nutrient enrichment can alter food sources in these generally low-nutrient streams.

Fish ALU scores generally were High or Exceptional with the exception of five samples categorized as Intermediate that were all collected from group NWW streams. Three samples, two in group WW streams and one in a group NWW stream, were categorized as Exceptional in 2005, but were reduced to High in 2006. The reason for these reductions is not clear, but streamflows in these streams during 2006 were lower relative to 2005.

Spearman's rank correlation indicated fish ALU scores were positively correlated with three nutrient measures (total nitrogen, total phosphorus, and nitrite plus nitrate) and the percentage of survey points classified in the highest macroalgae cover categories, MacA >75% and MacS >75%. Results from correlation using individual fish metrics indicated that fish species richness had a strong influence on ALU scores. Total fish species, like the ALU scores, was significantly positively correlated with total nitrogen, total phosphorus, nitrite plus nitrate, and MacA >75%. However, graphical analyses indicated that the correlation between total fish species and total phosphorus was related to the larger total phosphorus concentrations found in the group WW streams and that the correlation between total fish species and nitrite plus nitrate was more continuous across the suite of study streams. The increased species richness found in these streams is likely the result of increased productivity related to nutrient enrichment. Other related factors affecting species richness might be increased habitat heterogeneity associated with increased algal abundance and consistently stable streamflow associated with wastewater input.

Mean concentrations of the nitrogen constituents in group LD streams were consistent with the USEPA recommendations, but mean total phosphorus was less than the USEPA recommendation. Mean total phosphorus in this study might have been reduced in comparison to the USEPA recommendation because this study only sampled two subregions of the Edwards Plateau in summer. In contrast, the USEPA developed their recommendations from data covering the entire ecoregion and all seasons.

Both benthic and planktonic chlorophyll-*a* measures were related to nutrients, but this study indicates that benthic chlorophyll-*a* was the better choice for monitoring nutrient enrichment because (1) the strength of the relation between nutrients and benthic chlorophyll-*a* was stronger, and (2) a strong relation between benthic chlorophyll-*a* and nutrients persisted after removal of the sites influenced by wastewater effluent, which indicates superior ability of benthic chlorophyll-*a* to discriminate between conditions at lower nutrient concentrations.

The algal abundance estimate technique is a useful tool for identifying eutrophic conditions, assessing nuisance algal growth, and making broad comparisons among sites, but it appears to lack the fine resolution to identify lesser degrees of nutrient enrichment. Better resolution with the algal abundance estimate technique might be possible by stratifying estimates of algal cover by geomorphic channel units.

Several individual benthic invertebrate and fish metrics were correlated with nutrient conditions, but correlations were generally positive and the reverse of what would be expected when nutrient enrichment causes a proliferation of algal growth and stream degradation. However, the benthic invertebrate functional feeding group metrics showed some promise as measures of nutrient condition. Changes in benthic invertebrate functional feeding group percentages, especially the percentage of scrapers, were clearly related to both nutrient concentrations and algal conditions in the study streams.

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Appendixes 1–6

Appendix 1. Summary of macroalgae survey results for percent coverage by area in selected small streams, Edwards Plateau, Central Texas, 2005–06.

[Values are proportion of points assigned to each category from a 100-point transect. %, percent; <, less than; > greater than; Comp, calculated composite macroalgae cover score]

Site short name (table 1)	0%	< 5%	5 to 25%	25 to 50%	50 to 75%	> 75%	Comp
				2005			
Bar	0.10	0.12	0.11	0.15	0.20	0.31	317
Bar duplicate	.06	.15	.12	.15	.17	.35	327
BJo	.66	.13	.07	.07	.06	.01	77
Bla	.28	.32	.19	.14	.06	.01	141
Bru	.59	.11	.02	.08	.09	.11	131
Bul	.08	.19	.13	.15	.22	.21	289
Bul duplicate	.06	.15	.08	.23	.19	.28	317
Cib	.04	.09	.20	.15	.20	.32	334
Cow	.02	.08	.23	.24	.30	.13	311
Cur	.66	.16	.11	.02	.05	0	64
Cy1	.35	.17	.15	.16	.09	.08	172
Cy2	.26	.15	.13	.18	.11	.17	224
Lic	.64	.13	.12	.02	.06	.03	82
Oni	.53	.20	.13	.13	0	.01	90
SGr	.31	.16	.17	.18	.12	.06	182
SRo	.10	.19	.20	.26	.22	.02	237
SSG	.26	.35	.26	.12	.01	0	128
				2006			
Bar	.05	.10	.21	.11	.27	.26	323
Bla	.56	.18	.09	.08	.05	.04	101
Bru	.21	.13	.14	.07	.16	.29	271
Bul	.03	.19	.25	.29	.19	.05	257
Cib	.05	.06	.04	.05	.12	.68	417
Cy2	.17	.19	.25	.10	.11	.18	233
SGr	.22	.13	.17	.20	.19	.09	228
SRo	.63	.12	.12	.07	.03	.03	84

Appendix 2. Summary of macroalgae survey results for percent coverage of loose substrate in selected small streams, Edwards Plateau, Central Texas, 2005–06.

[Values are proportion of points assigned to each category from a 100-point transect. %, percent; <, less than; > greater than; Comp, calculated composite macroalgae cover score]

Site short name (table 1)	0%	< 5%	5 to 25%	25 to 50%	50 to 75%	> 75%	Comp
				2005			
Bar	0.17	0.30	0.27	0.18	0	0.08	180
Bar duplicate	.10	.42	.20	.17	.03	.08	187
BJo	.70	.04	.12	.04	.06	.04	84
Bla	.48	.29	.11	.09	.03	0	89
Bru	.63	.09	.03	.04	.10	.12	124
Bul	.10	.29	.22	.17	.12	.10	222
Bul duplicate	.04	.40	.18	.12	.12	.14	230
Cib	.08	.11	.18	.27	.13	.23	294
Cow	.13	.27	.21	.23	.14	.03	206
Cur	.63	.25	.10	.01	.01	0	52
Cy1	.47	.22	.12	.12	.03	.05	117
Cy2	.17	.33	.33	.08	.08	0	158
Lic	.60	.10	.08	.02	.08	.10	119
Oni	.67	.14	.13	.04	0	.01	58
SGr	.23	.16	.14	.17	.16	.13	226
SRo	.19	.29	.20	.10	.16	.05	191
SSG	.36	.36	.19	.08	0	0	99
				2006			
Bar	.22	.20	.17	.12	.15	.14	220
Bla	.52	.25	.06	.09	.04	.04	99
Bru	.32	.19	.18	.14	.10	.07	174
Bul	0	.23	.30	.30	.15	.03	245
Cib	.31	.07	.08	.13	.18	.24	251
Cy2	.23	.30	.14	.20	.04	.09	177
SGr	.16	.16	.21	.15	.15	.18	251
SRo	.68	.11	.12	.05	.02	.02	70

Appendix 3. Summary of survey results for thickness of microalgae on loose substrate for selected small streams, Edwards Plateau, Central Texas, 2005–06.

[Values are proportion of points assigned to each category from a 100-point transect. <, less than; mm, millimeter; Comp, calculated composite macroalgae thickness score]

Site short name (table 1)	Rough	Slimy	Visible (<0.5 mm)	0.5 to 1 mm	1 to 5 mm	Comp
			2005			
Bar	0.53	0.45	0.02	0	0	24
Bar duplicate	.52	.47	.02	0	0	25
BJo	.24	.22	.22	.30	.02	99
Bla	.42	.27	.14	.18	0	63
Bru	.26	.16	.32	.18	.09	102
Bul	.06	.30	.40	.23	0	102
Bul duplicate	.17	.31	.31	.16	.05	94
Cib	.04	.69	.25	.02	0	64
Cow	.17	.14	.29	.19	.21	137
Cur	.15	.26	.30	.29	0	101
Cy1	.61	.39	0	0	0	19
Cy2	.35	.16	.41	.08	0	67
Lic	.17	.51	.32	0	0	56
Oni	.33	.22	.11	.24	.11	102
SGr	.08	.41	.40	.12	0	83
SRo	.03	.25	.39	.33	0	118
SSG	0	.01	.48	.49	.01	151
			2006			
Bar	.33	.49	.19	0	0	43
Bla	.15	.20	.48	.16	0	91
Bru	.25	.34	.18	.23	.01	83
Bul	0	.25	.45	.29	.01	119
Cib	.42	.21	.28	.08	0	55
Cy2	.34	.05	.16	.32	.13	121
SGr	.11	.21	.48	.21	0	99
SRo	.13	.33	.31	.23	0	93

Appendix 4. Results of Spearman rank correlation analyses for variables considered indirectly related to nutrient concentrations in selected small streams, Edwards Plateau, Central Texas, 2005–06.

[Results significant at 5-percent level ($p \leq .05$) in bold. TN, total nitrogen; rho, correlation coefficient; p, probability of Type I error in statistical results; N+N, nitrite plus nitrate; TP, total phosphorus; DO, dissolved oxygen; EPT, Ephemeroptera-Plecoptera-Trichoptera]

Response variable	TN rho	TN p	N+N rho	N+N p	TP rho	TP p
Diel DO mean	.09	.6837	.25	.2596	-.15	.5103
Diel DO minimum	-.15	.5094	.06	.8045	**-.52**	**.0134**
Diel DO maximum	.31	.1540	.17	.4398	.35	.1145
Diel DO range	.19	.3850	.01	.9582	**.43**	**.0446**
Diel pH mean	.19	.3992	.11	.6199	.05	.8337
Diel pH minimum	.13	.5629	.21	.3421	.06	.8045
Diel pH maximum	.26	.2504	.02	.9204	.14	.5477
Diel pH range	.28	.2124	-.04	.8720	.28	.2016
Invertebrate aquatic life use score	**.50**	**.0155**	.31	.1550	.21	.3258
Percent collector-gatherers	.26	.2220	-.04	.8419	.32	.1376
Percent scrapers	**.65**	**.0008**	**.43**	**.0431**	**.71**	**.0002**
Percent filter-collectors	-.27	.2149	-.03	.9037	-.33	.1222
Percent predators	-.25	.2597	-.29	.1852	-.36	.0941
Percent shredders	.21	.3318	-.16	.4694	.13	.5555
Total taxa richness	**.43**	**.0420**	.12	.5891	.26	.2399
EPT taxa rich	**.46**	**.0268**	.13	.5504	.18	.4016
Hilsenhoff Biotic Index	**-.50**	**.0141**	**-.47**	**.0225**	-.14	.5289
Percent Chironomidae	.04	.8579	-.06	.7689	-.25	.2559
Percent dominate taxon	-.13	.5530	.03	.8895	-.36	.0908
Percent dominate feeding group	-.04	.8649	.01	.9625	.13	.5523
Ratio of intolerant to tolerant taxa	**.53**	**.0096**	**.50**	**.0161**	.27	.2054
Percent Trichoptera as Hydropsychidae	**-.57**	**.0054**	-.32	.1434	-.35	.1069
Number of non-insect taxa	.20	.3701	.20	.3522	**.42**	**.0444**
Percent Elmidae	.27	.2046	.18	.4032	.28	.1881
Fish aquatic life use score	**.53**	**.0105**	.42	.0524	**.49**	**.0217**
Total fish individuals	.20	.3683	.20	.3663	.23	.3015
Total fish species	**.51**	**.0147**	**.54**	**.0091**	**.56**	**.0066**
Number native cyprinid species	.33	.1398	.36	.1031	.33	.1279
Number benthic invertivore species	.36	.0991	.28	.2103	.24	.2862
Number sunfish species	.15	.5034	.26	.2418	**.43**	**.0464**
Number intolerant fish species	**.60**	**.0032**	**.44**	**.0408**	**.63**	**.0017**
Percent tolerant individuals[1]	**.44**	**.0383**	.22	.3253	**.50**	**.0166**
Percent omnivore individuals[1]	-.12	.5939	-.06	.7814	-.24	.2708
Percent invertivore individuals[1]	.24	.2844	.19	.4028	.22	.3264
Percent piscivore individuals[1]	.14	.5226	-.05	.8084	.07	.7526
Percent non-native individuals[1]	.15	.4961	.09	.6830	.18	.4152

[1]Arcsine transformed data.

Appendix 5. Results of Spearman rank correlation analyses for variables considered indirectly related to measures of chlorophyll-a biomass in selected small streams, Edwards Plateau, Central Texas, 2005–06.

[Results significant at 5-percent level ($p \leq .05$) in bold. Chl_B, benthic algal chlorophyll-*a*; rho, correlation coefficient; p, probability of Type I error in statistical results; Chl_P; phytoplankton chlorophyll-*a*; AFDW, ash-free dry weight; DO, dissolved oxygen; EPT, Ephemeroptera-Plecoptera-Trichoptera]

Response variable	Chl_B		Chl_P		AFDW	
	rho	p	rho	p	rho	p
Diel DO mean	.26	.2483	.04	.8491	.35	.1138
Diel DO minimum	.20	.3627	-.23	.3044	.42	.0522
Diel DO maximum	.07	.7473	.27	.2181	-.20	.3711
Diel DO range	-.15	.5161	.32	.1407	-.37	.0881
Diel pH mean	.01	.9702	.22	.3336	-.13	.5595
Diel pH minimum	.11	.6221	.14	.5264	-.11	.6115
Diel pH maximum	-.15	.4997	.27	.2165	-.35	.1132
Diel pH range	-.23	.2973	.28	.2082	**-.54**	**.0102**
Invertebrate aquatic life use score	.06	.7846	.11	.6029	-.34	.1120
Percent collector-gatherers	-.03	.8826	.40	.0610	-.07	.7470
Percent scrapers	.18	.3998	**.54**	**.0080**	-.37	.0834
Percent filter-collectors	.22	.3236	**-.65**	**.0008**	-.03	.8861
Percent predators	-.39	.0686	.20	.3560	**.42**	**.0478**
Percent shredders	-.28	.2036	.32	.1318	-.32	.1425
Total taxa richness	.02	.9160	.27	.2158	-.21	.3452
EPT taxa rich	.02	.9261	.03	.8917	-.30	.1684
Hilsenhoff Biotic Index	-.34	.1080	.01	.9606	.12	.5977
Percent Chironomidae	-.08	.7032	.22	.3052	.21	.3374
Percent dominate taxon	.18	.4168	-.09	.6674	.30	.1652
Percent dominate feeding group	.17	.4518	-.04	.8486	.06	.8020
Ratio of intolerant to tolerant taxa	**.48**	**.0206**	-.02	.8999	-.17	.4394
Percent Trichoptera as Hydropsychidae	.04	.8751	**-.43**	**.0438**	.32	.1470
Number of non-insect taxa	.19	.3802	**.56**	**.0058**	-.20	.3701
Percent Elmidae	.27	.2046	.10	.6576	.14	.5113
Fish aquatic life use score	.35	.1101	.21	.3574	**-.42**	**.0497**
Total fish individuals	-.06	.7933	.34	.1266	-.33	.1375
Total fish species	.42	.0511	.28	.2086	-.40	.0687
Number native cyprinid species	.28	.2128	.13	.5772	-.40	.0681
Number benthic invertivore species	.18	.4273	.11	.6292	-.30	.1778
Number sunfish species	.39	.0734	.11	.6230	-.08	.7155
Number intolerant fish species	.31	.1603	.36	.1030	-.53	.0107
Percent tolerant individuals	-.14	.5459	.41	.0577	-.23	.2963
Percent omnivore individuals	.10	.6472	-.09	.6773	.06	.8010
Percent invertivore individuals	.27	.2254	.32	.1407	.13	.5766
Percent piscivore individuals	-.07	.7511	-.02	.9243	.16	.4902
Percent non-native individuals	.26	.2352	.09	.6844	.15	.5156

Appendix 6. Results of Spearman rank correlation analyses for variables considered indirectly related to measures of algal abundance in selected small streams, Edwards Plateau, Central Texas, 2005–06.

[Results significant at 5-percent level ($p \leq .05$) in bold. MacA, composite macroalgae cover by area; rho, correlation coefficient; p, probability of Type I error in statistical results; MacA>75%, macroalgae cover by area greater than 75-percent category; MacS, composite macroalgae cover of loose substrate; MacS>75%, macroalgae cover of loose substrate greater than 75-percent category; MicT, thickness of microalgae cover on loose substrate; DO, dissolved oxygen; EPT, Ephemeroptera-Plecoptera-Trichoptera]

Response variable	MacA		MacA>75%		MacS		MacS>75%		MicT	
	rho	p	rho	p	rho	p	rho	p	rho	p
Diel DO mean	-.14	.5458	.08	.7318	.10	.6617	.07	.7603	-.13	.5662
Diel DO minimum	**-.47**	**.0259**	-.31	.1540	-.34	.1229	-.33	.1362	.02	.9205
Diel DO maximum	.38	.0840	**.52**	**.0122**	**.45**	**.0366**	**.51**	**.0160**	-.21	.3548
Diel DO range	**.43**	**.0459**	**.44**	**.0396**	.40	.0682	**.49**	**.0220**	-.09	.7060
Diel pH mean	.27	.2255	.35	.1153	.07	.7664	-.12	.6040	.14	.5460
Diel pH minimum	.13	.5629	.29	.1958	-.08	.7097	-.16	.4865	.04	.8477
Diel pH maximum	.30	.1794	.34	.1190	.08	.7227	.00	.9940	.12	.6327
Diel pH range	**.44**	**.0402**	.36	.1007	.28	.2134	.27	.2228	-.01	.9582
Invertebrate aquatic life use score	-.23	.2841	-.15	.4830	-.24	.2634	.13	.5493	-.27	.2143
Percent collector-gatherers	**.47**	**.0235**	**.45**	**.0297**	**.62**	**.0017**	**.47**	**.0233**	.22	.3057
Percent scrapers	.26	.2384	**.58**	**.0041**	.25	.2460	**.57**	**.0041**	-.33	.1210
Percent filter-collectors	**-.43**	**.0401**	-.41	.0544	**-.51**	**.0136**	-.39	.0648	.03	.8826
Percent predators	-.18	.4064	-.34	.1087	-.21	.3374	**-.48**	**.0200**	.22	.3236
Percent shredders	-.06	.7972	.04	.8690	.09	.6685	.14	.5139	.16	.4620
Total taxa richness	.13	.5670	.17	.4373	.19	.3907	.35	.0995	.12	.5982
EPT taxa rich	-.21	.3449	-.14	.5104	-.15	.4848	.06	.7705	.12	.5874
Hilsenhoff Biotic Index	.24	.2626	.30	.1707	.33	.1288	.14	.5212	.02	.9411
Percent Chironomidae	.32	.1429	.06	.7809	.36	.0938	-.10	.6367	.09	.6866
Percent dominate taxon	.33	.1289	.29	.1719	.38	.0750	.15	.4929	.05	.8089
Percent dominate feeding group	.31	.1524	.24	.2630	**.42**	**.0438**	.38	.0742	.04	.8685
Ratio of intolerant to tolerant taxa	-.12	.5821	-.11	.6289	-.16	.4572	.02	.9142	-.14	.5245
Percent Trichoptera as Hydropsychidae	-.17	.4555	-.06	.7755	-.12	.5956	-.13	.5617	.29	.1900
Number of non-insect taxa	**.56**	**.0054**	**.60**	**.0023**	**.69**	**.0003**	**.63**	**.0013**	-.14	.5219
Percent Elmidae	.21	.3420	.23	.2721	.25	.2480	.22	.3144	.12	.5776
Fish aquatic life use score	.29	.1961	**.50**	**.0188**	.30	.1737	**.51**	**.0156**	-.29	.1952
Total fish individuals	.07	.7511	-.03	.8907	-.15	.5161	.10	.6505	-.16	.4681
Total fish species	.34	.1183	**.55**	**.0074**	.21	.3466	.40	.0675	**-.54**	**.0102**
Number native cyprinid species	.16	.4832	.35	.1101	.02	.9387	.18	.4302	-.35	.1067
Number benthic invertivore species	.18	.4132	.37	.0941	.24	.2915	.42	.0534	-.33	.1344
Number sunfish species	.27	.2190	.40	.0617	.34	.1245	.32	.1532	-.11	.6298
Number intolerant fish species	.28	.2040	**.51**	**.0153**	.30	.1731	**.50**	**.0167**	**-.56**	**.0064**
Percent tolerant individuals	.02	.9463	.02	.9264	.15	.5095	.36	.1044	-.34	.1171
Percent omnivore individuals	.01	.9781	.11	.6252	-.06	.7779	-.10	.6433	.19	.3906
Percent invertivore individuals	.39	.0726	.41	.0585	.37	.0945	.12	.5831	.07	.7664
Percent piscivore individuals	-.16	.4870	-.24	.2872	.13	.5493	.02	.9383	.25	.2549
Percent non-native individuals	**.49**	**.0213**	**.55**	**.0076**	**.50**	**.0189**	.23	.2999	-.15	.4993

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