

IMPACTS OF NONPOINT-SOURCE POLLUTION ON PERIPHYTON CHARACTERISTICS IN THE WEST FORK OF THE RED RIVER IN NORTH-CENTRAL TENNESSEE

JEFFERSON LEBKUECHER¹, JESSIE CRAFT, RICHARD HANKENSON,
JESSICA JOHNSON, and JONATHAN MARTIN

Biology Department
Austin Peay State University
Clarksville, Tennessee 37044

¹lebkuecherj@apsu.edu

ABSTRACT

Approximately 60% of the Red River Watershed is used to produce agricultural products and is impacted by nonpoint-source pollution. The water quality of the West Fork of the Red River was evaluated by examining concentrations of soluble reactive phosphorous and NO₂ + NO₃ nitrogen, periphyton biomass, photoautotrophic periphyton physiological status, and the structure of the diatom assemblage. A high concentration of NO₂ + NO₃ nitrogen (4.9 mg/L) and a high concentration of photoautotrophic periphyton (101 ± 43 mg chlorophyll *a*/m² stream bottom) indicate the stream site sampled was impacted by nutrient enrichment. The three most abundant diatom taxa were *Cocconeis placentula* var. *euglypta* Erenb. (38%), *Gomphonema brasiliense* Kütz. (16%), and *Navicula minima* Grun. (4%). Low values for the Siltation Index and Organic Pollution Index for the diatom assemblage reveal the assemblage was not negatively impacted by siltation nor high concentrations of dissolved organics. Our results indicate the West Fork of the Red River should be targeted for habitat improvement by reduction of nutrient enrichment.

KEY WORDS: water pollution, periphyton, primary production, algae, diatoms, Tennessee

Nutrient enrichment and sediments from non-point sources are most responsible for the biological impairment of United States waters (Irvine & Murphy 2009). Assessments of pollution are prerequisites to developing watershed management plans to protect aquatic ecosystems (Smucker & Vis 2009). Biological monitoring is essential to characterize and quantify the influences of water quality. Periodic water sampling for chemical analyses alone may not reveal the impact of nonpoint-source pollution because pollutants from nonpoint sources often enter waterways in pulses. In addition, chemical analyses do not reveal the impacts of pollution on biological integrity (Taylor et al. 2007). We used multiple approaches to evaluate the impacts of non-point source pollution, including determinations of nutrient concentrations in water samples, evaluations of periphyton biomass and physiological status, and analyses of the structure of the diatom assemblage.

Photoautotrophic periphyton are the most important primary producers in the majority of wadeable streams (Lambert & Steinman 1997). Nutrient enrichment of streams changes photoautotrophic periphyton characteristics and affects whole-stream ecological relationships. The composition, biomass, and physiological status of photoautotrophic periphyton are excellent indicators of water quality and are used universally to follow changes in aquatic environments (Eaton et al. 2005).

The West Fork of the Red River is a fourth-order stream at the site sampled and joins the Red River two km upstream of the confluence of the Red and Cumberland Rivers in Clarksville, Tennessee (Fig. 1). The Red River Watershed is in the Western Pennyroyal Karst Level IV Ecoregion. The geologic base of the watershed is Mississippian-age limestone and includes some chert, shale, siltstone, sandstone, and dolomite. The soils are a thin loess mantle, highly erodible, and

very fertile (Baskin et al. 1997). Forests are Western Mesophytic and consist largely of *Quercus* and *Carya* species (Baskin et al. 1997). Approximately 60% of the watershed is used to produce agriculture products including tobacco, corn, soybean, and livestock (TDEC 2009). The cumulative effects of erosion, agricultural runoff, livestock access to streams, and poorly functioning sewage systems result in poor quality water.

Methods

Periphyton and water were sampled in the West Fork of the Red River at Billy Dunlop Park in Clarksville, Tennessee on Sept. 11, 2013 following 2 weeks without significant precipitation. Two transects from the waterlines of opposing banks, 10 m apart, were established at the sampling site. Transect widths and stream depths at 1/3 intervals of each transect were determined. Stream velocity was determined as the time required for a density-neutral object to travel 10 m downstream. Stream discharge was calculated as: $\text{Discharge} = \text{Width} \cdot \text{Depth} \cdot \text{Velocity} \cdot 0.9$ (Robins & Crawford 1954).

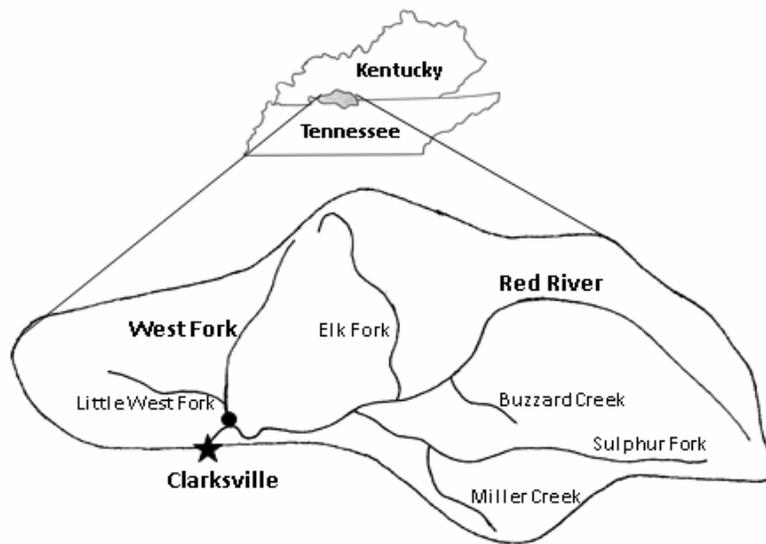


Figure 1. Location of the Red River Watershed (dark area) and location of the sampling site (dark circle).

Water samples to determine nitrogen and phosphorous concentrations were collected midstream, 5 cm below the surface. Concentrations of soluble reactive phosphorous and $\text{NO}_2 + \text{NO}_3$ nitrogen were determined with a Lachat QuickChem 8000 Flow Injection Analyzer (Lachat Instruments, 5600 Lindbergh Dr., Loveland, CO 80538).

Five midstream plots in the 10-m reach were established with 0.25 m^2 wire frames. The fractions of cobble and very coarse gravel (considered stable substrate) and coarse gravel plus smaller substrate (considered unstable substrate) in each plot were recorded. Two cobbles nearest the plot center were removed. One cobble was used for determination of periphyton dry weight, determinations of pigment concentrations, and ash-free periphyton dry weight. One cobble was used to evaluate diatom composition. One sample of unstable substrate from each plot was removed with a core sampler (30 cm^2) for determinations of pigment concentrations associated with unstable substrate.

Laboratory methods for measurements of ash-free periphyton dry weight and concentrations of periphyton chlorophyll (chl) *a* and pheophytin (pheo) *a* are described in Eaton et al. (2005). The surface area of cobble from which periphyton was removed was calculated by covering the upper

surface with aluminum foil, weighing the foil, and extrapolating weight to surface area (Hauer & Lamberti 2006). The autotrophic index (AI) was calculated using the equation of Crossy and LaPoint (1988): $AI = [\text{Ash-free periphyton dry weight (g m}^{-2})]/[\text{chlorophyll } a \text{ (g m}^{-2})]$. Identification of epilithic diatoms and calculation of diatom indices followed the methods described in KDOW (2002).

Results and Discussion

Stream site morphological characteristics were determined to provide detail of the abiotic characteristics of the sampling site (Table 1). Stable substrate for periphyton growth (cobble and very coarse gravel) comprises approximately 1/3 of the benthic environment.

Table 1. Abiotic characteristics of the West Fork of the Red River at the site sampled at Billy Dunlop Park in Clarksville, Tennessee.

Assay	Mean \pm SE
Width (m)	19.0 \pm 1.5
Depth (m)	0.3 \pm 0.1
Velocity (m/s)	0.3 \pm 0.0
Discharge	1.9 \pm 0.0
Percent benthic substrate	
Stable substrate (cobble + very coarse gravel)	34% \pm 21.0
Unstable substrate (substrate smaller than very coarse gravel)	66% \pm 21.0

The concentration of soluble reactive phosphorous (Table 2) was in the range symbolic of mesotrophic water (moderately nutrient enriched), whereas the concentration of $\text{NO}_2 + \text{NO}_3$ nitrogen (Table 2) was well above the boundary delineating mesotrophic and eutrophic water (Table 3). Nutrient enrichment increases periphyton biomass. Periphyton biomass, estimated as the concentration of chl *a* (mg/m^2 stream bottom) at the sampling site (Table 2) is typical of periphyton biomass in other wadeable streams in Tennessee impacted by nonpoint-source pollution (i.e., Lebkuecher et al. 2000) and is greater than the mesotrophic-eutrophic boundary suggested by Dodds et al. (1998).

Table 2. Concentrations of soluble reactive phosphorous and $\text{NO}_2 + \text{NO}_3$ nitrogen (mg L^{-1}) and characteristics of periphyton sampled from the West Fork of the Red River at Billy Dunlop Park in Clarksville, Tennessee.

Assay	Mean + SE
Soluble reactive phosphorous (mg/L)	0.04
$\text{NO}_2 + \text{NO}_3$ nitrogen (mg/L)	4.93
Chl <i>a</i> (mg/m^2 stream bottom)	101.5 \pm 42.9
Chl <i>a</i> (mg/m^2 cobble and very coarse gravel)	102.8 \pm 39.9
Chl <i>a</i> (mg/m^2 coarse gravel, sand, silt)	82.8 \pm 40.2
Pheo <i>a</i> (mg/m^2 stream bottom)	0.4 \pm 0.6
Ash-free periphyton (g/m^2 cobble)	4.9 \pm 2.7
Autotrophic Index	47.4 \pm 12.2

Stable substrate typically supports a greater periphyton biomass relative to unstable substrate (Myers et al. 2007). The biomass of photoautotrophic periphyton associated with substrate smaller than very coarse gravel (considered unstable substrate) was 81% of the biomass associated with cobble and very coarse gravel (Table 2). This result illustrates that small substrate can be an important substrate for primary production in streams with heterogeneous benthic environments.

Table 3. Suggested boundaries for the trophic classification levels of streams by total phosphorous (TP), total nitrogen (TN), and chlorophyll (chl) *a* (Dodds et al. 1998).

Trophic level	TP (mg/L)	TN (mg/L)	Chl <i>a</i> (mg/m ²)
Oligotrophic	≤ 0.025	≤ 0.7	> 20
Mesotrophic	0.026 - 0.075	0.7 - 1.5	20 - 70
Eutrophic	> 0.075	> 1.5	> 70

Measurements of the concentration of benthic pheophytin (pheo) *a* reveal the health of photoautotrophic periphyton. Chl *a* is degraded to pheo *a* as photoautotrophic periphyton senesce, hence high concentrations of pheo *a* indicate poor physiological condition. The low concentration of pheo *a* relative to the concentration of chl *a* indicates (Table 2) the photoautotrophic periphyton were in excellent physiological condition (Eaton et al. 2005). This result suggests the absence of chemical pollutants such as herbicides that adversely affect the health of photoautotrophic periphyton.

Organic pollution results from erosion of organic soil, input of manure or sewage, and overgrowth of algae due to nutrient enrichment (Van Dam et al. 1994). An increase of organic debris can change several biotic characteristics of aquatic environments. Excessive concentrations of decay byproducts such as ammonia and hydrogen sulfide may be harmful to aquatic organisms. The autotrophic index (AI) is a ratio of periphyton biomass (g dry wt m⁻²) to photoautotrophic-periphyton biomass (mg chl *a*/m²). The AI reveals the trophic nature of the periphyton community and is affected by the concentration of organics (Vannote et al. 1980). AI values typically range from 30 to 300; larger values indicate heterotrophic dominance associated with high concentrations of dissolved organics (Torres-Ruiz et al. 2007). The AI value of the West Fork of the Red River (Table 2) indicates that organic pollution did not impact the structure of the periphyton community.

Thirty-eight diatom taxa in 16 genera were identified from cobble sampled in the West Fork of the Red River (Table 4). The three most abundant diatom taxa were *Cocconeis placentula* var. *euglypta* Erenb. (38%), *Gomphonema brasiliense* Kütz. (16%), and *Navicula minima* Grun. (4%). The relatively low Shannon Diversity Index for the diatom assemblage (Table 5) results from the high evenness value, which in turn is due to the high relative abundance of *Cocconeis placentula* var. *euglypta*.

The Siltation Index (SI) is the percentage of motile diatoms (Bahls 1993). Motile diatoms are able to avoid being buried and are tolerant of sedimentation. SI values ≥ 50 denote severely degraded habitat by excessive sediments. The low SI value of the diatom assemblage (Table 5) indicates the reach sampled was not sediment impaired. The organic pollution Index (OPI) is the percentage of taxa tolerant of organic pollution (Kelly 1998). OPI values ≤ 20 indicate the absence of significant organic pollution, 21–40 infers some organic pollution present, and values > 40 suggest a significant influence of organic pollution. The low OPI value of the diatom assemblage (Table 5) indicates the absence of organic pollution. This conclusion is supported by the low value for the Autotrophic Index (Table 2).

Our results indicate the West Fork of the Red River is impacted by nutrient enrichment. This conclusion is supported by the high concentration of NO₂ + NO₃ nitrogen of the water and concentrations of photoautotrophic-periphyton biomass above the mesotrophic-eutrophic boundary suggested by Dodds et al. (1998). The structure of the diatom assemblage indicates the assemblage was not impacted by siltation nor dissolved organics. The results suggest the West Fork of the Red River should be targeted for habitat improvement by reduction of nutrient enrichment.

Table 4. Diatom taxa and percent taxon composition sampled from cobble in the West Fork of the Red River listed in alphabetical order.

Taxon name	Percent composition
<i>Achnanthes pinnata</i> Hust.	0.4
<i>Achnanthidium deflexa</i> -Reimer	0.4
<i>Achnanthidium minutissimum</i> (Kütz.) Czarn.	0.4
<i>Achnanthidium rivulare</i> Potapova and Ponander	5.4
<i>Achnanthidium</i> sp.	1.8
<i>Amphora veneta</i> Kütz.	0.4
<i>Cocconeis pediculus</i> Ehrenb.	3.6
<i>Cocconeis placentula</i> Ehrenb.	2.7
<i>Cocconeis placentula</i> var. <i>euglypta</i> Ehrenb.	37.9
<i>Cymbella affinis</i> Kütz.	0.9
<i>Gomphonema brasiliense</i> Grun.	16.1
<i>Gomphonema gracile</i> Ehrenb.	0.4
<i>Gomphonema pseudoaugur</i> Lange-Bert.	0.4
<i>Gomphonema pumilum</i> (Grun.) Reichardt and Lange-Bert.	0.4
<i>Gomphonema</i> sp.	1.8
<i>Gyrosigma scalproides</i> (Rabenh.) Cleve	2.2
<i>Luticola goeppertiana</i> (Bleish) D.G. Mann	0.9
<i>Navicula capitatoradiata</i> Germ.	0.9
<i>Navicula cryptotenella</i> Lange-Bert.	1.3
<i>Navicula elginensis</i> Greg.	0.4
<i>Navicula minima</i> Grun.	4.0
<i>Navicula menisculus</i> Schum.	0.9
<i>Navicula</i> sp.	2.2
<i>Navicula subrotundata</i> Hust.	0.4
<i>Navicula tripunctata</i> (Müller) Bory	1.8
<i>Nitzschia amphibia</i> Grun.	0.4
<i>Nitzschia disputata</i> (Kütz) Grun.	0.4
<i>Nitzschia frustulum</i> (Kütz.) Grun.	1.3
<i>Nitzschia</i> sp.	0.9
<i>Planothidium lanceolatum</i> Bréb.	1.3
<i>Planothidium lanceolatum</i> var. <i>dubia</i> Grun.	1.3
<i>Psammothidium curtissimum</i> (Carter) Aboal	1.8
<i>Reimeria sinuata</i> (Greg.) Kociolek and Stoermer	1.3
<i>Rhoicosphenia curvata</i> (Kütz.) Grun.	0.9
<i>Sellaphora pupula</i> Kütz.	0.4
<i>Stephanodiscus</i> sp.	1.8
<i>Achnanthidium</i> sp.	1.8
<i>Amphora veneta</i> Kütz.	0.4
<i>Cocconeis pediculus</i> Ehrenb.	3.6
<i>Cocconeis placentula</i> Ehrenb.	2.7

Table 5. Metrics and indices of the diatom assemblage sampled in the West Fork of the Red River. The index scale is the range of values possible from very good to very poor quality water.

Index	Index scale	Index value
Taxa richness		38
Genus richness		16
Shannon Diversity Index		2.5
Evenness	1 - 0	0.70
Siltation Index	0 - 100	17.4
Organic Pollution Index	0 - 100	8.9

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LITERATURE CITED

- Bahls, L.L. 1993. Periphyton Bioassessment Methods for Montana Streams. Water Quality Bureau, Department of Health and Environmental Sciences, Helena, Montana.
- Baskin, J.A., E.W. Chester, and C.C. Baskin. 1997. Forest vegetation of the Kentucky karst plain (Kentucky and Tennessee): review and synthesis. *J. Torrey Bot. Soc.* 24: 322–335.
- Crossey, M.J. and T.W. LaPointe. 1988. A comparison of periphyton community structural and functional responses to heavy metals. *Hydrobiologia* 162: 109–121.
- Dodds, W.K., J.R. Jones, E.B. Welch. 1998. Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorous. *Water Resources* 32: 1455–1462.
- Eaton, A.D., L.S. Clesceri, E.W. Rice, and A.E. Greenberg (eds.). 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st ed. American Public Health Association, Washington, D.C.
- Irvine, I.N. and T.P. Murphy. 2009. Assessment of eutrophication and phytoplankton community impairment in the Buffalo River area of concern. *J. Great Lakes Res.* 35: 83–93.
- KDOW. 2002. *Methods for assessing biological integrity of surface waters in Kentucky*. Department for Environmental Protection, Division of Water, Frankfort, Kentucky. <<http://water.ky.gov/Pages/SurfaceWaterSOP.aspx>>
- Kelly, M.G. 1998. Use of the trophic diatom index to monitor eutrophication in rivers. *Water Resources* 32: 236–242.
- Lamberti, G.A. and A.D. Steinman. 1997. A comparison of primary production in stream ecosystems. *J. North Amer. Benth. Soc.* 16: 95–104.
- Lebkuecher, J.G., A.S. Flynt, C.M. Loreant. 2000. Relationships between primary photochemistry and primary production in streams with differing water qualities. *Southern Assoc. Agricultural Sci. Bull. Biochem. Biotechn.* 13: 63–68.
- Myers, A.K., A.M. Marcarelli, C.D. Arp, M.A. Baker, and W.A. Wurtsbaugh. 2007. Disruptions of stream sediment size and stability by lakes in mountain watersheds: potential effects on periphyton biomass. *J. North Amer. Benth. Soc.* 26: 390–400.
- Robins, C.R. and R.W. Crawford. 1954. A short accurate method for estimating the volume of stream flow. *J. Wildl. Mgt.* 18: 366–369.
- Smucker, N.J. and M.L. Vis. 2009. Use of diatoms to assess agricultural and coal mining impacts on streams and a multiassemblage case study. *J. North Amer. Benth. Soc.* 28: 659–675.
- Taylor, J.C., M.S.J. van Vuuren, and A.J.H. Pieterse. 2007. The application and testing of diatom-based indices in the Vaal and Wilge Rivers, South Africa. *Water South Africa* 33: 51–59.

- TDEC. 2009. Red River Watershed (05130206) of the Cumberland River Basin Watershed Water Quality Management Plan. Tennessee Department of Environment and Conservation, Division of Water Pollution Control, Nashville.
<<http://www.tn.gov/environment/watersheds/four/redriver/index.shtml>>
- Torres-Ruiz, M., J.D. Wehr, and A.A. Perrone. 2007. Trophic relations in a stream food web: Importance of fatty acids for macroinvertebrate consumers. *J. North Amer. Benth. Soc.* 26: 509–522.
- Van Dam, H., A. Mertens, and J. Sinkeldam. 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Neth. J. Aquat. Ecol.* 28:117–133.
- Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The river continuum concept. *Can. J. Fish Aq. Sci.* 37:130–137.