CHANGES IN PERIPHYTON FATTY ACID COMPOSITION IN CHLORINE-POLLUTED STREAMS

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Changes in periphyton fatty acid composition in chlorine-polluted streams

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Abstract. A manipulative experiment was conducted to evaluate a biochemical method for using periphyton in water quality assessment. Periphyton assemblages that developed in situ on ceramic tiles placed in a reference site in one stream were transferred into a reference site and a polluted site in each of three streams in eastern Tennessee. Samples of periphyton from each site were analyzed for chlorophyll *a* content, rate of photosynthesis (by ^{14}C uptake), and fatty acid composition, 3, 17, and 35 days after being transferred to the sites. Chlorophyll *a* and photosynthetic rates per unit of chlorophyll at polluted sites were generally lower than at corresponding reference sites. More than 50 different fatty acids were separated and identified in the periphyton phospholipids; 12 of the 50 accounted for more than 90% of the total fatty acids. Periphyton fatty-acid biomarkers revealed differences in the periphyton taxonomic composition between reference and polluted sites. Fatty acid profiles indicated a shift from diatom to green alga-dominated assemblages in reference and chlorine contaminated sites respectively. Individual fatty-acid biomarkers specific for green algae (18:3ω3 or α-linolenic acid) and diatoms (20:5ω3 or eicosapentaenoic acid) proved particularly useful in quantifying the periphyton response to chlorine. The use of algal signature fatty acids may evolve into a quantitative automated method for measuring chlorine effect on stream periphyton.

Key words: periphyton, fatty acids, lipids, stream biological monitoring, water quality, chlorophyll.

Increasing efforts are being devoted to the use of periphyton for environmental assessment. This tendency is a consequence of periphyton's ubiquity, sensitivity to environmental perturbations, and high turnover rate. Because periphyton is the foundation of many food chains, factors that control its dynamics may have important implications for entire aquatic ecosystems. Natural and artificial substrates supporting complex periphytic assemblages can be collected and transported easily with little disturbance, and thus are useful for toxicity studies. Historically, periphyton assemblage composition, chlorophyll content, and photosynthetic rate have been the principal parameters used for environmental evaluation (e.g., Patrick 1978, Mulholland et al. 1986, Genter et al. 1988, Boston et al. 1991, Steinman et al. 1992).

Lipid analyses can provide important physiological and environmental information related to ecosystem function and to the distribution of lipophilic contaminants (Hadley 1985, 1989, Napolitano et al. 1992). Lipids are a heterogenous group of biogenic hydrophobic compounds used for energy storage, reproduction, and growth. Polar lipids (e.g., phospholipids and glycolipids) are key structural components of cellular membranes. Phospholipids occur in both plasma and intracellular membranes, while glycolipids are concentrated in chloroplast membranes (Kates 1970). Neutral lipids, in the form of triacylglycerols, are an efficient and common form of storage material in the cytosol of many algae, especially diatoms (Wood 1988). Phospholipid and triacylglycerol molecules contain a fixed number but a wide variety of fatty acids. Therefore, fatty acid analyses of lipid classes in microorganisms allow quantification of biomass (Tunlid and White 1992), and at the same time they may provide taxonomic information (Lechevalier and Lechevalier 1988).

McIntire et al. (1969) used an analysis of pe-

riphyton fatty acids to assess assemblage structure. Fatty acid and amino acid analyses also have been used to detect physiological and compositional changes of stream periphyton in response to grazing pressure and irradiance (Steinman et al. 1987, 1988). Using lipid analyses to evaluate periphyton assemblages in an industrially contaminated stream, Guckert et al. (1992) showed that membrane-to-storage lipid ratio (MEM/STO) of periphyton declined with distance downstream from a contamination source. This increase in MEM/STO was interpreted as recovery from physiological stress.

Here we investigate the relationship between conventional parameters (chlorophyll *a* density, primary production) and fatty acid analysis of periphyton in three eastern Tennessee streams affected by industrial operations. The purpose of this study was to explore the value of using the changes in the fatty acid composition of periphyton to assess environmental pollution in stream ecosystems.

Methods

Study site

East Fork Poplar Creek (EFPC) is a 3rd-order stream near the northeastern boundary of the U.S. Department of Energy Oak Ridge Reservation (Fig. 1). The stream originates within the Oak Ridge Y-12 Plant; it receives inputs from more than 200 outfall pipes from the plant operation before being intercepted by a settling basin (Lake Reality). The stream then flows to its confluence with Poplar Creek, a tributary of the Clinch River. Algal, invertebrate, and fish populations in EFPC have been studied intensively as part of the Y-12 Plant Biological Monitoring and Abatement Program (e.g., Hinzman 1991). The two sites on EFPC selected for this study were located upstream and downstream from Lake Reality; these sites were designated as "polluted" (poll) and "reference" (ref) sites, respectively. The two sites were similar with respect to ambient light and nutrient concentrations, but differed in other important parameters (Table 1). Chlorine concentrations in the EFPC-poll site often exceed 150 μg/L. Periphyton assemblages at this site were characterized by low taxonomic diversity (Boston et al. 1991). The EFPC-ref site was ~200 m downstream from Lake Reality. Although some species of pollution-sensitive fish and invertebrates were lacking at this site, chlorine and other contaminants were substantially lower at this site than at the polluted site (Table 1). White Oak Creek (WOC) is a 2nd-order stream that flows through the Oak Ridge National Laboratory (Fig. 1). The principal sources of the base flow in upper WOC are springs. The reference site in WOC was inside the ORNL grounds, and was upstream of most known sources of contaminants. In contrast, WOC-poll site was downstream of a number of outfalls, including those containing blowdown from cooling-tower operations, tap water used as once-through coolant, and storm drainage. Mitchell Branch (MB) is a small stream running along the boundary of the Department of Energy's K-25 Site (Fig. 1). This stream flows 1.5 km from its headwaters to its confluence with Poplar Creek. The reference and polluted sites on this stream were upstream and downstream respectively of the K-25 Site. Concentrations of total residual chlorine were below detection at both sites. However, activities within the K-25 Site released various organic compounds, mercury, copper, nickel, zinc, and silver to MB. Bioaccumulation studies have shown that MB was a source of chlorinated hydrocarbons (PCBs) to the biota. Although PCBs have rarely been detected in K-25 Site effluents, PCBs were still present in MB sediments (Martin Marietta Energy Systems, Inc. 1991).

Deployment and recovery of tiles

Unglazed ceramic tiles (2.4 × 2.4 × 0.58 cm) were placed in the WOC-ref site on 6 December 1991 for colonization by periphyton. On 24 February 1992 most colonized tiles were transferred from the WOC-ref site to WOC-poll, or to reference and polluted sites of EFPC and MB. Tiles were recovered from all sites on 3 d (27 February), 17 d (12 March) and 35 d (31 March) following the transfer. Tiles collected from each site were carefully placed in buckets with stream water and transported to the laboratory.

Carbon uptake

In the laboratory, tiles were placed into 2-L glass incubation chambers in which 1 L of stream water from the site was recirculated by a pump. Fifteen tiles from each site were placed in each chamber. Incubation was carried out under a constant illumination (400 μmol quanta $m^{-2}s^{-1}$)

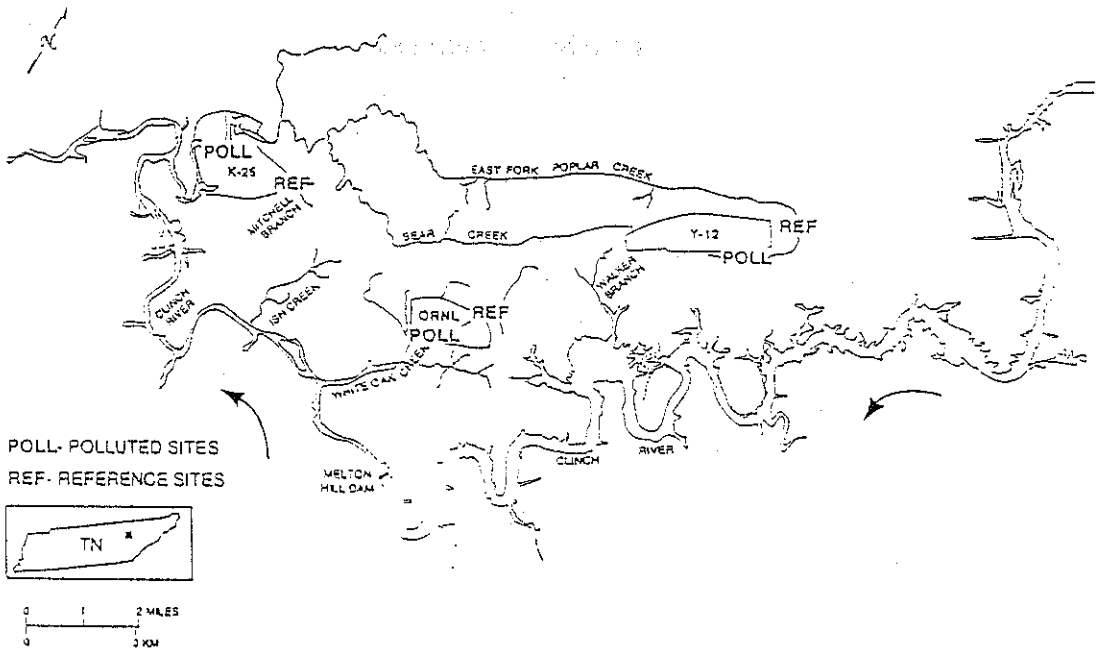

FIG. 1. Study area showing White Oak Creek, East Fork Poplar Creek, Mitchell Branch, and the location of reference and polluted sites in relationship to Oak Ridge National Laboratory, the Y-12 Plant, and the K-25 Site in Tennessee, USA. Arrows show flow direction in the Clinch River.

provided by a 1000-W metal halide lamp. Water temperature at each of the six sites differed slightly, so the temperature for the incubations was set at 12°C, which was within 5°C of all of the sites. Ten μCi $NaH^{14}CO_3$ (specific activity = 1.2 μCi/mmol; New England Nuclear) was added to each chamber, and the tiles were incubated in the radiolabeled water for 45 min. The incubations were terminated by rinsing the tiles twice with fresh stream water and immediately placing them into solvents for the extractions of lipids and chlorophyll. Three of the 15 tiles from each chamber were placed in individual bottles containing 10 mL of dimethylsulfoxide (DMSO) and extracted overnight. DMSO extracts both chlorophyll *a* and recently-labelled photosynthate products (Palumbo et al. 1987). Chlorophyll *a* was analyzed spectrophotometrically at 664 nm before and after acidification with three drops of 1 N HCl. The remaining 12 tiles in the chamber were divided into three groups of four tiles each, and the lipids of each of the three groups were extracted for lipid analysis in separate containers.

TABLE 1. Mean (±1 SD) values for water quality data at reference and polluted sites in White Oak Creek, East Fork Poplar Creek and Mitchell Branch. March and February 1992 averages.

Stream	pH	Conductivity (μS/cm)	Alkalinity (mg $CaCO_3$/L)	Hardness (mg $CaCO_3$/L)	Total residual chlorine (μg/L)
WOC-ref	8.16 ± 0.1	234 ± 42	103 ± 15	129 ± 20	0.00
-poll	8.13 ± 0.2	295 ± 43	103 ± 10	131 ± 17	14 ± 9
EFPC-ref	8.12 ± 0.07	497 ± 39	109 ± 4	198 ± 5	0.00
-poll	8.11 ± 0.1	394 ± 133	100 ± 8	200 ± 67	118 ± 30
MB-ref	7.71 ± 0.1	124 ± 3	57 ± 2	69 ± 10	0.00
-poll	8.02 ± 0.1	324 ± 27	129 ± 11	167 ± 10	0.00

TABLE 2. Periphytic algae observed in White Oak Creek (WOC) -ref (Feb 26), and in WOC, East Fork Poplar Creek (EFPC) and Mitchell Branch (MB) 17 days (March 12) and 35 days (March 31) after transfer to the reference and polluted streams.

Site	Day 3		Day 17		Day 35	
	Diatoms	Green algae	Diatoms	Green algae	Diatoms	Green algae
WOC-ref	*Gomphonema* *Cymbella* *Nitzchia* *Enootia* *Surirella*	*Stigeoclonium*	—	—	—	—
WOC-ref, EFPC-ref, MB-ref	—	—	*Achnanthes* *Surirella* *Gomphonema*	Unidentified unicellular,	*Nitzchia* *Achnanthes* *Surirella* *Navicula* *Gomphonema* *Cymbella*	*Stigeoclonium* (basal cells) "
WOC-poll EFPC-poll	—	—	—	*Stigeoclonium* (basal cells)	—	*Stigeoclonium* (basal cells and short filaments)
MB-poll	—	—	*Nitzchia* *Navicula* *Achnanthes* *Surirella*	A few *Oedogonium*	*Navicula* *Achnanthes* *Surirella*	A few *Oedogonium*

[1] Some blue green algae (*Pitormidium*) were observed in these samples.

Lipid extraction

Tiles with radiolabeled periphyton were extracted for lipids with a mixture of chloroform and methanol, using the method of Bligh and Dyer (1959). The lower organic phase containing the total lipid fraction was drained from the original extraction jar into a round-bottom flask and the solvents were removed under low pressure at 40°C. Polar lipids (consisting of phospholipids and glycolipids) and triacylglycerols were separated by preparative column chromatography for fatty acid composition and incorporation of radiolabel precursors (Guckert et al. 1992). An aliquot (10% of the total) from each lipid class was assayed for ^{14}C by liquid scintillation spectroscopy. These samples were photobleached by exposing them to a 1000-W lamp overnight (~400 μmol quanta m^{-2} s^{-1}) before analysis to reduce color quenching. Fatty acid methyl esters were prepared from phospholipids by mild alkaline methanolic transesterification (Christie 1982). The esters were separated and identified by capillary gas chromatography and mass spectrometry as described by Guckert and Cooksey (1990).

The fatty acid nomenclature used here is of the form 18:2ω6, where "18" designates the total number of carbon atoms, "2" the number of *cis* double bonds, and "ω6" the position (6) closest to the aliphatic (ω) end of the molecule.

Statistical analysis

Statistical comparisons were made between sites within each stream. We acknowledge that such comparisons test only for differences between the sites, regardless of the source of the difference, and do not test for the effect of pollution per se. Because of the lack of adequately replicated, similar pairs of polluted/reference sites, inferences about causal factors are weaker than if each site served as an experimental unit. However, true replication of pollution effects is difficult to obtain in nonexperimental studies, and experimental studies suffer from a lack of realism. Our statistical comparisons are meant to provide the necessary first step of determin-

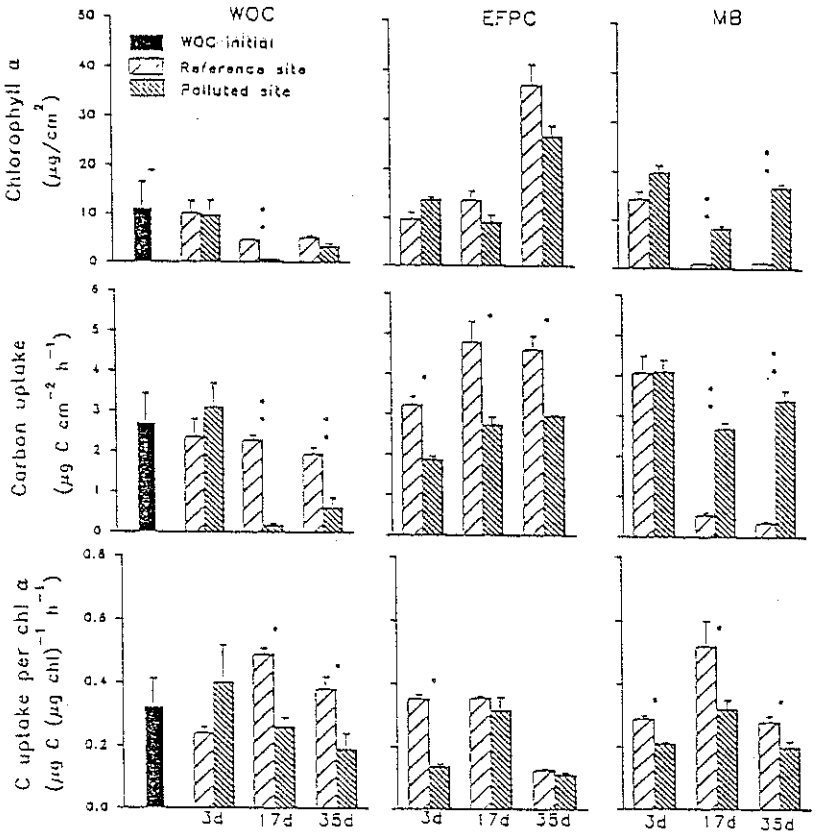

FIG. 2. Mean (+1 SD) values of chlorophyll *a*, carbon uptake, and chlorophyll-specific carbon uptake of periphyton from reference and polluted sites in White Oak Creek, East Fork Poplar Creek, and Mitchell Branch (* $p < 0.05$, ** $p < 0.01$, $n = 3$).

ing if there is a difference between sites within each stream. We attribute differences to pollution effects, but not in a formal, statistical context. Experimental units for chlorophyll *a* and carbon uptake analysis were individual tiles, replicated three times at each site. Three groups of four tiles apiece served as the experimental units in the lipid analyses (including the ^{14}C-labelled lipids). Significant differences ($p < 0.05$, unless specified otherwise) between reference and impacted periphyton at each pair of sites were analyzed by *t*-tests.

Results

Periphyton

Qualitative microscopic examination of periphyton samples scraped from the tiles revealed a large number of diatoms at the beginning of the experiment (Table 2). Filaments of *Stigeoclonium* sp. (a green alga) also were present in WOC-ref. By day 35, *Stigeoclonium* basal cells were still present at all sites, while diatoms were no longer observed in the WOC-poll and EFPC-poll locations. At MB, diatoms were abundant in the periphyton assemblages both at MB-ref and MB-poll sites (Table 2).

Chlorophyll a content and carbon uptake

At day 3, values for chlorophyll *a* at all the reference sites were not significantly different ($p > 0.05$) from these of the corresponding polluted site (approximately 10 $\mu g/cm^2$) (Fig. 2). Chlorophyll *a* content of periphyton in WOC-ref and WOC-poll decreased to < 6 $\mu g/cm^2$ during the longer exposure times; in EFPC-ref chlorophyll *a* increased to 37.0 $\mu g/cm^2$. At days 17 and 35, chlorophyll *a* concentrations in ref-

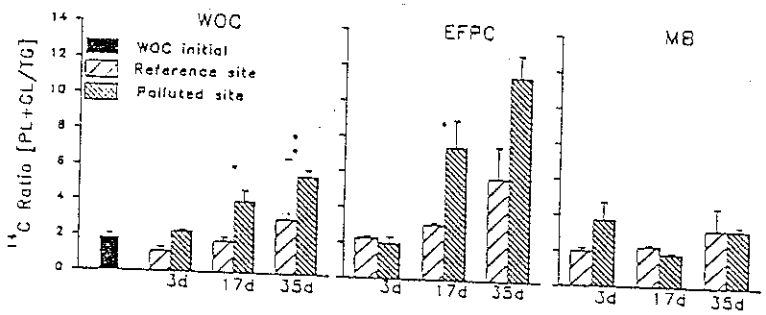

FIG. 3. Ratios of membrane lipid (phospholipid [PL] and glycolipids [GL]) to storage lipid (triacylglycerols [TG]) in periphyton from reference and polluted sites in White Oak Creek, East Fork Poplar Creek and Mitchell Branch as determined by ^{14}C-$NaHCO_3$ incorporation (means ± 1 SD, * $p < 0.05$, ** $p < 0.01$, $n = 3$).

erence sites typically were greater ($p < 0.05$) than in the polluted sites both in WOC and EFPC (Fig. 2). In MB, though, the opposite trend was noted. There was more chlorophyll *a* ($p < 0.05$) at the polluted site than in the reference site at day 17 (Fig. 2).

Carbon uptake by periphyton at the reference WOC site was 2.7 μg C cm^{-2} h^{-1} on day zero, and decreased slightly during the experiment (Fig. 2). Changes in total C incorporation at all sites exhibited similar trends to the changes in chlorophyll *a* (Fig. 2). Carbon uptake by periphyton in the WOC- and EFPC-ref sites was greater than by periphyton in polluted sites in these streams ($p < 0.05$). Carbon uptake by periphyton in MB, however, was lower at the reference site than at the polluted site ($p < 0.05$) (Fig. 2).

Consistent relationships between reference and polluted sites in the three streams were found in chlorophyll-specific rates of carbon uptake (Fig. 2). Carbon uptake rates per unit of chlorophyll *a* in reference sites, including MB, were greater ($p < 0.05$) than in the polluted sites in eight of the nine site-date combinations (WOC-ref/poll pair at day 3 was the sole exception).

Radiolabeling of lipids

Incorporation of ^{14}C into membrane (MEM) lipids (phospholipids and glycolipids) and storage (STO) lipids (triacylglycerols) varied among streams and sites (Fig. 3). No significant differences in MEM/STO based on the incorporation of radioactive precursors into different lipid fractions were observed between reference and polluted sites at day 3 ($p > 0.05$). However, MEM/STO of periphyton in WOC and EFPC-poll sites by day 17 tended to be much greater ($p < 0.05$) than in their reference counterparts. These differences persisted through day 35. MEM/STO in reference and polluted periphyton in MB on day 17 and day 35 varied less (from 2.0 to 3.5) and the differences were not significant on either date.

Phospholipid fatty acid (PLFA) composition

Periphyton phospholipids contained fatty acids with chain lengths ranging from 14 to 24 atoms of carbon (C14–C24) and containing from zero to six double bonds. More than 50 different fatty acids were separated and identified from these lipid fractions (e.g., Table 3). Twelve of the 50 fatty acids accounted for 90% of the total, on a mole percent basis. The dominant PLFAs in the periphyton were: 16:0 (palmitic), 16:1ω7 (palmitoleic), 20:5ω3, 18:3ω3 (α-linolenic), 18:2ω6 (linoleic), 18:1ω7 (*cis*-vaccenic), 18:1ω9 (oleic), 14:0 (myristic), 20:4ω6 (arachidonic), *trans*-3-16:1, 18:4ω3, 18:0 (stearic) and 16:3ω4.

Periphyton PLFAs of the three streams at day 3 showed non-significant differences ($p > 0.05$) between reference and polluted sites; on days 17 and 35, WOC and EFPC periphyton from reference and polluted sites differed considerably in their proportions of about 10 PLFAs (Figs. 4, 5, day 17 and 35). Some of these fatty acids (16:1ω7, 16:2ω6, 20:3ω3, 20:4ω6, 20:5ω3 and 22:6ω3) increased in WOC and EFPC-ref sites, while others (*trans*-3-16:1, 10Me18:0, 18:1ω9, 18:3ω3) increased at the polluted sites (Figs. 4, 5). An important fatty acid showing significant differences ($p < 0.05$) between reference and polluted sites in WOC and in EFPC was eicosa-

pentaenoic acid (20:5ω3). For example, 20:5ω3 concentrations were three to four times greater at EFPC-ref 17 and 35 days after the experiment had started. The concentrations of 20:5ω3 in the phospholipids of the periphyton from WOC-ref and EFPC-ref sites were about 14% and 10% respectively, while in WOC-poll and EFPC-poll sites concentrations decreased to about 2.0% by day 35. In contrast to 20:5ω3, α-linolenic acid (18:3ω3) increased significantly at polluted sites, so that by day 35, it was twice as prevalent at WOC and EFPC-poll sites.

In contrast to EFPC and WOC, phospholipid fatty acids in the periphyton of the reference and polluted sites in MB showed a few minor and inconsistent differences during the experiment (Fig. 6). The % of α-linolenic acid did not differ significantly between the MB-ref and MB-poll sites, while 20:5ω3 increased significantly in the polluted site.

The MEM/STO obtained for the reference and polluted sites of the three streams were compared with an index of periphyton taxonomic composition. This taxonomic index was defined as the ratio between the concentrations of 18:3ω3 and 20:5ω3 (characteristic fatty acids of green algae and diatoms respectively) in the periphyton phospholipids. The MEM/STO and 18:3ω3/20:5ω3 ratios were very highly correlated in WOC and in EFPC. The range of the ratios was much smaller in MB, and there was no significant correlation between them (Fig. 7).

TABLE 3. Mean and SD of periphyton phospholipid fatty acids of periphyton (mole %) from the White Oak Creek colonization site on 24 February 1992 (n = 3).

Shorthand designation	Trivial name	Mean	SD
i14:0	—	0.14	0.01
14:0	myristic	3.59	0.09
Me14:0	—	0.29	0.04
i15:0	—	0.58	0.05
a15:0	—	0.26	0.04
15:0	—	0.36	0.03
16:3ω4	—	1.50	0.03
16:2ω6	—	0.56	0.10
16:2ω4	—	0.63	0.02
C16PUFA	—	1.62	0.09
16:1ω9	—	0.57	0.04
16:1ω7	palmitoleic	16.05	0.33
16:1ω6	—	1.19	0.20
16:1ω5	—	0.42	0.04
trans-3-16:1	—	2.29	0.27
16:0	palmitic	22.12	0.57
i17:0	—	0.07	0.01
a17:0	—	0.19	0.02
17:0	margaric	0.27	0.03
C18PUFA	—	0.17	0.01
18:3ω6	γ-linolenic	0.68	0.04
18:4ω3	—	2.06	0.26
18:2ω6	linoleic	4.82	0.10
18:3ω3	α-linolenic	10.61	0.40
18:1ω9	oleic	3.79	0.13
18:1ω7	*cis*-vaccenic	3.99	0.23
18:0	stearic	1.61	0.20
10Me18:0	—	0.84	0.05
20:4ω6	arachidonic	2.10	0.09
20:5ω3	—	14.06	1.43
20:3ω6	—	0.19	0.01
20:4ω3	—	0.28	0.03
20:2ω3	—	0.12	0.04
20:3ω3	—	0.15	0.04
20:1ω9	gondoic	0.14	0.04
20:0	arachidic	0.26	0.04
22:5ω6	—	0.08	0.02
22:6ω3	—	0.41	0.05
22:4ω6	—	0.08	0.02
22:5ω3	—	0.52	0.15
22:0	behenic	0.10	0.01
24:0	lignoceric	0.26	0.07

Discussion

Periphyton

Water chemistry data indicated that chlorine was a major contaminant at WOC-poll and EFPC-poll, but not at MB-poll. High levels of total residual chlorine were recorded in these two polluted sites during the course of this experiment (Table 1). Moreover, monitoring programs in this laboratory have characterized these streams as chronically contaminated with chlorine (Hinzman 1991). Low taxonomic diversity and the presence of certain green algal species (such as *Stigeoclonium* and a small unicellular *Pleurococcus*-like alga) previously were observed in chlorine-polluted streams (Murray 1980, Boston et al. 1991). Although algal taxonomic composition was not examined quantitatively, the scarcity of diatoms in qualitative observations of the periphyton at the chlorine-polluted sites (Table 2) is consistent with previous studies (Boston et al. 1991). Interestingly, the opposite trend (i.e., diatoms replacing green algal species) was observed in periphyton after

pH. Environmental Toxicology and Chemistry 7:723–733.

GUCKERT, J. B., AND K. E. COOKSEY. 1990. Triglyceride accumulation and fatty acid profile changes in *Chlorella* (Chlorophyta) during high pH-induced cell cycle inhibition. Journal of Phycology 26:72–79.

GUCKERT, J. B., S. C. NOLD, H. L. BOSTON, AND D. C. WHITE. 1992. Periphyton response in an industrial receiving stream: lipid-based physiological stress analysis and pattern recognition of microbial community structure. Canadian Journal of Fisheries and Aquatic Sciences 49:2579–2587.

HADLEY, N. F. 1985. The adaptive role of lipids in biological systems. Wiley-Intersciences, New York.

HADLEY, N. F. 1989. Lipid water barriers in biological systems. Progress in Lipid Research 28:1–33.

HILL, W. R., AND H. L. BOSTON. 1991. Community development alters photosynthesis-irradiance relations in stream periphyton. Limnology and Oceanography 36:1375–1389.

HINZMAN, R. L. 1991. Second report on the Y-12 Plant Biological Monitoring and Abatement Program. Oak Ridge National Laboratory, Oak Ridge, Tennessee.

KATES, M. 1970. Plant phospholipids and glycolipids. Advances in Lipid Research 8:225–267.

KAYAMA, M., S. ARAKI, AND S. SATO. 1990. Lipids of marine plants. Pages 3–58 *in* R. G. Ackman (editor). Marine biogenic lipids, fats, and oils. CRC Press, Boca Raton, Florida.

KOHRING, L. L. 1993. Quantitative multi-species toxicity assessment by analysis of freshwater stream periphyton pigments and lipids. M.Sc. Thesis, University of Tennessee, Knoxville.

LECHEVALIER, H., AND M. P. LECHEVALIER. 1988. Chemotaxonomic use of lipids—an overview. Pages 869–902 *in* G. Ratledge and S. G. Wilkinson (editors). Microbial lipids. Academic Press, New York.

MARTIN MARIETTA ENERGY SYSTEMS, INC. 1991. Oak Ridge K-25 Site. Toxicity control plan for Mitchell Branch. K-25 Plant Internal Report K/HS-354, Oak Ridge, Tennessee.

MCINTIRE, C. D., I. J. TINSLEY, AND R. R. LOWRY. 1969. Fatty acids in lotic periphyton: another measure of community structure. Journal of Phycology 5:26–32.

MULHOLLAND, P. J., J. W. ELWOOD, A. V. PALUMBO, AND R. J. STEVENSON. 1986. Effect of stream acidification on periphyton composition, chlorophyll, and productivity. Canadian Journal of Fisheries and Aquatic Sciences 43:1846–1858.

MURRAY, S. A. 1980. Periphyton response to chlorination and temperature. Pages 641–648 *in* R. L. Jolley, W. A. Brungs, R. B. Cumming, and V. A. Jacobs (editors). Water chlorination. Environmental impact and health effects, Volume 3. Ann Arbor Science, Ann Arbor, Michigan.

NAPOLITANO, G. E., R. G. ACKMAN, AND C. C. PARRISH. 1992. Lipids and lipophilic pollutants in three species of migratory shorebirds and their food in Shepody Bay (Bay of Fundy, New Brunswick). Lipids 27:785–790.

NAPOLITANO, G. E., R. G. ACKMAN, AND M. N. RATNAYAKE. 1990. Fatty acid composition of three cultured algal species (*Isochrysis galbana*, *Chaetoceros gracilis* and *Chaetoceros calcitrans*) used as food for bivalve larvae. Journal of the World Aquaculture Society 21:122–130.

PALUMBO, A. V., P. J. MULHOLLAND, AND J. W. ELWOOD. 1987. Extraction with DMSO to simultaneously measure periphyton photosynthesis, chlorophyll, and ATP. Limnology and Oceanography 32:464–471.

PARRISH, C. C., AND P. J. WANGERSKY. 1987. Particulate and dissolved lipid classes in cultures of *Phaeodactylum tricornurum* grown in cage culture turbidostats with a range of nitrogen supply rates. Marine Ecology Progress Series 35:119–128.

PATRICK, R. 1963. The structure of diatom communities under varying ecological conditions. Annals of the New York Academy of Science 108: 359–364.

PATRICK, R. 1978. Effect of trace metals in the aquatic ecosystem. American Scientist 66:185–191.

PIORRECK, M., K-H. BAASCH, AND P. POHL. 1984. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. Phytochemistry 23:207–216.

STEINMAN, A. D., C. D. MCINTIRE, AND R. R. LOWRY. 1987. Effects of herbivore type and density on chemical composition of algal assemblages in laboratory streams. Journal of the North American Benthological Society 6:189–197.

STEINMAN, A. D., C. D. MCINTIRE, AND R. R. LOWRY. 1988. Effects of irradiance and age on chemical constituents of algal assemblages in laboratory streams. Archiv für Hydrobiologie 114:45–61.

STEINMAN, A. D., P. J. MULHOLLAND, A. V. PALUMBO, D. L. DEANGELIS, AND T. E. FLUM. 1992. Lotic ecosystem response to a chorine disturbance. Ecological Applications 2:341–355.

TUNLID, A., AND D. C. WHITE. 1992. Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. Pages 229–262 *in* G. Stotzky, and J-M. Bollag (editors). Soil biochemistry, Volume 7. Marcel Dekker, Inc., New York.

VECHTEL, B., W. EICHENBERGER, AND H. G. RUPPEL. 1992. Lipid bodies in *Eremosphaera viridis* De Bary (Chlorophyceae). Plant Cell Physiology 33:41–48.

VOLKMAN, J. K., S. W. JEFFREY, P. D. NICHOLS, G. I.

ROGERS, AND C. D. GARLAND. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. Journal of Experimental Marine Biology and Ecology 128:219–240.

WOOD, B. J. B. 1988. Lipids of algae and protozoa. Pages 807–865 *in* G. Ratledge and S. G. Wilkinson (editors). Microbial lipids, Volume 1. Academic Press, New York.

WOOD, L. W. 1965. Chloroform-methanol extraction of chlorophyll *a*. Canadian Journal of Fisheries and Aquatic Sciences 42:38–43.

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