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Periphyton Response in an Industrial Receiving Stream: Lipid-based Physiological Stress Analysis and Pattern Recognition of Microbial Community Structure

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Periphyton were collected from three physically similar sites on an industrial receiving stream which differed in their distances from the discharge. Previous studies had established that biological effects diminished with distance downstream from the discharges. In this study, a multivariate analysis of periphyton membrane lipid profiles quantitatively verified site-specific differences in time-independent microbial community structure qualitatively suggested by algal taxonomic analyses. There were no differences in periphyton abundance among sites. Periphyton physiology was evaluated using a ^{14}C-labeled bicarbonate and ^{3}H-labeled amino acids dual-label incubation, with subsequent analysis of carbon partitioning into lipid compartments. Total radiolabel uptake was similar for all sites. Physiological stress was shown to be highest at the site closest to the discharges using a membrane to storage lipid synthetic ratio. High values of this ratio indicated that both the phototrophic and heterotrophic constituents were stressed, and this stress declined with distance downstream. The ability to measure algal and bacterial abundance, community structure, activity, and physiological status from a single extraction provides a powerful method to evaluate periphyton which can serve as a useful biomonitoring and toxicity assessment tool for aquatic ecosystems.

Dans un cours d'eau récepteur d'effluents industriels, nous avons prélevé du périphyton à trois endroits physiquement similaires mais situés à différentes distances du point de rejet. Des études antérieures avaient établi que les effets biologiques diminuaient proportionnellement à l'augmentation de la distance en aval par rapport aux points de rejet. Dans la présente étude sur le périphyton, une analyse multidimensionnelle des profils lipidiques de la membrane cellulaire confirme quantitativement les différences particulières aux sites dans la structure de la communauté microbienne, indépendamment du temps; l'hypothèse relatives à ces différences était issue d'analyses taxinomiques qualitatives des algues. Nous n'avons noté aucune différence entre les endroits étudiés relativement à l'abondance du périphyton. La physiologie du périphyton a été évaluée sur des échantillons étiquetés en double et incubés avec du bicarbonate marqué au ^{14}C et des acides aminés marqués au ^{3}H; on a ensuite analysé la répartition du carbone entre les sites lipidiques. L'apport total en éléments radiomarqués était équivalent pour tous les sites. En utilisant le rapport entre les lipides de la membrane et les lipides intracellulaires, on a obtenu un niveau maximal de stress physiologique à l'endroit le plus rapproché du point de rejet. Les valeurs élevées de ce rapport indiquaient que les composants phototrophes et hétérotrophes subissaient un stress, et que ce stress diminuait en fonction de la distance en aval. La capacité de déterminer l'abondance, la structure des communautés, l'activité et la condition physiologique des algues et des bactéries à partir d'une seule extraction représente une méthode efficace pour l'évaluation du périphyton, qui peut être utile comme outil de surveillance biologique et d'évaluation de la toxicité des écosystèmes aquatiques.

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Periphyton is the complex microbial biofilm which develops on submerged surfaces. Because of their importance in lotic ecosystems, periphyton analyses have been an important aspect of stream ecology research. In addition, their ubiquitous nature makes periphyton communities excellent integrating "monitors" for water quality in receiving rivers and streams (e.g. Weitzel 1979; APHA 1989; Boston et al. 1991).

Using periphyton for biological monitoring presents the same challenges as any in situ analysis of complex microbial communities (White 1985). The methods and measurements ("endpoints") chosen to assess these communities determine the quality of results available for interpretation. Complex periphyton communities are often assessed with an emphasis on algal constituents (Aloi 1990), either from a viewpoint of biomass/community structure (e.g. Chessman 1985) or total photosynthesis (e.g. Blanck 1985). Periphytic bacterial populations are analyzed much less often (Dean-Ross 1990; Hudson et al. 1990), and only a few studies have monitored both algae and bacteria simultaneously (Stock and Ward 1989). Given the diversity of periphyton communities available in situ for water quality assessment studies, methods which provide an analysis of an entire assemblage might markedly improve resolution and sensitivity to anthropogenic chemicals.

The extraction of cellular lipids provides a quantitative and nonselective method of analysis for all viable microorganisms within complex communities (Vestal and White 1989). The fatty acid profiles of the plasma membrane phospholipids provide a multivariate index from all microbial populations (algal, bacterial, protozoan, fungal, etc.) within the community (Guckert and White 1986). Analysis of radiotracer incorporation into lipids can provide estimates of microbial activity, in terms of lipid synthesis, and physiological status, as carbon allocation patterns between storage lipids and membrane lipids (Findlay et al. 1990).

Total fatty acid profiles have been reported previously for laboratory stream periphyton; these profiles have distinct relationships to community structure (McIntire et al. 1969) and nutritional quality (Steinman et al. 1987, 1988). Although total fatty acid profiles are useful, even more information is available through fractionation of these into lipid classes. Algal lipids can be divided into three major classes: neutral lipids, which contain the storage lipid triglyceride; glycolipids, which generally have highly polyunsaturated fatty acids as the major membrane lipid of the chloroplast membranes; and polar lipids, which are dominated by the phospholipids from the plasma membrane (Guckert and Cooksey 1990). Fatty acid profile analyses in microbial ecology have focused on the polar lipid fraction due to its high extracellular turnover rate and constant relative amounts in microbial plasma membranes (Vestal and White 1989).

In this study, lipid analyses along with an algal/heterotrophic dual-label radiotracer were used as biochemical monitors to study periphyton physiological stress and community structure in an industrial receiving stream. This stream has been the focus of biological monitoring including periphyton, invertebrates, and biomarker analysis in fish.

Description of Study Site

East Fork Poplar Creek is a third-order stream located near the northern boundary of the Department of Energy's Oak Ridge National Laboratory (ORNL) Reservation in eastern Tennessee, USA. The headwaters of this creek lie within the Oak Ridge Y-12 nuclear weapons component manufacturing facility where the creek receives diverse industrial effluents (see Hill 1992 for a map of the watershed). The algal, invertebrate, and fish communities in this creek have been studied extensively through a Biological Monitoring and Abatement Program to assess the possible effects of unique contaminants from the Y-12 plant (e.g. Loar et al. 1989; Stewart et al. 1990, 1992; Boston et al. 1991; Hinzman 1991).

East Fork Poplar Creek receives effluent from more than 200 individual discharges before leaving the Y-12 plant. The creek then flows into a 2.2-ha catchment basin designed for effluent pH equilibration, sediment retention, and spill control (Kingrea 1986). From the outfall of this basin, designated Lake Reality, the creek flows a distance of 23.7 km to its confluence with Poplar Creek, a tributary of the Clinch River. Study sites are designated by their kilometre distance above this confluence. Effluent discharges of 388 $L \cdot s^{-1}$ from the Y-12 plant constitute 25% of the mean annual flow of the creek at the 5.3-km gauging station. The stream also receives urban runoff and some agricultural runoff between sites 22.7 and 7.7 (Jimenez et al. 1990).

The water and sediments of East Fork Poplar Creek contain metals, organic chemicals, and radionuclides discharged during the operation of the Y-12 plant. However, monthly testing of water from the Lake Reality outfall (site 23.4) has provided little evidence for acute or chronic toxicity to *Ceriodaphnia* or fathead minnow (*Pimephales promelas*) larvae (Loar et al. 1989; Kszos et al. 1992). Whole-fish bioaccumulation results indicated high concentrations of contaminants such as mercury and PCBs that decreased with distance downstream (Adams et al. 1990; Stewart and Loar 1993). Community analyses and fish biomarker assessment also indicated poor biotic conditions at upstream sites (Loar et al. 1989; Jimenez et al. 1990).

Three sites along East Fork Poplar Creek were selected because they were similar with respect to physical parameters (ambient light intensities, stream flow, nutrient concentrations) but different with respect to biological Y-12 effluent effects (Loar et al. 1989). Site 24.4 is located upstream of Lake Reality. Chlorine concentrations at this site often exceed 150 $\mu g \cdot L^{-1}$ (A. J. Stewart, ORNL, pers. comm.). Algal periphyton is physiologically stressed and exhibits low species diversity (Boston et al. 1991). Few fish or invertebrate species have been found at this site (Loar et al. 1989).

Site 23.2, located about 500 m downstream from Lake Reality, is characterized by greater periphytic algal taxonomic diversity than site 24.4; however, pollution-sensitive fish and invertebrate species are absent (Loar et al. 1989). Fish that are present at this site are physiologically stressed relative to unpolluted reference sites, based on biomarkers such as induction of the hepatic mixed-function oxidase system components (Adams et al. 1990; Jimenez et al. 1990) and carbohydrate/protein metabolism and membrane/storage lipid metabolism (Adams et al. 1990).

At site 13.8 the biological communities and physicochemical conditions are typical of nonpolluted streams in eastern Tennessee. The invertebrate communities contain fewer oligocheates and have about 10 times more insect biomass (areal basis) than the upstream sites (Loar et al. 1989). Additionally, the fish are less stressed physiologically than they are in the upstream sites (Adams et al. 1990; Jimenez et al. 1990).

Materials and Methods

Periphyton Sampling

At each sampling site, 20 relatively flat small rocks (average $\pm$ SD upper surface area = 45 $\pm$ 12 cm^2) with their attached

periphytic communities were collected from shallow riffles (<30 cm deep) on each of three dates (March 22, April 4, and May 9, 1990). The rocks were immediately placed in opaque containers with water from the collection site for transport to the laboratory.

Eight rocks from each site were randomly chosen and placed into a 2-L recirculating chamber with 1 L of stream water from that site within 30 min of collection. The recirculation chambers have been described elsewhere (Mulholland et al. 1986; Boston and Hill 1991). The remaining 12 rocks were stored in the dark at approximately in situ temperatures (15–20°C) for no more than 2 h for the following assays: bacterial cell density, organic carbon content, and algal taxonomic composition.

Radiolabel Incubation

The rocks within the recirculating chambers were incubated under constant illumination (approximately 400 μmol quanta·m^{-2}·s^{-1} photosynthetically active radiation, ≈16% full sun) provided by a 1000-W metal halide lamp. During the incubation, the water temperature was maintained within 4°C of ambient stream temperature and the water in the incubation chambers was circulated constantly by submersible pumps to simulate natural conditions (Boston and Hill 1991).

We used a dual-label incubation method to simultaneously measure photoautotrophic (^{14}C-labeled dissolved inorganic carbon (DIC)) and heterotrophic (^{3}H-labeled dissolved organic carbon (DOC)) radiolabel uptake/incorporation. Initially, 10 μCi of $NaH^{14}CO_3$ (1 μCi = 37 kBq) (specific activity = 1.2 mCi·$mmol^{-1}$, New England Nuclear NEC-086S) was added to each chamber. After 45 min, 20 μCi of a mixture of amino acids (approximately 54 mCi·$mmol^{-1}$, New England Nuclear NEC-250) was added. The rocks were removed after an additional 45 min and rinsed twice in distilled water just prior to extraction.

DMSO Extraction

Four of the rocks from the recirculating chambers were each placed in a container with 30 mL of dimethylsulfoxide (DMSO) in the dark for 24 h. The material extracted by this technique was used to simultaneously measure chlorophyll *a*, ATP, [^{14}C] DIC uptake/incorporation and [^{3}H] DOC uptake/incorporation for each replicate rock (Palumbo et al. 1987).

Chlorophyll *a* was used as an estimate of algal biomass. Five millilitres of DMSO extract was diluted 1:1 with acetone and chlorophyll *a* concentration determined spectrophotometrically with corrections made for phaeopigments (Boston et al. 1991).

ATP was measured to provide an estimate of community biomass. ATP was assayed in an aliquot of DMSO extract using Turner reagents and a Turner ATP photometer. Phosphate was added to DMSO (20 mM final concentration) prior to extraction to prevent loss of ATP due to phosphatase activity (Palumbo et al. 1987).

^{14}C and ^{3}H contents in the DMSO extract were determined by liquid scintillation counting (LSC). A 500-μL aliquot of extract was added to 10 mL of scintillation cocktail (Aquasol, New England Nuclear) and assayed in a Packard Tri-Carb scintillation spectrometer using a dual-label protocol. The protocol used an external standard ratio quench correction, and counting times continued until a constant variance was achieved (σ = 1.5%).

Lipid Extraction

The remaining four rocks from each recirculating chamber were extracted for lipids using a chloroform/methanol/phosphate buffer procedure as described in Guckert et al. (1985). Following phase separation, a 1-mL aliquot was removed from the aqueous phase and added to 10 mL of Aquasol for LSC. The organic (lipid) phase was drained into a round-bottom flask for removal of solvents under vacuum (<37°C). Ten percent of the total lipid was then removed to a LSC vial. The samples were photobleached (1000-W metal halide lamps used in incubation) overnight prior to Aquasol (10 mL) addition to reduce color quench for LSC. The dual-label LSC protocol was used for all lipid LSC. The remaining lipid was stored under nitrogen at −20°C for later analysis.

The total lipids were separated into three general lipid classes by silicic acid column chromatography (0.5 g of Unisil, 100–200 mesh) using a series of mobile phases of increasing polarity: neutral lipids, 10 mL of chloroform; glycolipids, 10 mL of acetone; and polar lipids, 10 mL of methanol (Guckert et al. 1985). Following solvent removal under a nitrogen purge, a 10% aliquot from each lipid class was removed for dual-label LSC as described above. Fatty acid methyl esters were prepared from the esterified lipids in the polar lipid fraction by mild alkaline methanolic transesterification (Guckert et al. 1985). Methods for the separation, quantification, and identification of the fatty acids in each lipid class by capillary gas chromatography (GC) and mass spectrometry are described in Guckert and Cooksey (1990) and Guckert et al. (1985). Equivalent chain lengths were used to assist in the identification of fatty acid methyl esters by GC (Kates 1986).

Fatty acid nomenclature used in this study was of the form "A:BωC" where "A" designates the total number of carbon atoms, "B" the number of double bonds, with the position closest to the aliphatic (ω) end of the molecule indicated as "C" with the geometry suffix "c" for *cis* and "t" for *trans*. The prefixes "i" and "a" refer to iso and anteiso methyl-branching, respectively.

Bacterial Density and Organic Carbon Content

Four of the rocks collected and stored in the dark for each study site were used for these assays. Periphyton was brushed from the rock surfaces with a toothbrush into a known volume of water. This periphyton suspension was diluted 1:10 with distilled water for bacterial cell enumeration with acridine orange stain (ASTM 1987). Equal parts of diluted suspension and acridine orange stain (0.01%, w/v) were mixed and filtered through a black polycarbonate membrane filter (Nuclepore 0.2-μm pore size). Stained bacteria were counted in 20 grids at 1000× power using a Nikon Labophot microscope equipped with a mercury vapor lamp (HBO 50 W). Organic carbon content was also determined as a measure of community biomass for these rocks. A 50-mL aliquot of the above periphyton suspension was filtered through an ashed, preweighed Whatman G6 glass fiber filter. The filter was dried for 24 h at 65°C and weighed. The filter was then combusted at 500°C for 1 h, after which ash weight was determined (APHA 1989).

Algal Taxonomic Composition

Periphyton was brushed from the remaining rocks stored after collection and examined microscopically to provide a qualitative assessment of algal taxonomic composition.

Rock Surface Areas

The area of the rock that was covered with periphyton was determined by covering the upper surface of the rock with alu-

minum foil, determining the weight of the foil, and converting to surface area based on a known weight per unit area of foil (Boston et al. 1991). This procedure was repeated twice for each rock. All parameters, except the fatty acid profiles, were normalized to periphyton (top) area; fatty acid profiles were normalized to the entire rock surface area.

Statistical Analysis

Data are presented as arithmetic means with the standard deviations. Differences among sites were evaluated with an ANOVA (mainframe SPSSx Version 3.0). Ratio data (e.g. membrane to storage lipid ratios and fatty acid mole percents) were arcsin-square root transformed before testing (Winer 1971). Multivariate analysis of fatty acid profiles was done with the pattern recognition software package Ein*Sight Version 2.5 (Infometrix, Inc.). Dendrograms were constructed with the Ein*Sight cluster analysis using a complete linkage, farthest neighbor method as well as all other clustering algorithms available with Ein*Sight or SPSSx. For principal component analysis, the raw fatty acid mole percent data were first autoscaled to a mean of 0 and constant variance. Loadings were generated for both the samples and the individual fatty acids in the profile.

Results

Periphyton Abundance and Activity

Periphyton biomass estimates (ATP, chlorophyll *a*, bacterial cell density) were conducted over the first 7 mo of 1990 for sites 24.2, 23.3, and 13.8. Algal periphyton coverage averaged 49.5 ± 10.3% of the total rock surface area at all sites. No consistent trends in biomass estimates between sites were evident. The chlorophyll content was 10–30 μg Chl $a \cdot cm^{-2}$ and bacterial cell density was consistently about 3×10^{10} cells$\cdot cm^{-2}$ when sampled in March, April, and May (Fig. 1). Photosynthetic carbon (^{14}C in DMSO) fixation varied over the times sampled (18–96 mg $C \cdot m^{-2} \cdot h^{-1}$). There was a trend for rates to be lower at the impacted upstream site 24.4, but most of the differences were not significant (Fig. 1). The rate of algal activity (both photosynthesis and total lipid synthesis) was higher ($\alpha = 0.05$) at the nonpolluted site 13.8 on April 4 as compared with the impacted site 24.4 (Fig. 1). The date of this increase corresponded to an observed *Gomphenema* bloom at site 13.8 (W. R. Hill, ORNL, unpubl. data).

Heterotrophic uptake (^{3}H content of either DMSO or total lipid extracts) was consistent over all sites and times. The heterotrophic community assimilated approximately 0.35 pmol amino acids$\cdot cm^{-2} \cdot h^{-1}$ (Fig. 1).

Evaluating Physiological Stress

The radiolabel content of the total lipid extracts (Fig. 1) is useful for comparisons with the DMSO extracts. When the dpm recovered in DMSO was compared with the dpm recovered in lipids, there was 6.3 (±5.3) times more ^{3}H in DMSO than in lipids and 2.0 (±0.8) as much ^{14}C. This value decreased when the dpm recovered in the aqueous phase of the lipid extraction was included (dpm DMSO/dpm lipid extraction ratios: 2.4 ± 2.6 for ^{3}H, 1.5 ± 0.6 for ^{14}C).

The lipid assays also provided additional information by evaluating the radiotracer incorporation into lipid classes. Virtually 100% of both ^{3}H (106 ± 19%) and ^{14}C (102 ± 9%) applied to the silicic acid columns as total lipid extracts was recovered in the neutral, glyco-, and polar lipid classes. Since total lipid synthesis results agreed with DMSO results and there was no loss of material during lipid class separation, the lipid class patterns provide an analysis of carbon allocation within the periphyton.

Examination of the phototrophic lipid class patterns (defined as the ^{14}C content of lipid classes) by study site and sampling revealed that the plasma membrane polar lipid ^{14}C content was about the same for all samples (Fig. 2). Periphyton from site 24.4 had smaller amounts of ^{14}C in the storage neutral lipids relative to the other sites. Sites 23.2 and 13.8 had larger, but variable, quantities of ^{14}C in the neutral lipid fraction. At all sites, the amount of ^{14}C incorporated into chloroplast membrane glycolipid exceeded the amount of ^{14}C in the polar lipid fraction (Fig. 2).

The heterotrophic community consistently incorporated the majority of the ^{3}H from amino acids (DOC) into plasma membrane polar lipids (Fig. 2). The ^{3}H content of polar lipids was similar for all sites and all times. Bacteria generally do not contain neutral lipids, although we always detected some ^{3}H in this lipid class at site 13.8. Bacterial storage lipids were found in the glycolipid fraction, principally in the form of poly-β-hydroxybutyrate. The ^{3}H content of this lipid class was highly variable, but tended to be higher for sites 23.2 and 13.8 than for site 24.4.

The heterotrophic membrane to storage lipid ratios were defined as the ^{3}H content of the polar lipid fraction to the glycolipid fraction. The phototrophic ratios were defined as the ^{14}C content of the polar and glycolipid fractions to the neutral lipid fraction. We found a consistent and statistically significant ($\alpha = 0.05$) longitudinal trend for both the heterotrophic and phototrophic membrane to storage lipid ratios, with the highest values occurring in periphyton from site 24.4 in each sampling period (Fig. 3).

Periphyton Community Structure

Periphyton fatty acid profiles were analyzed in a three-step process for community structure assessment: (1) data set exploration using multivariate cluster analysis to suggest structure in the data set; (2) fatty acid profiles (average ± SD) reported for grouped data; and (3) multivariate principal components analysis for the final analysis.

The periphyton fatty acid profiles were grouped by cluster analysis based primarily on location in the stream. Since the periphyton fatty acid profile (= community structure) appeared to be most consistent by study site over the sampling period, fatty acid profiles were grouped by site to investigate trends (Table 1). Total fatty acid content of the periphyton was not significantly different for any site, which was consistent with other biomass/abundance estimates. The fatty acid profiles of site 24.4 were dominated by 16- and 18-carbon fatty acids such as 18:1ω9c (31%), 16:0 (20%), 16:1ω7c (10%), 18:2ω6 (9%), and 18:1ω7c (6%) (Table 1). Site 23.2 also contained high levels of 16:1ω7c (20%) and 16:0 (17%), along with equivalent amounts of the long-chain polyunsaturated fatty acids 20:4ω6 (10%) and 20:5ω3 (10%). In addition, the profiles from this site contain fatty acids common to bacterial membranes such as i18:0 (6%), 16:1ω5c (6%), and 18:1ω7c (5%) (Table 1). A fatty acid common in diatom membranes, 20:5ω3, dominated the profiles of site 13.8 (23%). Other major fatty acid included 16:0 (18%), 16:1ω7c (12%), i18:0 (7%), and 18:2ω6 (5%).

Principal components analysis was used to view the discrimination of the East Fork Poplar Creek periphyton communities

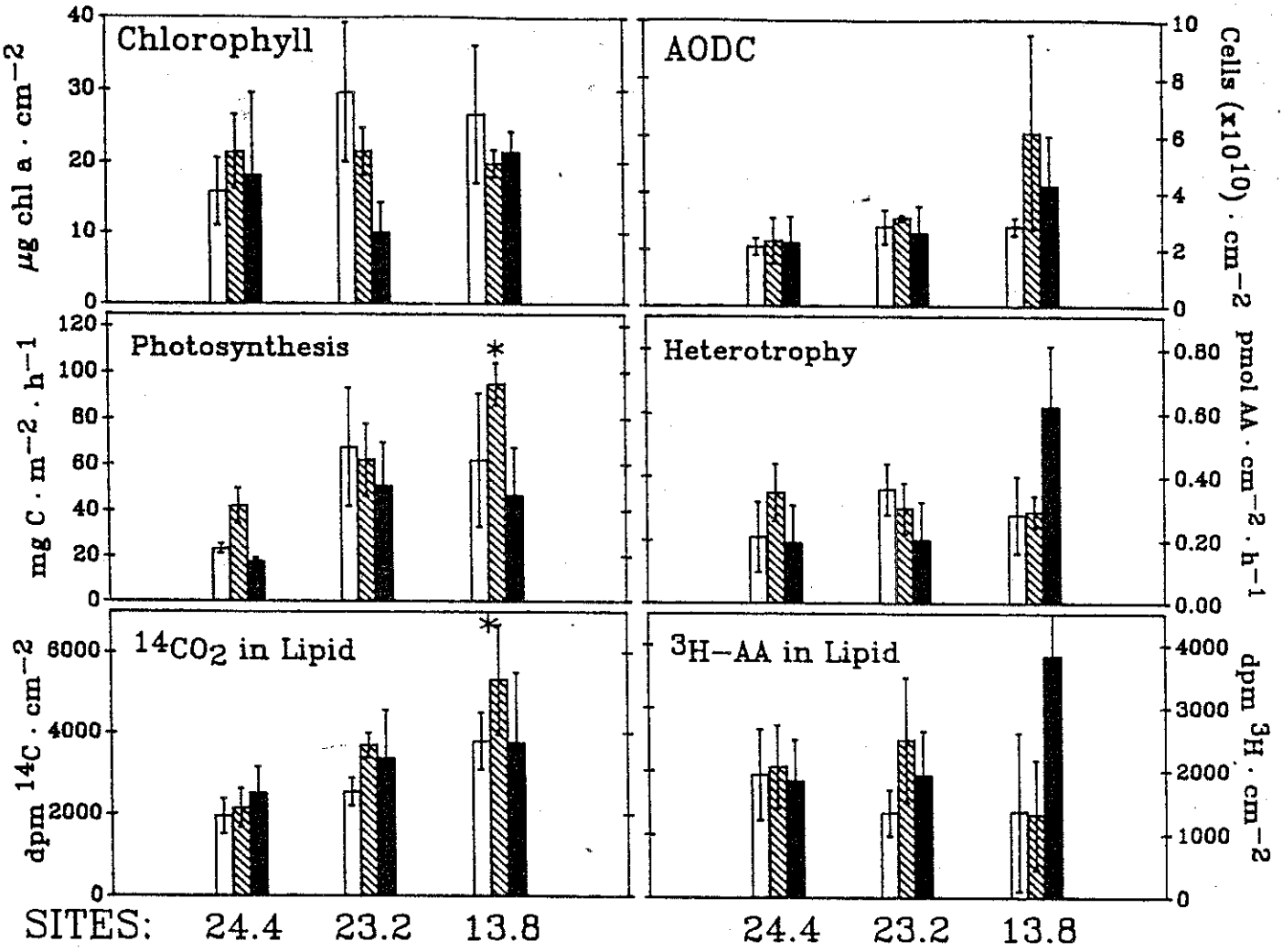

FIG. 1. Average (±SD) East Fork Poplar Creek periphyton biomass and activity estimates for March 22 (open bars), April 4 (hatched bars), and May 5 (solid bars), 1990, samplings. Algal periphyton values (chlorophyll, photosynthesis, $^{14}CO_2$ in lipid) are shown in the left-hand panels. Heterotrophic values (cell counts by AODC, heterotrophy, ^{3}H in lipid) are on the right. An asterisk indicates a value significantly different ($\alpha = 0.05$) from the corresponding value at site 24.4.

by fatty acid profiles and to evaluate the influence of fatty acid patterns in the separation of study sites. The separation of the replicates by the first two principal components (PC) (Fig. 4) accounted for 71% of the variability within the data set (PC1 = 51%, PC2 = 20%). The third PC accounted for 8% of the variability, and a scree plot analysis indicated that no further information would be gained by evaluating the fourth principal component (PC4 = 6%). The study sites separated clearly based on PC1 and PC2 (Fig. 4). This separation was essentially identical to that achieved by cluster analysis.

The loadings of the fatty acids for PC1-separated site 24.4 in a positive loading (Fig. 4) were highly positive with fatty acids common to green algae (18:1ω9c, 16:4ω3, 18:2ω6, and 16:1ω13t) (Table 2). PC2, which separated site 13.8 (positive loadings) and site 23.2 (negative loadings) (Fig. 4), had positive fatty acid loadings commonly found in diatoms and other microeukaryotes (22:4ω6, 14:0, 20:5ω3, 18:3ω6) and negative loadings with mostly bacterial monounsaturated fatty acids (16:1ω5c, 16:1ω7c, 18:1ω5c) as well as 20:4ω6 (Table 2). PC3, which separated the April 4 samples of sites 23.2 and 13.8 from the other sampling times (negative loadings, data not shown), was dominated by 22:6ω3 in the negative direction, although other diatom fatty acids such as 20:5ω3 were also contributing to the separation (Table 2).

Discussion

Periphyton communities are useful for the evaluation of aquatic toxicity (Boston et al. 1991). The periphyton communities of East Fork Poplar Creek were shown to be physiologically stressed at the upstream site 24.4 when compared with other sites. The stress, as measured with a membrane to storage lipid synthetic ratio for both the phototrophic and heterotrophic constituents, declined with distance downstream from the source of the industrial effluents and was consistent over three sampling periods. These results agreed with previous periphyton stress analyses conducted at these sites (Boston et al. 1991). This work suggests that the use of lipid-based assays along with a simultaneous phototroph/heterotroph activity analysis can provide further insight into this ecologically important community. This detailed information will improve the use of periphyton as a biomonitoring and aquatic toxicity assessment tool.

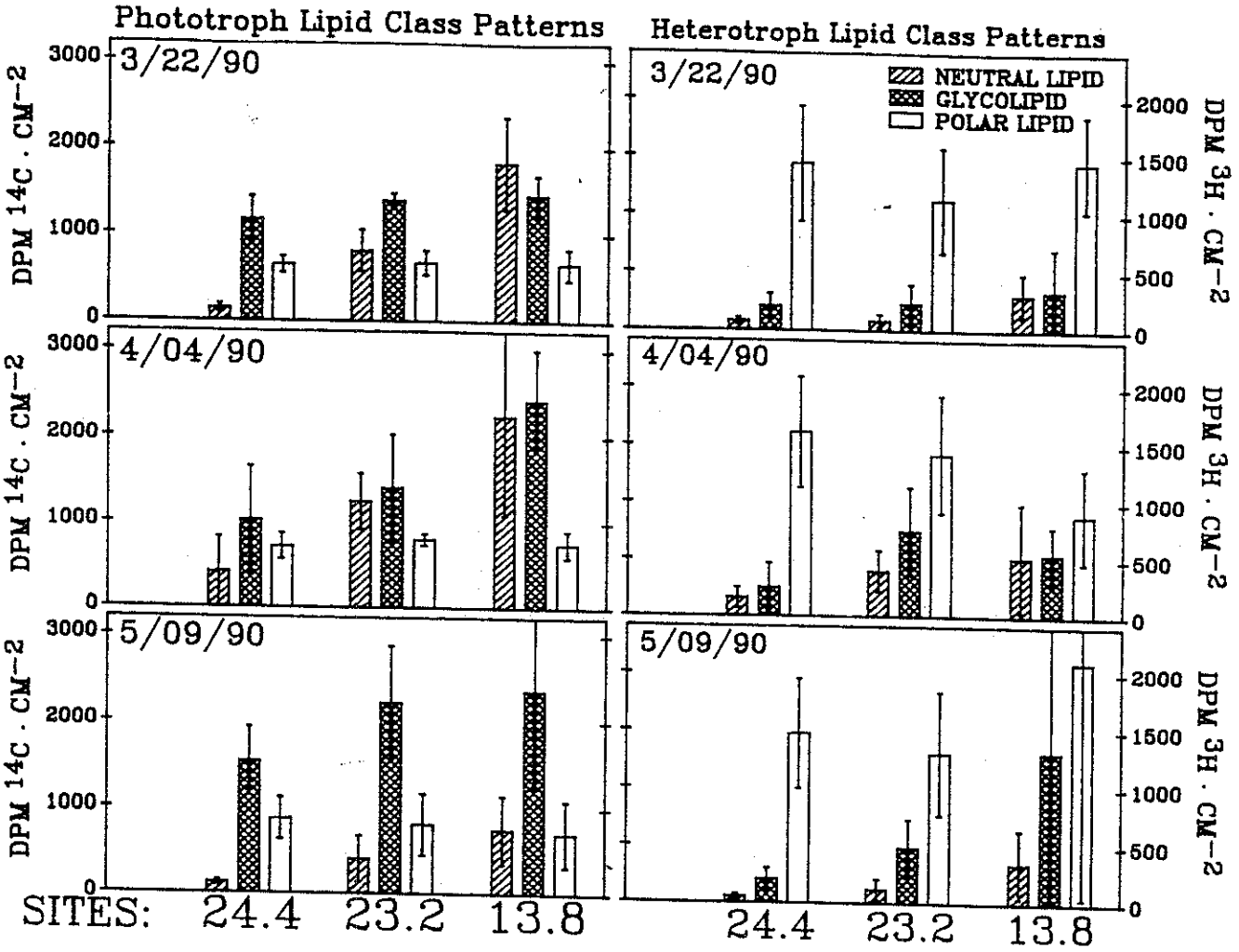

FIG. 2. Average (±SD) East Fork Poplar Creek periphyton lipid class radiolabel incorporation patterns including neutral lipids (hatched bars), glycolipids (cross-hatched bars), and polar lipids (open bars). Phototrophic (^{14}C incorporation) patterns for the three sampling dates are shown in the left-hand panels. Heterotrophic (^{3}H incorporation) patterns are on the right. Sample dates are indicated for each set of graphs.

Evaluating Physiological Stress

Microbial activities are often measured by the quantity of radiotracer carbon assimilated over time. Physiological status must then be inferred by a comparison of activities or the calculation of biomass-normalized activities (e.g. chlorophyll-adjusted photosynthetic rates (Boston et al. 1991)). Analysis of radiotracer carbon assimilated into total lipid is an additional measure of activity, and its partitioning into lipid classes provides an improved method to estimate physiological status.

Other macromolecules could be evaluated for carbon allocation such as proteins or carbohydrates (Amblard et al. 1990). An evaluation of membrane versus storage lipid synthesis rates, however, has the advantage of using a common lipid precursor pool. This simplifies physiological interpretations because differences of radiotracer incorporation into separate macromolecules can be both a function of synthetic rates and differences in precursor pool sizes. Also, the membrane and storage lipid information can be obtained simultaneously from one extraction, which simplifies the procedures.

Increased membrane to storage lipid synthesis ratios have been previously reported for bacterial responses to physical perturbations in stratified estuarine sediments (Findlay et al. 1990). This same response can be detected for both phototrophic and heterotrophic benthic microbial communities in the work of Dobbs et al. (1989) if their data are replotted as membrane to storage lipid ratios versus level of disturbance due to radiolabel introduction into sediment cores (injection < pore-water replacement < slurry). Our results with periphyton are the first to describe the increased membrane to storage lipid ratios for microbial communities exposed to industrial effluent.

Elevated membrane to storage lipid ratios due to toxicant exposure have been reported in macrofauna. Fraser (1989) reported increased membrane sterol to triglyceride ratios in both juvenile lobsters and blue crabs with increasing concentrations of crude oil. The most significant example for this work, however, are the changes in lipid content which occur in East Fork Poplar Creek fish with distance downstream. Adams et al. (1990) reported decreases in phospholipid and increases in triglyceride (i.e. membrane to storage lipid ratios decline) for fish caught near site 13.8 when compared with fish caught at several sites further upstream. These results suggest that the microbial membrane to storage lipid ratios might be predictive of similar macrofaunal responses to water quality.

There are ecological implications of consistently high membrane to storage lipid synthetic ratios in periphyton communities. Periphyton biomass (abundance) was similar for the

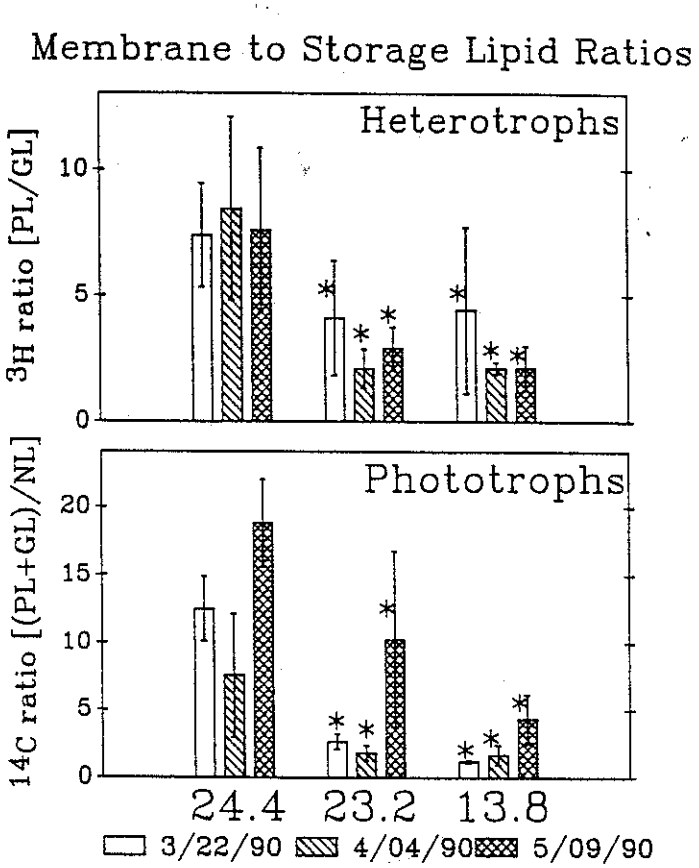

FIG. 3. Average (±SD) for heterotrophic (^{3}H ratio on top) and phototrophic (^{14}C ratio on bottom) membrane to storage lipid synthetic ratios (PL = polar lipids; GL = glycolipids; NL = neutral lipids). Values are given by East Fork Poplar Creek site and sampling date (March 22 = open bars, April 4 = hatched bars, May 5 = cross-hatched bars). All values for sites 23.2 and 13.8 were significantly lower (α = 0.05, marked with an asterisk) than site 24.4 values on each sampling day.

three study sites (Fig. 1; Table 1), and total carbon uptake/incorporation had only a slight trend of increase with distance downstream. The communities of the impacted upstream site 24.4 were, therefore, consistently using a higher proportion of their assimilated carbon for membrane synthesis without subsequent increases in biomass. A community under chronic stress would be expected to increase its carbon allocation to biomass components at the expense of storage materials (Odum 1985). As a consequence, the periphyton communities of site 24.4 may be more vulnerable to other stresses and perturbations due to lower energy reserves. In addition, since periphyton serves as an important food resource in streams, communities not accumulating as much carbon in lipid storage pools are more likely to be of lower food quality to grazers. In fact, Hill (1992) reported that periphyton from East Fork Poplar Creek site 13.8 (site EF2 in his study) is a better food resource than periphyton from site 23.2 for the snail *Elimia clavaeformis* and the caddis fly larva *Neophylax etnieri*. In a laboratory feeding experiment, snails fed periphyton from site 13.8 tended to have higher growth rates and neutral lipid content than those fed periphyton from site 23.2. Caddis fly larvae had similar trends along with a significantly faster developmental cycle when fed periphyton from site 13.8 (Hill 1992).

Heterotrophic Activities within Periphyton

Another important aspect of this work was the simultaneous evaluation of phototrophic (^{14}C-labeled bicarbonate) and heterotrophic (^{3}H-labeled amino acids) activity. Amino acid mixtures have been shown to be readily utilized by bacterial periphyton (Dean-Ross 1990). Our use of [^{14}C] DIC and [^{3}H] DOC provides a more complete view of periphyton activities and physiological status.

Few prokaryotic heterotrophs synthesize significant amounts of neutral lipid. The source of the ^{3}H recovered in the neutral lipid fraction from site 13.8 is unknown. Additional time course analyses have indicated that this pattern is established early in the incubation time course (within 5 min), suggesting that potential eukaryotic direct incorporation rather than incorporation into a bacteriovore may be occurring (S. C. Nold, unpubl. data).

TABLE 1. Phospholipid, ester-linked fatty acid profiles of East Fork Poplar Creek periphyton by study site (n = 12). Results are expressed as average (±SD) percentages of total fatty acid recovered.

Fatty acid[a]	ECL[b]	Site 24.4	Site 23.2	Site 13.8
14:0	14.00	0.8 ± 0.3	2.0 ± 0.7	4.2 ± 1.2
16:4ω3	15.47	1.3 ± 0.2	0.4 ± 0.1	0.3 ± 0.2
i16:0	15.63	1.1 ± 0.1	0.5 ± 0.3	0.3 ± 0.2
16:1ω7c	15.75	9.8 ± 1.7	19.7 ± 2.2	11.9 ± 1.9
16:1ω5c	15.84	1.6 ± 0.9	6.5 ± 1.9	0.9 ± 0.2
16:1ωi3t	15.92	4.1 ± 0.5	2.0 ± 0.3	1.8 ± 0.4
16:0	16.00	20.5 ± 2.0	16.9 ± 1.9	18.0 ± 3.5
Unknown	17.43	0.3 ± 0.1	0.9 ± 0.1	1.2 ± 0.3
18:3ω6	17.46	2.8 ± 0.4	1.5 ± 0.2	2.4 ± 0.5
18:2ω6	17.62	8.6 ± 0.7	3.6 ± 0.8	5.0 ± 1.3
i18:0	17.66	0.0 ± 0.0	6.1 ± 2.0	6.8 ± 2.9
18:1ω9c	17.71	30.9 ± 2.8	2.5 ± 0.4	4.5 ± 2.2
18:1ω7c	17.76	5.9 ± 1.1	5.4 ± 1.3	4.7 ± 1.0
18:1ω5c	17.86	1.0 ± 0.3	1.0 ± 0.2	0.7 ± 0.2
18:0	18.00	2.1 ± 0.6	1.3 ± 0.4	2.0 ± 0.8
20:4ω6	19.21	1.7 ± 0.6	9.8 ± 3.2	4.0 ± 1.0
20:5ω3	19.28	3.0 ± 0.8	9.8 ± 2.2	22.8 ± 8.5
22:6ω3	21.10	0.0 ± 0.0	0.8 ± 0.3	1.0 ± 0.9
22:4ω6	21.24	0.0 ± 0.0	0.5 ± 0.2	1.5 ± 0.7
Σ nmol·cm^{-2}		28.0 ± 8.0	32.7 ± 9.6	29.5 ± 9.3

[a]Phospholipid, ester-linked fatty acids.
[b]Equivalent chain length.

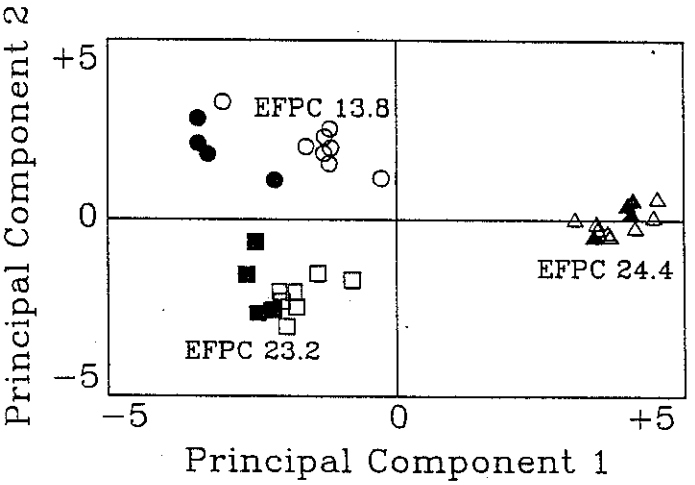

FIG. 4. Sample loadings for the first two principal components (PC) of East Fork Poplar Creek (EFPC) periphyton phospholipid fatty acid profiles. Site 24.4 (triangles) is separated from the other downstream sites by PC1. Sites 23.2 (squares) and 13.8 (circles) are more closely related, but are separated by PC2. The April 4 samples are represented by solid symbols.

TABLE 2. Principal component (PC) loadings for fatty acid. Highest loading is highlighted (asterisk) for each PC. Loading value corresponds to placement onto the PC axes.

Fatty acid	PC1	PC2	PC3
18:1ω9c	+0.314*	+0.045	−0.133
16:4ω3	+0.305*	+0.0005	−0.115
18:2ω6	+0.301*	+0.140	+0.072
16:1ω13t	+0.299*	−0.038	−0.186
i16:0	+0.284*	−0.121	−0.047
Unknown	−0.270*	+0.096	+0.131
16:1ω5c	−0.113	−0.443*	+0.059
16:1ω7c	−0.204	−0.376*	−0.042
22:4ω6	−0.195	+0.313*	+0.144
14:0	−0.225	+0.302*	+0.084
20:4ω6	−0.215	−0.292*	−0.088
18:1ω5c	+0.054	−0.286*	+0.109
20:5ω3	−0.238	+0.284*	−0.197
18:3ω6	+0.198	+0.274*	−0.070
i18:0	−0.228	+0.031	+0.497*
18:1ω7c	+0.144	−0.126	+0.487*
16:0	+0.202	+0.020	+0.400*
22:6ω3	−0.236	+0.127	−0.316*
18:0	+0.130	+0.263	+0.264*

Periphyton Community Structure based on Lipids

Another benefit of the lipid-based method is that periphyton community structure, based on fatty acid profiles, can be obtained with little additional effort. Algal communities can be evaluated taxonomically, but much of the microbial community (e.g. bacteria) cannot be identified or quantified accurately. Thus, fatty acid profiles can serve at least three purposes in periphyton evaluations, as follow.

(1) Fatty acid profiles are nontaxonomic descriptions, but comparable in data structure with algal taxonomic lists (McIntire et al. 1969). The fatty acid profiles can be used to confirm trends noted in the taxonomic evaluations, such as the green algal dominance at site 24.4 and the diatom dominance at site 13.8.

(2) The fatty acid profiles provide a quantitative index of the entire periphytic community, which is not available by any other method. This includes the heterotrophic bacteria which, especially for site 23.2, had not been previously recognized as being significantly different from the other sites as indicated by the multivariate analysis (Fig. 4; Table 2). For the East Fork Poplar Creek periphyton, the fatty acid analysis indicated stable separation of community structure by study site. The most impacted site (24.4) was easily separated from other downstream sites. Interestingly, the *Gomphenema* bloom on April 4 only resulted in a temporary change in the site 13.8 fatty acid profiles which was absent at the next sample period (Fig. 4). The stability of the fatty acid profiles suggests that patterns of these profiles may be used to classify the degree of chronic impact for any given site.

(3) The fatty acid profiles may be useful for discussing food quality issues (although total lipid profiles would be more appropriate). The profiles shown in Table 1 and loadings shown in Table 2 suggest that for the same amount of biomass, the periphyton of site 13.8 is clearly superior as a source of long-chain (>18 carbons) ω-3 polyunsaturated fatty acids. This is not a definitive measure of food quality but does suggest an important hypothesis to test regarding the increased grazer biomass at this site and may help to explain the results of Hill (1992) which showed that a diet of periphyton from East Fork Poplar Creek site 13.8 improved growth, development, and neutral lipid content in the grazers.

In conclusion, the lipid analyses outlined here for radiotracer-incubated stream periphyton would be an excellent complement to more traditional endpoints evaluated. The lipid data will help to confirm and refine results for total DMSO-soluble material (biomass, activity) as well as algal taxonomy (community structure). Physiological status of the periphyton can be obtained through the carbon allocation patterns of membrane and storage lipids. This level of biological evaluation is essential if periphyton is to further develop into a biomonitoring and toxicity assessment tool for aquatic ecosystems.

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