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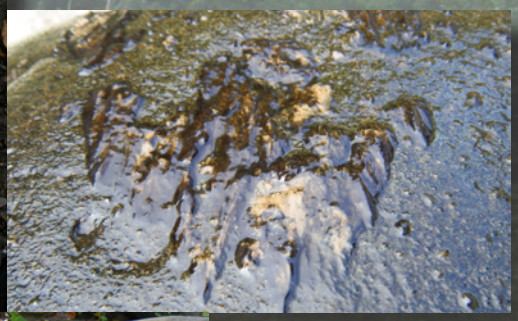
Response of Periphyton Fatty Acid Composition to Supplemental Flows in the Upper Esopus Creek, Catskill Mountains, New York



Scientific Investigations Report 2015–5161

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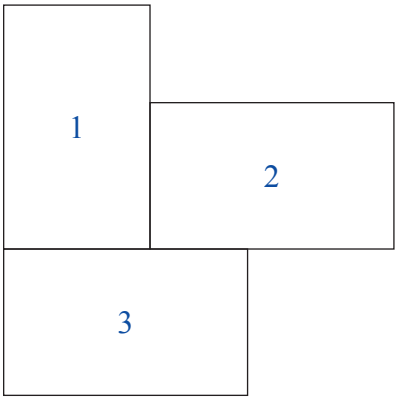

Cover. Background photograph: Waters from the Shandaken portal entering the upper Esopus Creek in August 2009.
1. Filtering equipment used for collecting quantitative periphyton standing crop samples.
2. Periphyton growing on a rock from the upper Esopus Creek, New York.
3. Qualitative periphyton samples for fatty acid analysis.

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Conversion Factors

International System of Units to Inch/Pound

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
	Area	
square kilometer (km^2)	247.1	acre
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
liter (L)	33.82	ounce, fluid (fl. oz)
	Flow rate	
cubic meter per second (m^3/s)	35.31	cubic foot per second (ft^3/s)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
	Surface density	
milligram per square centimeter (mg/cm^2)	0.000227573	ounce per square inch (oz/in^2)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as

$°F = (1.8 \times °C) + 32$.

Datum

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

Supplemental Information

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C). Suspended sediment concentrations are given in milligrams per liter (mg/L).

Nephelometric turbidity unit (NTU) is a measure of turbidity in a water sample, roughly equivalent to the formazin turbidity unit (FTU) and Jackson turbidity unit (JTU).

Abbreviations

AFDM	ash-free dry mass
ANOSIM	analysis of similarity
ANOVA	analysis of variance
chl *a*	chlorophyll *a*
DAP	diatom assessment profile
FAME	fatty acid methyl esters
MDS	nonmetric multidimensional scaling
MUFA	monounsaturated fatty acid
NTU	nephelometric turbidity units
PUFA	polyunsaturated fatty acid
SAFA	saturated fatty acid

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Abstract

Fatty acid analysis of periphyton is an emerging tool for assessing the condition of a stream ecosystem on the basis of its water quality. The study presented in this report was designed to test the hypothesis that periphyton communities have a fatty acid profile that can detect excessive turbidity and suspended sediment. The fatty acid composition of periphyton was assessed during two seasons upstream and downstream from an underground aqueduct that provides supplemental flows, which are a potential source of turbidity and suspended sediment on the upper Esopus Creek, New York. These data were compared with measurements of periphyton standing crop, diatom community structure and integrity, and basic water-quality parameters. Periphyton standing crop and diatom community integrity indicated little evidence of impairment from the supplemental flows. The relative abundances of two physiologically important fatty acids, γ-linolenic acid (18:3ω6) and eicosapentaenoic acid (20:5ω3), were significantly lower downstream from the supplemental flows and multivariate analyses of fatty acid profiles identified significant differences between sites upstream and downstream from the supplemental flows. Individual fatty acids and summary metrics, however, were not significantly correlated with turbidity or suspended sediment. Together, these results indicate that the supplemental flows may cause some measurable effects but they do not constitute a major disturbance to the periphyton community on the upper Esopus Creek. Fatty acid analysis may have potential as a tool for monitoring changes in periphyton nutritional composition that may reflect water quality and ecosystem health but needs to be further evaluated around a more definitive source of water-quality impairment.

Introduction

The U.S. Geological Survey and the New York State Department of Environmental Conservation used fatty acid analysis of periphyton to characterize the local water quality and ecosystem condition of the upper Esopus Creek, New York (fig. 1) in August and November 2009. The study detailed in this report evaluated the hypothesis that periphyton communities (composed of benthic algae and microorganisms) have a fatty acid profile that allows for the detection of water-quality impairment, specifically from excessive turbidity and suspended sediment. Periphyton fatty acid composition and standing crop (ash-free dry mass [AFDM] and chlorophyll *a* [chl *a*]), structure and integrity of diatom communities, and water-quality parameters were analyzed and compared upstream and downstream from the Shandaken portal, New York (fig. 1), which provides supplemental flows to the upper Esopus Creek that are sometimes turbid in nature.

The portal is the terminus of a 29-kilometer (km) underground aqueduct, the Shandaken Tunnel, which diverts water from the Schoharie Reservoir in the Mohawk River drainage into the upper Esopus Creek as part of the New York City water supply system. Discharge from the portal can increase natural flows on the upper Esopus Creek by a factor of two or more (Burns and Gazoorian, 2015) and is often more turbid than ambient receiving waters. The portal is a source of controversy among local residents and anglers who perceive it as delivering turbid water that degrades the aesthetic and biological integrity of the stream (Cornell Cooperative Extension of Ulster County and others, 2007). Assessments of diatoms and macroinvertebrates conducted by the New York State Department of Environmental Conservation, however, have produced inconsistent results concerning the effect of the portal on these assemblages (Bode and others, 1995, 2001; Smith, 2013). Consequently, the overall effect of the altered suspended sediment and flow regimes on aquatic biota remains unclear. The contents of this report will help bridge this knowledge gap by analyzing the fatty acid content of periphyton, which comprises the base of the aquatic food web, throughout the watershed.

The condition of aquatic ecosystems is often assessed by using various biological components of the food web, such as invertebrates or fish (Barbour and others 1999; Moulton and others, 2002). Although these methods are very useful for predicting water quality, such assemblages are only surrogates for the essential nutrients present in the

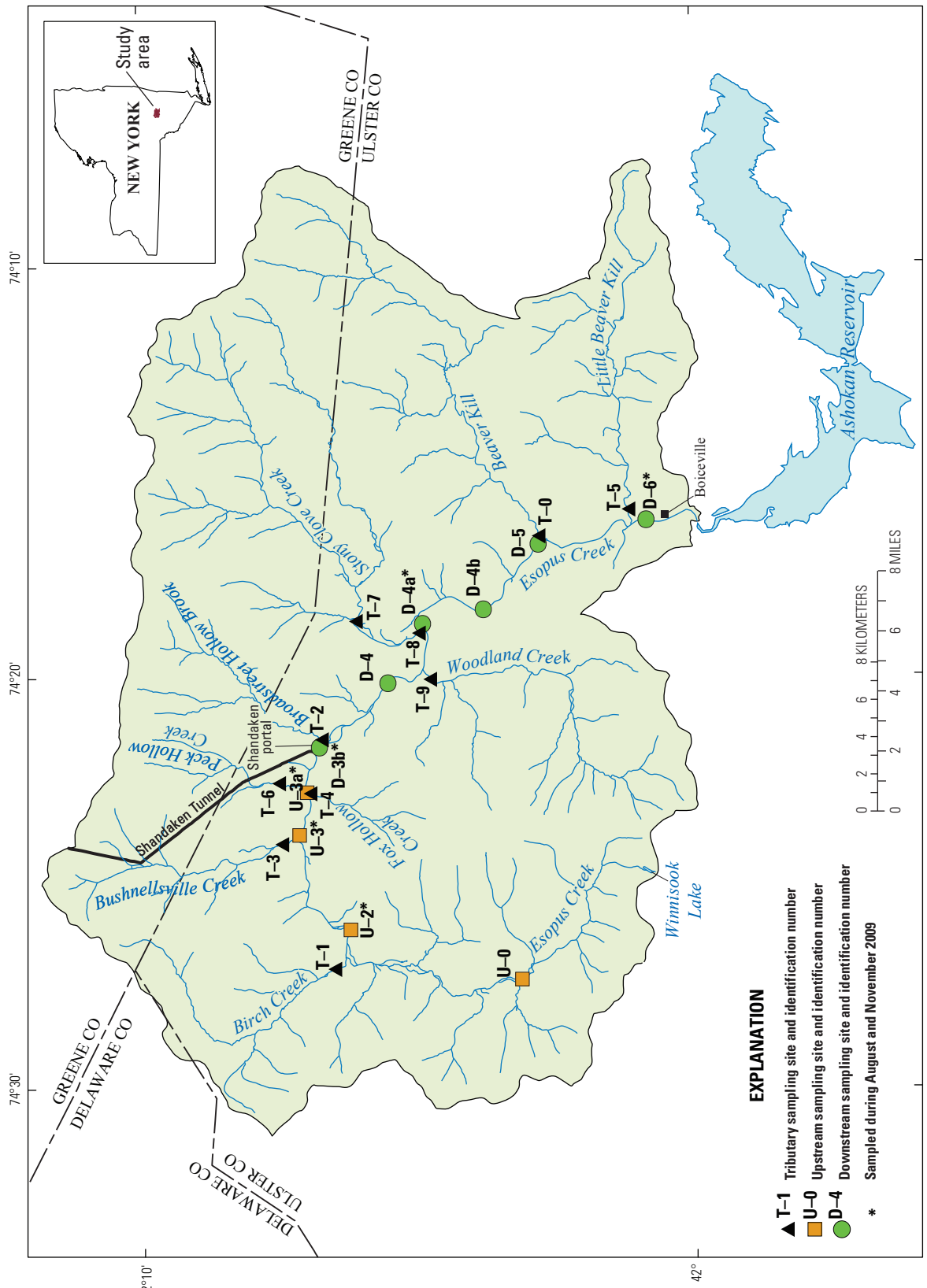

Figure 1. Location of 20 sampling sites on the main stem and tributaries of the upper Esopus Creek in the Catskill Mountains, southeastern New York. Sites indicated with U are upstream from the Shandaken portal, New York, D are downstream from the portal, and T are at tributaries of the upper Esopus Creek.

system. A nutrient is considered essential to an organism when it cannot be synthesized internally and must be obtained from an external source (Arts and others, 2001; Hill and others, 2011) and each biological entity within the food web has specific nutritional requirements. If required essential nutrients are not produced by another organism, such as a primary producer, or if production of the essential nutrient is constrained by the physical habitat or anthropogenic factors, then the species will not be present.

Fatty Acids

Polyunsaturated fatty acids (PUFAs) are an example of essential nutrients for consumers, and primary producers are a key source of these essential fatty acids. Fatty acids are long amphipathic molecules composed of a carboxyl head and an aliphatic tail that, in aquatic organisms, generally ranges from 12 to 24 carbon atoms in chain length (Napolitano, 1999). Fatty acids can be divided into the following classes based on the number of double bonds in the carbon chain: saturated fatty acids (SAFAs) contain no double bonds between carbon atoms, monounsaturated fatty acids (MUFAs) contain one double bond in the carbon chain, and PUFAs contain more than one double bond in the carbon chain. Several of the PUFAs such as α-linolenic acid and linoleic acid (and their elongation products), are considered to be dietary essential fatty acids for most consumers (Arts and others, 2001; Parrish, 2009; Hill and others, 2011). Herein however, essential fatty acids will be referred to as physiologically important to avoid confusion between an indispensable and conditionally indispensable fatty acid (Cunnane, 2003).

Advances in fatty acid analysis that allow for separation, identification, and quantification of individual long-chained fatty acids have enabled the use of fatty acid analysis in many biological and ecological applications. For example, identifying bacterial species using fatty acid methyl esters (FAME) is now a routine methodology (Moss, 1981; Miller, 1982; Miller and Berger, 1985; Welch, 1991). Fatty acids have been used successfully to describe food-web dynamics (Alfaro and others, 2006) and to determine the diet of top predators (Budge and others, 2006). Knowledge of the fatty acid composition of algal taxa is also being used to optimize production in aquaculture (Patil and others, 2007).

The use of fatty acid analysis of periphyton in freshwaters is emerging as a tool for assessing algal community structure and ecosystem health on the basis of water quality. McIntire and others (1969) provided one of the first lotic freshwater studies linking proportions of individual fatty acids to specific components of the periphyton community. The presence and relative abundance of different algal classes can now be determined using associated fatty acid biomarkers (Napolitano, 1999). For example, palmitic acid (16:0), palmitoleic acid (16:1ω7), and eicosapentaenoic acid (20:5ω3) are considered to be markers of diatom dominance, and α-linolenic acid (18:3ω3) is a marker of green algae dominance (Napolitano, 1999; Hill and others, 2011). Because algae are the main source of physiologically important fatty acids in aquatic ecosystems (Parrish, 2009), and because water quality strongly influences the health and diversity of the algal community (Collins and Weber, 1978; Barbour and others, 1999; Larned, 2010), it follows that impaired water quality should lessen the diversity and quality of fatty acids available to consumers. Napolitano and others (1994) determined that chlorine-polluted streams had an altered fatty acid profile that indicated a shift in dominance from diatoms to green algae. Two recent studies were also completed on the response of periphyton fatty acid profiles in acid mine streams. Genter and Lehman (2000) determined that the fatty acid profiles of periphyton communities were different upstream and downstream from point-source metal inputs because of decreases in particular fatty acids. Similarly, Honeyfield (Dale C. Honeyfield, U.S. Geological Survey, unpub. data, 2010–14) determined that acid mine streams contained a limited number of individual fatty acids and either none or extremely inadequate amounts of physiologically important fatty acids compared with unaffected trout streams, which contained two to three times the number of fatty acids and included the entire physiologically important omega-3 and omega-6 fatty acid families. Together, these investigations suggest that fatty acid analysis of periphyton can be used to assess water quality, although the sensitivity, and therefore overall utility of this method remains understudied and unclear.

Study Objective and Design Concepts

The primary objective of this study is to determine whether water delivered through the Shandaken portal has a deleterious effect on the nutritional quality of the food-web base (primary producers) in the upper Esopus Creek. It was hypothesized that if the portal is adversely affecting aquatic biota, then the fatty acid profile of periphyton downstream from the portal would reflect this impairment. Additionally, major tributaries to the upper Esopus Creek have a wide range of suspended sediment concentrations. If turbidity or suspended sediment affects the nutritional quality of periphyton, then changes in the proportions of physiologically important fatty acids may exist across a gradient of suspended sediment concentrations. This impairment, whether attributable to waters entering the upper Esopus Creek through the portal or to a general gradient of suspended sediment throughout the basin, could result in reduced diversity of periphyton fatty acids or a shift in fatty acid structure indicative of an altered periphyton community. Although the relation between suspended sediment and periphyton fatty acid composition has not been investigated, some studies have assessed the effect of different light levels on the fatty acid profile of periphyton. Cashman and others (2013) determined that greater light caused an increase in the proportions of MUFAs and short-chain PUFAs and a decrease in the

proportions of SAFAs and long-chain PUFAs. Somewhat in contrast, Hill and others (2011) determined that greater light levels caused an increase in the proportions of SAFAs and MUFAs and a decrease in the proportions of PUFAs. Because reduced penetration of light is considered the primary effect of suspended sediment on periphyton (Newcombe and Macdonald, 1991), periphyton fatty acid composition at the most turbid sites in this study might be expected to respond similarly to the low-light conditions in one of these experiments.

Purpose and Scope

The purpose of this report is to provide data for SAFAs, MUFAs, and PUFAs detected in periphyton at 20 sites sampled in August 2009 and at 6 sites sampled in November 2009. Diatom identifications and two measures of periphyton standing crop are also reported along with limited data for five water-quality parameters. Results are presented from univariate and multivariate statistical tests assessing spatial and temporal differences in periphyton communities. Limitations of the study and recommendations for future work are also presented.

Methods

Study Scope and Area

The upper Esopus Creek is in the Catskill Mountain region of southeastern New York and follows a 41.8-km semicircular clockwise course from its headwaters at Winnisook Lake to its impoundment downstream from Boiceville, where it forms the Ashokan Reservoir (fig 1). The watershed of the upper Esopus Creek drains 497.3 square kilometers, much of which is rugged and mountainous terrain (Cornell Cooperative Extension of Ulster County and others, 2007). Nine major tributaries deliver waters to the upper Esopus Creek in addition to the Shandaken portal, which enters the upper Esopus Creek approximately 20 km upstream from the Ashokan Reservoir. The portal's contribution to the overall sediment load in the upper Esopus Creek is considered minimal (Cornell Cooperative Extension of Ulster County and others, 2007) because the portal is not operated during storm events, during which time tributaries supply high sediment loads (McHale and Siemion, 2014). Under base flows, however, waters from the portal are often more turbid than the receiving waters of the upper Esopus Creek. For example, the median turbidity value observed at the portal from 1987 to 2005 of 8.8 nephelometric turbidity units (NTU) was the highest in the basin (Cornell Cooperative Extension of Ulster County and others, 2007).

Periphyton samples were collected at 20 sites in the watershed (fig. 1; table 1) in August 2009. Ten sites were on the main stem of the upper Esopus Creek, with four upstream and six downstream from the portal. Ten other sites were on nine major tributaries to the upper Esopus Creek (two were on Stony Clove Creek [fig. 1]). To provide information on seasonal changes, six main stem sites—three upstream and three downstream from the portal—were sampled again in November 2009. Throughout this report, the six sites sampled during both periods are referred to as seasonal sites, the main stem sites upstream from the portal are referred to as upstream sites, and the main stem sites downstream from the portal are referred to as downstream sites.

Periphyton Sampling

Collection of periphyton samples was similar to that described in the U.S. Environmental Protection Agency periphyton protocols for sampling single habitats (Stevenson and Bahls, 1999). Periphyton samples were collected exclusively from riffles using rocks with flat surfaces that were classified as large cobble or boulder using the Wentworth scale (Cummins, 1962). A single, pooled qualitative periphyton sample that was representative of the entire stream channel was collected at each site for fatty acid analysis. Periphyton was scraped from sets of three rocks that were collected from the left, right, and center thirds of the streambed for a total of nine rocks per pooled sample. Each sample was stored in an 0.53 L Nasco Whirl-Pak bag and kept in the dark on ice until the sample could be frozen and stored at -80 degrees Celsius (°C).

At the same time and sites where fatty acid samples were collected for qualitative analysis, quantitative periphyton samples were collected to determine standing crop (chl *a* and AFDM) and to identify attached diatoms. Three replicate samples (replicates) were collected at each site by combining the scrapings from a delineated area of the surface of three rocks from one stream third (left, right, and center). A fourth replicate was collected for diatom identification during the August 2009 survey at each site using one rock from each stream third. The volume of slurry from each replicate was measured, and subsamples were taken for determination of chl *a* and AFDM and for diatom identification. For chl *a* and AFDM, the sample was mixed thoroughly with an electric mixer, and a 5-milliliter (mL) subsample was vacuumed through an Advantec Grade GF-75 glass fiber filter. Each filter was placed in a petri dish, covered with aluminum foil, and kept on ice until the filter could be frozen in the laboratory. For diatom identification, the sample was mixed using an electric mixer and a 20-mL subsample was pipetted into a glass vial and preserved with formalin. The scraped area of each rock was outlined in chalk and photographed with a wire screen overlain of known mesh size. Area was calculated from these photographs using Analyzing Digital Images software (Pickle, 2008).

Table 1. Stream name, site identification number, drainage area, and location of 20 sampling sites along the main stem and tributaries of the upper Esopus Creek, New York.

[Sites identified as U–[X] are upstream of the entrance of the Shandaken portal, which diverts waters from the Schoharie Reservoir to the upper Esopus Creek; sites identified as D–[X] are downstream of the portal; sites identified as T–[X] are on tributaries to the Esopus Creek. * indicates sites that were sampled in both August and November 2009. Latitude and longitude are in decimal degrees and are from the North American Datum of 1983. ID, identification number; km^2, square kilometer]

Stream name	Site ID	Drainage area (km^2)	Town/village	Latitude	Longitude
Upper Esopus Creek	U–0	30.3	Oliverea	42.0656	-74.4606
Upper Esopus Creek	U–2*	111.9	Big Indian	42.1042	-74.4367
Upper Esopus Creek	U–3*	152.0	Shandaken	42.1194	-74.3975
Upper Esopus Creek	U–3a*	165.0	Allaben	42.1143	-74.3666
Upper Esopus Creek	D–3b*	181.0	Allaben	42.1134	-74.3619
Upper Esopus Creek	D–4	215.7	Upstream of Phoenicia	42.0922	-74.3364
Upper Esopus Creek	D–4a*	357.4	Phoenicia	42.0819	-74.3119
Upper Esopus Creek	D–4b	365.2	Phoenicia	42.0636	-74.3064
Upper Esopus Creek	D–5	373.0	Mount Pleasant	42.0467	-74.2803
Upper Esopus Creek	D–6*	497.3	Boiceville	42.0039	-74.2683
Beaver Kill	T–0	64.7	Mount Tremper	42.0474	-74.2762
Birch Creek	T–1	32.4	Big Indian	42.1076	-74.4504
Broadstreet Hollow	T–2	23.7	Allaben	42.1126	-74.3585
Bushnellsville Creek	T–3	29.5	Shandaken	42.1209	-74.3989
Fox Hollow	T–4	10.3	Allaben	42.1161	-74.3806
Little Beaver Kill	T–5	42.7	Beechford	42.0195	-74.2697
Peck Hollow	T–6	12.3	Allaben	42.1180	-74.3783
Stony Clove Creek	T–7	80.0	Chichester	42.1020	-74.3109
Stony Clove Creek	T–8	83.9	Phoenicia	42.0831	-74.3148
Woodland Creek	T–9	53.4	Phoenicia	42.0813	-74.3317

Fatty Acid Analysis

The FAME analysis is a three step process: gravimetric extraction, derivatization, and quantification on a gas chromatograph. A subsample of freeze-dried periphyton was extracted three times by grinding freeze-dried materials with a Teflon mortar in a glass tube to which a 2:1 chloroform-to-methanol solution was added (Folch and others, 1957). After centrifugation at 600 times gravity centrifugal force to remove most nonlipid material, the supernatant was transferred to acid-washed 15-mL centrifuge tubes and rinsed with the chloroform-to-methanol solution. This procedure was followed by a salt wash (0.9 percent aqueous sodium chloride solution) to remove lipophilic proteins before the samples were evaporated to 2 mL using a stream of nitrogen gas. From this volume, duplicate 0.2-mL aliquots (10 percent of the sample extract) were weighed on an electronic microbalance (0.001 milligrams) to provide a gravimetric measurement of total lipid concentration (lipid mass per dry-weight tissue extracted). The remaining 1.6 mL of each extract was evaporated to dryness with nitrogen gas, tightly capped, and stored at -80 °C for later gas chromatography analyses of FAME. A synthetic lipid (cholestane), detectable by gas chromatography analysis along with FAME, was added to all samples as an internal standard to determine extraction efficiency (Sigurgisladottir and others, 1992). Immediately prior to derivitization, samples were removed from the -80 °C freezer and warmed to room temperature in the dark. Hexane (2 mL) was added to redissolve the extract. Derivitization of FAME was based on the sulfuric acid methanol method of Schlechtriem and others (2008). Gas chromatograph separation and quantification of fatty acids employed an Agilent 6890N gas chromatograph fitted with a Supelco SP–2560 (Bellefonte, PA) 100-meter × 0.25-millimeter-internal diameter × 0.20-micrometer-thick film. The oven temperature of the gas chromatograph was set at 100 °C for 1 minute before increasing by 5 °C per minute to 240 °C, which was held for 31 minutes; helium carrier gas flow rate was set at 20 centimeters per second; peak detection was by flame ionization with detector temperature

set at 260 °C; injector temperature was set at 260 °C; and total run time was 60 minutes per sample. Docosapentaenoic acid (Supleco #47563–U) was added to the 37-component FAME standard (Supelco #47885–U). The identity of eluted peaks in samples was determined by comparing sample peak retention times with retention time of the FAME standards. A four-point standard calibration curve was used to quantify the concentration of each fatty acid peak identified.

The area under each fatty acid peak was calculated as a percentage of the total area of all fatty acid peaks in the chromatogram and quantitatively as micrograms of fatty acid per milligram of periphyton. Both measurements gave similar results (coefficient of determination [r^2] = 0.95); therefore, only the percentage data are presented in this report. Fatty acids are reported in the shorthand form *x*:*aωb*, where *x* is the number of carbons in the acyl chain, *a* is the total number of double bonds, and *b* is the position of the first double bond from the methyl end of the molecule (Allan and others, 2010); the suffix *c* or *t* indicates the *cis* or *trans* isomer, respectively, where applicable. In addition to calculating the relative abundance of each fatty acid, the following summary metrics were also calculated: sum of SAFAs, sum of MUFAs, and sum of PUFAs.

Standing Crop and Diatom Analysis

Total chl *a* was determined using standard methodology for fluorometric determination of chl *a* with a pheophytin *a* correction (Rice and others, 2012). The hold time for chl *a* samples was 470 days for samples from August 2009 and 398 days for samples from November 2009, which exceeded the advised hold time. Therefore, the utility of the chl *a* data is only to identify relative differences within the study area. Filters for AFDM were oven dried at 100 °C for 24 hours, weighed, ashed at 500 °C for 2 hours, and reweighed to determine AFDM (Steinman and others, 1996), which was calculated as the difference between the dried weight and the ashed weight of each replicate.

Periphyton samples for identification of diatoms were shipped to the Rhithron Associates, Inc. laboratory in Missoula, Montana and identified to the lowest possible taxonomic resolution (generally to species). Diatoms were identified because of their role in the biological assessment of surface waters in New York State. The New York State Department of Environmental Conservation has developed several diatom-based community metrics to assess water-quality conditions (Passy and Bode, 2004; New York State Department of Environmental Conservation, 2012). Therefore, it was important to derive a connection between the fatty acid content of the periphyton community and the diatom assemblages used in the assessment of surface waters in New York State. Permanent diatom slides were prepared from acid-washed subsamples from each replicate at each site. A transect was scribed on each slide, and the first 600 valves along the transect line were identified.

Water Quality

Average daily stream discharge for each seasonal site was obtained from existing U.S. Geological Survey streamgages or, in the absence of streamgages, estimated by a drainage-area adjustment of nearby observed discharges. Additionally, a limited number of suspended sediment, turbidity, and water chemistry samples were collected at the seasonal sites for an independent project beginning in August 2009. Water samples were collected on three occasions: August 26–27, September 23, and November 3 (the date of fatty acid sampling), meaning that three samples were collected at each site during a 2 to 3 month period immediately before the collection of the November fatty acid samples. Because insufficient water-quality data were available before the August fatty acid sampling, relations between water-quality parameters and fatty acids were only evaluated for the November samples, and only the fatty acids that were detected from at least three of these sites were included. Pearson product-moment correlations (*r*) were used to identify significant relations between mean water-quality parameters from each site and the relative abundance of individual fatty acids and summary metrics.

Statistical Analysis

The hypothesis that the fatty acid content and standing crop of periphyton differed by location (upstream, downstream, and tributary) was evaluated using one-way analysis of variance (ANOVA) with Tukey's test of multiple comparisons. The fatty acid dataset was tested using percentages of individual fatty acids and summary metrics from the pooled samples, whereas standing crop was tested using the mean values of chl *a* and AFDM from the three replicates collected at each site. Samples from each period were tested separately, which enabled the use of the tributary data in the August dataset. A one-way ANOVA was also used to identify differences in the fatty acid and standing crop datasets between the August (excluding tributaries) and November samples. All subsequent analyses between seasons also exclude tributaries; thus, 10 sites are considered in August and 6 sites are considered in November.

Fatty acid composition was also evaluated using nonmetric multidimensional scaling (MDS) ordinations (Shepard, 1962; Kruskal, 1964) with Primer-E version 6 software (Clarke and Gorley, 2006). The raw data (as proportions) were arcsine-square-root-transformed, and Bray-Curtis similarities were used to form a resemblance matrix comparing all samples (Clarke and Warwick, 2001). Analysis of similarity (ANOSIM) was applied to the resemblance matrix to test the null hypothesis of no difference between the fatty acid composition of sites grouped by location (upstream, downstream, and tributary) or season (August or November). Although the ANOSIM test produces a *P* value, the *R*-test statistic is considered more informative and is interpreted as

follows: $R > 0.5$ indicates separate but overlapping groups and $0.75 < R < 1$ indicates strong separation between groups (Clarke and Warwick, 2001; Ramette, 2007). Two ANOSIM tests were performed: a two-way crossed analysis of a combined dataset (upstream and downstream for August and November) that excluded tributaries and a one-way analysis of the August dataset (upstream, downstream, and tributary) alone.

The condition of the diatom community (as an indicator of water quality) was assessed using an index of biological integrity, the diatom assessment profile (DAP; New York State Department of Environmental Conservation, 2012). A DAP score was produced for each sample at each site, and a one-way ANOVA with Tukey's test of multiple comparisons was used to test for differences in mean DAP score between locations and seasons. Diatom community structure was also assessed using MDS ordinations in order to identify sites (or groups of sites) with distinctive relative abundances of species. Identification results (species counts) for all replicates from each site were combined to obtain the most representative sample. The data were square-root-transformed to reduce the influence of highly abundant taxa, Bray-Curtis similarities were used to plot the MDS ordination (Clarke and Warwick, 2001), and ANOSIM tests were performed, as described above, to determine if assemblages differed by location or season.

Results

Fatty Acids

Percentages of individual fatty acids and summary metrics at each site are presented in appendix 1. The fatty acids 21:0, 14:1ω5, and 17:1 as well as the *trans* isomers 18:1ω9t and 18:2ω6t were not detected in any sample and therefore are not included in any tables or analyses. When all sites and both seasons were considered, the fatty acid indicators of diatom dominance (16:0, 16:1ω7, and 20:5ω3) accounted for an average of 60.3 percent (range of 35.5 to 77.9 percent) of the fatty acids present, and the three major classes of fatty acids occurred in similar mean abundances: SAFA, 37.9 percent; MUFA, 32.4 percent; and PUFA, 29.7 percent.

When samples from August 2009 were considered alone, summary metrics did not differ significantly by location; however, the percentages of some individual fatty acids did differ significantly by location. Percentages of the PUFAs 18:3ω6 and 20:5ω3 differed significantly ($P < 0.001$ and $P = 0.019$, respectively), and each was more abundant at upstream sites than at downstream sites (table 2). The PUFA 22:5ω3 also differed significantly by location ($P = 0.013$), with greater abundance at tributary sites than at downstream sites. Differences in the relative abundance of 22:6ω3 also approached significance ($0.05 < P < 0.1$) with no presence at downstream (0.00 percent) sites and low abundance at upstream (0.28 percent) and tributary (0.21 percent) sites. The only SAFA to differ significantly by location was 14:0 ($P = 0.025$), which was more abundant at tributary sites than at upstream sites. No MUFA varied significantly by location, although differences in 22:1ω9 and 24:1ω9 each approached significance ($0.05 < P < 0.1$). When samples from November 2009 were considered alone, neither summary metrics nor any individual fatty acids differed significantly by location (table 2). The relative abundance of fatty acids changed little between sampling seasons; thus, tabular results of those comparisons are not presented. None of the summary metrics differed significantly between seasons, and only one individual fatty acid, 22:5ω3, differed significantly ($P = 0.033$) between samples from August (mean 0.14 percent) and November (mean 0.59 percent). Additionally, the PUFA 22:6ω3 was only detected at 2 out of 10 main stem sites in August, whereas it was present at 5 out of 6 main stem sites in November; however, these differences were not significant.

Results from the multivariate analyses indicate that the portal may have had an effect on the fatty acid composition of periphyton. When samples from August were analyzed alone, sites clustered weakly by location (fig. 2). A one-way ANOSIM test indicated that upstream sites were significantly different from downstream ($R = 0.492$, $P = 0.010$) and tributary ($R = 0.451$, $P = 0.020$) sites while tributary and downstream sites were not significantly different ($R = 0.086$, $P = 0.208$). When both periods (excluding tributaries) were analyzed together, the samples again clustered weakly by location (fig. 3), and the two-way ANOSIM test indicated that fatty acid composition of upstream and downstream sites was significantly different ($R = 0.372$, $P = 0.008$). Sites downstream from the portal clustered more tightly while a few upstream sites exhibited distinctive fatty acid signatures. The two-way ANOSIM test also indicated that samples from August and November did not differ significantly ($R = 0.156$, $P = 0.168$), although samples from November, with the exception of site U–2, appeared to group more closely in the ordination (fig. 3).

Water Quality and Fatty Acid Relations

Water-quality parameters from samples at individual sites did not exhibit much variability during the collection period (August 26 to November 3, 2009; appendix 2). As expected, however, waters downstream from the portal were generally more turbid and contained higher concentrations of suspended sediment than upstream sites during stable flows. Mean values of turbidity ranged from 1.3 NTU at site U–3a to 5.0 NTU at site D–3b, the sampling site immediately downstream from the portal, and mean turbidity at downstream sites (4.0 NTU) was significantly greater ($P = 0.012$) than that of upstream sites (1.5 NTU; table 3). Mean values of suspended sediment ranged from 0.7 milligrams per liter (mg/L) at site U–3a to

Table 2. Summary of one-way analysis of variance results comparing the relative abundance of individual fatty acids and summary metrics for periphyton from sites along the main stem and tributaries of the upper Esopus Creek, New York.

[Samples are from upstream, downstream, and tributary sites during the August 2009 survey and upstream and downstream sites during the November 2009 survey. Significant *P* values are shown in bold; the significant groupings are denoted by letters (groups that do not share a letter are significantly different). *n*, number of samples; SD, standard deviation; <, less than]

Fatty acid	August							November				
	Upstream sites (*n* = 4)		Downstream sites (*n* = 6)		Tributary sites (*n* = 10)			Upstream sites (*n* = 3)		Downstream sites (*n* = 3)		
	Mean	SD	Mean	SD	Mean	SD	*P* value	Mean	SD	Mean	SD	*P* value
Saturated fatty acids												
12:0	0.18	0.37	0.00	0.00	0.00	0.00	0.134	0.00	0.00	0.00	0.00	1.000
14:0	5.88 a	1.23	6.55 ab	1.10	7.96 b	1.39	**0.025**	7.00	1.79	7.23	1.52	0.875
15:0	0.07	0.14	0.00	0.00	0.03	0.09	0.477	0.24	0.23	0.00	0.00	0.150
16:0	23.13	1.99	27.04	2.22	26.73	3.05	0.069	23.88	3.73	23.24	1.91	0.805
17:0	0.42	0.38	0.35	0.18	0.31	0.29	0.832	0.58	0.08	0.63	0.27	0.745
18:0	5.24	3.35	3.64	0.48	3.55	0.83	0.190	7.91	5.63	4.28	0.95	0.333
20:0	0.11	0.23	0.19	0.15	0.36	0.17	0.059	0.37	0.40	0.00	0.00	0.184
22:0	0.05	0.10	0.09	0.10	0.07	0.10	0.783	0.21	0.23	0.07	0.13	0.425
23:0	0.09	0.18	0.00	0.00	0.00	0.00	0.134	0.05	0.09	0.00	0.00	0.374
24:0	0.08	0.16	0.00	0.00	0.00	0.00	0.134	0.00	0.00	0.00	0.00	1.000
Total	35.25	1.94	37.87	3.49	39.01	3.95	0.228	40.23	2.91	35.47	3.43	0.140
Monounsaturated fatty acids												
15:1	0.10	0.20	0.05	0.13	0.05	0.15	0.855	0.00	0.00	0.21	0.37	0.374
16:1ω7	23.61	12.06	27.71	6.85	26.85	7.25	0.728	17.36	5.94	27.79	8.96	0.168
18:1ω9c	5.50	1.88	5.15	0.61	5.08	0.97	0.809	8.41	5.19	5.71	1.37	0.434
20:1ω9	0.19	0.13	0.23	0.16	0.27	0.29	0.844	3.16	5.06	0.09	0.16	0.353
22:1ω9	0.54	0.28	0.34	0.05	0.37	0.09	0.080	0.26	0.23	0.49	0.30	0.351
24:1ω9	0.05	0.10	0.18	0.14	0.33	0.23	0.056	0.16	0.14	0.27	0.24	0.526
Total	29.98	12.16	33.66	6.86	32.95	6.93	0.766	29.35	5.17	34.57	8.00	0.396
Polyunsaturated fatty acids												
18:2ω6c	4.75	2.02	5.74	1.26	5.34	1.77	0.666	5.48	1.63	4.21	0.82	0.294
18:3ω3	13.48	7.85	13.23	6.62	9.73	4.45	0.406	10.50	6.90	9.54	4.82	0.853
18:3ω6	1.07 a	0.26	0.61 b	0.05	0.89 a	0.15	**<0.001**	0.77	0.40	0.72	0.10	0.845
20:2ω6	1.49	1.14	0.99	0.17	1.34	0.31	0.318	1.46	0.75	1.65	0.29	0.707
20:3ω3	0.12	0.14	0.55	0.09	0.04	0.09	0.448	0.14	0.13	0.07	0.12	0.508
20:3ω6	0.05	0.09	0.05	0.12	0.01	0.03	0.575	0.00	0.00	0.00	0.00	1.000
20:4ω6	0.80	0.64	0.61	0.22	0.81	0.24	0.512	0.68	0.38	0.53	0.34	0.622
20:5ω3	12.20 a	3.74	6.78 b	1.52	8.94 ab	2.70	**0.019**	8.41	5.02	12.23	6.00	0.445
22:2ω6	0.29	0.24	0.35	0.02	0.42	0.11	0.222	0.22	0.20	0.42	0.03	0.161
22:5ω3	0.25 ab	0.18	0.07 a	0.10	0.32 b	0.16	**0.013**	0.79	0.81	0.38	0.18	0.440
22:6ω3	0.28	0.32	0.00	0.00	0.21	0.19	0.072	1.97	2.71	0.22	0.20	0.327
Total	34.77	10.96	28.48	6.31	28.04	5.99	0.290	30.42	7.92	29.96	5.10	0.937

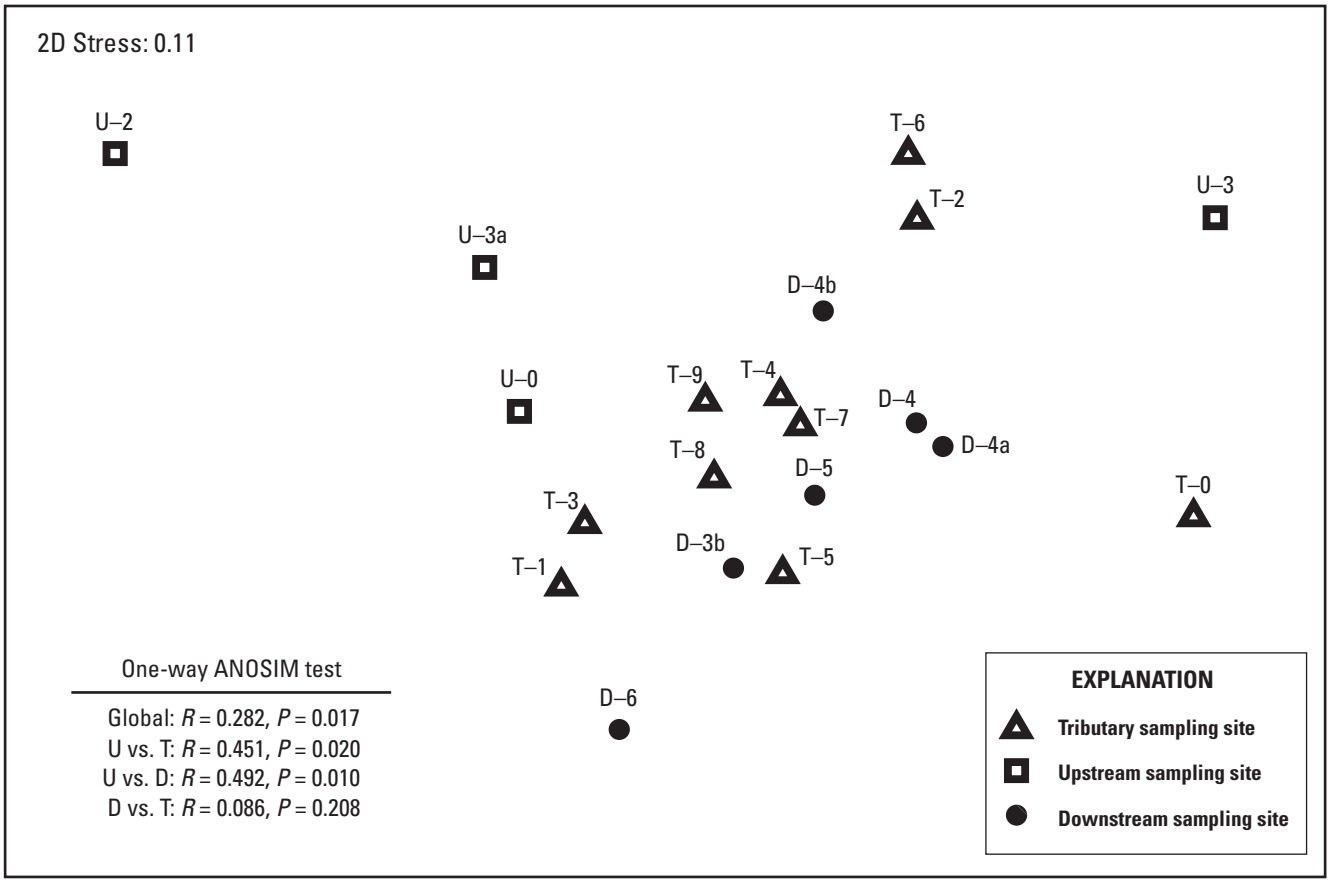

Figure 2. Nonmetric multidimensional scaling ordination based on arcsine-square-root-transformed proportion data of periphyton fatty acid content collected during August 2009 from sites upstream or downstream from the Shandaken portal, or on a tributary of the upper Esopus Creek, New York. Site locations and identification numbers are identified in table 1. Results of the one-way analysis of similarity (ANOSIM) test show the significance of the three levels of the location factor. Two-dimensional (2D) stress is a measurement of the goodness of fit of the arrangement of points in the ordination. U, upstream; T, tributary; D, downstream; vs., versus.

4.0 mg/L at site D–3b, and differences in mean suspended sediment at upstream sites (0.9 mg/L) and downstream sites (2.8 mg/L) approached significance ($0.05 < P < 0.1$). Each parameter peaked immediately downstream from the portal and decreased moving downstream. Mean specific conductance, pH, and stream discharge were also significantly higher ($P < 0.05$) at downstream sites, whereas differences in nitrate were not significant.

Relations between mean water-quality parameters and individual fatty acids or summary metrics were generally weak. None of the individual fatty acids or summary metrics were significantly correlated with turbidity or suspended sediment. The correlations between suspended sediment and 22:1ω9 ($r = 0.75$, $P = 0.087$) and 20:5ω3 ($r = 0.72$, $P = 0.108$) were the closest to reaching significance, but most correlations between fatty acids and suspended sediment or turbidity did not exceed $r = 0.5$. Nitrate was significantly correlated with 17:0 ($r = 0.91$, $P = 0.012$), pH was significantly correlated with 22:2ω6 ($r = 0.83$, $P = 0.041$), and there were no significant correlations with specific conductance or stream discharge.

Although turbidity, specific conductance, and pH were all significantly greater at downstream sites than at upstream sites, water-quality parameters generally exhibited low variability during the study and did not reach levels that would be expected to cause impairment to the periphyton community. For example, the highest mean turbidity value recorded was only 5.0 NTU (at site D–3b) which was well below most thresholds for impairment of lotic biota (Lloyd, 1987; Ryan, 1991). As a result, water-quality parameters generally did not explain much variation in the fatty acid dataset from November and there were no significant correlations between fatty acids and turbidity or suspended sediment. Additionally, the significant correlations identified with nitrate and pH may not be biologically meaningful because of the low variation in these parameters. It is noteworthy that mean daily stream

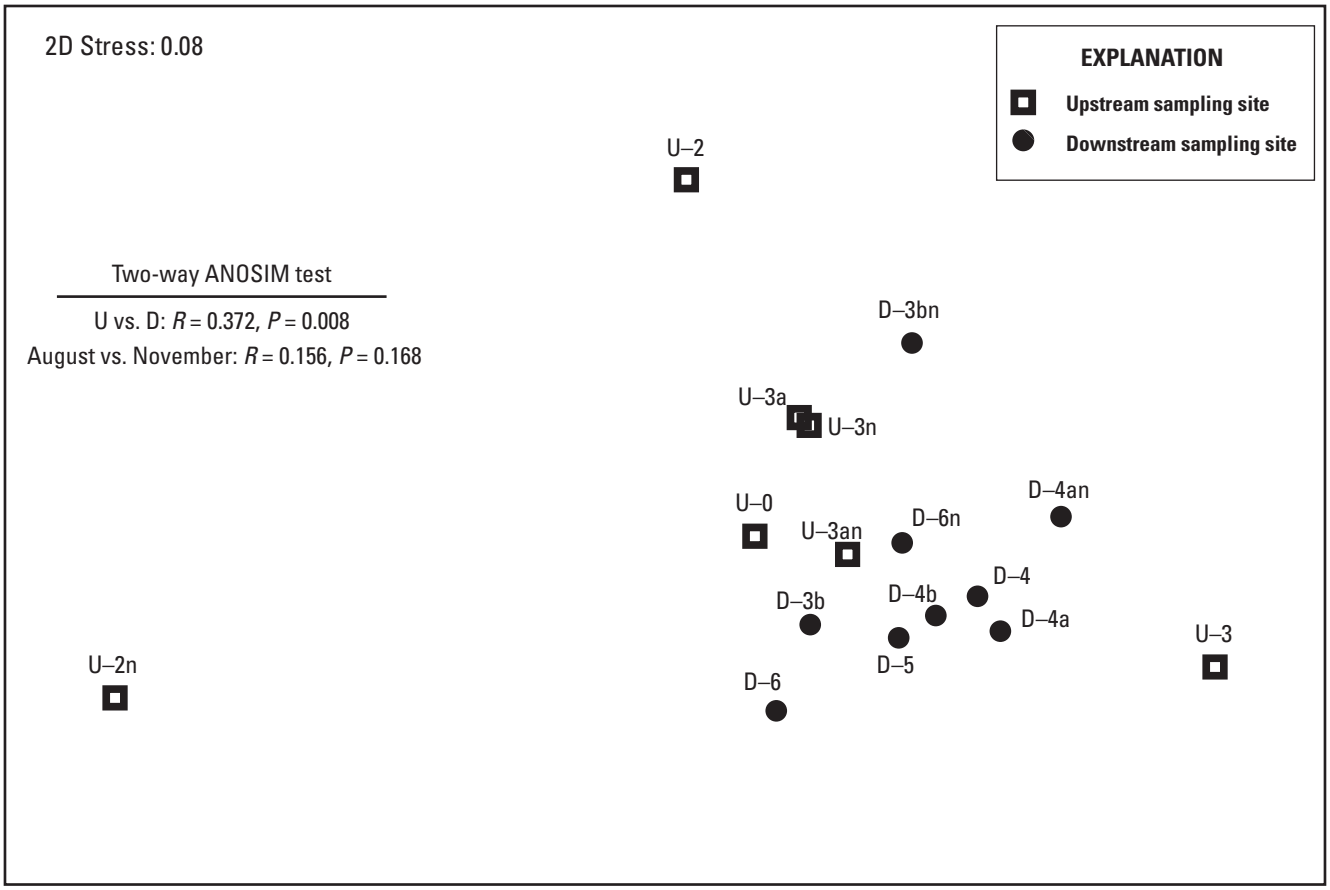

Figure 3. Nonmetric multidimensional scaling ordination based on arcsine-square-root-transformed proportion data of periphyton fatty acid content collected during August and November 2009 from sites on the upper Esopus Creek upstream or downstream from the Shandaken portal, New York. Site identification numbers ending with n denote samples from November. Site locations and identification numbers are identified in table 1. Results of the two-way analysis of similarity (ANOSIM) test show the significance of the two factors (location [upstream or downstream] and season [August or November 2009]). Two-dimensional (2D) stress is a measurement of the goodness of fit of the arrangement of points in the ordination. U, upstream; D, downstream; vs., versus.

discharge across the 70-day period before the November survey increased from 2.2 cubic meters per second at site U–3a to 8.9 cubic meters per second at site D–3b, an increase of approximately 300 percent in less than 1 km (primarily because of inputs from the portal). In contrast, mean discharge at site D–6 (approximately 17 km downstream from the portal) during the same period was only 63 percent greater than at site D–3b. This observed increase in discharge between sites U–3a and D–3b is consistent with results from a recent study indicating that under low and moderate flows, the portal generally increased natural flows on the upper Esopus Creek by more than 100 percent (Burns and Gazoorian, 2015). These results indicate that supplemental flows from the portal contribute substantially to the discharge at all downstream sites and that any differences in periphyton between upstream and downstream sites cannot simply be attributed to normal longitudinal changes.

Standing Crop

The chl *a* and AFDM data were significantly correlated ($r = 0.91$; $P < 0.001$) and are presented in table 3 as the results of the one-way ANOVA and in appendix 3 as the means of all three samples (left, right, and center of stream). The chl *a* data differed significantly by location when August samples were analyzed alone ($P = 0.019$). Mean chl *a* at tributary sites (5.38 micrograms per square centimeter [$\mu g/cm^2$]) differed ($P < 0.05$) from that of upstream (2.57 $\mu g/cm^2$) and downstream (2.93 $\mu g/cm^2$) sites (table 3). Although samples from upstream and downstream sites were not significantly different ($P > 0.05$), chl *a* dropped sharply from 4.53 $\mu g/cm^2$ at site U–3a (immediately upstream from the portal) to 1.97 $\mu g/cm^2$ at site D–3b (immediately downstream from the portal) and remained low at sites D–4 and D–4a. In November, chl *a* data from upstream and downstream sites

Table 3. Summary of one-way analysis of variance results comparing periphyton standing crop, diatom community integrity, and water-quality parameters from sites along the main stem and tributaries of the upper Esopus Creek, New York.

[Samples are from upstream, downstream, and tributary sites during the August and November 2009 surveys and for water-quality parameters and daily stream discharge at upstream and downstream sites during the 70 days before the November 2009 survey. Significant *P* values are shown in bold; the significant groupings are denoted by letters (groups that do not share a letter are significantly different). *n*, number of samples; SD, standard deviation; --, not available]

	August							November				
	Upstream sites (*n* = 4)		Downstream sites (*n* = 6)		Tributary sites (*n* = 10)			Upstream sites (*n* = 3)		Downstream sites (*n* = 3)		
	Mean	SD	Mean	SD	Mean	SD	*P* value	Mean	SD	Mean	SD	*P* value
Chlorophyll *a*, in micrograms per square centimeter	2.57 b	1.52	2.93 b	1.17	5.38 a	2.18	**0.019**	12.26	0.92	12.84	7.24	0.897
Ash-free dry mass, in milligrams per square centimeter	1.00	0.58	1.06	0.55	1.03	0.28	0.979	2.62	0.31	3.75	2.12	0.412
Diatom assessment profile score	7.19	0.16	7.02	0.27	6.95	0.36	0.450	6.35	0.13	6.43	0.37	0.735
Nitrate, in micromoles per liter	--	--	--	--	--	--	--	7.4	0.6	7.1	1.3	0.785
pH	--	--	--	--	--	--	--	7.05	0.05	7.19	0.03	**0.021**
Specific conductance, in microsiemens per centimeter at 25 degrees Celsius	--	--	--	--	--	--	--	50.3	2.5	60.1	2.7	**0.010**
Stream discharge, in cubic feet per second	--	--	--	--	--	--	--	68.7	13.3	420.4	99.5	**0.004**
Total suspended sediment, in milligrams per liter	--	--	--	--	--	--	--	0.9	0.2	2.8	1.2	0.051
Turbidity, in nephelometric turbidity units	--	--	--	--	--	--	--	1.5	0.2	4.0	1.0	**0.012**

(mean values of 12.26 and 12.84 µg/cm^2, respectively) were also not significantly different ($P = 0.897$; table 3), but a spike from 13.10 to 20.77 µg/cm^2 was observed between sites U–3a and D–3b. The chl *a* data did differ significantly ($P < 0.001$) between seasons (excluding tributaries) irrespective of location: mean chl *a* in August was 2.79 µg/cm^2 compared to 12.55 µg/cm^2 in November.

The AFDM data had a similar pattern to that of the chl *a* data but did not differ significantly by location during August ($P = 0.979$). Mean AFDM in August was 1.00 milligrams per square centimeter (mg/cm^2) at upstream sites, 1.06 mg/cm^2 at downstream sites, and 1.03 mg/cm^2 at tributary sites (table 3). Similar to chl *a*, AFDM decreased by more than 50 percent between sites U–3a and D–3b and remained low until site D–5. During November, mean AFDM was not significantly different ($P = 0.412$) at upstream (2.62 mg/cm^2) and downstream (3.75 mg/cm^2; table 3) sites but did increase sharply from 2.95 to 5.98 mg/cm^2 between sites U–3a and D–3b. The AFDM data did differ significantly ($P = 0.001$) between seasons as mean AFDM was 1.03 mg/cm^2 in August compared with 3.19 mg/cm^2 in November.

Diatoms

Diatom identifications were used to produce a DAP score at each site. When August sites were considered alone, DAP scores at the three locations were not significantly different ($P = 0.450$). The mean DAP score was 7.19 at upstream sites, 7.02 at downstream sites, and 6.95 at tributary sites (table 3). Unlike measurements of standing crop, the DAP score did not drop sharply between sites U–3a and D–3b. In November, the mean DAP score was 6.35 at upstream sites and 6.43 at downstream sites and also did not differ significantly ($P = 0.735$). However, the DAP score did differ significantly between seasons ($P < 0.001$) as sites sampled in August had a mean DAP score of 7.09 compared with 6.39 in November.

Results of the diatom identifications were also expressed as MDS ordinations. When sites sampled in August were analyzed alone (fig. 4), downstream sites grouped tightly and were significantly different from upstream sites ($R = 0.567$, $P = 0.005$) and tributary sites ($R = 0.331$, $P = 0.009$) whereas upstream and tributary sites were not significantly different ($R = -0.039$, $P = 0.538$). Community structure was most variable at tributary sites. When main stem sites from both periods were analyzed together (fig. 5), the samples clustered significantly by location ($R = 0.595$, $P = 0.001$) and by season ($R = 0.989$, $P = 0.001$). Species assemblages from all sites sampled in August and November were at least 60 percent similar to each other (fig. 5); however, no single pair of November and August assemblages reached this degree of similarity.

Response of Periphyton Fatty Acid Composition to Supplemental Flows

The primary purpose of the study presented in this report was to gain a more comprehensive understanding of how waters diverted from the Schoharie Reservoir through the Shandaken Tunnel affect periphyton in the upper Esopus Creek. Measurements of periphyton standing crop and fatty acid composition, diatom community structure and integrity, and basic water-quality parameters were characterized upstream and downstream from the portal during two seasons and at tributary sites during one season. This combination of spatial and seasonal measurements helped to determine the effect of supplemental flows from the portal on the fatty acid composition of periphyton, assess the utility of fatty acid analysis as a tool for evaluating ecosystem condition, and explore spatial and temporal differences in the periphyton community.

Spatial Patterns

The effects of waters from the portal on periphyton communities were not as pronounced as anticipated. Overall, measurements of standing crop and multivariate analysis of diatom assemblages provided little evidence that the inflow of portal waters affected periphyton. A comparison of standing crop measurements at the sites immediately upstream (U–3a) and downstream (D–3b) of the portal indicates that the portal may have locally affected standing crop differently during each season. The chl *a* and AFDM values decreased sharply between sites U–3a and D–3b in August but each increased sharply between sites U–3a and D–3b in November. However, these effects were observed primarily at the sites nearest to the portal, and mean AFDM, chl *a*, and DAP score did not vary significantly upstream and downstream from the portal during either season. In contrast, multivariate analysis of the diatom identifications lent stronger support to a portal-driven influence. Analyses of the August and combined datasets each indicated that diatom community structure at upstream sites was significantly different from that of downstream sites.

The minor effect of the portal on periphyton communities and water-quality parameters during the study period provided a poor opportunity to test the hypothesis that fatty acid analysis of periphyton is sensitive to differences in water quality or ecosystem condition. A few analyses, however, did detect changes in the fatty acid composition of periphyton downstream from the portal during August. Multivariate analysis indicated that fatty acid composition differed significantly between upstream and downstream sites. It is also noteworthy that the sum of PUFAs decreased from 42.4 percent at site U–3a to 27.4 percent at site D–3b, and some individual PUFAs were more abundant upstream from the portal than downstream. Within the omega-6 family of fatty acids, 18:3ω6 was significantly more abundant upstream

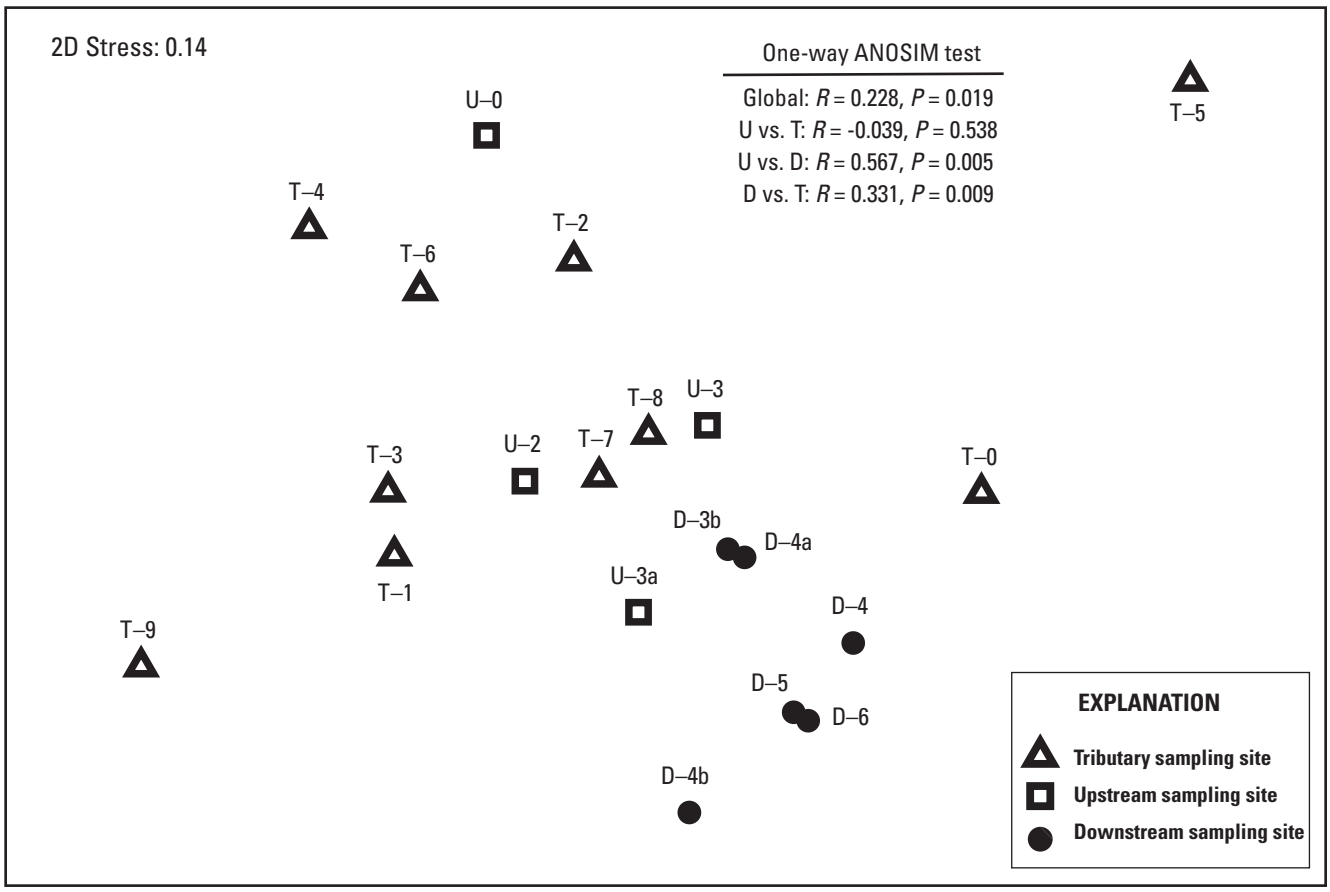

Figure 4. Nonmetric multidimensional scaling ordination based on square-root-transformed species abundance data of diatom assemblages collected during August 2009 from sites upstream or downstream from the Shandaken portal, or on a tributary of the upper Esopus Creek, New York. Site locations and identification numbers are identified in table 1. Results of the one-way analysis of similarity (ANOSIM) test show the significance of the three levels of the location factor. Two-dimensional (2D) stress is a measurement of the goodness of fit of the arrangement of points in the ordination. U, upstream; T, tributary; D, downstream; vs., versus.

from the portal than downstream, and the physiologically important 20:4ω6 (arachidonic acid), which is the elongation product of 18:3ω6, was also more abundant (although not significantly) upstream from the portal. Within the omega-3 family, 20:5ω3, 22:5ω3, and 22:6ω3 were detected in greater relative abundances upstream from the portal than downstream, although not all differences were significant. Brett and Müller-Navarra (1997) determined that the nutritional quality of periphyton can be evaluated by the abundance of particular PUFAs and specifically, that the availability of 20:5ω3 and 22:6ω3 in primary producers is critical to the growth and condition of associated consumers. Therefore, these findings suggest the nutritional quality of periphyton may be higher upstream from the portal than downstream, and in turn may explain why the New York State Department of Environmental Conservation determined that diatom and macroinvertebrate assemblages upstream from the portal were healthier than those downstream from the portal in some years (Bode and others, 2001; Smith, 2013).

Seasonal Differences

Although spatial variation in the periphyton community was less than expected, significant seasonal changes were observed in the periphyton community between the August and November samples. Mean chl *a* increased from 2.79 µg/cm^2 in August to 12.55 µg/cm^2 in November, and mean AFDM increased from 1.03 mg/cm^2 in August to 3.19 mg/cm^2 in November. These significant increases in standing crop coincided with a significant decrease in mean DAP score from 7.09 in August to 6.39 in November. The increase in standing crop and decrease in DAP score are all consistent with the results of a companion study (George and Baldigo, 2015) that identified the bloom-forming diatom *Didymosphenia geminata* in significantly greater densities during November. During August, *D. geminata* was not detected upstream from the portal and had a mean cell density of 51 cells per square centimeter at sites downstream from the portal. In contrast, the mean cell density

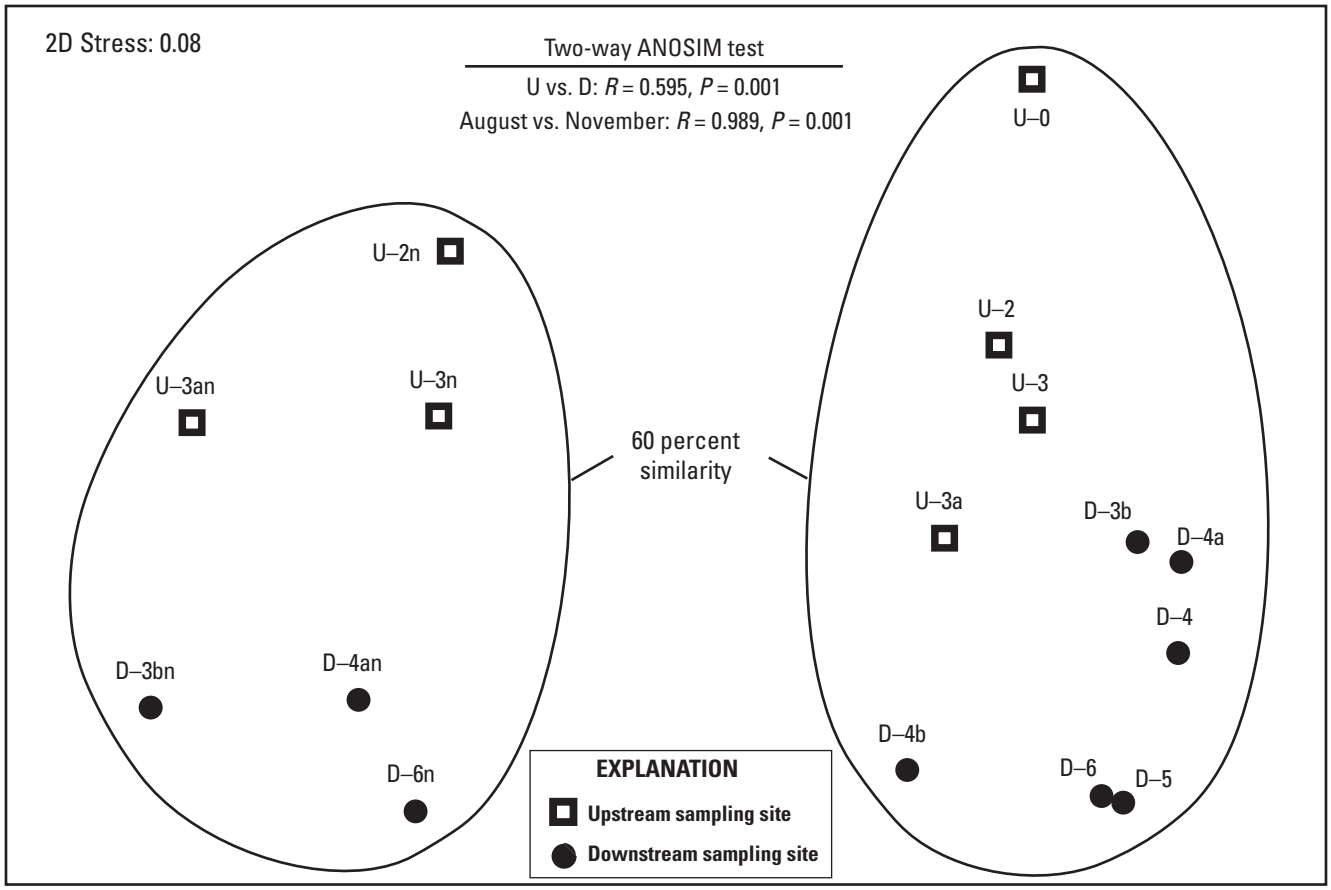

Figure 5. Nonmetric multidimensional scaling ordination based on square-root-transformed species abundance data of diatom assemblages collected during August and November 2009 from sites on the upper Esopus Creek upstream or downstream from the Shandaken portal, New York. Site identification numbers ending with n denote samples from November; bubbles indicate 60 percent similarity. Site locations and identification numbers are identified in table 1. Results of the two-way analysis of similarity (ANOSIM) test show the significance of the two factors (location [upstream or downstream] and season [August or November 2009]). Two-dimensional (2D) stress is a measurement of the goodness of fit of the arrangement of points in the ordination. U, upstream; D, downstream; vs., versus.

of *D. geminata* in November was 8 cells per square centimeter upstream from the portal and 1,352 cells per square centimeter downstream from the portal. Streams with severe blooms of *D. geminata* often exhibit vast increases in algal standing crop (Spaulding and Elwell, 2007; Kilroy and others, 2009). This phenomenon may explain the large increase in chl *a* and AFDM observed in the Esopus Creek between August and November. Diatom species ordinations showed strong clustering by season (fig. 5), and assemblages at all sites sampled in November were at least 60 percent similar to each other, yet no single pair of November and August assemblages reached this degree of similarity. This result strongly indicates that seasonal influences on the diatom community exceeded any effects from the supplemental flows from the portal.

Despite these notable changes between the August and November samples, fatty acid analysis failed to differentiate between the two sampling seasons. None of the summary metrics differed significantly by season, and only the relative abundance of the PUFA 22:5ω3 differed significantly between August (0.14 percent) and November (0.59 percent) samples. Multivariate analysis of fatty acid assemblages also indicated that the August and November groups were not significantly different. Honeyfield and Maloney (2014), however, recently reported seasonal changes in the fatty acid content of periphyton in six Pennsylvania streams that included significant differences between summer and fall samples. The reason that the fatty acid data from the upper Esopus Creek did not differentiate by season is unknown but it is likely that changes in other components of the periphyton community that were not directly assessed, such as filamentous algae, also affect the proportions of available fatty acids in the upper Esopus Creek.

Limitations of Study and Recommendations for Future Work

Several limitations of this initial study should be considered and may benefit future research in this emerging field. Although results of this study did not indicate a direct relation between turbidity or suspended sediment and individual fatty acids or summary metrics, the values of these parameters were low for all samples. In studies with known impairment of water quality, such as acid mine drainage (Genter and Lehman, 2000) or chlorine-contamination (Napolitano and others, 1994), a link between water quality and fatty acid composition of periphyton has been noted. To identify a similar relation between fatty acids and turbidity or suspended sediment (if it exists), future studies will require a greater range in the measured values of these parameters. Additionally, periphyton communities have been observed to exhibit patchiness (Kawata and others, 2003) similar to that observed in terrestrial plant communities. This patchiness may explain why fatty acid composition was so distinctive at a few of the sites presented in this report. To overcome the problem of patchiness, periphyton samples should be collected following the U.S. Geological Survey protocol (Moulton and others, 2002) or a similar robust methodology that will account for spatial variability in community types. Additionally, a complete assessment of the algal community should be performed rather than an assessment of diatoms alone. A complete taxonomic assessment of the benthic algal community might have improved the interpretation of the fatty acid dataset in this study. Despite the limitations of the initial study in this report, fatty acid analysis may have potential as a tool for the assessment of water quality and aquatic ecosystem health.

Summary

The U.S. Geological Survey and the New York State Department of Environmental Conservation used fatty acid analysis of periphyton to characterize the local water quality and ecosystem condition of the upper Esopus Creek, New York in August and November 2009. The Schoharie Reservoir is in the Mohawk River drainage and provides supplemental flows to the upper Esopus Creek through a 29-kilometer underground aqueduct known as the Shandaken Tunnel as part of the New York City water supply system. The terminus of the Shandaken Tunnel is the Shandaken portal which has been the focus of controversy among local residents and anglers because the supplemental flows are sometimes turbid and are thought by some to negatively affect the aesthetic and biological integrity of the stream. Because the perceived effects of these supplemental flows are speculative, however, the overall effect of the altered suspended sediment and flow regimes on aquatic biota remains unclear. The study detailed in this report evaluated the hypothesis that periphyton communities have a fatty acid profile that allows for the detection of excessive turbidity and suspended sediment. To this end, periphyton fatty acid composition and standing crop (ash-free dry mass and chlorophyll *a*), structure and integrity of diatom communities, and water-quality parameters were analyzed and compared upstream and downstream from the Shandaken portal.

The fatty acid composition of periphyton in the upper Esopus Creek was assessed during two seasons upstream and downstream from the portal. These data were compared with measurements of periphyton standing crop, diatom community structure and integrity, and basic water-quality parameters. Periphyton samples were collected at 20 sites in the watershed in August 2009 and at 6 sites in November 2009. This combination of spatial and seasonal measurements helped to determine the effect of supplemental flows from the portal on the fatty acid composition of periphyton, assess the utility of fatty acid analysis as a tool for evaluating ecosystem condition, and explore spatial and temporal differences in the periphyton community.

The effects of the supplemental flows from the portal on periphyton communities of the upper Esopus Creek were not as pronounced as anticipated. Measurements of periphyton standing crop and diatom community integrity indicated little evidence of impairment from the supplemental flows. The relative abundances of two physiologically important fatty acids, γ-linolenic acid (18:3ω6) and eicosapentaenoic acid (20:5ω3), however, were significantly lower downstream from the portal. Multivariate analyses of fatty acid profiles indicated significant differences between sites upstream and downstream from the portal, but individual fatty acids and summary metrics were not significantly correlated with turbidity or suspended sediment. Comparisons between seasons indicated differences in periphyton standing crop and diatom community structure and integrity, although the fatty acid profiles did not detect temporal changes.

Turbidity, specific conductance, and pH were all significantly greater at downstream sites than at upstream sites; however, none of the water-quality parameters reached levels that would be expected to cause a measurable response from the periphyton community. The minor effect of the portal on water-quality parameters during the study period provided a poor opportunity to test the hypothesis that fatty acid analysis of periphyton is sensitive to excessive turbidity, suspended sediment, or general changes in water quality. Despite this, a few analyses did detect changes in the fatty acid composition of periphyton downstream from the portal.

The study described herein provides only limited evidence that periphyton communities are responding to some characteristic of waters from the portal. Although some differences between upstream and downstream sites can be attributed to portal inflows, and the nutritional quality of periphyton may be slightly poorer downstream from the portal, the results in this report generally indicate that the Shandaken portal was not a source of impairment to

periphyton in the upper Esopus Creek during the study period. Although results of this study did not identify a direct relation between turbidity or suspended sediment and individual fatty acids or summary metrics, the values of these parameters were low for all samples. Despite the limitations of this initial study, fatty acid analysis may have potential as a tool to monitor periphyton nutritional composition that may reflect water quality and ecosystem health but needs to be further evaluated around a more definitive source of water-quality impairment.

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Appendixes

Appendix 1. Fatty Acid Composition of Periphyton at Sites Sampled on the Main Stem and Tributaries of the Upper Esopus Creek, New York

Appendix 2. Water-Quality Parameters and Daily Stream Discharge Collected between August 26 and November 3, 2009, at Six Sites on the Upper Esopus Creek, New York

Appendix 3. Chlorophyll *a*, Ash-Free Dry Mass, and Diatom Assessment Profile Score for Sites Sampled on the Main Stem and Tributaries of the Upper Esopus Creek, New York

Table 1–1. Fatty acid composition of periphyton at sites sampled on the main stem and tributaries of the upper Esopus Creek, New York.

[Fatty acid composition is in percent of contribution. Site identification numbers and locations are listed in table 1. --, not detected]

Fatty acid	Sites sampled in August 2009																				Sites sampled in November 2009					
	U–0	U–2	U–3	U–3a	D–3b	D–4	D–4a	D–4b	D–5	D–6	T–0	T–1	T–2	T–3	T–4	T–5	T–6	T–7	T–8	T–9	U–2	U–3	U–3a	D–3b	D–4a	D–6
Saturated fatty acids																										
12:0	--	0.7	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
14:0	6.3	4.4	5.6	7.3	8.6	6.4	6.7	6.1	6.3	5.3	6.4	9.8	8.8	8.5	8.5	9.7	7.0	8.5	5.8	6.7	4.9	8.1	8.0	8.6	7.5	5.6
15:0	--	--	--	0.3	--	--	--	--	--	--	0.3	--	--	--	--	--	--	--	--	--	0.5	0.3	--	--	--	--
16:0	21.8	22.1	26.1	22.5	29.8	25.8	27.8	23.9	26.0	29.1	29.7	26.2	21.5	28.1	25.8	30.3	22.4	27.1	30.2	26.0	20.7	22.9	28.0	24.4	21.0	24.3
17:0	0.8	0.2	--	0.6	0.5	0.4	0.4	--	0.4	0.4	0.3	--	--	0.7	0.6	0.5	--	0.5	--	0.6	0.6	0.7	0.5	0.9	0.6	0.4
18:0	5.7	9.7	1.9	3.7	4.4	3.2	3.7	3.1	3.8	3.6	2.5	3.7	2.8	4.4	3.6	3.7	2.2	3.9	4.9	3.7	14.4	5.0	4.3	5.3	4.2	3.4
20:0	0.5	--	--	--	0.4	--	0.3	--	0.3	0.3	0.2	0.4	0.4	0.5	0.4	0.3	--	0.5	0.6	0.4	0.8	--	0.3	--	--	--
22:0	--	0.2	--	--	--	0.2	0.2	0.2	--	--	0.1	--	--	--	--	--	--	0.1	0.3	0.2	0.5	--	0.2	0.2	--	--
23:0	--	0.4	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.2	--	--	--	--	--
24:0	--	0.3	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Total	35.1	38.0	33.5	34.4	43.6	35.9	39.0	33.3	36.8	38.6	39.5	40.1	33.3	42.3	38.9	44.5	31.6	40.6	41.7	37.6	42.5	37.0	41.3	39.4	33.4	33.6
Monounsaturated fatty acids																										
15:1	--	--	0.4	--	--	--	0.3	--	--	--	--	--	--	--	--	0.5	--	--	--	--	--	--	--	--	0.6	--
16:1ω7	18.5	17.4	41.7	16.9	21.7	35.8	34.2	29.5	26.8	18.4	41.8	17.6	31.4	17.6	28.1	24.2	32.0	28.4	23.6	23.8	11.2	17.9	23.0	18.2	36.0	29.1
18:1ω9c	7.7	3.1	5.6	5.7	6.2	4.9	5.0	5.3	5.2	4.3	3.6	4.2	7.1	4.7	5.4	4.6	6.2	5.0	5.1	4.9	14.4	5.6	5.2	7.3	4.9	5.0
20:1ω9	0.3	0.3	--	0.2	0.5	0.3	0.2	0.2	--	0.3	--	0.7	--	0.7	0.2	0.5	--	0.3	0.3	--	9.0	0.2	0.2	--	--	0.3
22:1ω9	0.5	0.9	0.3	0.5	0.4	0.3	0.3	0.3	0.3	0.3	0.2	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.4	0.3	--	0.5	0.3	0.8	0.4	0.3
24:1ω9	--	0.2	--	--	0.3	0.3	0.3	--	--	0.2	0.2	0.7	--	0.6	--	0.4	0.4	0.4	0.5	0.3	0.2	0.3	--	--	0.4	0.4
Total	27.0	21.9	47.9	23.2	29.0	41.6	40.2	35.4	32.3	23.5	45.7	23.7	38.8	24.0	34.1	30.5	39.0	34.5	29.9	29.3	34.8	24.5	28.8	26.4	42.4	35.0
Polyunsaturated fatty acids																										
18:2ω6c	7.7	3.9	3.1	4.3	6.5	5.0	3.8	5.5	6.3	7.4	2.4	9.0	5.6	7.2	4.6	5.3	4.4	4.5	5.6	4.7	7.2	3.9	5.4	4.2	3.4	5.0
18:3ω3	16.1	16.8	1.9	19.2	9.9	7.5	6.9	15.2	15.3	24.5	2.8	17.0	6.4	14.8	8.4	10.2	5.7	8.1	9.7	14.1	2.7	15.7	13.2	6.0	7.6	15.0
18:3ω6	0.9	1.4	0.9	1.0	0.6	0.6	0.7	0.7	0.6	0.5	0.7	0.8	1.1	0.8	0.8	0.8	1.1	0.9	0.9	1.1	0.3	1.1	0.8	0.8	0.6	0.8
20:2ω6	2.7	--	1.4	1.8	1.1	0.9	1.2	1.1	1.0	0.7	1.0	1.2	1.3	1.6	1.6	1.2	2.0	1.1	1.2	1.2	1.1	2.3	1.0	2.0	1.5	1.5
20:3ω3	0.2	--	--	0.3	0.2	--	--	--	--	0.2	--	--	--	0.2	--	--	--	--	--	0.2	--	0.3	0.2	--	--	0.2
20:3ω6	--	0.2	--	--	0.3	--	--	--	--	--	0.1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
20:4ω6	0.7	0.4	1.7	0.4	1.0	0.6	0.5	0.5	0.5	0.5	0.7	0.9	1.3	0.6	1.0	0.6	0.9	0.8	0.8	0.5	1.1	0.4	0.6	0.9	0.3	0.4
20:5ω3	8.8	16.5	9.4	14.1	7.3	7.4	7.3	8.0	6.9	3.8	6.4	5.9	11.6	7.2	9.6	6.2	14.3	8.9	9.1	10.4	3.6	13.6	8.0	19.0	10.2	7.6
22:2ω6	0.6	--	0.2	0.4	0.3	0.3	0.4	0.4	0.4	0.3	0.2	0.4	0.4	0.5	0.5	0.3	0.6	0.4	0.4	0.6	--	0.4	0.3	0.4	0.5	0.4
22:5ω3	0.3	0.4	--	0.4	0.2	0.2	--	--	--	--	0.2	0.6	--	0.5	0.3	0.2	0.4	0.3	0.4	0.3	1.7	0.4	0.3	0.6	0.3	0.3
22:6ω3	--	0.5	--	0.6	--	--	--	--	--	--	0.2	0.5	--	0.4	0.4	0.3	--	--	0.4	--	5.1	0.5	0.3	0.4	--	0.3
Total	38.0	40.1	18.6	42.4	27.4	22.5	20.8	31.3	30.9	37.9	14.7	36.2	27.8	33.7	27.0	25.1	29.4	24.9	28.4	33.1	22.8	38.6	29.9	34.2	24.3	31.4

Table 2–1. Water-quality parameters and daily stream discharge collected between August 26 and November 3, 2009, at six sites on the upper Esopus Creek, New York.

[Number of samples (*n*) = 3 for water-quality parameters at all sites except for site U–3 where *n* = 2. Site identification numbers and locations are listed in table 1. SD, standard deviation]

Parameter	Site identification number											
	U–2		U–3		U–3a		D–3b		D–4a		D–6	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Nitrate, in micromoles per liter	7.5	4.7	7.9	5.3	6.7	3.5	8.4	2.9	7.3	1.4	5.7	0.5
pH	7.00	0.29	7.06	0.28	7.10	0.19	7.16	0.15	7.18	0.18	7.22	0.21
Specific conductance, in microsiemens per centimeter at 25 degrees Celsius	49.3	10.7	48.4	7.6	53.1	8.8	61.6	14.2	56.9	13.3	61.7	12.2
Stream discharge, in cubic meters per second	1.5	1.6	2.1	2.1	2.2	2.3	8.9	3.0	12.3	6.5	14.5	9.2
Total suspended sediment, in milligrams per liter	1.0	0.9	1.0	0.0	0.7	0.3	4.0	4.4	2.7	1.2	1.7	1.2
Turbidity, in nephelometric turbidity units	1.4	0.7	1.7	0.2	1.3	0.3	5.0	5.0	4.1	1.2	3.0	1.2

Table 3–1. Chlorophyll *a*, ash-free dry mass, and diatom assessment profile score for sites sampled on the main stem and tributaries of the upper Esopus Creek, New York.

[Number of samples per site (*n*) = 3 except diatom assessment profile scores in August where *n* = 4. Site identification numbers and locations are listed in table 1. µg/cm^2, microgram per square centimeter; mg/cm^2, milligram per square centimeter; SD, standard deviation]

Site identification number	Chlorophyll *a*, in µg/cm^2		Ash-free dry mass, in mg/cm^2		Diatom assessment profile score	
	Mean	SD	Mean	SD	Mean	SD
Sites sampled in August 2009						
U–0	0.82	0.23	0.36	0.12	7.23	0.25
U–2	2.50	1.77	0.80	0.39	7.03	0.33
U–3	2.43	1.62	1.08	0.81	7.09	0.31
U–3a	4.53	3.04	1.75	1.21	7.39	0.25
D–3b	1.97	0.35	0.78	0.13	7.20	0.34
D–4	1.84	1.01	0.67	0.26	6.50	0.24
D–4a	1.80	0.45	0.47	0.06	7.07	0.53
D–4b	4.22	1.12	0.96	0.33	7.25	0.36
D–5	3.81	1.84	1.62	0.33	7.04	0.20
D–6	3.95	0.18	1.85	0.78	7.05	0.27
T–0	4.06	1.97	1.05	0.36	7.24	0.14
T–1	5.93	2.77	1.40	0.63	6.83	0.14
T–2	9.63	1.14	1.37	0.13	7.21	0.29
T–3	3.16	1.67	0.77	0.25	6.72	0.41
T–4	6.15	3.55	1.33	0.71	6.61	0.28
T–5	6.48	2.86	0.92	0.34	6.76	0.09
T–6	3.36	0.43	0.73	0.09	7.13	0.23
T–7	2.67	0.16	0.62	0.08	7.36	0.22
T–8	7.45	3.49	1.18	0.31	7.35	0.14
T–9	4.87	0.91	0.89	0.17	6.30	0.37
Sites sampled in November 2009						
U–2	11.27	5.80	2.59	1.28	6.36	0.18
U–3	12.40	1.94	2.33	0.16	6.48	0.20
U–3a	13.10	3.48	2.95	0.95	6.21	0.24
D–3b	20.77	6.66	5.98	2.54	6.51	0.31
D–4a	11.15	3.43	3.49	1.01	6.03	0.16
D–6	6.59	1.49	1.78	0.24	6.76	0.33

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