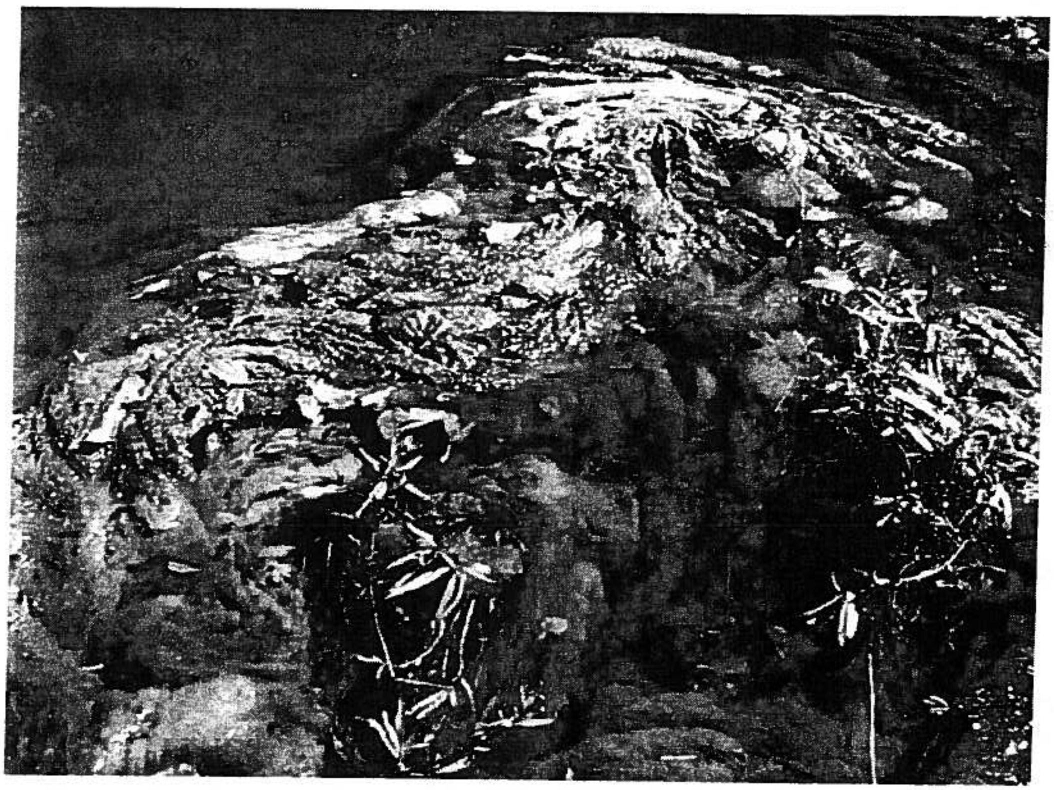
COMPARISON OF NUTRIENT LEVELS, PERIPHYTON DENSITIES AND DIURNAL DISSOLVED OXYGEN PATTERNS IN IMPAIRED AND REFERENCE QUALITY STREAMS IN TENNESSEE



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COMPARISON OF NUTRIENT LEVELS, PERIPHYTON DENSITIES AND DIURNAL DISSOLVED OXYGEN PATTERNS IN IMPAIRED AND REFERENCE QUALITY STREAMS IN TENNESSEE

by

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TDEC
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EXECUTIVE SUMMARY

This study used federal nutrient criteria development funds to conduct algal density field surveys and nutrient sampling for comparison to diurnal dissolved oxygen patterns in reference and impaired streams in 16 ecological subregions. This report is in partial fulfillment of grant X974588-02-0 which enabled nutrient sample collections and algal surveys to be performed at the same time diurnal dissolved oxygen monitoring was being conducted as part of a previously funded study (CP-97449702-0). The results from the diurnal dissolved oxygen portion of the study were published in January 2003.

The periphyton community is made up of sessile algae that inhabit the surfaces of underwater rocks and other stable substrates. Algae are the primary producers in a stream ecosystem, turning nutrients into food for aquatic macroinvertebrates and fish. Since periphyton are sedentary, they are sensitive to changes in water quality. A diverse community can be found in healthy streams. Nuisance blooms are usually symptoms of a system stressed by factors such as elevated nutrients and high temperatures.

This study focused on the density of periphyton rather than the community composition. Taxonomic identification would provide more information. However, sample collection and analysis are time consuming and would require expertise and training in algae taxonomy. It was hoped that a rapid field method requiring no laboratory analysis and limited taxonomic ability would result in a cost effective method that could supplement macroinvertebrate stream surveys and water quality monitoring whenever nutrient enrichment and algae blooms are suspected to be problems.

An additional goal of this study was to determine how nutrient levels affected periphyton densities in different ecoregions. Background levels of total phosphorus and nitrate+nitrite vary in different ecoregions throughout the state with some regions naturally higher in nutrients (Denton et. al., 2001). Nutrients are one of the factors vital to algal growth. By comparing nutrient levels to algal density, this study hoped to find a relationship between nutrients and algal density on a regional basis.

A third goal of this study was to observe the relationship between diurnal dissolved oxygen patterns and the density of periphyton present in the streams. Due to photosynthesis and respiration, it is likely that in streams with an abundance of algae, diurnal oxygen shifts would be more pronounced. Extreme fluctuations in oxygen levels have the potential to stress aquatic fauna.

Thirty-five test sites and 44 reference sites located in 15 ecoregions were surveyed for periphyton. Since the parent study focused on dissolved oxygen, test sites were selected that had been assessed as impaired due to organic enrichment and/or low dissolved oxygen rather than nutrients according to the 2002 assessment database. However, it should be noted that the presence of organic enrichment generally results in higher nutrient levels and increased algal growth while conversely low DO is often just a symptom. Seven of the test sites had also been assessed as impaired due to nutrients and two were fully supporting.

Based on this preliminary study, the rapid periphyton method appeared to be most useable in middle and east Tennessee streams with generally rocky substrates. This type of periphyton survey does not seem to be a viable option for water quality assessments in most west Tennessee subregions. Except for the Transition Hills (65j) and the Bluff Hills (74a), west Tennessee streams usually have shifting sand bottoms with limited stable rock substrate. Other methods, such as the use of artificial substrates or collection of other habitats with taxonomic identification, may yield better results but would not provide a rapid field based assessment. These more intensive methods may prove cost and time prohibitive and would require specialized staff training in algal taxonomy.

In most regions with suitable substrate, very little periphyton was measured in reference streams. The mean density of microalgae did not exceed a thickness rank of 0.5 (slimy substrate but no visual accumulation) in most ecoregions. The only exceptions were the Inner (71i) and Outer (71h) Nashville Basins of the Interior Plateau where the mean density approached 1.0 (thin layer of algae visible but no measurable accumulation). These were also the only regions, along with the Southern Limestone/Dolomite Valleys and Low Rolling Hills (67f), where macroalgae were found in reference streams.

Periphyton densities were not always a good predictor of nutrient levels even in regions with little algae present in reference conditions. At many test sites, nutrient levels were elevated, but periphyton abundance was similar to reference levels. This is because other factors, including sunlight and warm temperatures also influence the growth rates of algae. Many streams, especially small ones, have canopies that block sunlight and keep water temperatures down. In addition, the abundance of grazing animals, such as snails, can have an impact on algal density. However, periphyton densities did correspond with elevated nutrients at some sites. The best response was seen at three streams in the Eastern Highland Rim (71g).

Dissolved oxygen levels appeared to be affected by the amount of periphyton present in the streams. Although levels generally stayed above regional criteria, diurnal fluctuations were more pronounced when algal densities were above reference stream conditions. Extreme changes in dissolved oxygen levels are believed to have a detrimental affect on aquatic life even when criteria for minimum concentrations are met. The type or periphyton (macroalgae or microalgae) did not appear to be as strong an influence on dissolved oxygen fluctuations as the abundance.

This study demonstrated the value of using canopy measurements and macroinvertebrate collections (grazer abundance) when conducting this type of periphyton survey. Due to funding constraints of the grant, neither of these measures was used as part of this project. This information gap limited interpretive ability in streams where algae were not present. Partially as a result of insight gained during this project, rapid periphyton assessments have been added to two additional 104(b) grants the Division has recently been awarded. As the data set grows, the information will help clarify where and when periphyton surveys are most useful and how best to interpret results.

1. INTRODUCTION

This study used federal nutrient criteria development funds to conduct algal density field surveys and nutrient sampling for comparison to diurnal dissolved oxygen patterns in reference and impaired streams in 15 ecological subregions. This report is in partial fulfillment of grant X974588-02-0 which enabled nutrient sample collections and algal surveys to be performed at the same time diurnal dissolved oxygen monitoring was being conducted as part of a previously funded study (CP-97449702-0). The results from the diurnal dissolved oxygen portion of the study were published in January 2003

The results from both studies will help guide assessment decisions for the 2004 305(b) report of the Status of Water Quality in Tennessee as well as provide additional insight into the continuing development of regional nutrient and dissolved oxygen criteria. The work undertaken for this project is specific to wadeable streams. Any results or conclusions contained within the report have limited application to other waterbody types, such as large, nonwadeable streams, lakes and wetlands.

1.0 Periphyton and Water Quality

The periphyton community is made up of sessile algae that inhabit the surfaces of underwater rocks and other stable substrates. They are the primary producers in the stream ecosystem, turning nutrients into food for aquatic macroinvertebrates and fish. For the purposes of this study, periphyton can be divided into two broad categories. Macroalgae are long filamentous strands of algae such as *Cladophora* or *Spirogyra* spp. Microalgae are primarily single celled algae which coat the substrate and are generally composed of diatoms and soft algae such as blue-green algae.

Due to the sedentary nature of periphyton, the community composition and biomass are sensitive to changes in water quality. A diverse community of periphyton can be found in healthy streams. Nuisance blooms are usually symptoms of a system stressed by factors such as excessive nutrients, elevated temperatures, or stagnant conditions.

Excessive algal growth can reduce biodiversity by making habitat unsuitable for benthic fish and macroinvertebrates and by altering diurnal dissolved oxygen patterns. Excessive algae levels are generally associated with an increase in tolerant macroinvertebrates. Grazers (scrapers) such as snails generally dominate a benthic community influenced by excessive algae growth.

This study focused on the density of periphyton rather than the community composition. Taxonomic identification would provide more information. However, collection and analyses are time consuming and would require expertise and training in algae taxonomy. It was hoped that a rapid field method requiring no laboratory analysis or taxonomic expertise would result in a cost effective method that could supplement macroinvertebrate stream surveys and water quality monitoring whenever nutrient enrichment and algae blooms are suspected to be a problem.

One limitation of using benthic algae as an indicator of nutrient enrichment is that densities can be affected by many other factors. Algal growth is influenced by factors such as canopy cover (sunlight), time available to grow since the last flood, streambed stability, substrate size, water velocity, nutrients and grazing by aquatic fauna.

The number of animals that eat periphyton (grazers and scrapers) increases as algae becomes more abundant. These organisms, in turn, reduce the amount of algae. Streams that are heavily grazed may not have abundant periphyton although conditions are right for growth. For this reason, algal surveys are best performed in conjunction with macroinvertebrate sampling.

Sunlight is necessary for algal growth. Streams with heavy canopy or high turbidity will not support abundant periphyton. Weather is another important factor. Several days of rain or cloud cover that blocks sunlight will temporarily reduce algal growth.

Suitable stable substrate is also essential. Gravel must be at least two cm for periphyton to grow (Barbour et al, 1999). Streams with shifting sand bottoms are not suitable for colonization. If storm events have caused flooding, it can take several weeks for periphyton to return to former levels. However, recolonization will be faster in streams with nutrient enrichment.

1.1 Nutrient Enrichment and Density of Periphyton

Another goal in this study was to see how nutrient levels affected periphyton densities in different ecoregions. Background levels of total phosphorus and nitrate+nitrite vary in different ecoregions throughout the state with some regions being naturally high in nutrients (Denton et. al., 2001). Nutrients are vital to algal growth. By comparing nutrient levels to algal density, this study hoped to find a relationship between nutrients and algal density on a regional basis.

Nutrients are generally considered a secondary stressor rather than a direct toxicant. Elevated nutrients, under certain conditions, promote the growth of algae. However, elevated nutrient levels do not always stimulate algal growth. As mentioned earlier, many other factors, especially sunlight, are necessary for algae to thrive. Therefore, it cannot be assumed that nutrient enrichment always results in nuisance levels of algae.

1.2 Density of Periphyton and Dissolved Oxygen

A third goal of this study was to observe the relationship between diurnal dissolved oxygen patterns and the density of periphyton. Oxygen is produced during photosynthesis and consumed during respiration and decomposition. Because photosynthesis requires light, it occurs only during daylight hours. At night, in the absence of photosynthesis, DO levels decline due to respiration and decomposition.

Due to the effects of photosynthesis, it is likely that in streams with an abundance of algae, diurnal oxygen shifts will be more pronounced. Daylight monitoring of dissolved oxygen may miss water quality problems since levels are at their highest during daylight hours. Even if nighttime lows remain above criteria, extreme diurnal fluctuations appear to have a stressful effect on aquatic life (Arnwine and Denton, 2003).

2 SITE SELECTION

The nutrient and periphyton portion of this study was added to an existing dissolved oxygen study (Arnwine and Denton, 2003). The initial dissolved oxygen study plan targeted 72 reference streams and 72 test streams in 16 ecological subregions. Of these, many were dry at the time of scheduled sample collections, while others were inappropriate for rapid periphyton density surveys due to deep non-wadeable water, shifting sand substrates, or turbid conditions. Thirty-four test sites and 44 reference sites in 15 subregions were surveyed for periphyton (Figure 1). One of the original 16 subregions, the Sequatchie Valley (68b), was dropped completely as all targeted sites were dry during the study period.

Since the parent study focused on dissolved oxygen, test sites were selected based on impairment due to low dissolved oxygen rather than nutrient enrichment according to the 2002 assessment database. Of these, seven had also been assessed as impaired due to nutrients. Two additional test sites which were fully supporting were also selected. The test sites fell within nine subregions.

An ecoregion is a relatively homogenous area defined by similarity of climate, landform, soil, potential natural vegetation, hydrology and other ecologically relevant variables. Ecoregions are further delineated into subregions to provide more clear boundaries and homogeneity in factors that can affect water quality and biotic characteristics than the national-scale ecoregions (Griffith et al., 1997). Tennessee has been divided into 25 ecological subregions (Figure 2), which were defined during the ecoregion delineation project (Arnwine et al, 2000).

Established reference sites located within 15 of the targeted subregions were monitored to provide baseline information on periphyton and diurnal dissolved oxygen. Nutrient data at these sites have been collected on a routine basis since 1996. A complete site list (reference and test) is provided in Table 1.

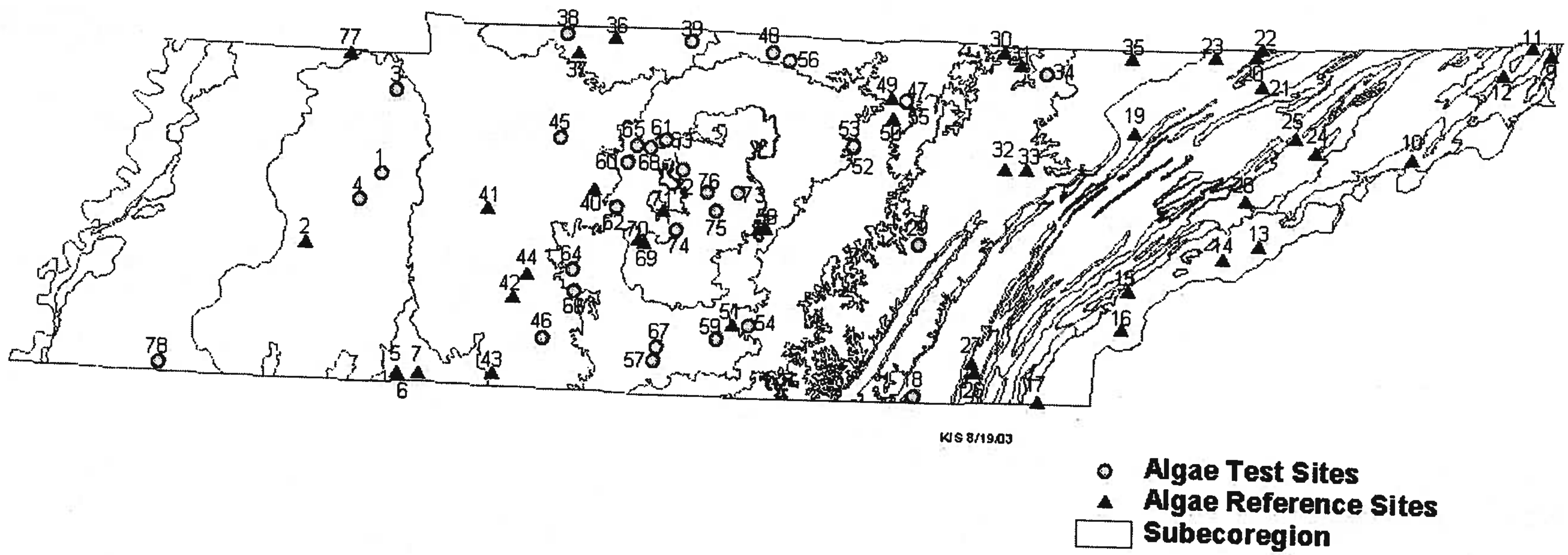
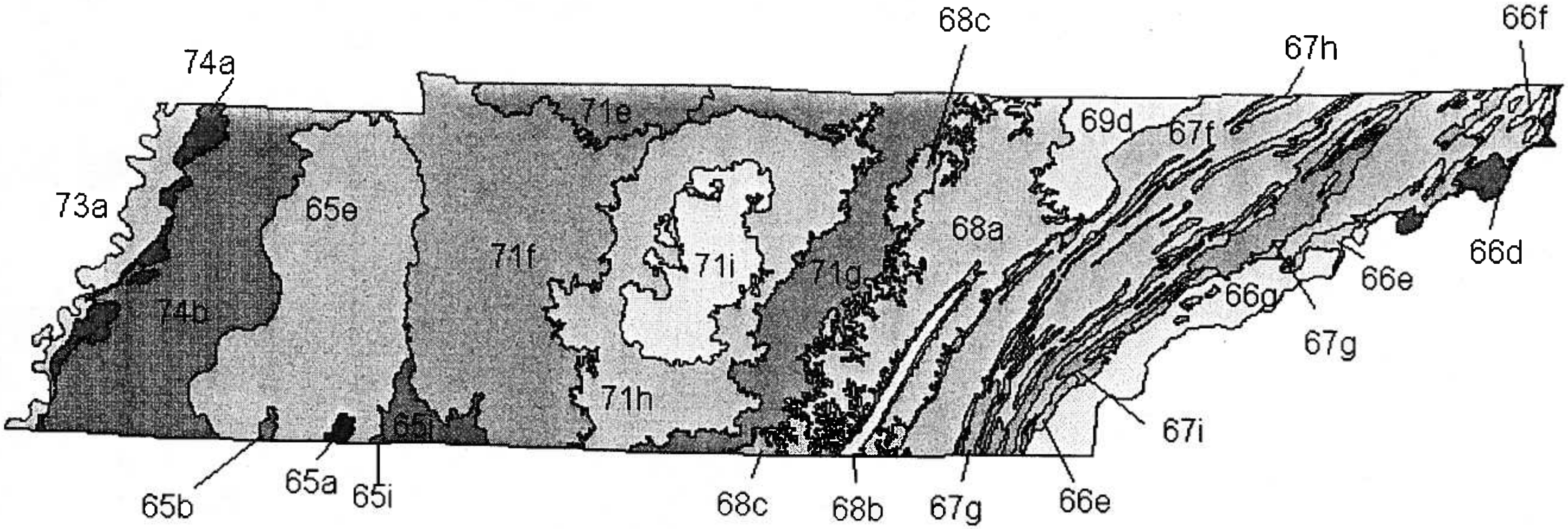


Figure 1: Location of reference sites and test sites where periphyton surveys were conducted (August – October, 2002).



65a Blackland Prairie
65b Flatwoods/Alluvial Prairie Margins
65e Southeastern Plains and Hills
65i Fall Line Hills
65j Transition Hills
66d Southern Igneous Ridges and Mtns
66e Southern Sedimentary Ridges
66f Limestone Valleys and Coves
66g Southern Metasedimentary Mtns.
67f Southern Limestone/Dolomite Valleys and Low Rolling Hills
67g Southern Shale Valleys
67h Southern Sandstone Ridges
67i Southern Dissected Ridges & Knobs
68a Cumberland Plateau
68b Sequatchie Valley
68c Plateau Escarpment
69d Cumberland Mountains
71e Western Pennyroyal Karst
71f Western Highland Rim
71g Eastern Highland Rim
71h Outer Nashville Basin
71i Inner Nashville Basin
73a Northern Mississippi Alluvial Plain
74a Bluff Hills
74b Loess Plains

Figure 2: Ecological subregions of Tennessee

Table 1: Site List

MAP No.	ECO	STATION ID	STREAM	COUNTY	POLLUTANT 2002 Assessment
1	65e	BSAND036.4CR	Big Sandy River	Carroll	Org. Enr./Low DO Pathogens
2	65e	ECO65E08	Harris Creek	Madison	Reference
3	65e	HFORK004.0HN	Holly Fork Creek	Henry	Org. Enr./Low DO Habitat Alterations Pathogens
4	65e	MUD004.7CR	Mud Creek	Carroll	Org. Enr./Low DO Habitat Alterations Siltation
5	65j	ECO65J04	Pompeys Branch	Hardin	Reference
6	65j	ECO65J05	Dry Creek	Hardin	Reference
7	65j	ECO65J06	Right Fork Whites Creek	Hardin	Reference
8	65j	ECO65J11	Unnamed Trib Rt Fk Whites Cr.	Hardin	Reference
9	66e	ECO66E04	Gentry Creek	Johnson	Reference
10	66e	ECO66E11	Lower Higgens Creek	Unicoi	Reference
11	66f	ECO66F07	Beaverdam Creek	Johnson	Reference
12	66f	ECO66F08	Stoney Creek	Carter	Reference
13	66g	ECO66G04	Middle Prong Little Pigeon River	Sevier	Reference
14	66g	ECO66G05	Little River	Sevier	Reference
15	66g	ECO66G07	Citico Creek	Monroe	Reference
16	66g	ECO66G09	North River	Monroe	Reference
17	66g	ECO66G12	Sheeds Creek	Polk	Reference
18	67f	DOBBS000.3HM	Dobbs Branch	Hamilton	Org. Enr./Low DO Habitat Alterations Pathogens
19	67f	ECO67F06	Clear Creek	Anderson	Reference
20	67f	ECO67F14	Powell River	Hancock	Reference
21	67f	ECO67F17	Big War Creek	Hancock	Reference
22	67f	ECO67F23	Martin Creek	Hancock	Reference
23	67f	ECO67F25	Powell River	Claiborne	Reference
24	67g	ECO67G01	Little Chucky Creek	Greene	Reference
25	67g	ECO67G05	Bent Creek	Hamblen	Reference
26	67g	ECO67G08	Brymer Creek	Bradley	Reference
27	67g	ECO67G09	Harris Creek	Bradley	Reference

Table 1 cont.

MAP No.	ECO	STATION ID	STREAM	COUNTY	POLLUTANT 2002 Assessment
28	67g	ECO67G10	Flat Creek	Sevier	Reference
29	68a	BRADD000.2BL	Bradden Creek	Bledsoe	Org. Enr./Low DO Habitat Alterations
30	68a	ECO68A01	Rock Creek	Morgan	Reference
31	68a	ECO68A03	Laurel Fork Station Camp Creek	Scott	Reference
32	68a	ECO68A26	Daddys Creek	Cumberland	Reference
33	68a	ECO68A27	Island Creek	Morgan	Reference
34	68a	PINE006.0SC	Pine Creek	Scott	Low DO Nutrients Priority Organics Siltation Habitat Alterations Pathogens
35	69d	ECO69D01	No Business Branch	Campbell	Reference
36	71e	ECO71E09	Buzzard Creek	Robertson	Reference
37	71e	ECO71E14	Passenger Creek	Montgomery	Reference
38	71e	SPRIN009.8MT	Spring Creek	Montgomery	Org. Enr./Low DO Habitat Alterations Siltation
39	71e	SUMME008.6SR	Summers Branch	Sumner	Org. Enr./Low DO Nutrients Siltation Pathogens
40	71f	ECO71F12	South Harpeth Creek	Williamson	Reference
41	71f	ECO71F16	Wolf Creek	Hickman	Reference
42	71f	ECO71F19	Brush Creek	Lewis	Reference
43	71f	ECO71F27	Swanegan Branch	Wayne	Reference
44	71f	ECO71F28	Little Swan Creek	Lewis	Reference
45	71f	JONES014.4DI	Jones Creek	Dickson	Org. Enr./Low DO Siltation
46	71f	SHOAL055.4LW	Shoal Creek	Lawrence	Org. Enr./Low DO Ammonia Metals Pathogens
47	71g	CARR003.6OV	Carr Creek	Overton	Org. Enr./Low DO Pathogens
48	71g	CLEAR001.1CE	Clear Branch	Coffee	Org. Enr./Low DO Pathogens

Table 1 cont.

MAP No.	ECO	STATION ID	STREAM	COUNTY	POLLUTANT 2002 Assessment
49	71g	ECO71G03	Flat Creek	Overton	Reference
50	71g	ECO71G04	Spring Creek	Overton	Reference
51	71g	ECO71G10	Hurricane Creek	Moore	Reference
52	71g	MLICK014.7PU	Mine Lick Creek	Putnam	Fully Supporting Test Site
53	71g	MLICK015.5PU	Mine Lick Creek	Putnam	Org. Enr./Low DO Pathogens
54	71g	ROCK009.3FR	Rock Creek	Franklin	Org. Enr./Low DO Flow Alteration Thermal Siltation
55	71g	TOWN000.9OV	Town Creek	Overton	Org. Enr./Low DO Pathogens
56	71g	TOWN001.1MA	Town Creek	Macon	Org. Enr./Low DO Nutrients Ammonia Pathogens
57	71h	BROWN000.4DA	Browns Creek	Davidson	Org. Enr./Low DO Nutrients Habitat Alterations Pathogens Oil and Grease
58	71h	ECO71H09	Carson Creek	Cannon	Reference
59	71h	EFMUL010.4MR	East Fork Mulberry Creek	Moore	Org. Enr./Low DO Siltation
60	71h	LHARP001.8WI	Little Harpeth River	Williamson	Low DO Habitat Alterations Siltation
61	71h	MILL003.3DA	Mill Creek	Davidson	Org. Enr./Low DO Siltation
62	71h	RATTL000.2WI	Rattlesnake Branch	Williamson	Org. Enr./Low DO Habitat Alterations
63	71h	SIMS000.8DA	Sims Branch	Davidson	Org. Enr./Low DO Nutrients Habitat Alterations Pathogens
64	71h	SUGAF002.4MY	Sugar Fork	Maury	Fully Supporting Test Site
65	71h	SUGAR000.1DA	Sugartree Creek	Davidson	Org. Enr./Low DO Habitat Alterations Pathogens

Table 1 cont.

MAP No.	ECO	STATION ID	STREAM	COUNTY	POLLUTANT 2002 Assessment
66	71h	SUGAR000.2MY	Sugar Creek	Maury	Org. Enr./Low DO Habitat Alterations Siltation Unionized Ammonia Salinity/TDS/Chlorides
67	71h	SWAN008.0LI	Swan Creek	Lincoln	Org. Enr./Low DO Pathogens
68	71h	WFBRO000.1DA	West Fork Browns Creek	Davidson	Org. Enr./Low DO Pathogens
69	71i	ECO71I10	Flat Creek	Marshall	Reference
70	71i	ECO71I14	Little Flat Creek	Maury	Reference
71	71i	ECO71I15	Harpeth River	Williamson	Reference
72	71i	HURRI004.2RU	Hurricane Creek	Rutherford	Org. Enr./Low DO Nutrients Siltation
73	71i	JARMA000.3RU	Jarman Branch	Rutherford	Org. Enr./Low DO Habitat Alterations Siltation
74	71i	KELLY000.4RU	Kelley Creek	Rutherford	Org. Enr./Low DO Habitat Alterations Siltation
75	71i	LYTLE1T0.1RU	Unnamed trib to Lytle Creek	Rutherford	Org. Enr./Low DO Pathogens
76	71i	WFSTO008.3RU	West Fork Stones River	Rutherford	Organic Enrichment Nutrients Siltation
77	74b	ECO74B01	Terrapin Creek	Henry	Reference
78	74b	STOUT000.4FA	Stout Creek	Fayette	Org. Enr./Low DO Siltation

* Number in first column (MAP NO.) refers to station location in Figure 1

3. DATA COLLECTION

3.0 Field-Based Periphyton Surveys

The periphyton protocols for this study were adapted from the field-based rapid periphyton method developed by Stevenson and Bahls (Barbour et. al., 1999). The modified method provided information on periphyton densities in two broad categories: macroalgae and microalgae. Macroalgae are long filamentous strands of algae such as *Cladophora* or *Spirogyra* spp. Microalgae are primarily single celled algae that coat the substrate and are generally composed of diatoms and soft algae such as blue-green algae.

Three transects were surveyed at each site. Transects were located in riffle or run areas with the least amount of canopy in the stream reach. Three locations were then selected along each transect (right, middle and left bank). A gridded viewing bucket was positioned over the nine selected locations at each site. The viewing surface of the bucket was divided into a 50-dot grid for a total of 450 dots at each site.

Along each transect the following information was recorded:

a. The number of dots that occurred over macroalgae.
b. The number of dots where substrate was suitable for microalgal accumulation (gravel> 2 cm and not covered by macroalgae)
c. The thickness rank of microalgae under each dot was measured using the following thickness scale:

   0 – substrate rough with no evidence of microalgae
   0.5 – substrate slimy, but no visual accumulation of microalgae is evident
   1 - a thin layer of microalgae is visually evident, less than 0.5 mm thick
   2 - accumulation of microalgal layer from 0.5-1 mm thick is evident
   3 - accumulation of microalgal layer from 1 to 5 mm thick is evident
   4 - accumulation of microalgal layer from 5 mm to 2 cm thick is evident
   5 - accumulation of microalgal layer greater than 2 cm thick is evident.

The density of algae on substrate at each site was then statistically characterized by determining:

a. The percent of macroalgae present
b. The percent of substrate available for microalgae colonization
c. The maximum thickness rank of microalgae
d. The Mean thickness rank of microalgae (mean density)

$$\sum d_i r_i / d_t$$

Where: d_i = number of grid points (dots) over microalga of different thickness ranks
r_i = thickness rank of microalgae
d_t = total number of grid points over suitable microalgae substrate at the site

3.1 Nutrient Sample Collections

Total phosphorus and nitrate+nitrite samples were collected at the beginning and end of each seven-day sampling period at the test sites. Samples were collected using a modified clean hands technique established for the ecoregion monitoring project (Arnwine et al, 2000). Field personnel wore new disposable gloves while handling each sample. Samples were double bagged, iced and returned to the state laboratory for analysis within 4 days of collection. (The holding time for a nutrient sample is 28 days.)

Nutrient analyses were performed by the state environmental lab, Tennessee Department of Health (TDH) in Nashville. Total phosphorus was analyzed using method A.18.9.1 with a detection limit of 0.004 mg/l. Nitrate+nitrite was analyzed using method A.18.4 with a detection limit of 0.01 mg/l.

In accordance with the study plan, nutrient samples were not collected at reference sites. Reference nutrient guidelines for each ecoregion were determined using the 90th percentile of reference data collected from 1996 to 2001 (Denton et. al., 2001).

3.2 Diurnal Dissolved Oxygen Monitoring

The original study plan focused on diurnal dissolved oxygen patterns. A second grant was awarded to collect periphyton and nutrient samples at sites which had been selected for DO monitoring. Continuous monitoring dissolved oxygen and temperature probes were deployed at each reference and test site as part of the concurrent grant.

Each site was continuously monitored for approximately 168 hours (seven days) with readings recorded every 30 minutes. Graphs of the diurnal data from all monitored reference and test sites are provided in Appendix C of *Evaluation of Regional Dissolved Oxygen Patterns of Wadeable Streams in Tennessee Based on Diurnal and Daylight Monitoring* (Arnwine and Denton, 2003).

3.3 Quality Assurance

Algal surveys were performed by trained aquatic biologists following EPA field-based rapid periphyton survey methods (Barbour et al, 1999). The protocol was modified for this study in that all microalgae were grouped rather than splitting by type. Duplicate surveys were conducted by a second biologist at 10% of the sites.

A modified clean technique was used for the nutrient collections. Duplicates, trip blanks and field blanks were collected at 10% of the sites. Samples were preserved with sulfuric acid, iced and delivered directly to the lab within four days of collection by the samplers. Chain of Custody was maintained on all samples. All nutrient analyses were performed by chemists at the state laboratory who followed EPA approved analysis and quality assurance methods. Unique log numbers were assigned to all samples for tracking.

Stevens/Greenspan Model CS304 multi-parameter probes were used for diurnal monitoring. The accuracy of the dissolved oxygen probe was +/- 0.2 ppm. The probes were factory calibrated with pre and post calibration checks performed on each probe. Two probes were set simultaneously at 10 percent of the diurnal continuous monitoring sites.

4. RESULTS

4.0 Periphyton, Nutrients and Dissolved Oxygen in Reference and Impaired Streams of the Southeastern Plains and Hills (65e)

The majority of streams in the Southeastern Plains and Hills subregion do not have rock substrates suitable for periphyton colonization. Bottom substrate is characterized by shifting sand with particle sizes generally less than 2 cm. Only one of the four reference sites surveyed, Harris Creek (ECO65E08), had suitable rock substrate (Table 2). Eighteen percent of the available substrate at this site was covered by microalgae with no macroalgae observed. The microalgae were never more than a slime coating with no measurable thickness resulting in a mean density of 0.1 (Figure 3). Dissolved oxygen levels remained above 6 ppm throughout the seven-day study period. Possibly due at least in part to the lack of algal growth, diurnal fluctuations were limited. DO never dropped more than 2 ppm during any cycle and generally dropped less than 1 ppm.

Table 2: Periphyton and nutrient data for test and reference sites in the Southeastern Plains and Hills (65e).

Test Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	NO2+NO3 in mg/l (2 readings at beginning and end of monitoring week)		TP in mg/l (2 readings at beginning and end of monitoring week)	
BEAVE005.5CR	NA	NA	NA	NA	NA	0.16	0.14	0.11	0.12
BSAND036.4CR	12	0	18	0.5	0.1	0.3	0.26	0.01	0.03
CLEAR001.2CR	NA	NA	NA	NA	NA	0.86	0.52	0.29	0.17
HFORK004.0HN	1	0	0	0.0	0.0	0.48	0.40	0.002	0.09
MUD004.7CR	1	0	0	0.0	0.0	0.13	0.1	0.05	0.08
Reference Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	Regional NO2+NO3 Guidelines		Regional TP Guidelines	
ECO65E04	NA	NA	NA	NA	NA	0.34		0.04	
ECO65E06	NA	NA	NA	NA	NA				
ECO65E08	54	0	18	0.5	0.1				
ECO65E10	NA	NA	NA	NA	NA				

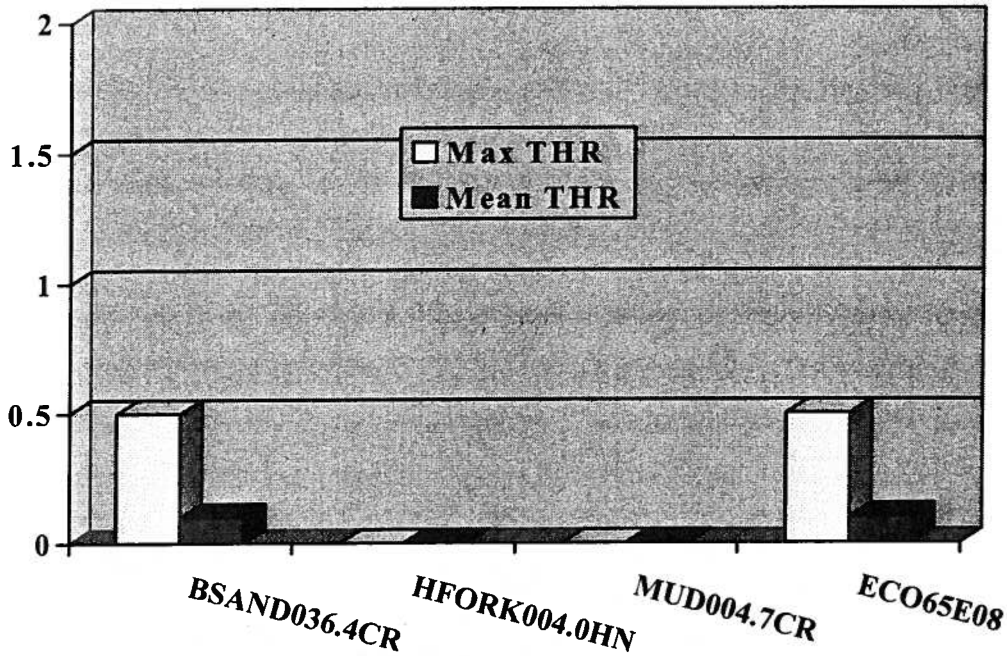


Figure 3: Maximum and mean thickness ranks (THR) of microalgae at reference and test sites in the Southeastern Plains and Hills (65e).

Only three of the five test sites surveyed had suitable rock substrate. The substrate was very sparse at 1 to 12%. The site with the most rock habitat (12%), Big Sandy Creek, had a slime layer of microalgae on 18% of the suitable substrate with a mean density of 0.1. Dissolved oxygen levels at this site were above 7 ppm throughout the study. Diurnal fluctuations were generally less than 1 ppm. Nutrients were not elevated during the study week.

Both nitrate+nitrite and total phosphorus were elevated at Holly Fork and total phosphorus was elevated at Mud Fork (Figures 4 and 5). However, these two sites only had 1% of bottom substrate stable enough for periphyton colonization and no periphyton were observed. Diurnal DO fluctuations at both sites were under 1 ppm except during a storm event where DO fell approximately 1.5 ppm. DO levels at both sites remained at or above 6 ppm.

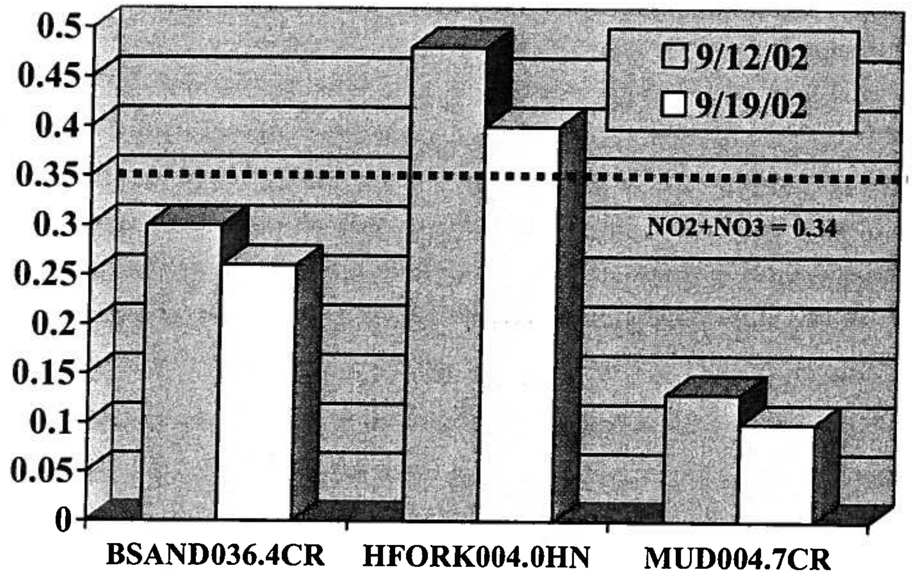


Figure 4: Nitrate+nitrite (mg/l) at beginning and end of 1-week survey period at 3 test sites in the Southeastern Plains and Hills (65e). Initial samples were collected on 9/12/02 and second samples were collected on 9/19/02. Dashed line is regional guideline (0.34 mg/l).

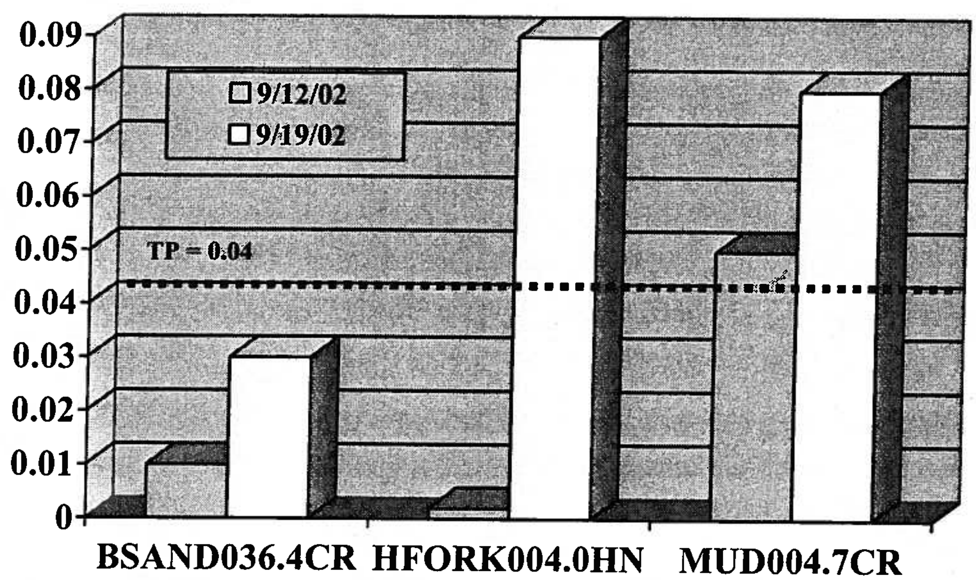


Figure 5: Total phosphorus (mg/l) at beginning and end of 1-week survey period at 3 test sites in the Southeastern Plains and Hills (65e). Initial samples were collected on 9/12/02 and second samples were collected on 9/19/02. Dashed line is regional guideline (0.04 mg/l).

Due to the limited stable rock substrate in streams in the Southeastern Plains and Hills, the periphyton method used in this study does not seem to be a viable option for water quality assessments. Other methods (artificial substrates or collection of other habitats with taxonomic identification) may yield better results but would not provide a rapid field-based assessment.

4.1 Periphyton, Nutrients and Dissolved Oxygen in Reference Streams in the Transition Hills (65j).

All four of the reference streams in the Transition Hills subregion of the Southeastern Plains had plentiful rock substrate suitable for periphyton colonization (54-96%). Even so, periphyton growth was not abundant with only 0 to 33% of the substrate covered by a thin layer of microalgae less than 0.5 mm thick (Table 3). Although some rocks had a density of 1, the mean thickness rank was low ranging from 0.0 to 0.2 (Figure 6). Macroalgae were not present at any reference site. Reference levels of nitrate+nitrite in this region are among the lowest in the state. This may help account for the limited periphyton community found in these streams.

Dissolved oxygen levels did not fall below 7 ppm at any of the reference sites and diurnal fluctuations were less than 1 ppm. Test sites were not monitored since there were no streams in this region assessed as impaired due to organic enrichment or low DO during the 2002 assessment process.

Table 3: Periphyton levels and nutrient guidelines based on reference stream monitoring in the Transition Hills (65j).

Reference Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	Regional NO2+NO3 Guidelines	Regional TP Guidelines
ECO65J04	96	0	32	0.5	0.2	0.22	0.04
ECO65J05	80	0	0	0.0	0.0		
ECO65J06	80	0	33	1.0	0.2		
ECO65J11	54	0	24	1.0	0.2		

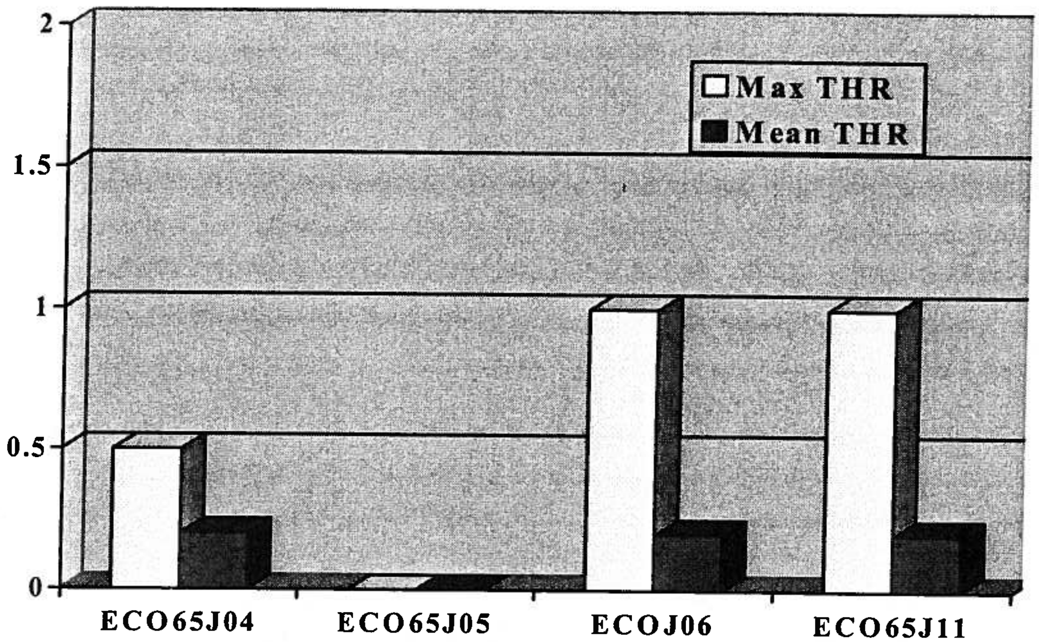


Figure 6: Maximum and Mean thickness ranks (THR) of microalgae at reference sites in the Transition Hills (65j).

4.2 Periphyton, Nutrients and Dissolved Oxygen in Reference Streams in the Southern Sedimentary Ridges (66e).

The majority of bottom substrate in reference streams in the Southern Sedimentary Ridges subregion of the Blue Ridge Mountains was suitable for periphyton colonization. However, periphyton coverage at reference sites did not exceed 10% (Table 4). Only a slime coating was evident on a few rocks with a mean thickness rank of 0.0 (Figure 7).

Table 4: Periphyton levels and nutrient guidelines based on reference stream monitoring in the Southern Sedimentary Ridges (66e).

Reference Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	Regional NO2+NO3 Guidelines	Regional TP Guidelines
ECO66E04	86	0	10	0.5	0.0	0.30	0.01
ECO66E11	90	0	6	0.5	0.0		

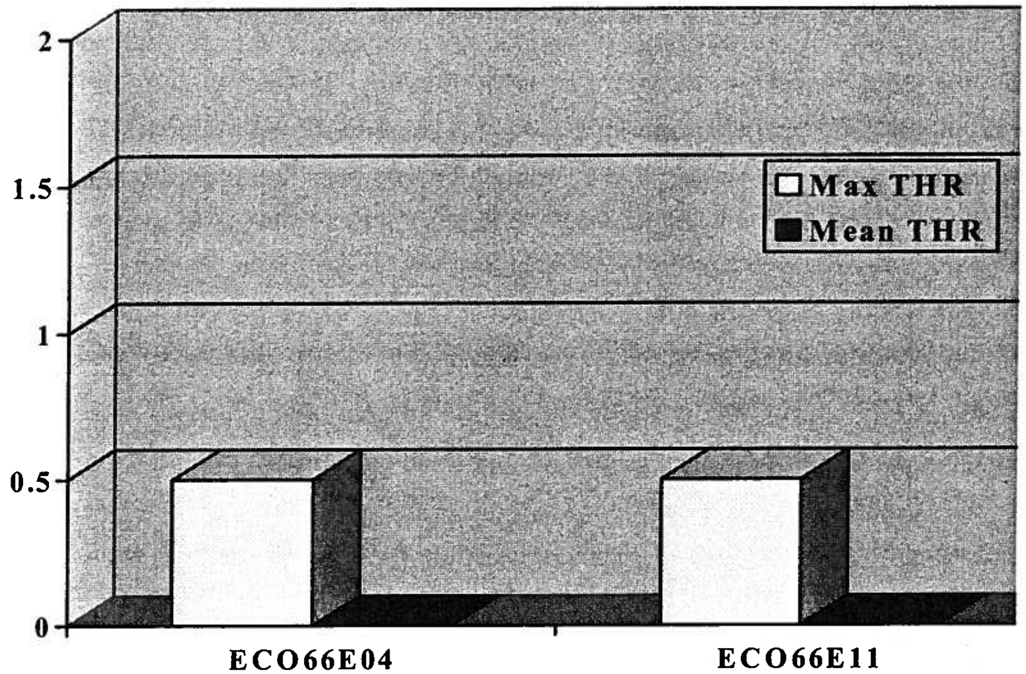


Figure 7: Maximum and mean thickness ranks (THR) of microalgae at reference sites in the Southern Sedimentary Ridges (66e).

Dissolved oxygen levels stayed above 7.7 ppm throughout the study period and did not fluctuate by more than 1 ppm during each diurnal cycle. Cold, turbulent water, rather than photosynthesis, is probably more important to oxygen generation in most Blue Ridge subregions. Reference total phosphorus levels in this subregion, along with two other Blue Ridge Mountain subregions, are the lowest in the state. Test sites were not monitored since there were no streams in this region assessed as impaired due to organic enrichment or low DO during the 2002 assessment process.

4.3 Periphyton, Nutrients and Dissolved Oxygen in Reference Streams in the Limestone Valleys and Coves (66f).

The majority of bottom substrate in reference streams in the Limestone Valleys and Coves subregion of the Blue Ridge Mountains was suitable for periphyton colonization (94-97%). Very little periphyton was present even though total phosphorus levels are generally higher in the Limestone Valleys and Coves than other Blue Ridge subregions. Only 6 – 22% of the substrate at the two reference streams sampled was covered by a slime layer of microalgae (Table 5). The mean thickness rank ranged from 0.0 to 0.1 (Figure 8). There were no macroalgae present.

Dissolved oxygen levels were relatively stable and did not vary by more than 1.5 ppm within any 24-hour period. The lowest value at either site was 7.5 ppm. Test sites were not monitored since there were no streams in this region assessed as impaired due to organic enrichment or low DO during the 2002 assessment process.

Table 5: Periphyton levels and nutrient guidelines based on reference stream monitoring in the Limestone Valleys and Coves (66f).

Reference Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	Regional NO2+NO3 Guidelines	Regional TP Guidelines
ECO66F07	97	0	22	0.5	0.1	0.30	0.03
ECO66F08	94	0	6	0.5	0.0		

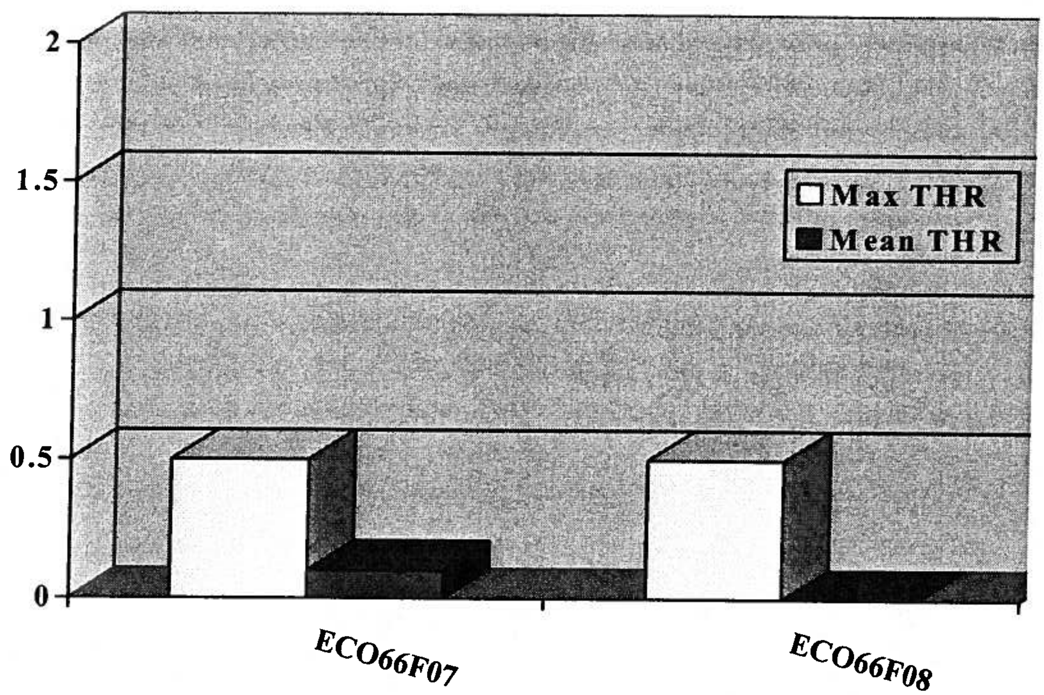


Figure 8: Maximum and mean thickness ranks (THR) of microalgae at reference sites in the Limestone Valleys and Coves (66f).

4.4 Periphyton, Nutrients and Dissolved Oxygen in Reference Streams in the Southern Metasedimentary Mountains (66g).

Most of the bottom substrate in streams in the Metasedimentary Mountains subregion of the Blue Ridge Mountains is suitable for periphyton colonization (74-94%). Periphyton were more abundant in reference streams here than in other Blue Ridge subregions even though total phosphorus levels were lower than those measured in the Limestone Valleys and Coves. Microalgae covered 33-85% of the rocks in the five reference streams surveyed (Table 6). However, density was still relatively low. The mean thickness rank ranged from 0.2 to 0.5 (Figure 9). There were no macroalgae present at any of the reference sites.

Table 6: Periphyton levels and nutrient guidelines based on reference stream monitoring in the Southern Metasedimentary Mountains (66g).

Reference Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	Regional NO2+NO3 Guidelines	Regional TP Guidelines
ECO66G04	94	0	72	1.0	0.5	0.30	0.01
ECO66G05	87	0	29	0.5	0.2		
ECO66G07	90	0	85	1.0	0.5		
ECO66G09	74	0	33	0.5	0.2		
ECO66G12	84	0	34	0.5	0.2		

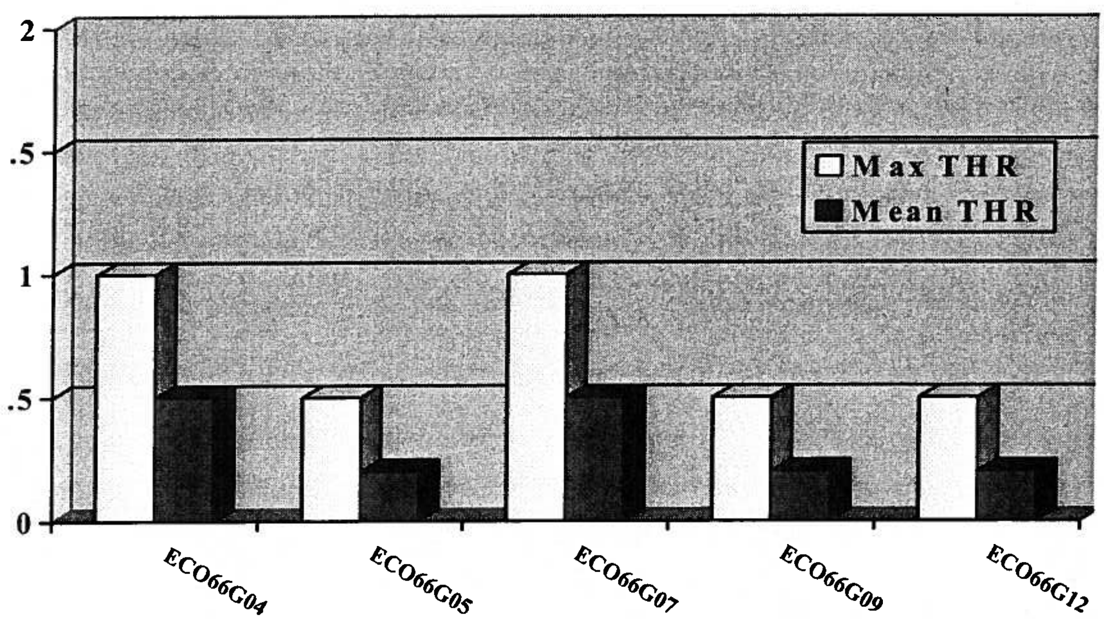


Figure 9: Maximum and mean thickness ranks (THR) of microalgae at reference sites in the Southern Metasedimentary Mountains (66g)

Even though periphyton densities in streams of this region were somewhat higher than the other Blue Ridge regions, diurnal dissolved oxygen patterns were similar. DO generally stayed above 8 ppm with diurnal fluctuations within 1 ppm. Test sites were not monitored since there were no streams in this region assessed as impaired due to organic enrichment or low DO during the 2002 assessment process.

4.5 Periphyton, Nutrients and Dissolved Oxygen in Reference and Impaired Streams in the Southern Limestone/Dolomite Valleys and Low Rolling Hills (67f).

Streams in this subregion of the Ridge and Valley ecoregion generally have plentiful rock substrate suitable for periphyton colonization. Although total phosphorus levels are comparable to other regions, nitrate+nitrite concentrations at reference streams are among the highest in the state (90th percentile = 1.22 mg/l). Even so, periphyton are not abundant at reference streams with three out of five having less than 10% of available substrate covered by a slime layer with the mean density ranging from 0.0 to 0.2 (Table 7 and Figure 10).

Table 7: Periphyton and nutrient data for test and reference sites in the Southern Limestone/Dolomite Valleys and Low Rolling Hills (67f).

Test Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	NO2+NO3 in mg/l (2 readings at beginning and end of monitoring week)	TP in mg/l (2 readings at beginning and end of monitoring week)
DOBBS000.3HM	36	0	44	0.5	0.5	0.22 0.53	0.292 0.05
Reference Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	Regional NO2+NO3 Guidelines	Regional TP Guidelines
ECO67F06	92	0	7	0.5	0.0	1.22	0.04
ECO67F14	83	0	15	1	0.1		
ECO67F17	90	0	5	0.5	0.0		
ECO67F23	82	0	8	0.5	0.0		
ECO67F25	74	10	31	1	0.2		

Three reference sites had less than 10% of the available substrate covered by a slime coat of microalgae. The mean density at these sites was 0.0 and no macroalgae were present. Dissolved oxygen levels generally fluctuated about 2 ppm during each diurnal cycle with minimum readings above 6.5 ppm.

Two reference stations (ECO67F14 and ECO67F25) had more abundant microalgae although the mean thickness rank was still low. Both of these sites are located on the Powell River. The river was also the only stream surveyed in this region where macroalgae was recorded (10%). This increase in periphyton production is probably a reflection of the river width. The wide river allowed more direct sunlight than is available at other, smaller reference streams with more canopy cover. Despite the difference in periphyton growth, diurnal dissolved oxygen patterns in the Powell River were similar to the smaller streams. Minimum readings were generally around 6.5 ppm with diurnal fluctuations of 2 ppm.

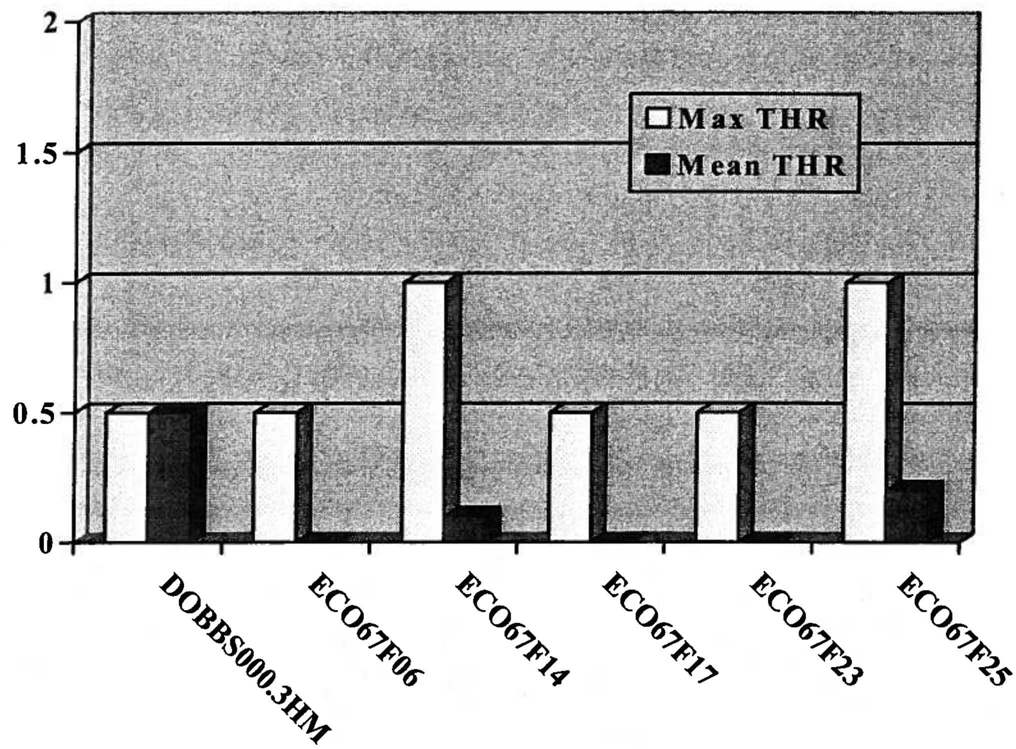


Figure 10: Maximum and mean thickness ranks (THR) of microalgae at reference and test sites in the Southern Limestone/Dolomite Valleys and Low Rolling Hills (67f).

One test site, Dobbs Branch (DOBBS000.3HM), was also surveyed. This site had elevated total phosphorus levels seven days prior to the periphyton survey (Figure 11). Levels had dropped but were still slightly elevated on the day the survey was conducted. Nitrate+nitrite levels met regional guidelines throughout the survey week.

Only 36% of the substrate was suitable for periphyton colonization at Dobbs Creek due to sediment covering the majority of rocks. Forty-four percent of the available substrate was covered by a slime layer of microalgae. There were no macroalgae observed at this site. Dissolved oxygen readings were erratic. The diurnal readings fluctuated between 0 and 5.2 ppm and were usually below 5 ppm.

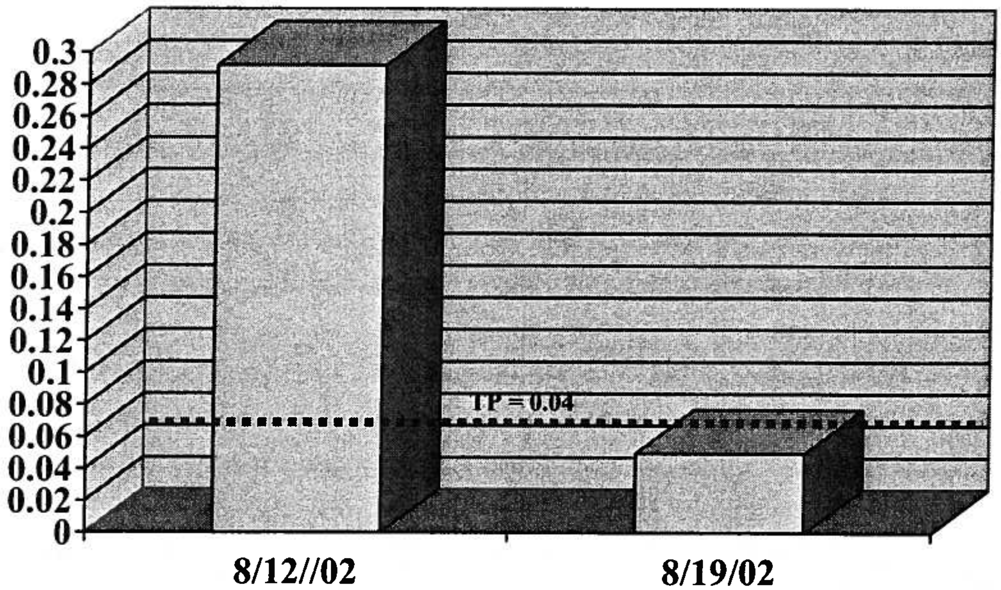


Figure 11: Total phosphorus (mg/l) at beginning and end of 1-week survey period at Dobbs Creek test site in the Southern Limestone/Dolomite Valleys and Low Rolling Hills (67f). Dashed line is regional guideline (0.04 mg/l).

4.6 Periphyton, Nutrients and Dissolved Oxygen in Reference Streams in the Southern Shale Valleys (67g).

Five reference streams were sampled in the Southern Shale Valleys subregion of the Ridge and Valley ecoregion. Rock substrate ranged from 63-96%. A slime layer of microalgae was present on 0-48% of the suitable substrate (Table 8). Density was low with the mean thickness rank ranging from 0.0 to 0.2 (Figure 12). Macroalgae were not present at any reference site.

Total phosphorus levels at reference sites were generally higher in this subregion (90th percentile = 0.09 mg/l) than other Ridge and Valley regions (90th percentile = 0.04 mg/l). Test sites were not monitored since there were no streams in this region assessed as impaired due to organic enrichment or low DO during the 2002 assessment process.

Table 8: Periphyton levels and nutrient guidelines based on reference stream monitoring in the Southern Shale Valleys (67g).

Reference Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	Regional NO2+NO3 Guidelines	Regional TP Guidelines
ECO67G01	63	0	0	0.0	0.0	1.22	0.09
ECO67G05	82	0	5	0.5	0.0		
ECO67G08	83	0	25	0.5	0.1		
ECO67G09	69	0	35	0.5	0.2		
ECO67G10	96	0	48	0.5	0.2		

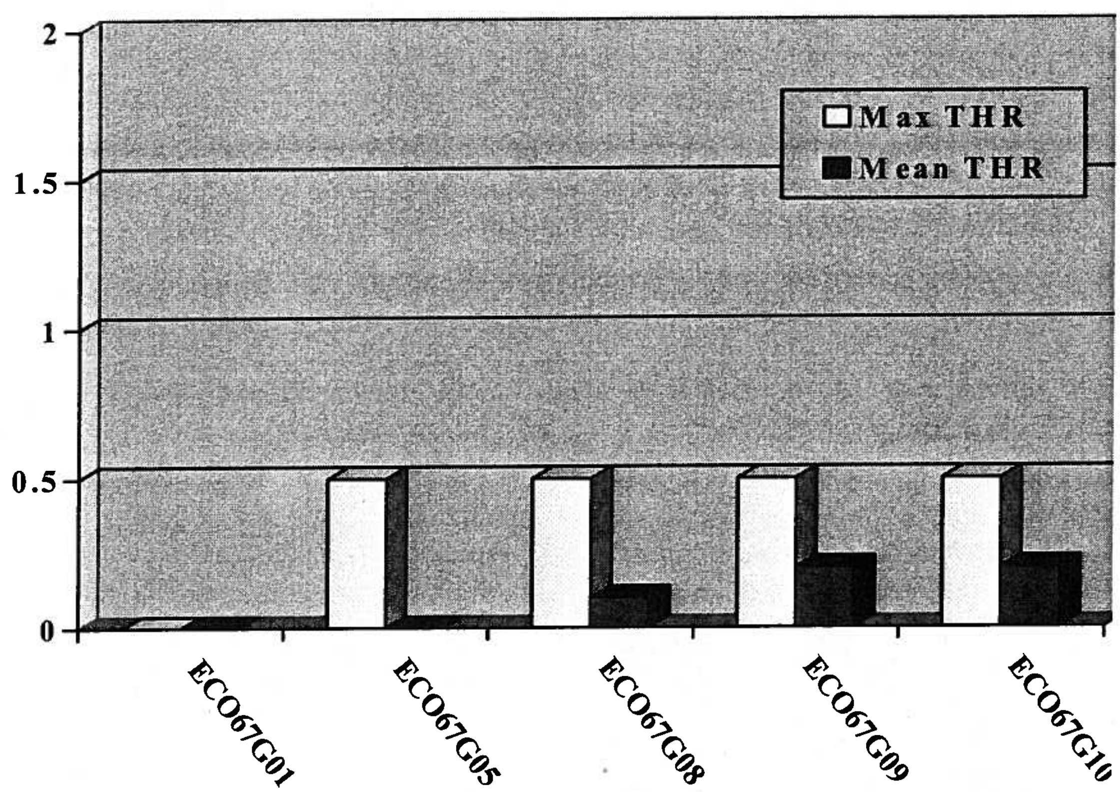


Figure 12: Maximum and mean thickness ranks (THR) of microalgae at reference sites in the Southern Shale Valleys (67g).

4.7 Periphyton, Nutrients and Dissolved Oxygen in Reference and Impaired Streams in the Cumberland Plateau (68a).

Four reference sites were sampled in the Cumberland Plateau subregion of the Southwestern Appalachians. Suitable rock substrate was plentiful, ranging from 46-91%. A slime coat of microalgae was visible on less than 10% of the rocks with no measurable density at three of the four reference sites monitored in this region (Table 9).

The fourth reference site, Daddys Creek (ECO68A26), had a slime layer of periphyton on 93% of the rock substrate. This may be a reflection of stream width and sunlight as this creek is larger than the other three reference sites surveyed. The station on Daddy's Creek has approximately 25% canopy cover while the other stations are 50-95% shaded.

Even though microalgae covered most of the substrate, it was mostly present as a slime layer yielding a mean thickness rank of 0.5 (Figure 13). Diurnal dissolved oxygen fluctuations were more pronounced at this creek (1.5 ppm) than the other reference streams, which generally fluctuated around 0.5 ppm during each cycle.

Table 9: Periphyton and nutrient data for test and reference sites in the Cumberland Plateau (68a).

Test Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	NO2+NO3 in mg/l (2 readings at beginning and end of monitoring week)		TP in mg/l (2 readings at beginning and end of monitoring week)	
BRADD000.2BL	92	4	32	1.0	0.2	0.1	0.03	0.038	0.08
PINE006.0SC	91	0	91	0.5	0.5	1.68	8.80	0.126	1.4
Reference Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	Regional NO2+NO3 Guidelines		Regional TP Guidelines	
ECO68A01	86	0	7	0.5	0.0	0.23		0.03	
ECO68A03	46	0	3	0.5	0.0				
ECO68A26	91	0	93	0.5	0.5				
ECO68A27	57	0	5	0.5	0.0				

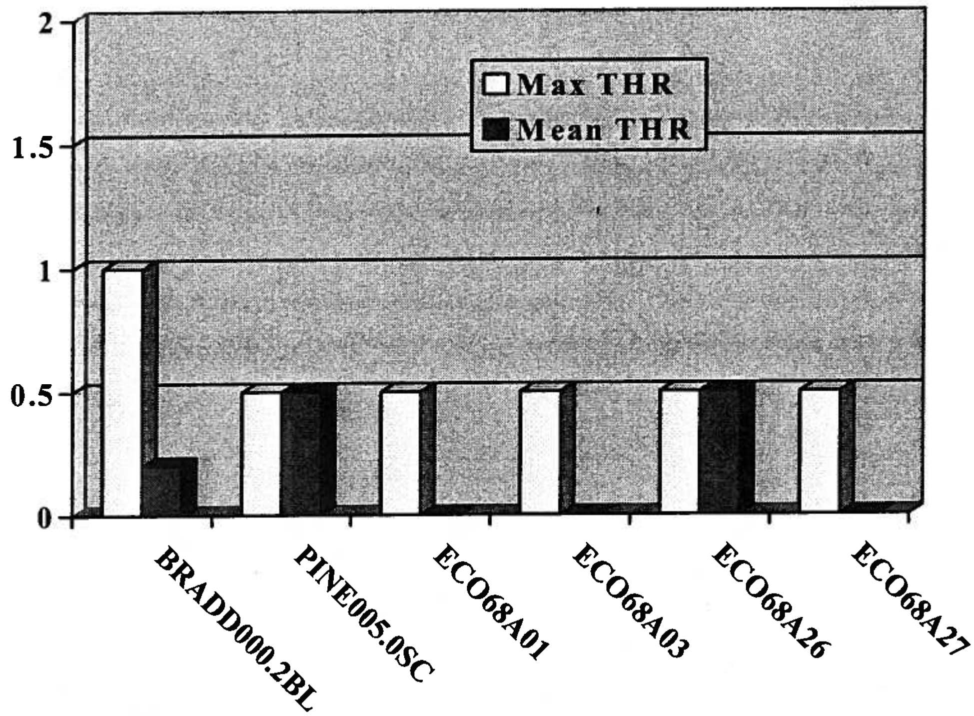


Figure 13: Maximum and mean thickness ranks (THR) of microalgae at reference and test sites in the Cumberland Plateau (68a).

Two test sites were also surveyed in the Cumberland Plateau. Bradden Creek had slightly elevated total phosphorus levels during the survey week (Figure 14). Thirty-two percent of the rock substrate was covered by a thin layer of microalgae up to 0.4 mm thick. The mean thickness rank was 0.2. This was the only stream surveyed in this region where macroalgae were present (4%). Unlike the reference streams, where DO generally fluctuated by 0.5 to 1.5 ppm, DO in this stream varied as much as 3 ppm during each diurnal cycle.

The second test site sampled in the Cumberland Plateau was on Pine Creek downstream of the Oneida STP. Both total phosphorus and nitrate+nitrite exceeded regional guidelines during the survey week (Figures 14 and 15). A slime coat of algae covered 91% of the substrate. The mean thickness rank was 0.5. Although this is comparable to the reference site on Daddy's Creek, Pine is a much smaller creek with a greater percent of canopy (45%). Periphyton densities would be expected to be more similar to the other reference sites. Similar to the other test site on Bradden Creek and unlike the reference sites, dissolved oxygen fluctuations were pronounced, varying up to 3 ppm during each diurnal cycle (Figures 16 and 17).

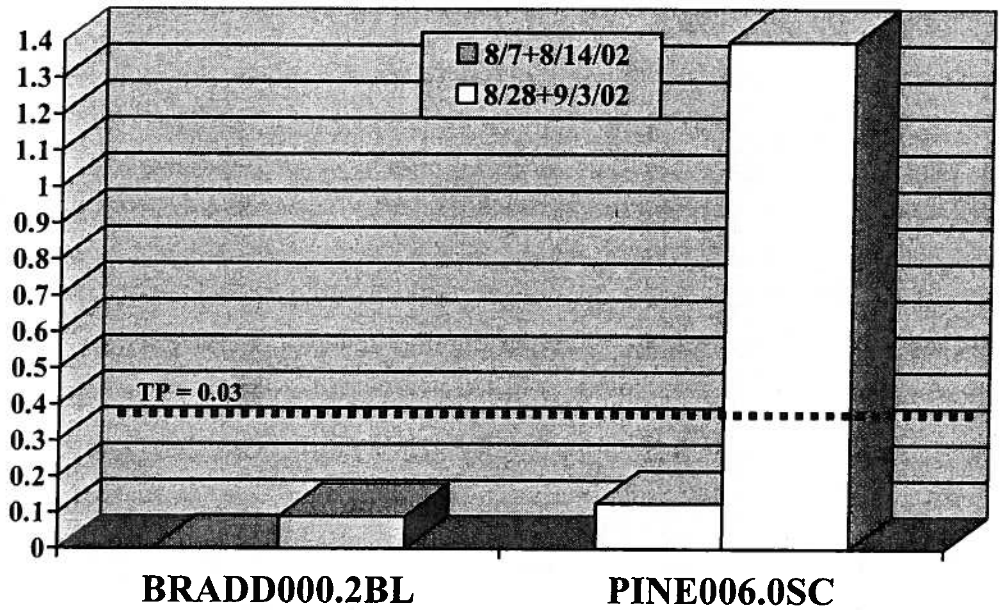


Figure 14: Total phosphorus (mg/l) at beginning and end of 1-week survey period at 2 test sites in the Cumberland Plateau (68a). Dashed line is regional guideline (0.03 mg/l).

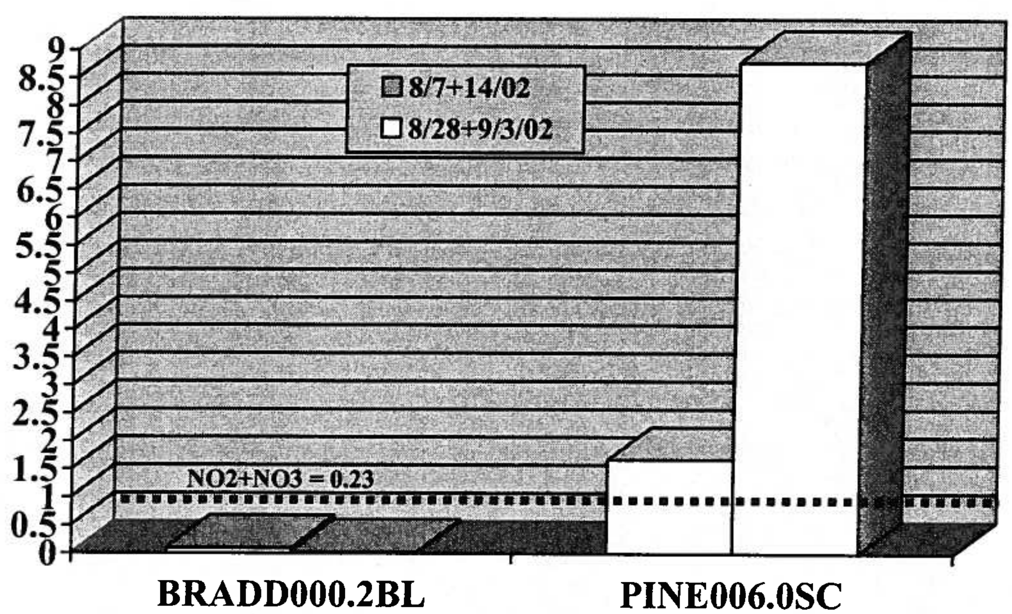


Figure 15: Nitrate+nitrite (mg/l) at beginning and end of 1-week survey period at 2 test sites in the Cumberland Plateau (68a). Dashed line is regional guideline (0.23 mg/l).

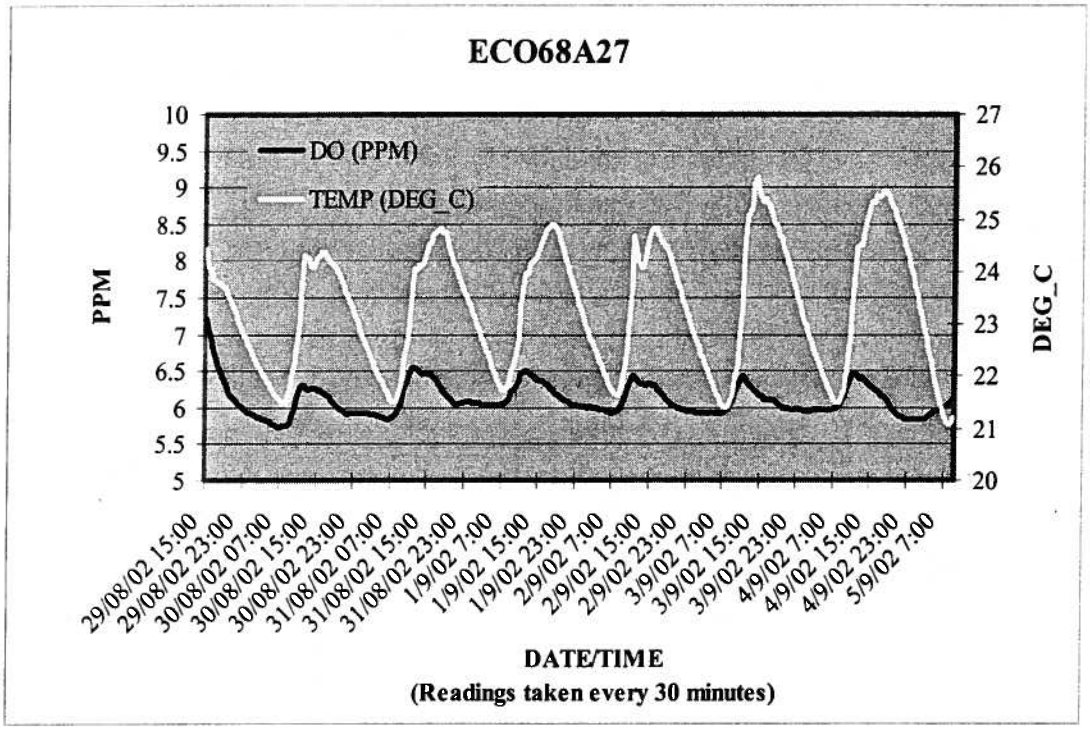


Figure 16: Diurnal dissolved oxygen and temperature data, Island Creek reference site, Cumberland Plateau (68a). Readings every 30 minutes for 162 hours.

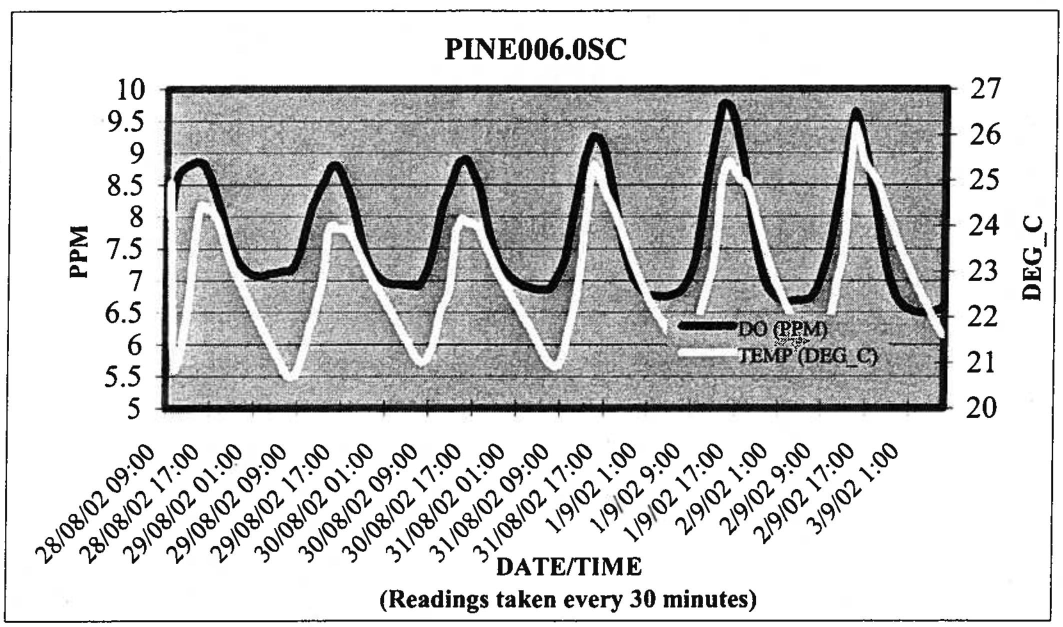


Figure 17: Diurnal dissolved oxygen and temperature data, Pine Creek test site, Cumberland Plateau (68a). Readings every 30 minutes for 142 hours.

4.8 Periphyton, Nutrients and Dissolved Oxygen in a Reference Stream in the Cumberland Mountains (69d)

A single reference site, No Business Branch (ECO69D01), was sampled in the Cumberland Mountains subregion of the Central Appalachians. The rest of the established reference sites had insufficient flow. This lack of steam flow is typical of streams in this subregion during the late summer/early fall season. Test sites were not monitored since there were no streams in this region assessed as impaired due to organic enrichment or low DO during the 2002 assessment process.

Fifty-nine percent of the bottom substrate at No Business Branch was suitable for periphyton colonization (Table 10). Only 13% of the rocks had a slime coating of microalgae with a mean thickness rank of 0.1 (Figure 18). Macroalgae were not present.

Diurnal dissolved oxygen swings were up to 2 ppm although this may not reflect a typical pattern since water levels fell dramatically during the week and the probe may have been partially exposed. Fluctuations were generally around 0.5 ppm during the first half of the week when water levels were higher.

Table 10: Periphyton levels and nutrient guidelines based on reference stream monitoring in the Cumberland Mountains (69d).

Reference Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	Regional NO2+NO3 Guidelines	Regional TP Guidelines
ECO69D01	59	0	13	0.5	0.1	0.27	0.02

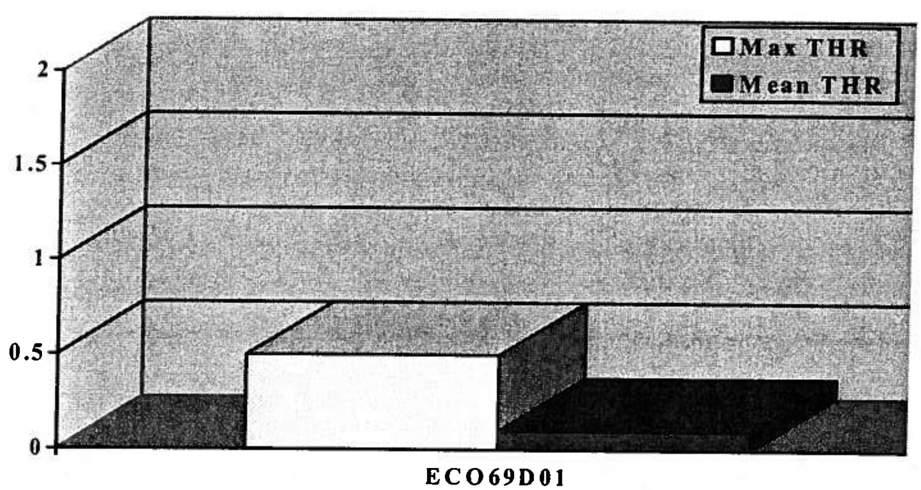


Figure 18: Maximum and mean thickness rank (THR) of microalgae at No Business Branch reference site in the Cumberland Mountains (69d).

4.9 Periphyton, Nutrients and Dissolved Oxygen in Reference and Impaired Streams in the Western Pennyroyal Karst (71e).

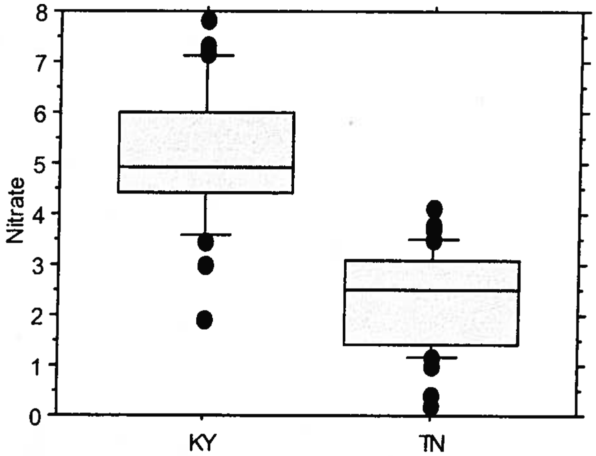
Two reference sites were sampled in the Western Pennyroyal Karst subregion of the Interior Plateau. Most of the bottom substrate (86-87%) was rock suitable for periphyton colonization (Table 11).

Despite the high background levels of nitrate+nitrite in this subregion, microalgae densities were similar to other regions in the state. A thin layer of microalgae (less than 0.5 mm thick) was present on 58% of the substrate at Passenger Creek (ECO71E14) while a slime surface was noted on 45% of the rocks at Buzzard Creek (ECO71E09). Mean density ranged from 0.2 to 0.4 at the two sites (Figure 20). Macroalgae were not present at either site.

Although reference total phosphorus levels are comparable to other Tennessee subregions, nitrate+nitrite levels in the Western Pennyroyal Karst are the highest of any region in the state (90th percentile = 3.48 mg/l). However, these levels are lower than those found in Kentucky reference streams in this subregion (Figure 19).

Table 11: Periphyton and nutrient data for test and reference sites in the Western Pennyroyal Karst (71e).

Test Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	NO2+NO3 in mg/l (2 readings at beginning and end of monitoring week)		TP in mg/l (2 readings at beginning and end of monitoring week)	
SPRIN009.8MT	60	0	0	0.0	0.0	4.4	3.4	0.02	0.08
SUMME008.6SR	56	0	66	1.0	0.4	1.79	1.38	2.27	0.7
Reference Stations	**% Substrate Available**	**% Macro algae**	**% Micro algae**	**Max Thickness Rank**	**Mean Thickness Rank (Mean Density)**	**Regional NO2+NO3 Guidelines**		**Regional TP Guidelines**	
ECO71E09	87	0	45	0.5	0.2	3.48		0.05	
ECO71E14	86	0	58	1.0	0.4				



	Kentucky	Tennessee
MN	5.15	2.28
SD	1.38	0.96
SE	0.26	0.16
COUNT	29	37
MIN	1.93	0.19
MAX	7.83	4.11
MED	4.92	2.52

Figure 19: Comparison of nitrate (KY) and nitrate+nitrite (TN) levels at reference streams in the Western Pennyroyal Karst (71e). Data provided by KY Department of Environmental Protection, Division of Water.

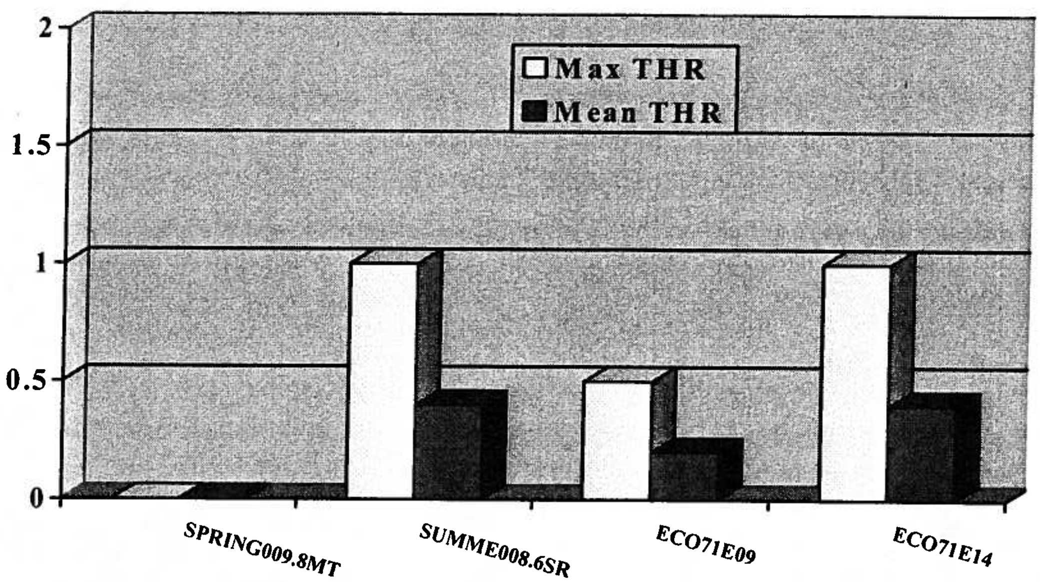


Figure 20: Maximum and mean thickness ranks (THR) of microalgae at reference and test sites in the Western Pennyroyal Karst (71e).

Diurnal dissolved oxygen fluctuations were pronounced at both reference sites. Passenger Creek fluctuated almost 3 ppm during some cycles while Buzzard Creek fluctuated around 2 ppm during each cycle.

Two test streams were also surveyed in this region. Periphyton were not observed at Spring Creek although nitrate+nitrite guidelines were exceeded at the beginning of the seven-day sampling period and total phosphorus was exceeded on the periphyton survey date (Figures 21 and 22). Habitat was not a limiting factor since 60% of the bottom substrate was suitable for colonization. However, substantial rains and high water were documented during the week prior to the survey, which may have affected periphyton growth. Dissolved oxygen levels stayed above 5.5 ppm with diurnal swings of 1.5 ppm, a smaller fluctuation than measured at reference sites where periphyton was present.

The second test site, Summers Branch had total phosphorus well above guidelines throughout the sampling period. Sixty-six percent of the rock substrate was covered by a thin layer of microalgae with a mean thickness rank of 0.4. This was similar to periphyton densities at the Passenger Creek reference site. Macroalgae were not present at either test site. Excessive sediment was noted at this site, which may account for the limited algal growth. Diurnal dissolved oxygen fluctuations were at least 2 ppm. Minimum DO values were 3 ppm at night and rose above 5 ppm only during the afternoon hours.

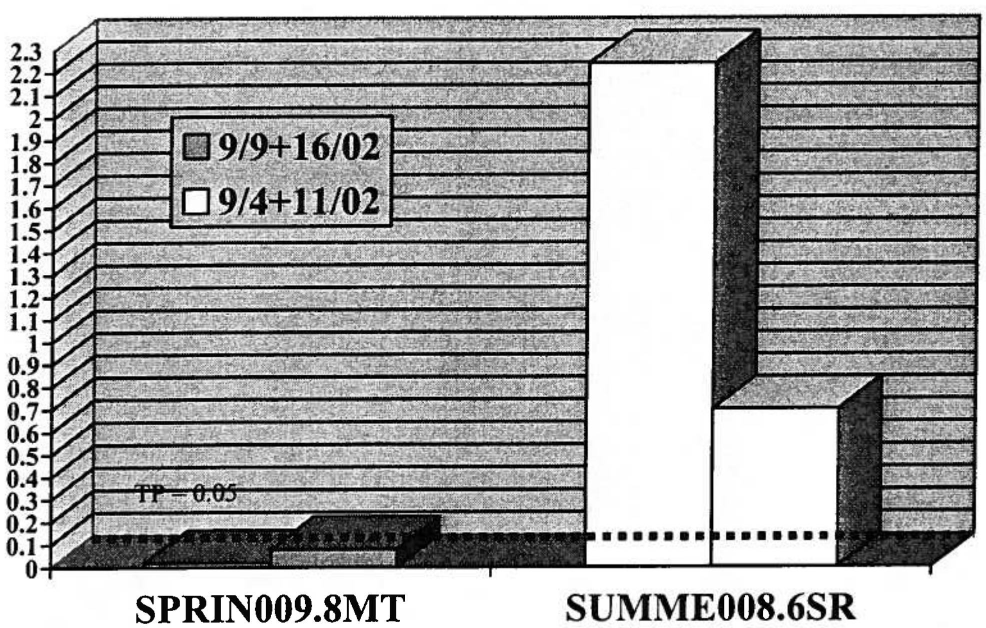


Figure 21: Total phosphorus (mg/l) at beginning and end of 1-week survey period at 2 test sites in the Western Pennyroyal Karst (71e). The dotted line represents regional guidelines (0.05 mg/l).

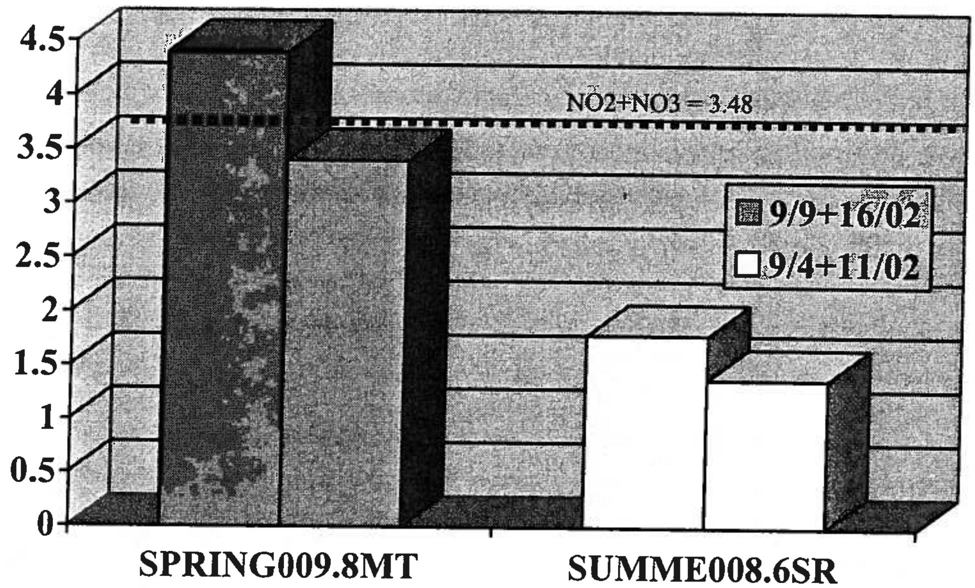


Figure 22: Nitrate+nitrite (mg/l) at beginning and end of 1-week survey period at 2 test sites in the Western Pennyroyal Karst (71e). The dotted line represents regional guidelines (3.48 mg/l).

4.10 Periphyton, Nutrients and Dissolved Oxygen in Reference and Impaired Streams in the Western Highland Rim (71f).

Five reference sites were surveyed in the Western Highland Rim subregion of the Interior Plateau. The streams had from 70 to 85% of the bottom substrate suitable for periphyton colonization (Table 12). Microalgae covered from 11 to 79% of the suitable substrate. Generally, the microalgae were in the form of a slime coating with no visible accumulation.

A thin layer of microalgae was noted on a few rocks at South Harpeth Creek (ECO71F12). Little Swan Creek (ECO71F28) was the reference site with the highest density of microalgae. Although microalgae were present on 79% of the suitable substrate, the mean density was low (0.4) since only a slime coat was observed (Figure 23). Macroalgae were not visible at any reference site in this region.

Reference dissolved oxygen levels stayed well above 6 ppm, with the lowest reading being 6.8 ppm at Swanegan Branch (ECO 71F27). The other reference sites stayed above 7 ppm. Diurnal fluctuations were typically less than 2 ppm although South Harpeth Creek (ECO71F12) varied by 3 ppm within a 24-hour period during a rain event.

Table 12: Periphyton and nutrient data for test and reference sites in the Western Highland Rim (71f).

Test Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	NO2+NO3 in mg/l (2 readings at beginning and end of monitoring week)		TP in mg/l (2 readings at beginning and end of monitoring week)	
JONES014.4DI	48	0	32	1.0	0.2	0.19	0.28	0.15	0.15
SHOAL055.4LW	64	0	59	0.5	0.4	1.56	1.72	0.014	0.07
Reference Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	Regional NO2+NO3 Guidelines		Regional TP Guidelines	
ECO71F12	78	0	44	1.0	0.3	0.38		0.05	
ECO71F16	78	0	51	0.5	0.2				
ECO71F19	85	0	54	0.5	0.2				
ECO71F27	70	0	11	0.5	0.1				
ECO71F28	83	0	79	0.5	0.4				

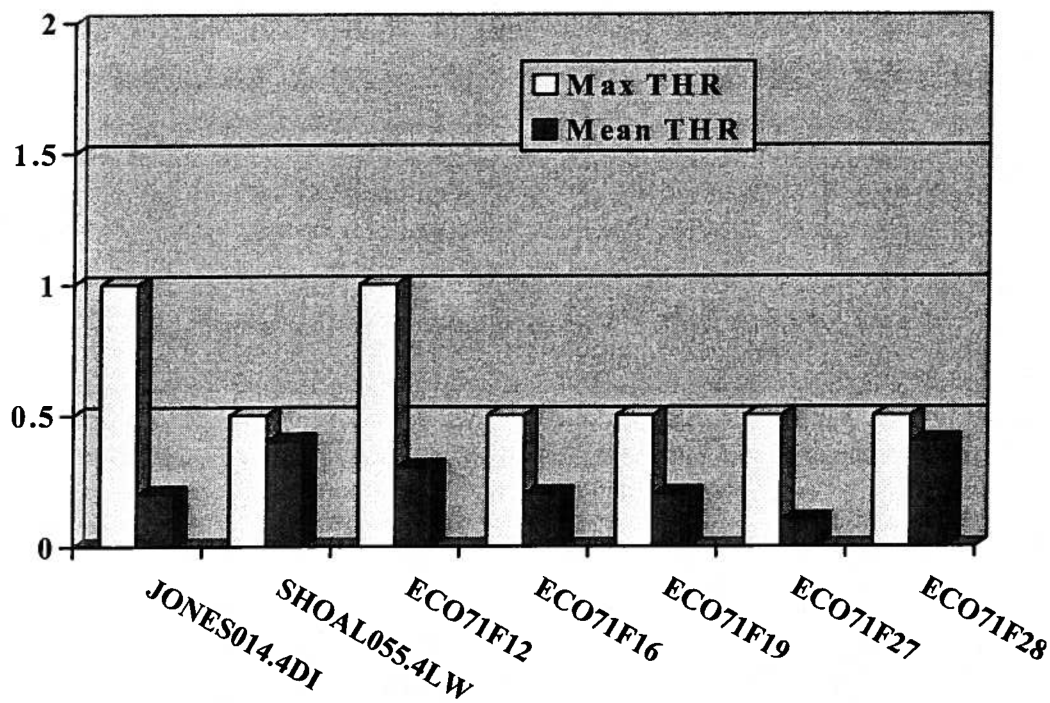


Figure 23: Maximum and mean thickness ranks (THR) of microalgae at reference and test sites in the Western Highland Rim (71f).

Two test sites were also sampled in the Western Highland Rim. Periphyton abundance was similar to reference sites with 32-59% of available substrate covered by a slime of periphyton with no visible accumulation. One site, Jones Creek, had substantially elevated total phosphorus throughout the sampling period (Figure 24). Excessive sediment was observed which might have retarded periphyton growth.

Although periphyton levels were similar to reference condition, diurnal dissolved oxygen swings were more pronounced with fluctuations of 4 ppm common during each cycle. The minimum DO hovered around the 5 ppm criterion level each evening.

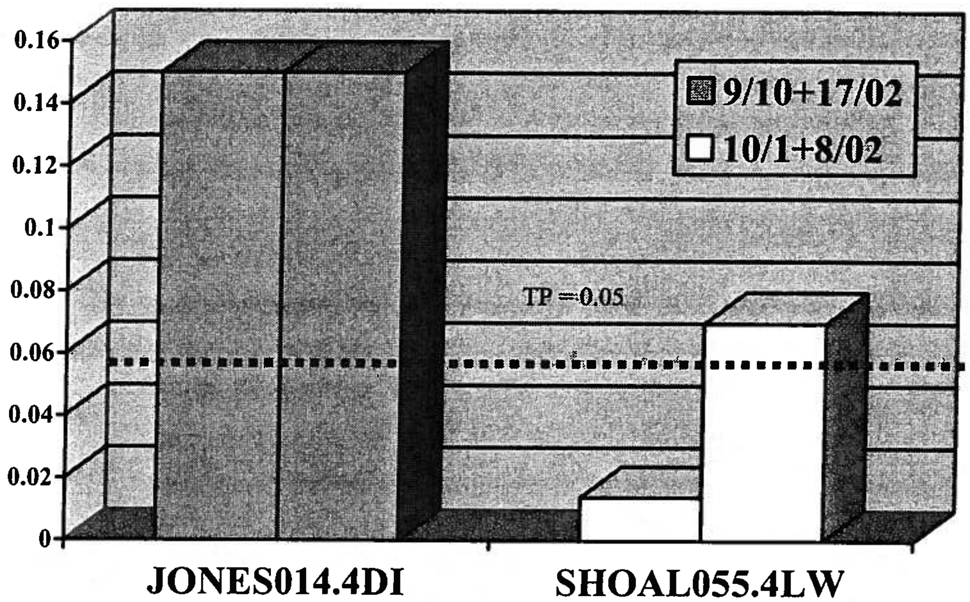


Figure 24: Total phosphorus (mg/l) at beginning and end of 1-week survey period at 2 test sites in the Western Highland Rim (71f). Patterned line depicts regional guidelines (0.05 mg/l).

The other test site, Shoal Creek, had elevated nitrate+nitrite throughout the study week (Figure 25). Total phosphorus slightly exceeded guidelines at the end of the week. The mean density of periphyton (0.4) was comparable to the Little Swan Creek reference site (ECO71F28). The water had been up due to heavy rains within seven days prior to the survey, which may have inhibited periphyton growth. However, macroinvertebrate samples passed biorecon guidelines at this site the previous year indicating that excessive algal growth is probably not a problem at this location. Diurnal dissolved oxygen readings during this seven-day study and during monitoring conducted in 2001 never fell below 7 ppm with fluctuations around 1.5 ppm.

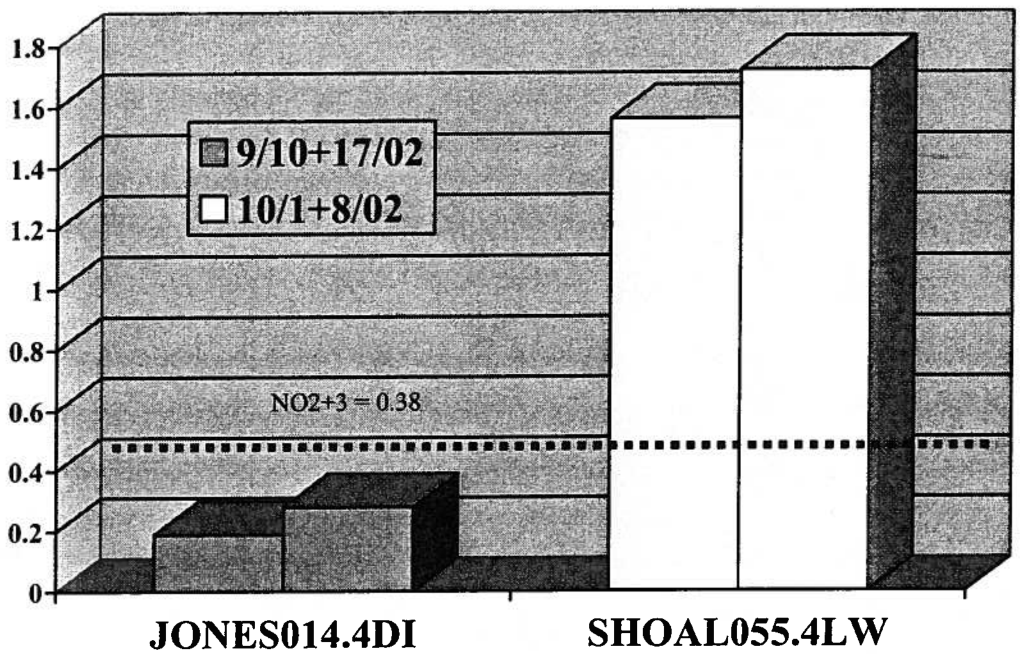


Figure 25 Nitrate+nitrite (mg/l) at beginning and end of 1-week survey period at 2 test sites in the Western Highland Rim (71f). Dotted line depicts regional guidelines (0.38 mg/l).

4.11 Periphyton, Nutrients and Dissolved Oxygen in Reference and Impaired Streams in the Eastern Highland Rim (71g).

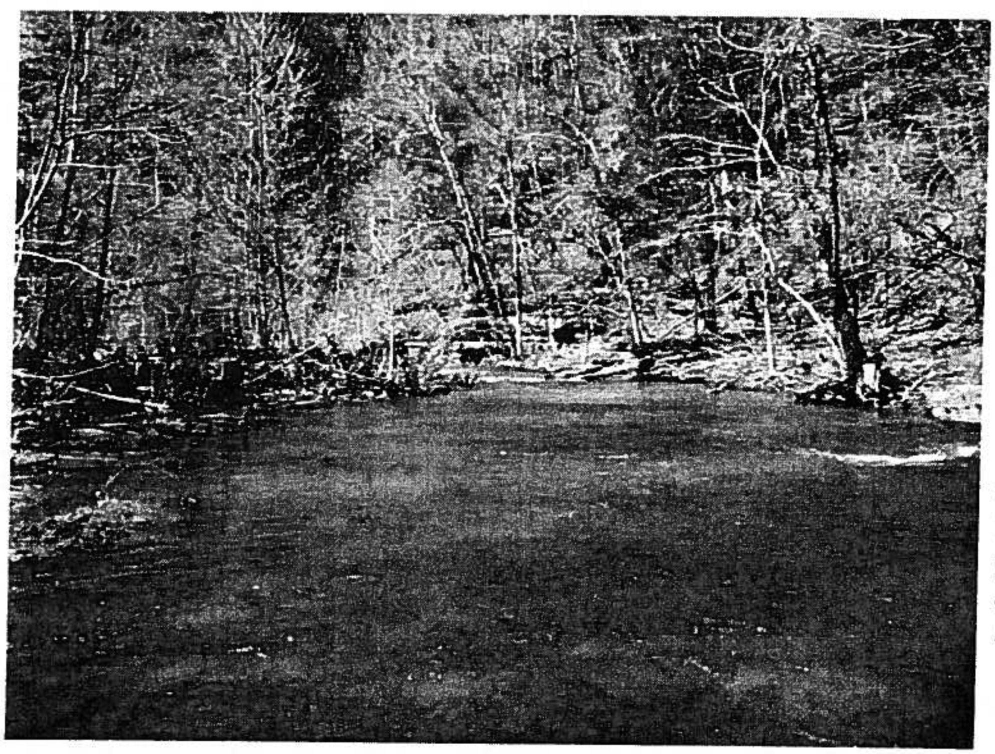
Three reference sites were surveyed in the Eastern Highland Rim subregion of the Interior Plateau. The periphyton community at one site, Flat Creek (ECO71G03), had a somewhat different structure than the others. This was the only site where macroalgae were observed, covering 21% of the substrate. Only 1% of the available substrate was covered by a slime of microalgae.

The other two reference sites did not have any macroalgae, but 52-81% of the rocks were covered by a microalgae slime (Table 13). Although the majority of rocks supported periphyton growth, density was not high with a mean thickness rank of 0.2 (Figure 26).

Despite the differences observed in the algae community, diurnal dissolved oxygen patterns were similar at all three sites. Minimum readings stayed above 5.5 ppm with diurnal fluctuations under 2 ppm.

Table 13: Periphyton and nutrient data for test and reference sites in the Eastern Highland Rim (71g).

Test Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	NO2+NO3 in mg/l (2 readings at beginning and end of monitoring week)		TP in mg/l (2 readings at beginning and end of monitoring week)	
CARR003.6OV	33	11	0	0.0	0.0	0.17	0.07	0.05	0.11
CLEAR001.1CE	100	53	47	1.0	1.0	0.12	0.09	2.0	0.66
MLICK014.7PU	97	0	100	0.5	0.5	0.34	0.22	0.21	0.02
MLICK015.5PU	69	0	0	0.0	0.0	0.45	0.33	0.07	0.01
ROCK009.3FR	62	0	45	0.5	0.2	4.6	5.7	1.3	0.64
TOWN000.9OV	41	0	11	0.5	0.0	1.13	1.44	0.45	0.5
TOWN001.1MA	82	47	30	0.5	0.1	2.2	NA	0.579	NA
Reference Stations	**% Substrate Available**	**% Macro algae**	**% Micro algae**	**Max Thickness Rank**	**Mean Thickness Rank (Mean Density)**	**Regional NO2+NO3 Guidelines**		**Regional TP Guidelines**	
ECO71G03	83	21	1	0.5	0.0	0.94		0.05	
ECO71G04	58	0	52	0.5	0.3				
ECO71G10	52	0	81	0.5	0.4				



Flat Creek (ECO71G03) is a reference stream in the Eastern Highland Rim. This reference site had a different periphyton community than other reference sites in 71g. *Photo provided by Jimmy Smith, NEAC*

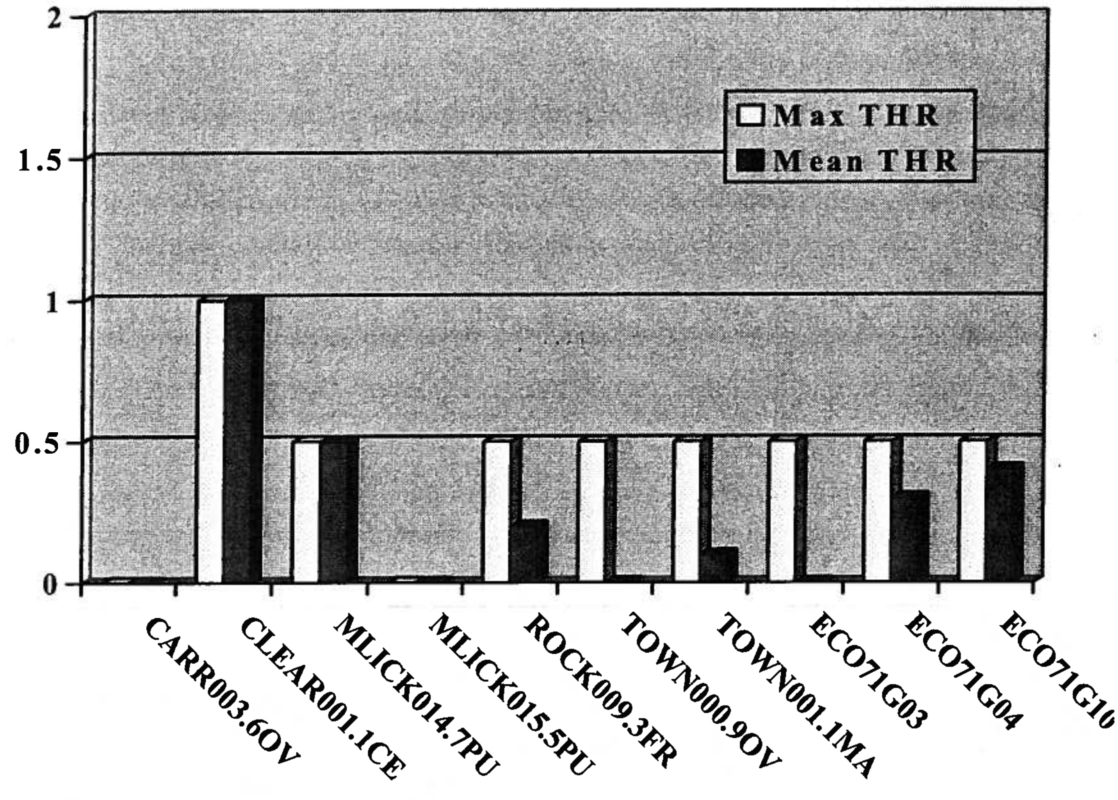


Figure 26: Maximum and mean thickness ranks (THR) of microalgae at reference and test sites in the Eastern Highland Rim (71g).

Seven test sites were also sampled in this subregion. Although all seven sites had elevated total phosphorus levels, only three had more dense periphyton than observed at the reference sites: Clear Creek, one site on Mine Lick Creek (mile 14.7) and Town Creek in Macon County.

Six of the test sites had been assessed as impaired while one site on Mine Lick Creek (MLICK014.7PU) is considered fully supporting based on macroinvertebrate data collected at the same site in 1998. This station is approximately 0.7 miles downstream of the Baxter STP. Although not dense, microalgae were widespread at this site with 100% of the available substrate covered by a slime coat with no visible accumulation.

Total phosphorus was substantially elevated seven days prior to the survey but had dropped below guidelines by the end of the week (Figure 27). Dissolved oxygen readings varied as much as 4 ppm during a diurnal cycle although readings stayed above 7 ppm.

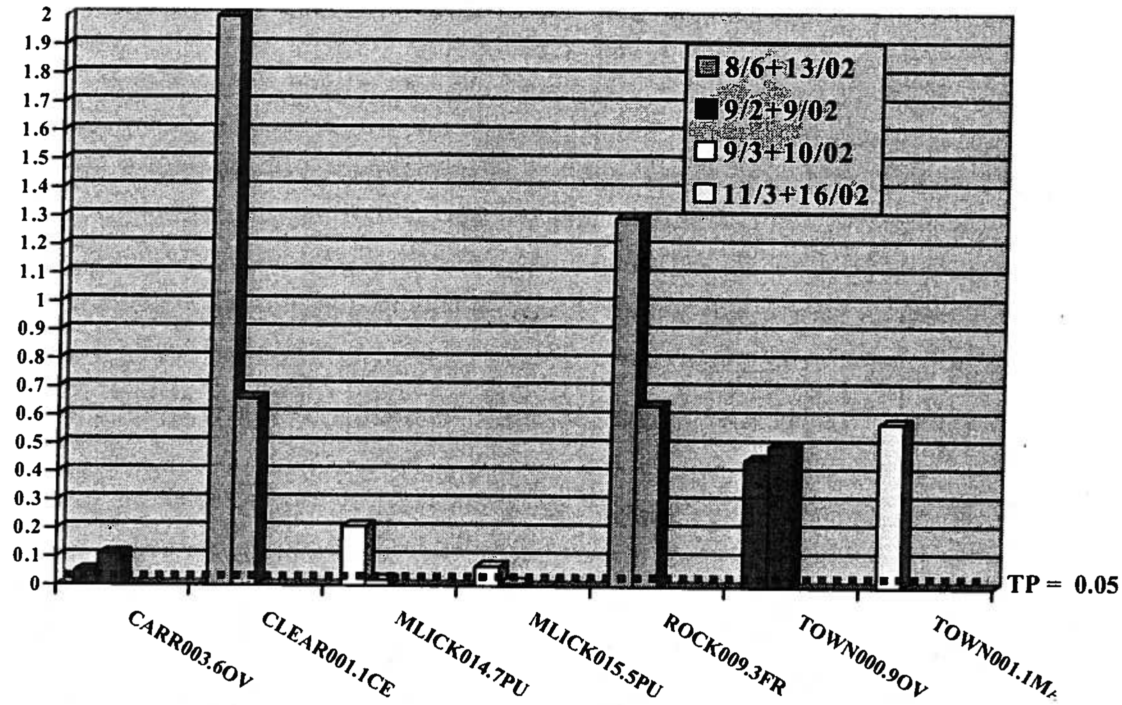


Figure 27: Total phosphorus (mg/l) at beginning and end of 1-week survey period at 7 test sites in the Eastern Highland Rim (71g). The dotted line represents regional guidelines (0.05 mg/l).

A second site on Mine Lick Creek (MLICK015.5PU), 0.8 mile upstream was also surveyed. This site is approximately 0.1 mile upstream of the Baxter STP. It lies within a 3.4 mile segment assessed as partially supporting based on biological data collected immediately downstream of the STP. Total phosphorus levels were slightly elevated seven days prior to the periphyton survey, but had dropped below regional guidelines on the day of the survey. No periphyton were present at this site. Diurnal dissolved oxygen fluctuations were less pronounced at this location than at the downstream site varying by 2 ppm. Minimum DO values stayed above 7 ppm.

Clear Branch (CLEAR001.1CE) had 100% of the rocks covered with periphyton. This was split between 53% macroalgae (Figure 28) and a thin layer of microalgae, which covered the other 47% of the substrate. Total phosphorus was substantially above regional guidelines seven days prior to the survey (2 mg/l). Although levels dropped during the week, they remained elevated (0.66 mg/l).

Diurnal dissolved oxygen values were not useable at the Clear Branch site as the probe was covered with filamentous algae that interfered with DO readings. An instantaneous reading at the time the periphyton survey was conducted yielded a DO of 1.5 ppm. This reading was recorded at 1230 pm so it is unlikely that values would rise substantially during the afternoon despite the abundant algal growth.

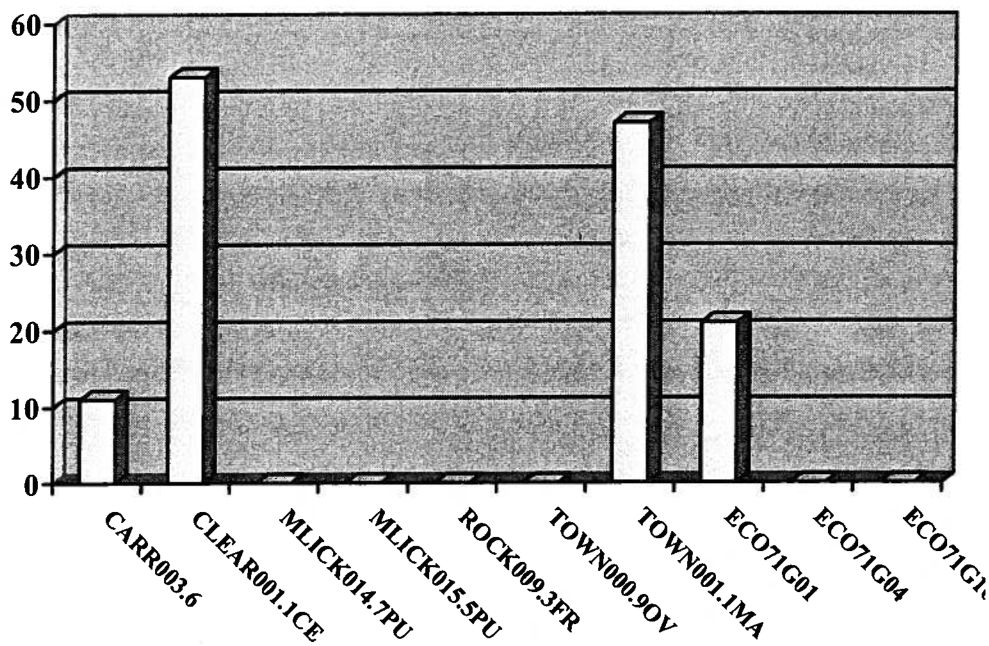


Figure 28: Percent of rock substrate covered by macroalgae at test and reference sites in the Eastern Highland Rim (71g).

No microalgae were present at Carr Creek (CARR003.6OV0) but 11% of the available substrate was covered by macroalgae. Excessive sediment was observed at this site, which may help account for the lack of microalgae. Total phosphorus was elevated on the periphyton survey date although regional guidelines were met seven days earlier. Dissolved oxygen readings never reached 5 ppm during the week, generally fluctuating between 3 ppm and 4.8 ppm.

Rock Creek (ROCK009.3FR) as well as Town Creek below the Livingston STP (TOWN000.9OV) had periphyton levels similar to the reference sites even though both nitrate+nitrite and total phosphorus exceeded regional guidelines (Figures 27 and 29). Elevated nutrient levels were especially evident in Rock Creek. Siltation was noted in both creeks, which may have affected periphyton growth. Diurnal dissolved oxygen readings at both sites generally fluctuated by 2 ppm. Rock Creek values regularly fell below 5 ppm each evening while Town Creek remained above 5 ppm.

A different Town Creek below the Lafayette STP (TOWN001.1MA) had abundant macroalgae (47%) as well as microalgae (30%) covering the rocks. Nitrate+nitrite and total phosphorus levels were elevated at this site when sampled seven days prior to the periphyton survey. (Nutrient data are not available for the survey date due to laboratory analysis error). This site is listed as impaired based on a history of elevated nutrient levels as well as an impaired macroinvertebrate community in 2000. DO readings were erratic, at times fluctuating as much as 4 ppm during a diurnal cycle. Readings were generally less than 5 ppm except during afternoon hours. Levels fell below 3 ppm at night on some occasions.

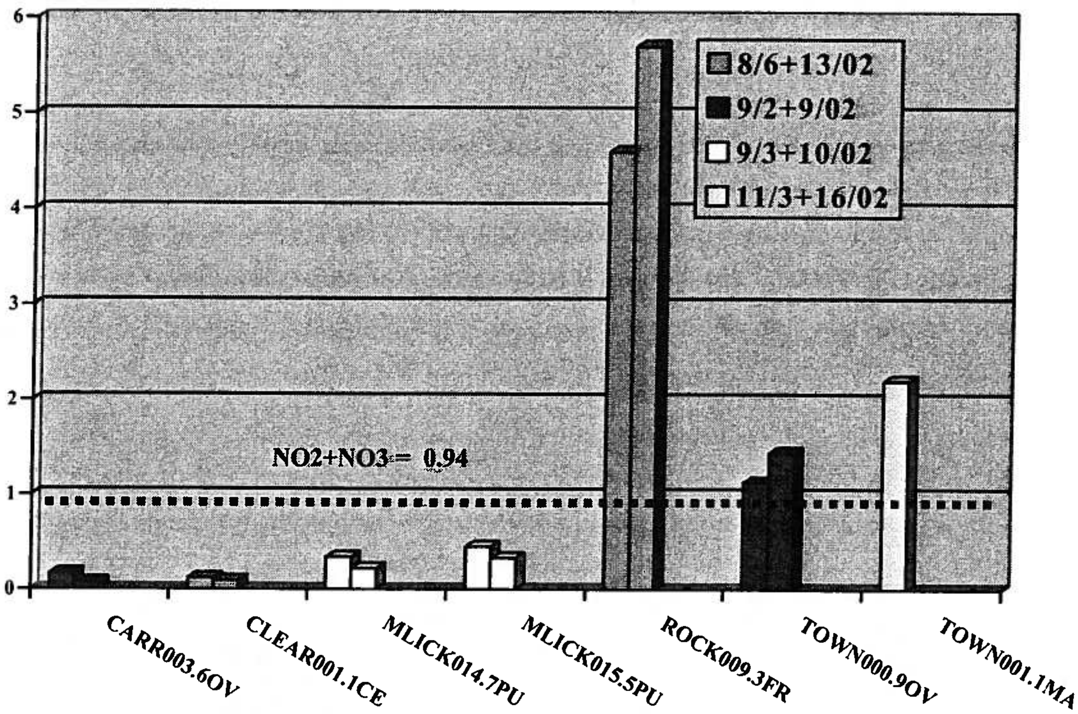


Figure 29: Nitrate+nitrite (mg/l) at beginning and end of 1-week survey period at 7 test sites in the Eastern Highland Rim (71g). The dotted line represents regional guidelines (0.94 mg/l).

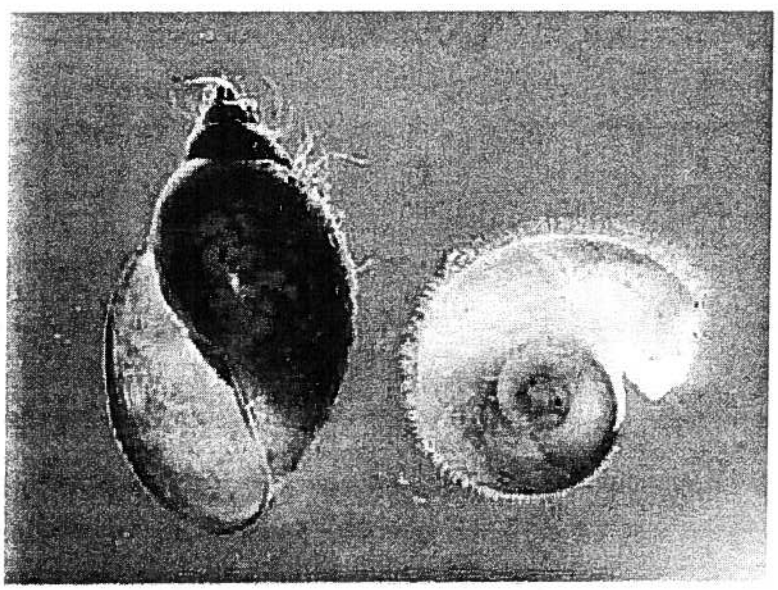
4.12 Periphyton, Nutrients and Dissolved Oxygen in Reference and Impaired Streams in the Outer Nashville Basin (71h).

Only one reference site in the Outer Nashville Basin subregion of the Interior Plateau, Carson Fork (ECO71H09), had sufficient flow for a periphyton survey during the study period. Microalgae were prevalent, covering 93% of the substrate (Table 14). The mean thickness rank was 0.8 (Figure 30).

Carson Fork had the highest reference stream microalgal density measured in any region except the Inner Nashville Basin (section 4.12). Macroalgae were not present (Figure 31). Dissolved oxygen levels generally fluctuated 2 to 3 ppm during each diurnal cycle. Values stayed above 5 ppm the first half of the week. Nightly lows fell below 5 ppm later in the week. However, data are questionable since an instantaneous reading using a different probe was over 4 ppm higher when the probe was picked up.

Table 14: Periphyton and nutrient data for test and reference sites in the Outer Nashville Basin (71h).

Test Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	NO2+NO3 in mg/l (2 readings at beginning and end of monitoring week)		TP in mg/l (2 readings at beginning and end of monitoring week)	
BROWN000.4DA	42	4	27	0.5	0.2	1.97	1.35	0.221	0.222
EFMUL010.4MR	62	0	28	0.5	0.1	NA	1.91	NA	0.397
LHARP001.8WI	81	0	33	1.0	0.2	1.53	1.27	0.15	0.13
MILL003.3DA	100	7	93	0.5	0.5	0.52	0.2	0.263	0.1
RATTL000.2WI	89	0	84	1.0	0.6	0.48	3.9	0.18	0.17
SIMS000.8DA	45	0	52	0.5	0.3	0.46	0.52	0.212	0.29
SUGAF002.4MY	89	0	36	1.0	0.2	1.47	2.39	0.2	0.27
SUGAR000.1DA	82	0	0	0.0	0.0	1.01	0.85	0.309	0.486
SUGAR000.2MY	82	0	20	1.0	0.1	1.76	NA	0.19	NA
SWAN008.0LI	100	39	18	0.5	0.1	0.03	0.08	0.341	0.4
WFBRO000.1DA	95	0	9	0.5	0.0	1.54	1.64	0.279	0.22
Reference Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	Regional NO2+NO3 Guidelines		Regional TP Guidelines	
ECO71H09	85	0	93	1.0	0.8	0.94		0.10	



Aquatic snails feed by scraping periphyton off rocks. Snails often become the dominant organisms in streams impacted by excessive algal growth.

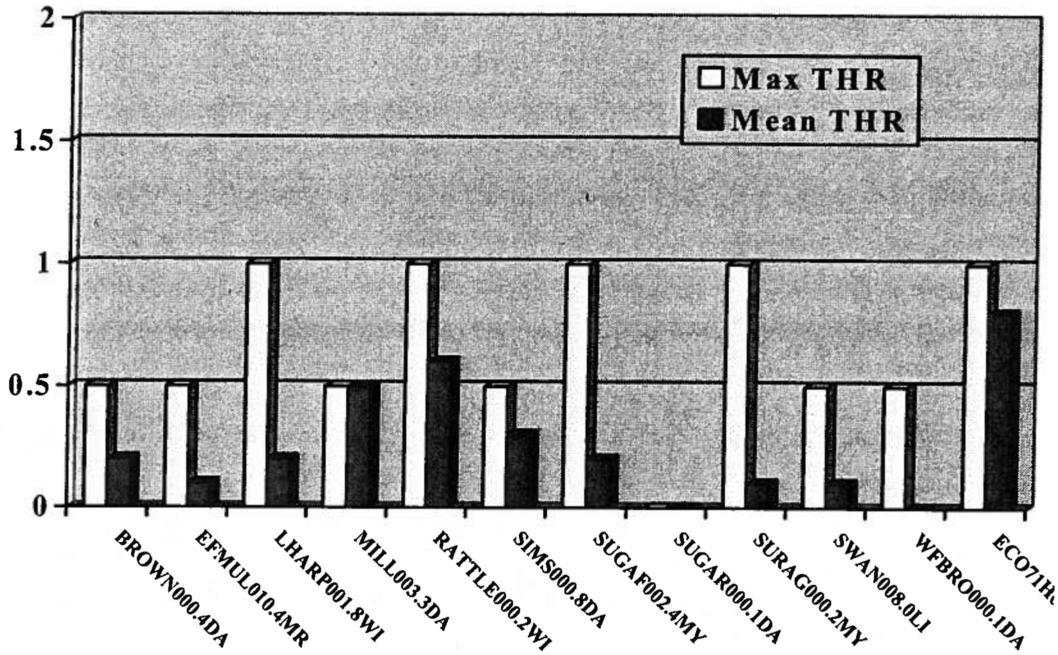


Figure 30: Maximum and mean thickness ranks (THR) of microalgae at reference and test sites in the Outer Nashville Basin (71h).

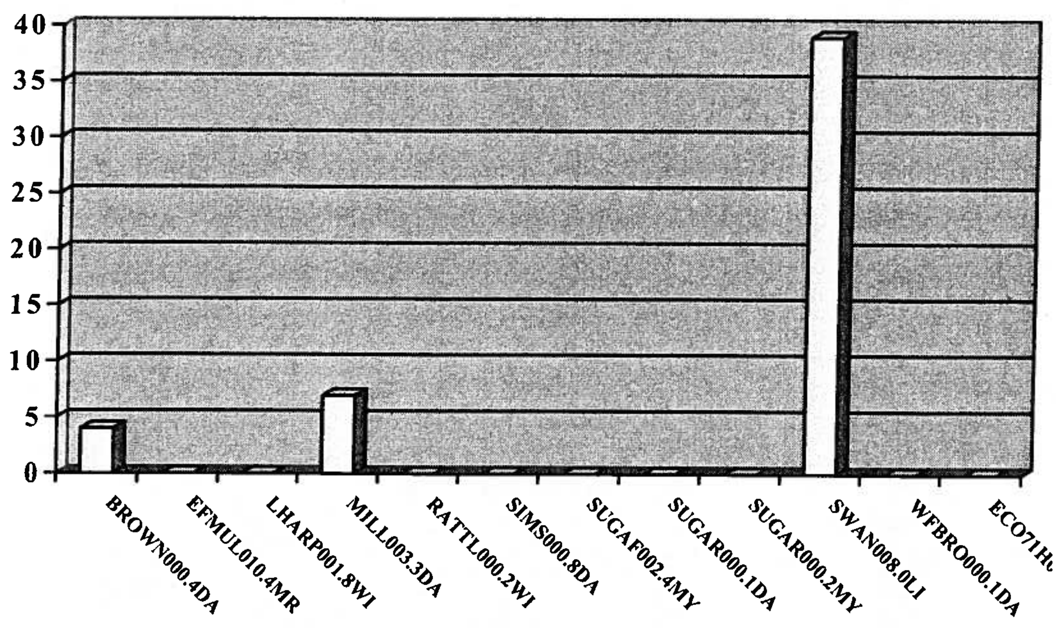


Figure 31: Percent of rock substrate covered by macroalgae at test and reference sites in the Outer Nashville Basin (71h).

Eleven test sites were sampled in the Outer Nashville Basin. All test sites have been assessed as impaired except Sugar Fork (SUGAF002.4MY), which is fully supporting based on a macroinvertebrate survey in 1999. Sugar Fork had elevated nitrate+nitrite and total phosphorus throughout the seven-day sampling period, but the presence of good canopy helped suppress periphyton growth. Microalgae covered 36% of the substrate with a mean thickness rank of 0.2. Macroalgae were not present. Diurnal dissolved oxygen levels never fell below 6.5 ppm and generally changed less than 2 ppm during each cycle.

All test sites had elevated total phosphorus (Figure 32). Only 3 creeks, Sims Branch, Mill Creek, and Swan Creek did not also have elevated nitrate+nitrite (Figure 33). Nutrient sampling the previous winter and spring on Sims Branch indicated elevated nitrate+nitrite during those seasons. A slime layer of periphyton covered 52% of the rock substrate at Sims Branch. Macroalgae were not present at this site. A typical diurnal dissolved oxygen cycle was not observed but readings were erratic, dropping to near 0 ppm at one point before climbing back to 4.5 ppm.

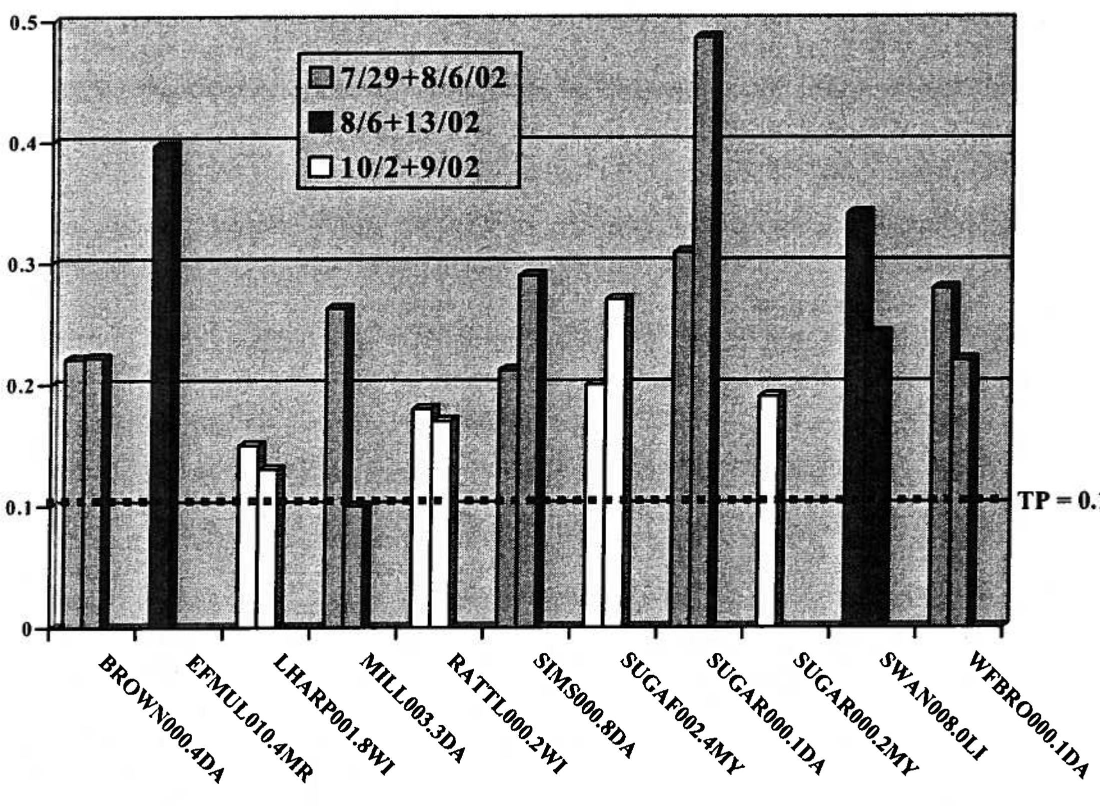


Figure 32: Total phosphorus (mg/l) at beginning and end of 1-week survey period at 11 test sites in the Outer Nashville Basin (71h). The dotted line represents regional guidelines (0.10 mg/l).

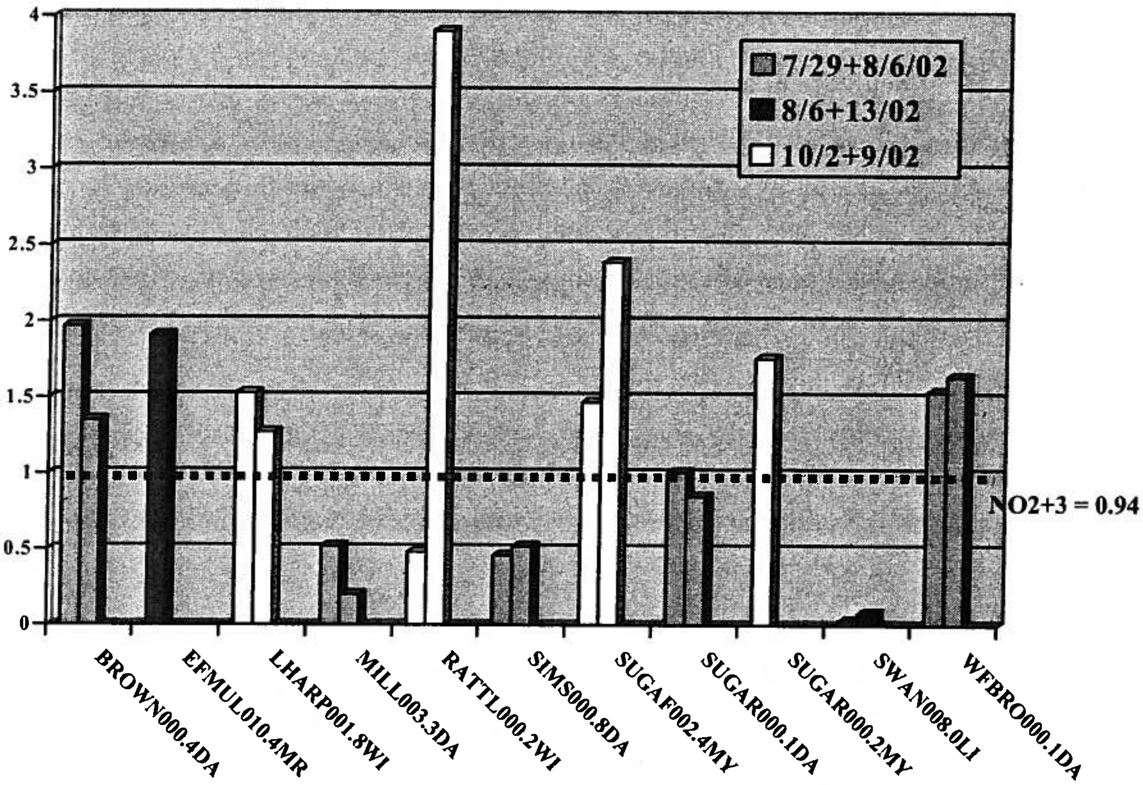


Figure 33: Nitrate+nitrite (mg/l) at beginning and end of 1-week survey period at 11 test sites in the Outer Nashville Basin (71h). The dotted line represents regional guidelines (0.94 mg/l).

Mill Creek had 100% suitable rock substrate for periphyton colonization. All of the substrate was covered by either a slime coat of microalgae (93%) or filamentous strands of macroalgae (7%). Mill Creek was one of only three sites in this subregion that had macroalgae present.

Mill Creek had the greatest diurnal dissolved oxygen fluctuation of any test site in this subregion. DO levels varied 5 ppm during most 24-hour periods. Lows rarely fell below 5 ppm (Figure 34).

Swan Creek was the only 71h test stream besides Sims and Mill that met nitrate+nitrite guidelines. This site was also one of the three with macroalgae and had the highest concentration in the region with 39% of the substrate covered. A slime layer of microalgae covered another 18%. Since two of the three sites with macroalgae did not have elevated nitrate+nitrite, it is possible that total phosphorus may be more critical to filamentous algae growth. The diurnal dissolved oxygen cycle was relatively consistent, cycling between 3.5 and 6.0 ppm during each 24-hour period.

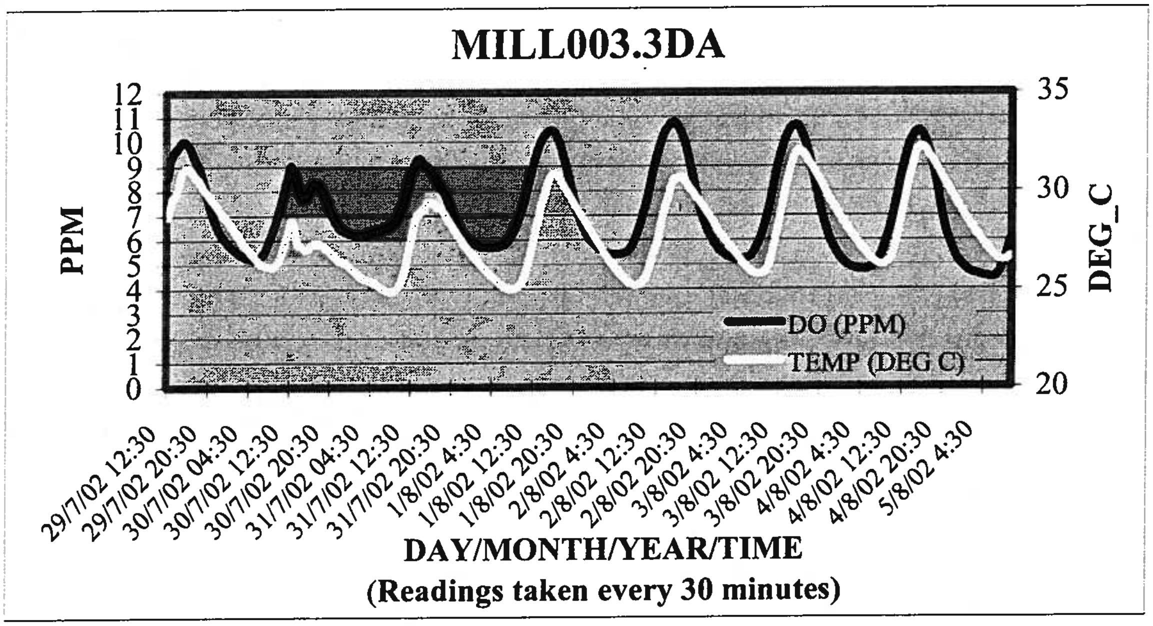


Figure 34: Diurnal dissolved oxygen fluctuations at Mill Creek test site, Outer Nashville Basin (71h). Readings every 30 minutes for 166 hours.

Like Mill and Swan, Brown Creek was one of the three test sites where macroalgae were present, although it had the least amount with 4% of the substrate covered. A slime layer of microalgae covered another 27% of the rock surface. Unlike the other two sites where only total phosphorus was elevated, both nitrate+nitrite and total phosphorus were elevated at this site. Dissolved oxygen was erratic fluctuating between 3.5 and 5.5 ppm for the first half of the week before dropping to values between 0 and 2.5 ppm.

One test site, Sugartree Creek (SUGAR000.1DA), had no periphyton present. However, the creek at this location is completely shaded so sunlight was not available for algal growth. Nitrate+nitrite levels were slightly elevated seven days prior to the algae survey but dropped below regional guidelines during the week. Total phosphorus levels remained elevated throughout the study period. Dissolved oxygen showed very little diurnal fluctuation, but dropped below 3 ppm toward the end of the week. A macroinvertebrate biorecon conducted at the same station the previous year demonstrated a stressed community with no EPT or intolerant families present.

Only one other test site , West Fork Browns Creek (WFBRO000.1DA), had a mean microalgal density of 0.0 although a limited amount of microalgae slime was present on 9% of the rock surface. This creek was also shaded. Both nitrate+nitrite and total phosphorus were elevated throughout the study period. DO levels did not drop below 6.7 ppm and varied about 2 ppm during each diurnal cycle. A previous macroinvertebrate survey conducted at this same site indicated a stressed benthic community with only five families including one EPT.

Rattlesnake Branch had the highest density of microalgae of any test site. A thin layer of microalgae covered 84% of the suitable substrate with a mean thickness rank of 0.6. This was the highest microalgal density measured at any test site in the region, although it was not as dense as the reference site (0.8). Both nitrate+nitrite and total phosphorus were elevated. Dissolved oxygen fluctuated between 6.5 and 8.0 ppm for most of the study period. Data for the latter part of the week are questionable since DO readings spiked to 24.5 ppm for one cycle. The probe may have been removed from the water or otherwise disturbed.

East Fork Mulberry Creek had little microalgae with a mean thickness rank of 0.1 even though both nitrate+nitrite and total phosphorus levels were elevated. This creek is impaired by siltation, which may have inhibited algal growth. Diurnal dissolved oxygen readings only varied by 2 ppm during each cycle, but values consistently fell below 5 ppm each night.

Measurements at the remaining two test sites Sugar Creek (SUGAR000.2MY) and Little Harpeth River (LHARP001.8WI), were similar. Both streams had elevated nitrate+nitrite and total phosphorus, although total phosphorus was only slightly elevated at the Little Harpeth River. The microalgal density was low in both streams, which are assessed as impaired by siltation. These two sites had the least diurnal dissolved oxygen fluctuations in the region with only 1 ppm variation during each 24-hour cycle. Nighttime lows approached 6.5 ppm at both sites.

Since only one reference site could be sampled in this region, it is not possible to generalize. However, if future sampling shows similar periphyton levels at reference sites, algal density may not be a viable assessment tool in this region since impaired sites had comparable levels.

4.13 Periphyton, Nutrients and Dissolved Oxygen in Reference and Impaired Streams in the Inner Nashville Basin (71i).

Four reference sites were surveyed in the Inner Nashville Basin subregion of the Interior Plateau. Periphyton covered from 0 to 100% of the rock substrate (Table 15). The mean thickness rank ranged from 0.0 to 0.9. None of the reference sites had macroalgae.

Reference diurnal dissolved oxygen cycles in this subregion are more extreme than any other region with levels sometimes fluctuating as much as 4 ppm during a 24-hour period. Nighttime lows recorded during this study generally stayed above 5 ppm.

Background levels of total phosphorus (90th percentile = 0.22 mg/l) in the Inner Nashville Basin are relatively high compared to other ecological subregions in Tennessee. Only one other subregion, the Northern Mississippi Alluvial Plain (73a), had higher reference levels.

Table 15: Periphyton and nutrient data for test and reference sites in the Inner Nashville Basin (71i).

Test Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	NO2+NO3 in mg/l (2 readings at beginning and end of monitoring week)		TP in mg/l (2 readings at beginning and end of monitoring week)	
HURRI004.2RU	97	66	18	2.0	0.9	0.36	0.12	0.309	0.148
JARMA000.3RU	97	72	15	0.5	0.3	2.2	0.61	0.47	0.053
KELLY000.4RU	40	14	15	0.5	0.1	0.36	0.23	0.056	0.085
LYTLE1T0.1RU	73	0	13	0.5	0.1	0.36	NA	0.075	NA
WFSTO008.3RU	100	0	100	2.0	2.0	1.23	1.76	0.47	2.04
Reference Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	Regional NO2+NO3 Guidelines		Regional TP Guidelines	
ECO71I03	23	0	0	0.0	0.0	0.94		0.22	
ECO71I10	90	0	94	1.0	0.8				
ECO71I14	100	0	99	1.0	0.9				
ECO71I15	96	0	100	1.0	0.5				

Five test sites were surveyed in this region. Two streams, Hurricane Creek and West Fork Stones River downstream of the Murfreesboro STP, have a history of elevated nutrients. Macroinvertebrate surveys conducted at the Hurricane Creek site in 1997 and the West Fork Stone River site in 2000 indicated stressed benthic populations.

The West Fork Stones River at this location had no macroalgae, however 100% of the substrate was covered by a uniform 1 mm thick layer of microalgae (Figure 35). The mean thickness rank of 2.0 was the highest density of microalgae at any site in any subregion. Both total phosphorus and nitrate+nitrite were elevated throughout the study period (Figures 36 and 37). Diurnal dissolved oxygen fluctuations exceeded 4 ppm during some cycles with lows dropping below 5 ppm toward the end of the week.

Hurricane Creek had abundant macroalgae with 66% of the substrate covered with filamentous strands up to 5 inches in length (Figure 38). A layer of microalgae up to 1 mm thick in places (mean thickness rank = 0.9) covered over half the remaining substrate. Despite the abundant filamentous algae, total phosphorus levels were only slightly elevated at this site at the beginning of the week with levels dropping by the survey date. Nitrate+nitrite met regional guidelines.

Only one other site, Jarman Branch, was dominated by macroalgae (72%). Both nitrate+nitrite and total phosphorus were elevated at this site seven days prior to the survey with levels meeting regional guidelines by the end of the week. Microalgae colonization was limited at this site. A slime coat with no visible accumulation was present on 15% of the rocks.

Diurnal dissolved oxygen fluctuations were the most extreme at these two macroalgae-dominated sites. Readings fluctuated by as much as 8 ppm (Figure 39). Daytime levels often reached super saturation, while nighttime lows dropped below 3 ppm.

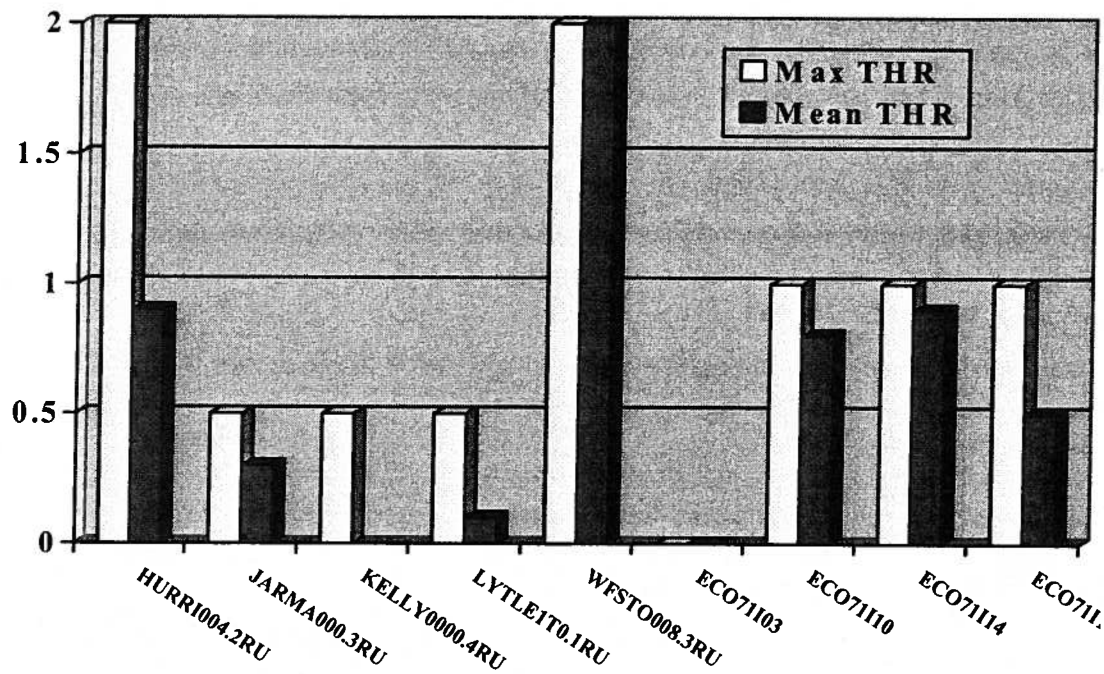


Figure 35: Maximum and mean thickness ranks (THR) of microalgae at reference and test sites in the Inner Nashville Basin (71i).

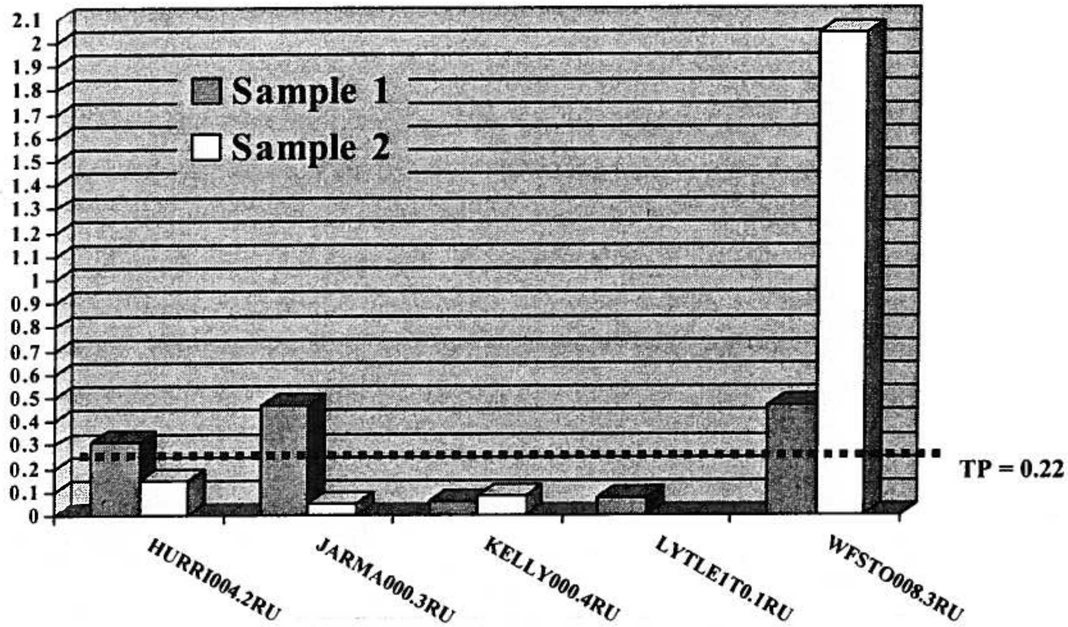


Figure 36: Total phosphorus (mg/l) at beginning and end of 1-week survey period at 5 test sites in the Inner Nashville Basin (71i). Initial samples collected 7/29 or 7/31 2002. Second samples collected between 8/6 and 8/9, 2002. The dotted line represents regional guidelines (0.22 mg/l).

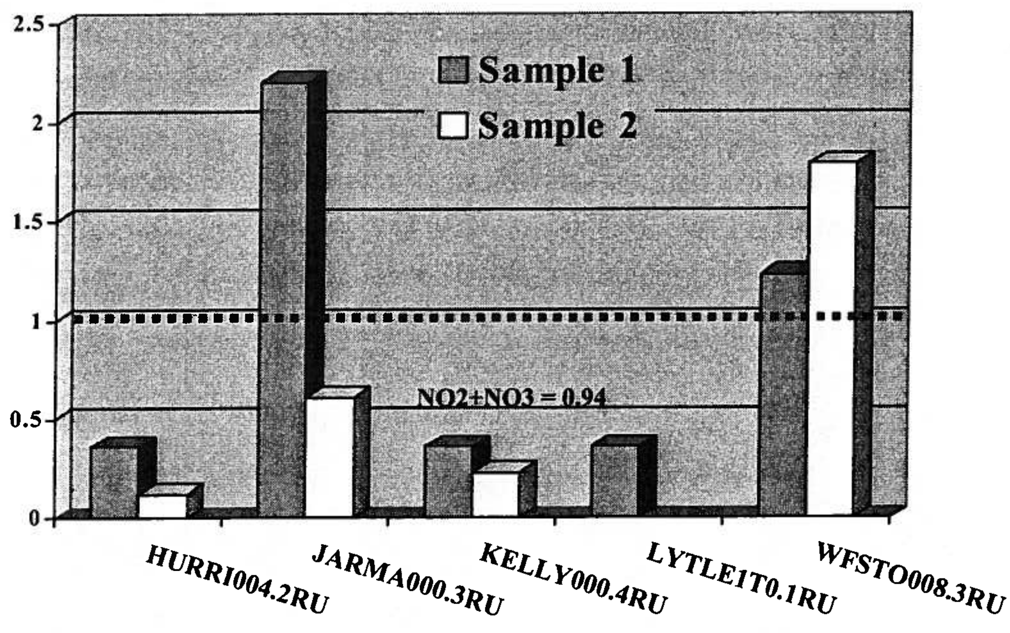


Figure 37: Nitrate+nitrite (mg/l) at beginning and end of 1-week survey period at 5 test sites in the Inner Nashville Basin (71i). Initial samples collected 7/29 or 7/31 2002. Second samples collected between 8/6 and 8/9, 2002. The dotted line represents regional guidelines (0.94 mg/l).

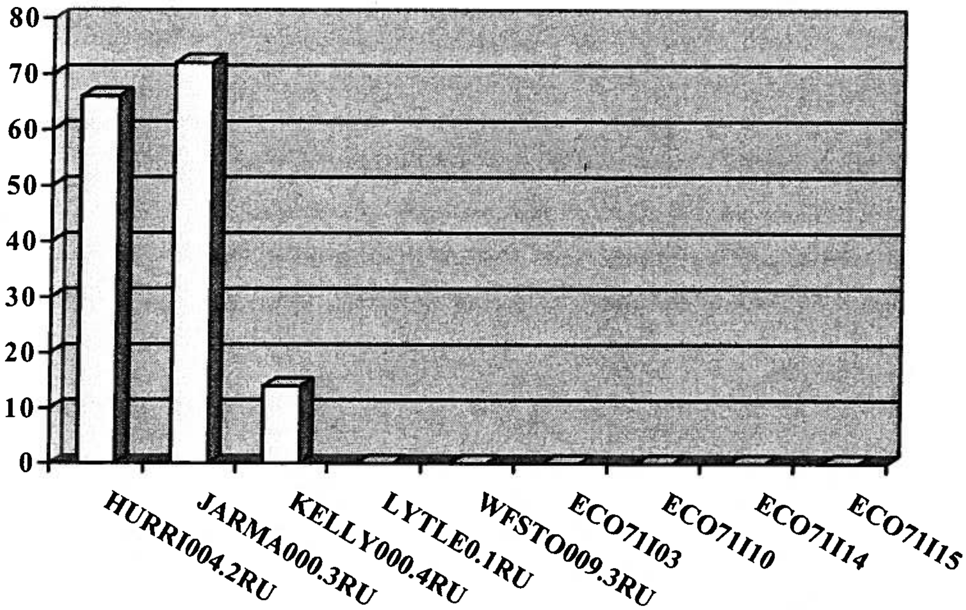


Figure 38: Percent of rock substrate covered by macroalgae at test and reference sites in the Inner Nashville Basin (71i).

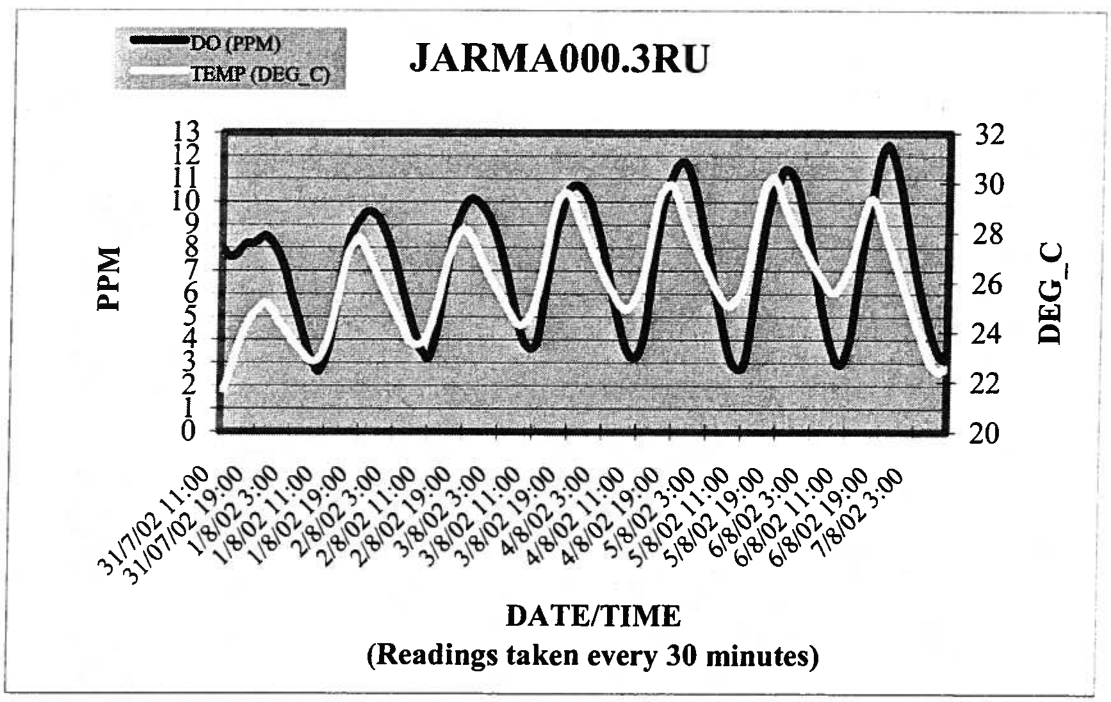


Figure 39: Diurnal dissolved oxygen and temperature readings at Jarman Branch test site in the Inner Nashville Basin (71i). Readings every 30 minutes for 164 hours.

Nutrient levels stayed within regional guidelines at two test sites, a tributary to Lytle Creek and Kelly Creek. The Lytle Creek tributary had very little periphyton present with only 13% of the available substrate having a slime coat with no visible accumulation. No macroalgae were present. Like the reference streams, diurnal dissolved oxygen fluctuations were generally around 3 ppm. However, nighttime lows reached 4 ppm.

The density of microalgae at Kelly Creek was similar, but this site also had 14% of the substrate covered by macroalgae. Diurnal dissolved oxygen fluctuations were also around 3 ppm at this site. Readings were generally less than 3 ppm during daylight hours and dropped below 1 ppm at night.

4.14 Periphyton, Nutrients and Dissolved Oxygen in Reference and Impaired Streams in the Loess Plains (74b).

Only one reference site in the Loess Plains subregion of the Mississippi Valley Loess Plains, Terrapin Creek (ECO74B01), had sufficient flow to conduct a periphyton survey. Very little rock substrate is available in the majority of streams in this region. The dominant bottom substrate in most Loess Plains streams is shifting sand not conducive to periphyton growth. Terrapin Creek had 18% suitable stable habitat with no periphyton colonization evident (Table 16). Dissolved oxygen levels stayed above 6.5 ppm with diurnal fluctuations around 2 ppm.

One test site, Stout Creek, was surveyed in this subregion. A limited amount of bottom substrate (8%) was suitable for periphyton. However, 100% of the available substrate was covered by a microalgae slime. Although abundant, the algae layer was not thick so density was low (Figure 40). Total phosphorus levels exceeded regional guidelines during the survey week (Figure 41). Dissolved oxygen had an erratic diurnal pattern. Values did not fluctuate more than 2 ppm but did fall below 5 ppm during one cycle.

Table 16: Periphyton and nutrient data for test and reference sites in the Loess Plains (74b).

Test Stations	% Substrate Available	% Macro algae	% Micro algae	Max Thickness Rank	Mean Thickness Rank (Mean Density)	NO2+NO3 in mg/l (2 readings at beginning and end of monitoring week)		TP in mg/l (2 readings at beginning and end of monitoring week)	
STOUT000.4FA	8	0	100	0.5	0.5	0.73	0.19	0.36	0.18
Reference Stations	**% Substrate Available**	**% Macro algae**	**% Micro algae**	**Max Thickness Rank**	**Mean Thickness Rank (Mean Density)**	**Regional NO2+NO3 Guidelines**		**Regional TP Guidelines**	
ECO74B01	18	0	0	0.0	0.0	1.10		0.11	

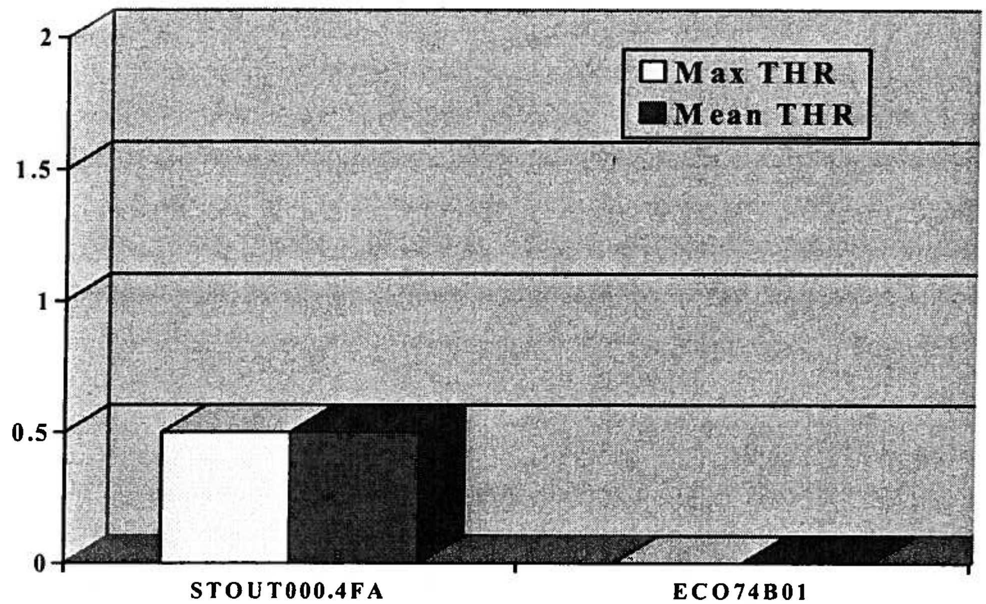


Figure 40: Maximum and mean thickness ranks (THR) of microalgae at Terrapin Creek reference site and Stout Creek test site in the Loess Plains (74b).

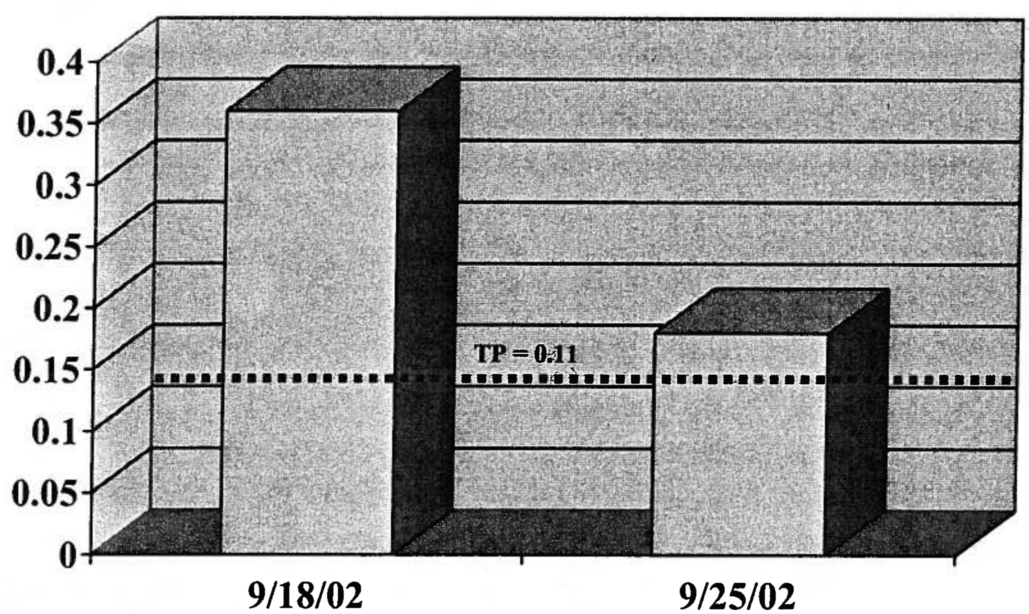


Figure 41: Total phosphorus (mg/l) at beginning and end of 1-week survey period at Stout Creek test site in the Loess Plains (74b). Dashed line represents regional guideline (0.11 mg/l).

5. SUMMARY

Based on this preliminary study, the rapid periphyton method appeared to be most useable in middle and east Tennessee streams with generally rocky substrates. This type of periphyton survey does not seem to be a viable option for water quality assessments in most west Tennessee subregions other than the Transition Hills (65j). Streams in other west Tennessee regions have shifting sand bottoms with limited stable rock substrate. Other methods (artificial substrates or collection of other habitats with taxonomic identification) may yield better results, but would not provide a rapid field-based assessment and may be cost and time prohibitive.

In regions with suitable substrate, very little periphyton was measured in reference streams. The mean density of microalgae did not exceed a thickness rank of 0.5 (slimy substrate but no visual accumulation) in most regions. The only exceptions were the Inner (71i) and Outer (71h) Nashville Basins where the mean density approached 1 (thin layer of algae visible but no measurable accumulation). These were also the only regions, along with the Southern Limestone/Dolomite Valleys and Low Rolling Hills (67f), where macroalgae were observed in reference streams.

Periphyton densities were not always a good predictor of nutrient levels even in regions with little algae present in reference conditions. At many test sites, nutrient levels were elevated but periphyton growth was not excessive compared to reference levels. This is because other factors, including sunlight and warm temperatures are needed for algae to grow.

Many streams, especially small ones, have canopy that blocks sunlight. In addition, the abundance of grazing animals, such as snails, can have an impact on algal density. This emphasizes the importance of using canopy measurements and macroinvertebrate collections (grazer abundance) when conducting this type of periphyton survey. Due to funding constraints of the grant, neither of these measures were used as part of this project. This limited interpretive ability in streams with elevated nutrients where algae were not present. However, periphyton densities did correspond with elevated nutrients at some sites. The best response was seen at three streams in the Eastern Highland Rim (71g).

Dissolved oxygen levels appeared to be affected by the amount of periphyton present in the streams. Algal abundance appeared to be more important than the type of algae (macroalgae or microalgae). Although lows often stayed above regional criteria, diurnal fluctuations were more pronounced when algal density exceeded the level measured at reference streams. Extreme changes in dissolved oxygen levels can have a detrimental affect on aquatic life even when minimum levels are maintained.

Partially as a result of this project, rapid periphyton assessments have been added to 2 additional 104(b) grants the Division has recently been awarded. As the data set grows, the information will help clarify where and when periphyton surveys are most useful and how best to interpret results.

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APPENDIX

PERIPHYTON DATA

Station ID	Date	ECO	Percent Macroalgae (Dm)	Maximum Length (in)	% Suitable Substrate (dt)	# Microalgae Points (di)	Microalgae Rank (ri)	$d_i r_i/d_t$*	Mean Thickness Rank $\sum d_i r_i/d_t$
BSAND036.4CR	9/19/02	65e	0	0	54	44	0	0.00	0.1
BSAND036.4CR	9/19/02	65e			54	10	0.5	0.09	
CLEAR001.2CR	9/19/02	65e	0	0	0	0	0	0.00	0.0
ECO65E04	9/23/02	65e	0	0	0	0	0	0.00	0.0
ECO65E06	9/23/02	65e	0	0	0	0	0	0.00	0.0
ECO65E08	9/24/02	65e	0	0	244	200	0	0.00	0.1
ECO65E08	9/24/02	65e			244	44	0.5	0.09	
ECO65E10	10/1/02	65e	0	0	0	0	0	0.00	0.0
HFORK004.0HU	9/18/02	65e	0	0	6	6	0	0.00	0.0
HFORK004.0HU-QC	9/18/02	65e	0	0	8	8	0	0.00	0.0
MUD004.7CR	9/19/02	65e	0	0	4	4	0	0.00	0.0
ECO65J04	10/1/02	65j	0	0	434	296	0	0.00	0.2
ECO65J04	10/1/02	65j			434	138	0.5	-0.16	
ECO65J05	10/7/02	65j	0	0	360	360	0	0.00	0.0
ECO65J06	10/2/02	65j	0	0	360	235	0	0.00	0.2
ECO65J06	10/2/02	65j			360	90	0.5	0.13	
ECO65J06	10/2/02	65j			360	35	1	0.10	
ECO65J06-QC	10/2/02	65j	0	0	370	255	0	0.00	0.2
ECO65J06-QC	10/2/02	65j			370	70	0.5	0.09	
ECO65J06-QC	10/2/02	65j			370	45	1	0.12	

Station ID	Date	ECO	Percent Macroalgae (Dm)	Maximum Length (in)	% Suitable Substrate (dt)	# Microalgae Points (di)	Microalgae Rank (ri)	$d_i r_i / d_t$*	Mean Thickness Rank $\sum d_i r_i / d_t$
ECO65J11	10/7/02	65j	0	0	245	185	0	0.00	0.2
ECO65J11	10/7/02	65j			245	45	0.5	0.09	
ECO65J11	10/7/02	65j			245	15	1	0.06	
ECO66E04	8/28/02	66e	0	0	385	345	0	0.00	0.0
ECO66E04	8/28/02	66e			385	40	0.5	0.05	
ECO66E11	8/27/02	66e	0	0	405	375	0	0.00	0.0
ECO66E11	8/27/02	66e			405	30	0.5	0.04	
ECO66E11-QC	8/27/02	66e			400	24	0.5	0.03	
ECO66E11-QC	8/27/02	66e	0	0	400	375		0.00	0.0
ECO66F07	8/28/02	66f	0	0	435	340	0	0.00	0.1
ECO66F07	8/28/02	66f			435	95	0.5	0.11	
ECO66F08	8/27/02	66f	0	0	425	400	0	0.00	0.0
ECO66F08	8/27/02	66f			425	25	0.5	0.03	
ECO66G04	8/26/02	66g	0	0	425	120	0	0.00	0.5
ECO66G04	8/26/02	66g			425	180	0.5	0.21	
ECO66G04	8/26/02	66g			425	125	1	0.29	
ECO66G05	8/16/02	66g	0	0	390	275	0	0.00	0.2
ECO66G05	8/16/02	66g			390	115	0.5	0.15	
ECO66G07	8/22/02	66g	0	0	403	60	0	0.00	0.5
ECO66G07	8/22/02	66g			403	268	0.5	0.33	
ECO66G07	8/22/02	66g			403	75	1	0.19	

Station ID	Date	ECO	Percent Macroalgae (Dm)	Maximum Length (in)	% Suitable Substrate (dt)	# Microalgae Points (di)	Microalgae Rank (ri)	$d_i r_i / d_t$*	Mean Thickness Rank $\sum d_i r_i / d_t$
ECO66G09	8/22/02	66g	0	0	335	225	0	0.00	0.2
ECO66G09	8/22/02	66g			335	110	0.5	0.16	
ECO66G12	8/21/02	66g	0	0	380	250	0	0.00	0.2
ECO66G12	8/21/02	66g			380	130	0.5	0.17	
DOBBS000.3HM	8/19/02	67f	0	0	160	89	0	0.00	0.2
DOBBS000.3HM	8/19/02	67f			160	71	0.5	0.22	
ECO67F06	9/2/02	67f	0	0	415	385	0	0.00	0.0
ECO67F06	9/2/02	67f			415	30	0.5	0.04	
ECO67F14	8/29/02	67f	0	0	375	310	0	0.00	0.1
ECO67F14	8/29/02	67f			375	60	0.5	0.08	
ECO67F14	8/29/02	67f			375	5	1	0.01	
ECO67F14-QC	8/29/02	67f	0	0	315	275	0	0.00	0.1
ECO67F14-QC	8/29/02	67f			315	40	0.5	0.06	
ECO67F17	8/28/02	67f	0	0	405	385	0	0.00	0.0
ECO67F17	8/28/02	67f			405	20	0.5	0.02	
ECO67F23	8/28/02	67f	0	0	370	340	0	0.00	0.0
ECO67F25	8/29/02	67f	9.7	3	335	230	0	0.00	0.2
ECO67F25	8/29/02	67f			335	90	0.5	0.13	
ECO67F25	8/29/02	67f			335	15	1	0.04	
ECO67G01	8/27/02	67g	0	0	285	285	0	0.00	0.0
ECO67G05	8/27/02	67g	0	0	370	360	0	0.00	0.0
ECO67G05	8/27/02	67g			370	10	0.5	0.01	

Station ID	Date	ECO	Percent Macroalgae (Dm)	Maximum Length (in)	% Suitable Substrate (dt)	# Microalgae Points (di)	Microalgae Rank (ri)	$d_i r_i / d_t$*	Mean Thickness Rank $\sum d_i r_i / d_t$
ECO67G08	8/20/02	67g	0	0	375	280	0	0.00	0.1
ECO67G08	8/20/02	67g			375	95	0.5	0.13	
ECO67G09	8/20/02	67g	0	0	310	200	0	0.00	0.2
ECO67G09	8/20/02	67g			310	110	0.5	0.18	
ECO67G10	8/22/02	67g	0	0	433	178	0	0.00	0.3
ECO67G10	8/22/02	67g			433	255	0.5	0.29	
ECO67G10-QC	8/22/02	67g	0	0	355	225	0	0.00	0.2
ECO67G10-QC	8/22/02	67g			355	130	0.5	0.18	
BRADD000.8BL	8/14/02	68a	3	1	400	250	0	0.00	0.2
BRADD000.8BL	8/14/02	68a			400	120	0.5	0.15	
BRADD000.8BL	8/14/02	68a			400	30	1	0.08	
BRADD000.8BL-QC	8/14/02	68a	4	1	390	285	0	0.00	0.2
BRADD000.8BL-QC	8/14/02	68a			390	85	0.5	0.11	
BRADD000.8BL-QC	8/14/02	68a			390	20	1	0.05	
ECO68A01	9/5/02	68a	0	0	388	362	0	0.00	0.0
ECO68A01	9/5/02	68a			388	26	0.5	0.03	
ECO68A03	9/3/02	68a	0	0	205	119	0	0.00	0.0
ECO68A03	9/3/02	68a			205	6	0.5	0.01	
ECO68A26	9/5/02	68a	0	0	410	30	0	0.00	0.5
ECO68A26	9/5/02	68a			410	380	0.5	0.46	
ECO68A27	9/5/02	68a	0	0	257	245	0	0.00	0.0
ECO68A27	9/5/02	68a			257	12	0.5	0.02	
PINE006.0SC	9/3/02	68a	0	0	410	35	0	0.00	0.5

Station ID	Date	ECO	Percent Macroalgae (Dm)	Maximum Length (in)	% Suitable Substrate (dt)	# Microalgae Points (di)	Microalgae Rank (ri)	d_ir_i/d_t*	Mean Thickness Rank $\sum d_ir_i/d_t$
PINE006.0SC	9/3/02	68a			410	375	0.5	0.46	
ECO69D01	9/2/02	69d	0	0	265	231	0	0.00	0.1
ECO69D01	9/2/02	69d			265	34	0.5	0.06	
ECO71E09	9/11/02	71e	0	0	390	215	0	0.00	0.2
ECO71E09	9/11/02	71e			390	175	0.5	0.22	
ECO71E14	9/16/02	71e	1.1	NA	380	160	0	0.00	0.4
ECO71E14	9/16/02	71e			380	145	0.5	0.19	
ECO71E14	9/16/02	71e			380	75	1	0.20	
SPRIN009.8MT	9/16/02	71e	0	0	273	273	0	0.00	0.0
SUMME008.6SR	9/11/02	71e	0	0	270	85	0	0.00	0.4
SUMME008.6SR	9/11/02	71e			270	130	0.5	0.24	
SUMME008.6SR	9/11/02	71e			270	55	1	0.20	
SUMME008.6SR-QC	9/11/02	71e	0	0	250	90	0	-0.00	0.4
SUMME008.6SR-QC	9/11/02	71e			250	120	0.5	0.24	
SUMME008.6SR-QC	9/11/02	71e			250	40	1	0.16	
ECO71F12	10/9/02	71f	0	0	350	195	0	0.00	0.3
ECO71F12	10/9/02	71f			350	125	0.5	0.18	
ECO71F12	10/9/02	71f			350	30	1	0.09	
ECO71F16	9/30/02	71f	0	0	353	173	0	0.00	0.2
ECO71F16	9/30/02	71f			353	180	0.5	0.25	
ECO71F19	9/30/02	71f	0	0	383	50	0	0.00	0.4
ECO71F19	9/30/02	71f			383	333	0.5	0.43	
ECO71F19-QC	9/30/02	71f	0	0	350	255	0	0.00	0.1

Station ID	Date	ECO	Percent Macroalgae (Dm)	Maximum Length (in)	% Suitable Substrate (dt)	# Microalgae Points (di)	Microalgae Rank (ri)	$d_i r_i / d_t$*	Mean Thickness Rank $\sum d_i r_i / d_t$
ECO71F19-QC	9/30/02	71f			350	70	0.5	0.10	
ECO71F27	10/8/02	71f	0	0	315	280	0	0.00	0.1
ECO71F27	10/8/02	71f			315	35	0.5	0.06	
ECO71F28	9/30/02	71f	0	0	373	78	0	0.00	0.4
ECO71F28	9/30/02	71f			373	295	0.5	0.40	
JONES014.4DI	9/17/02	71f	0	0	215	145	0	0.00	0.2
JONES014.4DI	9/17/02	71f			215	55	0.5	0.13	
JONES014.4DI	9/17/02	71f			215	15	1	0.07	
SHOAL055.4LW	10/8/02	71f	0	0	290	79	0	0.00	0.4
SHOAL055.4LW	10/8/02	71f			290	211	0.5	0.36	
SHOAL055.4LW-QC	10/8/02	71f	0	0	320	130	0	0.00	0.3
SHOAL055.4LW-QC	10/8/02	71f			320	190	0.5	0.30	
CARR003.6OV	9/9/02	71g	11.1	NA	100	100	0	0.00	0.0
CLEAR001.1CE	8/13/02	71g	53	3	212	212	1	1.00	1.0
ECO71G03	9/30/02	71g	21	3	280	275	0	0.00	0.0
ECO71G03	9/30/02	71g			280	5	0.5	0.01	
ECO71G04	9/9/02	71g	0	0	260	125	0	0.00	0.3
ECO71G04	9/9/02	71g			260	135	0.5	0.26	
ECO71G10	8/13/02	71g	0	0	235	45	0	0.00	0.4
ECO71G10	8/13/02	71g			235	190	0.5	0.40	
MLICK014.7PU	9/10/02	71g	0	0	438	438	0.5	0.50	0.5
MLICK015.5PU	9/10/02	71g	0	0	310	310	0	0.00	0.0

Station ID	Date	ECO	Percent Macroalgae (Dm)	Maximum Length (in)	% Suitable Substrate (dt)	# Microalgae Points (di)	Microalgae Rank (ri)	$d_i r_i / d_t$*	Mean Thickness Rank $\sum d_i r_i / d_t$
ROCK009.3FR	8/13/02	71g	0	0	280	155	0	0.00	0.2
ROCK009.3FR	8/13/02	71g			280	125	0.5	0.22	
TOWN000.9OV	9/9/02	71g	0	0	185	165	0	0.00	0.0
TOWN000.9OV	9/9/02	71g			185	20	0.5	0.05	
TOWN001.1MA	9/11/02	71g	46.6	4	160	120	0	0.00	0.1
TOWN001.1MA	9/11/02	71g			160	40	0.5	0.13	
BROWN000.4DA	8/6/02	71h	4.4	NA	167	117	0	0.00	
BROWN000.4DA	8/6/02	71h			167	50	0.5	0.15	
ECO71H09	9/11/02	71h	0	0	382	27	0	0.00	0.8
ECO71H09	9/11/02	71h			382	78	0.5	0.10	
ECO71H09	9/11/02	71h			382	277	1	0.73	
EFMUL010.1WR	8/13/02	71h	0	0	278	200	0	0.00	0.1
EFMUL010.1WR	8/13/02	71h			278	78	0.5	0.14	
LHARP001.8WI	10/9/02	71h	0	0	365	285	0	0.00	0.2
LHARP001.8WI	10/9/02	71h			365	105	0.5	0.14	
LHARP001.8WI	10/9/02	71h			365	15	1	0.04	
MILL003.3DA	8/5/02	71h	6.7	6	420	420	0.5	0.50	0.5
RATTL000.2WI	10/9/02	71h	0	0	400	65	0	0.00	0.6
RATTL000.2WI	10/9/02	71h			400	180	0.5	0.23	
RATTL000.2WI	10/9/02	71h			400	155	1	0.39	
SIMS000.8DA	8/5/02	71h	0	0	204	98	0	0.00	0.3
SIMS000.8DA	8/5/02	71h			204	106	0.5	0.26	

Station ID	Date	ECO	Percent Macroalgae (Dm)	Maximum Length (in)	% Suitable Substrate (dt)	# Microalgae Points (di)	Microalgae Rank (ri)	$d_i r_i / d_t$*	Mean Thickness Rank $\sum d_i r_i / d_t$
SUGAR000.1DA	8/6/02	71h	0	0	400	0	0	0.00	0.0
SUGAR000.2MY	10/29/02	71h	0	0	370	295	0	0.00	0.1
SUGAR000.2MY	10/29/02	71h			370	65	0.5	0.09	
SUGAR000.2MY	10/29/02	71h			370	10	1	0.03	
SUGAR002.4MY	10/9/02	71h	0	0	370	235	0	0.00	0.2
SUGAR002.4MY	10/9/02	71h			370	95	0.5	0.13	
SUGAR002.4MY	10/9/02	71h			370	40	1	0.11	
SWAN008.0LI	8/12/02	71h	38.8	3	275	196	0	0.00	0.1
SWAN008.0LI	8/12/02	71h			275	79	0.5	0.14	
WFBRO000.1DA	8/5/02	71h	0	0	428	388	0	0.00	0.0
WFBRO000.1DA	8/5/02	71h			428	40	0.5	0.05	
ECO71I03	8/8/02	71i	0	0	105	105	0	0.00	0.0
ECO71I09	8/12/02	71i	26	3	295	137	0	0.00	0.3
ECO71I09	8/12/02	71i			295	158	0.5	0.27	
ECO71I10	10/8/02	71i	0	0	405	25	0	0.00	0.8
ECO71I10	10/8/02	71i			405	90	0.5	0.11	
ECO71I10	10/8/02	71i			405	290	1	0.72	
ECO71I14	10/9/02	71i	0	0	450	80	0.5	0.09	0.9
ECO71I14	10/9/02	71i			450	360	1	0.80	
ECO71I15	10/9/02	71i	0	0	430	390	0.5	0.45	0.6
ECO71I15	10/9/02	71i			430	40	1	0.09	
FALL003.6RU	8/12/02	71i	450	24	0	0	0	0.00	0.0

Station ID	Date	ECO	Percent Macroalgae (Dm)	Maximum Length (in)	% Suitable Substrate (dt)	# Microalgae Points (di)	Microalgae Rank (ri)	$d_i r_i / d_t$*	Mean Thickness Rank $\sum d_i r_i / d_t$
HURRI004.2RU	8/7/02	71i	66	5	147	67	0	0.00	0.9
HURRI004.2RU	8/7/02	71i			147	9	0.5	0.03	
HURRI004.2RU	8/7/02	71i			147	17	1	0.12	
HURRI004.2RU	8/7/02	71i			147	54	2	0.73	
JARMA000.3RU	8/7/02	71i	72	12	120	53	0	0.00	0.3
JARMA000.3RU	8/7/02	71i			120	67	0.5	0.28	
KELLE000.4RU	8/6/02	71i	14.4	5	111	84	0	0.00	0.1
KELLE000.4RU	8/6/02	71i			111	27	0.5	0.12	
LYTLE1T0.1RU	8/8/02	71i	0	0	330	288	0	0.00	0.1
LYTLE1T0.1RU	8/8/02	71i			330	42	0.5	0.06	
WFSTO008.3RU	8/7/02	71i	0	0	450	450	2	2.00	2.0
ECO74B01	9/18/02	74b	0	0	80	80	0	0.00	0.0
STOUT001.2FA	9/25/02	74b	0	0	35	35	0.5	0.50	0.5
STOUT001.2FA-QC	9/25/02	74b	0	0	0	0	0	0.00	0.0

*$d_i r_i / d_t$

Where d_i = number of grid point over microalga of different thickness ranks
r_i = thickness rank of microalgae
d_t = total number of grid points over suitable microalgae substrate at the site