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An evaluation of periphyton community structure and function on tile and cobble substrata in experimental stream mesocosms

Rex L. Lowe[1], J. B. Guckert[2], S. E. Belanger[2], D. H. Davidson[2] & D. W. Johnson[1]
[1]*Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403, USA*
[2]*Environmental Science Department, Ivorydale Technical Center, The Procter and Gamble Company, 5299 Spring Grove Ave., Cincinnati, OH 45217, USA*

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Abstract

Periphyton communities are an ecologically important source of information when conducting experimental stream ecotoxicological studies. Experimental stream studies often utilize unreplicated systems in order to understand dose-response phenomena. The objective of this study was to examine the robustness of such a dose-response investigation. Autotrophic and heterotrophic periphyton were evaluated in replicated control experimental streams with open recruitment (once-through flow design). Measurements of population and community structure and community function over an eleven week colonization period were made on unglazed clay tile and cobble substrata. Several measures were significantly different during the study; however, most of these were a result of initial stochastic colonization events or associated with relatively rare algal populations. During the latter half of the study autotrophic and heterotrophic community measurements on tile and cobble substrata displayed similar trends in both control streams. Population and community metrics were significantly different for approximately 5% of the 400 measurements made after the initial sampling. These results strongly suggest that the driving forces behind structuring aquatic population and community responses will be test chemical exposure and not spuriously developed stream ecosystems with individual trajectories. Recruitment in these open systems is evenly distributed across the replicated experimental stream ecosystems.

Introduction

Periphyton have been employed extensively as water quality monitors (Whitton et al., 1991). The small size, quick developmental response, physiological and taxonomic diversity, and cosmopolitan distribution of periphyton make it an ideal community for evaluating environmental quality of aquatic habitats. It is critical, however, to control certain confounding environmental variables when assaying periphyton community response to target variables. For example, if one is measuring the community response of periphyton to the concentration of a dissolved chemical, it is essential to hold other potentially influential environmental parameters such as light intensity, current speed and substratum texture constant. The nature of the stream substratum is of critical importance to periphyton community development (Miller et al., 1987; Gale et al., 1979; Tuchman & Stevenson, 1980). Substrata that differ physically and chemically support distinct species assemblages (Pringle, 1985). The diversity of substrata in a natural stream may include plants, silt, sand, cobble, wood, etc., providing a variable and patchy environment for periphyton community development. It may be confusing to evaluate periphyton community response to changes in water quality across such variable substrata. The Diatometer® (Patrick van Hohn, 1956) or other standard artificial substrata (Aloi, 1990) have been successfully employed in natural streams to reduce substratum variability and allow biologists to evaluate more clearly periphyton community response to changes in water chemistry.

Stream mesocosms are becoming increasingly available and popular as tools for examining the response of aquatic biota to environmental stimuli (McIntire, 1968; Steinman & McIntire, 1986; Planas et al., 1989; Lamberti & Steinman, 1993; Belanger et al., 1994). It is possible in replicated stream mesocosms

to closely regulate environmental variables such as the substratum texture, manipulating one or two variables. In 1991, a comprehensive ecosystem-level investigation of aquatic population and community responses to C_{12}-Alkyl Sulfate (AS) was conducted at the Procter & Gamble Experimental Stream Facility (ESF). Autotrophic and heterotrophic periphyton, protozoan, and macro invertebrate communities were evaluated simultaneously. As part of the research to appraise periphyton community structure and functions in the ESF stream mesocosms, tile and cobble were evaluated for their suitability as substrata. In order to further appraise periphyton community replicability prior to and during the eight week period of exposure to AS, control periphyton communities from two streams not exposed to AS were evaluated in detail. The objective was to determine the structural and functional differences of periphyton collected from tile and cobble through an 11 week colonization period in the experimental stream facility.

Table 1. Background water quality characteristics of Lower East Fork of the Little Miami River water delivered to the P&G Experimental Stream Facility ($n = 9$)

Water Quality Parameter	Mean ± SD ($mg\ l^{-1}$)
Total Alkalinity	136±60
Ammonia-Nitrogen	0.8±0.4
Total Kjeldahl Nitrogen	1.5±1.8
Nitrate-Nitrogen	1.5±1.0
Ortho-Phosphorus	0.17±0.08
Total-Phosphorus	0.23±0.08
Dissolved Organic Carbon	6.2±3.2
Total Organic Carbon	4.8±0.9
Chloride	20±5
Sulfate	45±7
Potassium	7±6
Magnesium	12±4
Sodium	13±5

Materials and methods

The ESF is located on the Lower East Fork of the Little Miami River, a fifth order tributary to the Little Miami River. The Lower East Fork, which is 132 km long and drains an area of approximately 1,297 km^2, is classified as an exceptional warmwater fishery by the Ohio EPA using Ohio regional criteria (OEPA, 1985). The mainstem of the Little Miami River is classified as a state and national scenic river (Benke, 1990). The Clermont County Lower East Fork Regional Wastewater Treatment Plant is adjacent to the ESF. The treatment plant employs rotating biological contactor technology to process secondarily treated wastewater, dominated by domestic inputs, for discharge to the Lower East Fork. The ESF has the capability to obtain water from Lower East Fork and from various points in the wastewater treatment plant for use in experimentation. A detailed description of the Experimental Stream Facility and the Lower East Fork of the Little Miami River is presented in Belanger et al. (1994). Lower East Fork dissolved oxygen is typically near saturation. Temperature, pH and conductivity ranges (mean daily values) for the present study were 25.4–14.5 °C, 7.9–8.3 and 193–336 μmhos. Water quality varied seasonally from summer through fall. Table 1 summarizes other relevant water quality parameters.

Water flows from access points at the river or at the wastewater treatment plant to head tanks in the ESF building, where it is then distributed to individual streams. An on-site computer system utilizing CAMM Solutions, Inc. process control, data acquisition, and logging software with μMac mini-computer hardware controls and monitors critical environmental conditions in the ESF. Systems under computer control include water flow and distribution, light cycle and light intensity. Monitoring functions include water quality (pH, temperature, dissolved oxygen and conductivity), light intensity and water flow. An automatic alarm system identifies the condition of system functions which are potentially critical to the success of the experiment. These include river water intake pump status, flow to the ESF and individual streams and chemical dosing system status (Belanger et al., 1994).

The ESF contains eight replicate stream channels. Stream channels used in ecosystem studies are 12 m long and consist of five sections: a head box (142 l), a 4.3 by 0.29 m reach at 1% slope used primarily in microbial periphyton studies, a 1.0 m flare which leads to a 4.3 m by 0.53 m reach at 5% slope used for invertebrate and microbial periphyton studies, and a 1.2 by 0.6 m tail pool of 568 l. The tail pool accommodates automated water quality sensors and selected studies of protozoan communities and single species bioassays. Each stream receives unfiltered river water in a once through design. Periphyton communities were evaluated in the upper reach of each channel. An array (three columns by 44 rows) of terracotta clay tiles (8826.8

± 58.0 mm^2) were placed into the 1% slope region in each stream on 29 July, 1991.

Invertebrate communities are normally evaluated in the lower reach of the stream channels (Belanger et al., 1994, 1995a, 1995b) but in this investigation periphyton were also sampled from cobbles in lower reach for comparison to tile communities. The sampling unit used in this stream section was a polypropylene tray (dimensions of 26.0 cm long, 15.9 cm wide, 6.4 cm deep). Forty-five trays arrayed in 3 columns by 15 rows were placed in each stream. Trays were held in place by a lexan holder machine grooved to provide a near water tight seal between trays and the holder. Thus, water flows over the surface and percolates through the trays. Trays were filled with 2.0 cm gravel substratum prior to colonization. Interstitial spaces (i.e., between individual pieces of gravel) tend to fill with finer settled solids carried by river water during the course of experiments. At the intersecting corners of trays a 25.0 cm^2 square tile was placed to break up the flow of water which tends to channel along the edges of the trays. Water depth ranged from 2.0–3.0 cm through the cobble reach.

Tile and cobble sample collection

A synopsis of the sampling and experimental design is provided in Table 2. Figure 1 details the process of obtaining periphyton community data from sampled substrata. Tile and cobble substrata were sampled after 3, 4, 5, 7, 9 and 11 weeks of colonization from control streams 3 and 6 (hereafter referred to as such in tables and figures). Sampling locations of tile and cobble samples in each stream followed a stratified random sampling design developed using SAS (1990). Tiles were sampled and replaced with new tiles which were not to be resampled. Cobble trays were sampled more than once for periphyton, however, different pieces of cobble were selected on each sampling event. To accomplish this, trays were visually divided into right and left hand sides. One side of the trays was sampled only once during the experiment and never used for invertebrate studies. Cobble that was removed was replaced with new cobble. Sampling always proceeded downstream to upstream to minimize disturbance effects. Cobble samples from each tray were selected to approximate the two-dimensional surface area of tiles. All samples were removed from the stream mesocosm and placed into 1.5 l incubation chambers (37 cm inner diameter, 5 cm deep). Tile incubation chambers had 1.5 l of stream water from the appropriate stream

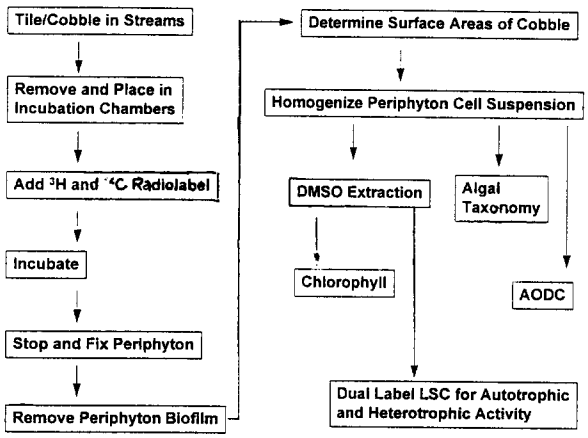

Figure 1. Diagrammatic representation of periphyton substrate sampling and experimental manipulations to derive community and population endpoints.

added to them. Cobble incubation chambers had 3.0 l of stream water to compensate for the occasionally greater substratum diameter of cobble. Tiles and cobbles were removed non-destructively and placed into the appropriate chamber immediately. Five replicate tiles and five cobble composites approximating a tile in area were taken from each of the two streams on each data for study.

Radiolabel incubation

Periphyton were allowed to acclimate in incubation chambers for 1.5–2.0 h prior to the start of radiolabel incubation. Calibrated Masterflex pumps (Cole Palmer Company, Niles, Illinois) recirculated water in each chamber at approximately 1.0 l min^{-1}. Temperature was controlled by placing chambers in a large water bath receiving ambient river water at 166 l min^{-1}. Temperature was monitored during the entire test via computer-interfaced temperature sensors (Belanger et al., 1993). Illuminating during the incubation was accomplished using the ESF lighting system and monitored continuously by LiQor light sensors (Belanger et al., 1994). A dual label incubation of ^{14}C-sodium bicarbonate (56 mCi $mmol^{-1}$) and ^3H-amino acid mixture (Amersham mixture #TRK.440, average specific activity of 44 Ci $mmol^{-1}$) was used (Palumbo et al., 1987).

Radioisotope additions were made at approximately the same time (12:37 PM to 1:12 PM for tile; 1:18 PM to 1:57 PM for cobble) throughout the study during maximum lighting intensity within the building. Back-

Table 2. Synopsis of sampling dates and periphyton endpoints used to assess cobble and tile periphyton communities in control streams during the 1991 AS experiment. In all situations $n = 5$ replicate substrates.

Date	Week	Endpoints	
		Autotrophic Community	Heterotrophic Community
20 Aug	3	Taxonomic identity	AODC (bacteria)
26 Aug	4	Taxa richness	Uptake of ^{3}H amino acids
3 Sept	5	Species diversity	
16 Sept	7	Total cell and biovolume density	
30 Sept	9	Community similarity	
14 Oct	11	Cell and biovolume density of dominant taxa	
		Community similarity	
		Chlorophyll-*a*	
		Uptake of ^{14}C-bicarbonate	

ground counts and radiolabel exhaustion were evaluated during incubation experiments to verify experimental protocols. Comparisons of radiolabel uptake and incorporation normalized to surface area or biomass and time of incubation were used to evaluate the replicability of periphyton function across samples. Calculations of photosynthetic carbon fixation corrected for alkalinity were not used for these studies. The alkalinity result would have applied equally to all data for a given week and therefore would not affect interpretations.

Preservation and removal of periphyton

After incubation, samples were transferred into ziplock bags with 10 ml of 3% glutaraldehyde solution and transported back to the laboratory for processing. Samples were stored at 4 °C until periphyton was removed from the substratum. Periphyton was scraped from tiles with a new razor blade and washed into a vial with fixative. Periphyton was removed from each cobble replicate with a stiff-bristle toothbrush and washed with fixative into a vial. Final volumes were usually 20 ml for tiles and 30 ml for cobbles.

Cobble surface area was determined using an aluminium foil wrapping technique. Foil was wrapped around each cobble to a unit layer thickness, removed, and weighed on a Mettler PM 200 4-place analytical balance. Regressions of aluminium foil weight versus known surface area was used to then estimate the surface area of cobble. Surface areas of small pieces of nearly spherical cobble were determined by a volume displacement technique. In all, 1562 pieces of cobble were brushed. Of these 689 were evaluated by the aluminium foil technique and 873 by volume displacement. Nearly all samples were a combination of both methods. The average cobble surface area for 420 samples was 11 401 $\pm$ 3596 mm^2 versus the tile surface area of 8827 $\pm$ 56 mm^2. Periphyton density values were normalized to the average surface area of the tiles; however, cobble periphyton values were normalized to the individual replicate surface area.

Periphyton endpoints

Aliquots of each sample were used to evaluate periphyton for various endpoints. In all cases, each periphyton endpoint was derived from the same sample (tile or cobble) unless otherwise stated. Samples were evaluated for algal community structure by the methods described in Belanger et al. (1994). Identifications of soft algae were made using a Palmer-Maloney counting chamber. Approximately 500 algal units were identified. Diatom densities at the generic level were analyzed in the counting chamber and species were later identified from cleaned diatom samples mounted in Naphrax (Northern Biological Supply, Ipswich, Great Britain). From these enumerations, species richness, species relative abundance and density (cell and biovolume), Shannon-Weaver species diversity (Shannon & Weaver, 1949) were derived. Taxa were considered dominant if they comprised an average of 1% or more of the total cell density or biovolume on any given week.

Bacterial cell counts were conducted using the acridine orange direct count method (Standard Method

9216B, APHA et al., 1989). Counts were normalized to substratum surface area. Aliquots of cell suspensions were extracted with DMSO to obtain material for chlorophyll and radioisotope uptake analyses (Mulholland et al., 1986; Palumbo et al., 1987). Periphyton suspensions were concentrated by centrifugation (1500 *g* for 15 min). Overlying supernatant was removed and 5 ml of DMSO was added to the periphyton pellet. The tube was vortexed, and the entire contents poured into a Liquid Scintillation Counter (LSC) vial. An aliquot of the DMSO-periphyton suspension was extracted with 90% acetone, mixed, and removed to a cuvette for reading on a HP 8452A Diode Array Spectrophotometer (Hewlett Packard, Palo Alto, CA) at 664 and 750 nm. The sample was then acidified with 100 μl 0.1N HCl, mixed, and allowed to set for 90 s and re-read on the spectrophotometer. Calculations of chlorophyll-*a* and phaeophytin-*a* were made by the formulae according to Standard Method H.2.b (APHA) et al., 1989). The remaining suspensions not analyzed for chlorophyll were allowed to continue DMSO extraction in a 65 °C oven overnight. Once cooled to room temperature, a 1.0 ml aliquot of the extract was put into a new LSC vial and 10 ml of Ultima-Gold XR LSC cocktail was added. A dual label analysis (^{14}C and ^{3}H) was conducted on a Packard model 2500 TR LSC (Hewlett Packard, Palo Alto, CA) using a transformed external standard spectrum quench correction method (Cauter and Roessler, 1991; Kessler, 1991). The Packard 2500 TR uses a windowless field spectrum dpm analysis to achieve accurate dual label evaluations. Expressions of periphyton activity were as dpm of label/min/mm^2 of surface area.

Data manipulation and statistical analysis

Data were first evaluated for equality of variance by Bartlett's Test for Homogeneity (Winer, 1971). Normally distributed data were analyzed parametrically by *t*-test, and data that were not normally distributed were evaluated using Wilcoxon's Rank Sum (Bhattacharya & Johnson, 1977; Holland & Wolfe, 1973). Similarity analyses were conducted using the permutation test described by Smith et al. (1990). The number of endpoints statistically evaluated from each sampling event varies due to varying numbers of dominant algal taxa across weeks.

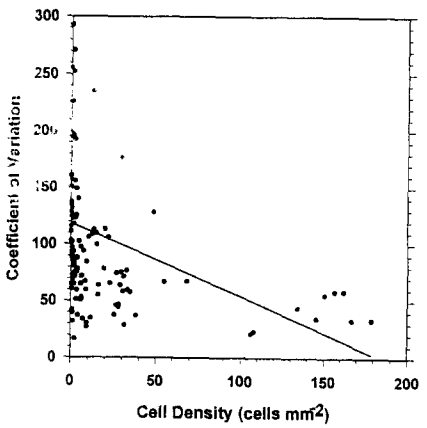

Figure 2. Relationship between algal population density and the coefficient of variation. $n = 5$ for all samples.

Results

General statistical trends

Relatively few differences were found when comparing periphyton communities in replicate streams. The greatest number of differences were found during Week 3, the first sampling date (Table 3). Eighteen of 40 endpoints were significantly different on tiles, but only two of 34 endpoints were different for cobbles. The majority (15) of the combined tile and cobble differences were due to differences in specific algal population responses (cells/mm^2 and μm^3/mm^2 of dominant species). By week 4, such differences were almost absent. Only 2 of 48 population level and 4 of 32 community level comparisons for tile and cobble substrata were significantly different. This pattern of relatively few differences was consistent through week 11. By the conclusion of the study 480 endpoints at the population and community level for autotrophic and heterotrophic periphyton were compared (Table 4). A total of 47 significant differences were noted (a total of 9.8%) for all weeks, but 42% of these occurred on week 3. Excluding week 3, significant differences were found only 5.6% of the time. This 5.6% value is similar to the number of significant differences that would be observed by chance alone given our 0.05 *p*-value for statistical significance.

Significant algal population-level differences were often associated with the less abundant taxa. To more fully evaluate this phenomenon, the coefficient of variation (CV), the ratio of the standard deviation and the mean expressed as a percent, was plotted against the

Table 3. Summary of periphyton parameters on week 3 of colonization at the Experimental Stream Facility. Sample size = 5 for each group. Means ± 1 SD are given for each group. The statistic used is given following the p-value in parentheses. T and W indicate t-test and Wilcoxon Rank Sum, respectively.

Endpoint (units)	Tile			Cobble		
	Stream 3	Stream 6	p-value	Stream 3	Stream 6	p-value
Community Level Analyses:						
AODC (cells × 10^4/mm^2)	8.17±4.17	9.38±3.06	0.613 (T)	40.06±20.59	39.90±6.53	0.841 (W)
Uptake of ^{3}H amino acids (dpm/min/mm^2)	0.12±0.03	0.048±0.008	0.013 (W)	0.280±0.149	0.286±0.098	0.950 (T)
Uptake of ^{3}H amino acids (dpm/min/cells × 10^6)	1.58±0.31	0.54±0.15	0.001 (T)	0.793±0.490	0.722±0.256	0.782 (T)
Chlorophyll *a* (μg/mm^2)	10.3±1.3	12.5±6.0	0.819 (W)	84.0±124.0	7.0±0.0	0.645 (T)
Uptake of ^{14}C-bicarbonate (dpm/min/mm^2)	0.023±0.009	0.019±0.003	0.449 (T)	0.037±0.005	0.037±0.009	0.937 (T)
Uptake of ^{14}C-bicarbonate (dpm/min/ng chl-a)	2.19±0.81	1.93±1.12	0.690 (T)	2.63±2.80	6.70±0.0	0.335 (T)
Uptake of ^{14}C-bicarbonate (dpm/min/cell × 10^4)	1.89±0.49	2.49±0.36	0.059 (T)	9.96±2.22	11.8±2.9	0.294 (T)
Uptake of ^{14}C-bicarbonate (dpm/min/μm^3 × 10^{-7})	5.58±1.04	7.07±1.39	0.091 (T)	44.6±9.3	56.9±12.8	
Algal taxa richness/sample	22.0±2.1	26.8±6.4	0.149 (T)	12.8±2.2	12.4±1.1	0.735 (T)
Algal species diversity (cell density data)	2.19±0.20	2.06±0.20	0.314 (T)	1.22±0.31	1.18±0.27	0.830 (T)
Algal species diversity (biovolume density data)	2.19±0.22	2.03±0.25	0.320 (T)	1.60±0.27	1.55±0.21	0.655 (T)
Algal total cell density (cells/mm^2)	120.0±30.0	79.0±18.4	0.033 (T)	38.2±5.8	34.4±11.9	0.532 (T)
Algal total biovolume density (mm^2)	40200±11700	28400±8700	0109 (T)	8400±900	7000±2300	0.245 (T)
Algal/bacteria biomass (AODC/ng chlorophyll)	0.79±0.37	0.87±0.51	0.768 (T)	2.47±2.31	6.24±0.0	0.293 (T)
Algal/bacteria activity ratio (aerial basis)	5.73±2.02	2.47±0.16	0.008 (W)	8.15±6.14	7.54±1.81	0.421 (W)
Algal/bacteria activity ratio (biomass basis)	0.81±0.32	0.34±0.14	0.019 (T)	2.06±3.30	0.10±0.0	0.658 (T)
Population Level Analyses						
Biddulphia laevis (cells/mm^2)	0.0±0.0	0.0±0.0	--	0.0±0.0	0.0±0.0	--
Cocconeis pediculus (cells/mm^2)	0.46±0.38	0.04±0.08	0.050 (W)	0.0±0.0	0.0±0.0	--
Cocconeis placentula (cells/mm^2)	34.38±16.77	20.64±3.76	0.310 (W)	25.70±6.12	22.42±7.73	0.478 (T)
Diatoma vulgare (cells/mm^2)	0.0±0.0	0.18±0.20	0.151 (W)	0.0±0.0	0.0±0.0	--
Gomphontrea parvulum (cells/mm^2)	3.84±2.38	0.92±0.34	0.008 (W)	0.52±0.17	0.58±0.10	0.540 (T)
Melosira varians (cells/mm^2)	6.38±4.05	1.0±0.45	0.008 (W)	0.34±0.54	0.18±0.40	0.612 (T)
Navicula cryptocephala (cells/mm^2)	1.22±1.43	6.06±4.29	0.044 (T)	0.80±0.25	0.72±0.40	0.720 (T)
Navicula salinarum (cells/mm^2)	1.36±1.63	1.92±1.42	0.590 (T)	0.0±0.0	0.0±0.0	--
Navicula symmetrica (cells/mm^2)	11.64±3.81	11.80±4.28	0.952 (T)	2.88±0.66	1.90±0.74	0.059 (T)
N. tripunctata v. *schizonemoides* (cells/mm^2)	9.58±13.17	22.74±6.13	0.077 (T)	2.36±0.79	1.58±0.71	0.141 (T)
Nitzschia dissipata (cells/mm^2)	8.78±4.12	3.22±1.81	0.025 (T)	0.0±0.0	0.0±0.0	--
Nitzschia palea (cells/mm^2)	2.58±0.32	1.88±0.40	0.017 (T)	0.58±0.28	0.12±0.21	0.021 (T)
Merismopedia sp. (cells/mm^2)	0.0±0.0	0.0±0.0	--	0.92±0.87	1.90±1.68	0.282 (T)
Rhodochorton violacea (cells/mm^2)	0.0±0.0	0.0±0.0	--	3.32±2.28	4.10±5.25	0.769 (T)
Schizothrix calcicola (cells/mm^2)	16.34±2.24	2.24±0.26	0.008 (W)	0.0±0.0	0.0±0.0	--

mean for all populations analyzed throughout the study (Figure 2). A clear, negative relationship between the CV and the mean cell density was observed with a correlation coefficient of -0.35 ($p < 0.001$). Therefore, as population density increased, the variation observed in the cell count declined. This relationship suggests that it would become 'easier' to detect significant population-level differences when populations (eventually translated to cell density) of a given taxon increased. The populations with the greatest density, *Cocconeis placentula* and *Navicula tripunctata* var. *schizonemoides* throughout the study, and taxa such as *Navicula symmetrica* and *N. cryptocephala* later in the study, were rarely significantly different when comparing the control streams. Given the relative lack of significant differences, additional comments on an endpoint-by-endpoint basis will not be developed here. The focus of the rest of the results will relate to overall trends in community and population development.

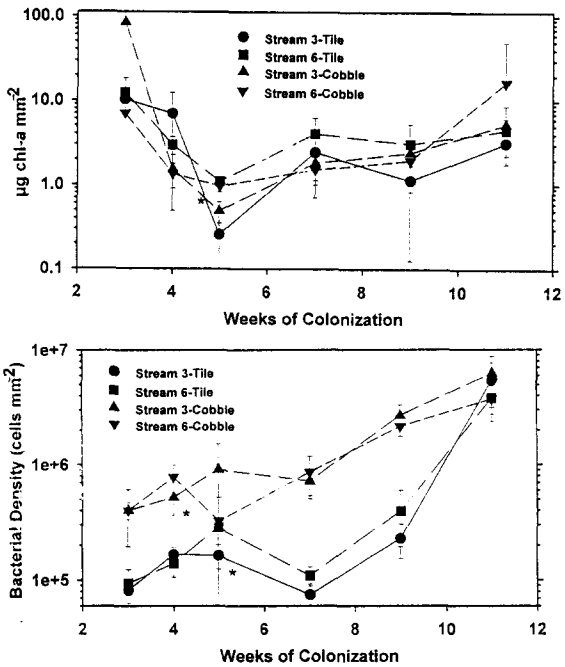

Figure 3. Heterotrophic biomass on tile and cobble estimated by AODC counts (upper graph) and algal biomass on tile and cobble estimated by cell density (lower graph).

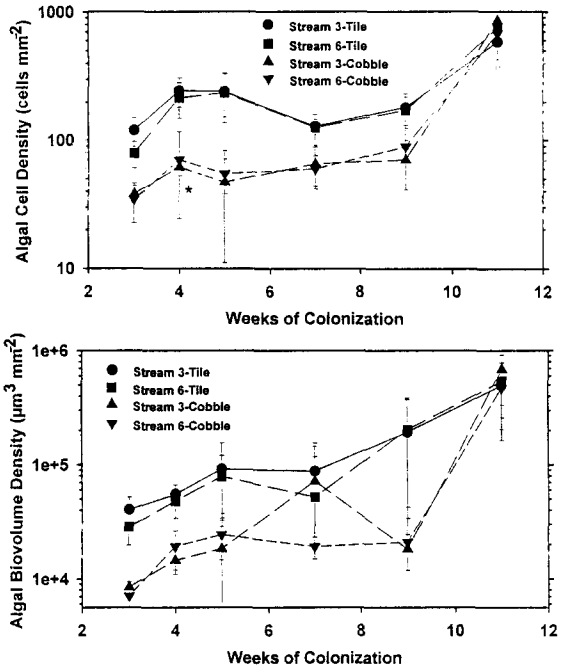

Figure 4. Algal cell density (upper graph) and biovolume density (lower graph) on tile and cobble.

Table 4. Incidence of significant differences in control tile and cobble periphyton end points from the Experimental Stream Facility.

Weeks of exposure	*n* of endpoints	Significant differences	% Significant differences
3	74	20	27.0
4	80	6	7.9
5	82	9	11.0
7	82	8	9.8
9	68	3	4.4
11	94	1	1.1
Sum of all weeks	480	27	9.8
Sum of all weeks – week 3	406	27	6.7

Periphyton biomass

Both bacterial and algal periphyton increased in biomass throughout the study. Bacterial periphyton densities increased an order of magnitude on tile and cobble (Figure 3). Biomass trends were consistent for both streams. Algal periphyton biomass was measured using chlorophyll-*a*, cell density, and biovolume density (Figures 3–4). Trends of all three estimators of algal biomass were consistent with increasing biomass accrued over time, particularly on week 11. Because chlorophyll-estimated biomass was close to detection limits, cell density and biovolume may be more accurate estimators of change through time. Week 11 tile samples were also evaluated for lipid biomass (total, neutral, polar and glycolipids of bacteria and algae) (Table 4). Relative to other biomass indicators it appears that lipid biomass was lower in variance. In spite of low variance, bacterial and algal communities were not significantly different for any of the 10 biomass endpoints considered.

Periphyton activity

Bacterial community activity increased on an areal basis throughout the study on tiles and was comparatively less variable and had smaller inter-stream differences than bacterial activity on cobble (Figure 5). When activity was adjusted on a biomass basis (cell density) cobble samples generally had somewhat lower activity than tiles in both streams. Algal community activity on an areal basis steadily increased throughout

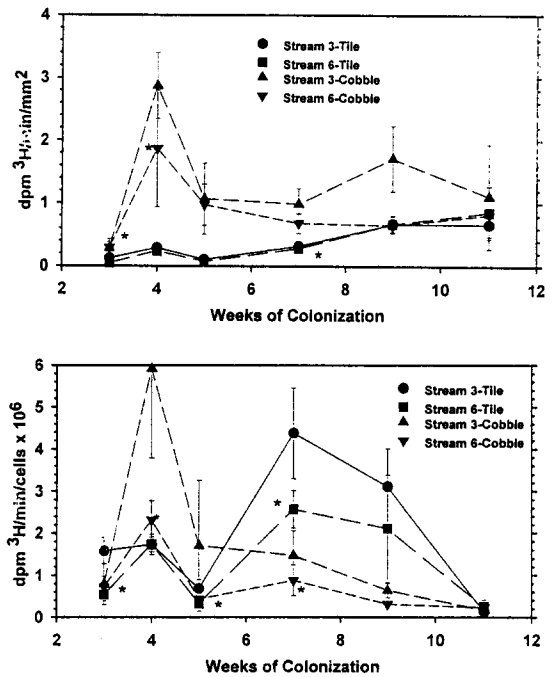

Figure 5. Heterotrophic activity on tile and cobble as measured by ^{3}H-amino acid uptake on an aerial basis (upper graph) and scaled by cells density (lower graph).

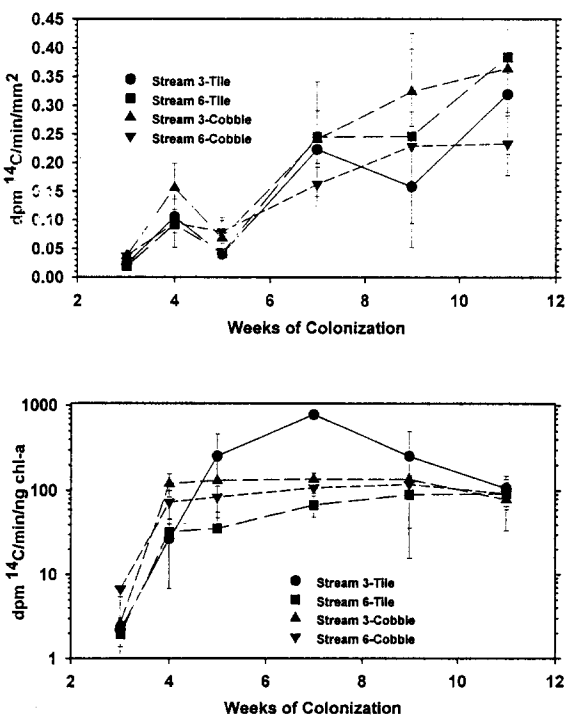

Figure 6. Algal activity on tile and cobble scaled by surface area (upper graph) and chlorophyll biomass (lower graph).

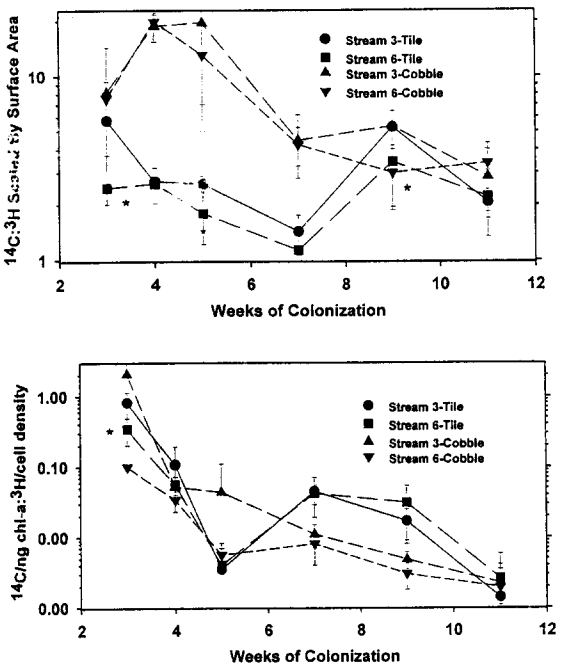

Figure 7. Algal: bacterial activity on tile and cobble scaled by surface area (upper graph) and microbial biomass (lower graph).

the study on both tile and cobble (Figure 6). Replicability between streams was high as shown by similar trends. Activity was relatively constant through the study when scaled by chlorophyll biomass, with very similar values for tile and cobble in both streams with the exception of an apparently spurious value from stream 3 tile on week 4. The same trends (relatively small changes over the duration of the study) for algal activity scaled by cell density and biovolume were found (data not shown). The relative changes of bacterial and algal activity through time can be evaluated by investigation of the ratio of the two metrics. Bacterial:algal activity on tiles was constant on an areal basis (Figure 7). Bacterial:algal activity on cobble was initially higher, but declined to levels observed on tiles by week 9. Bacterial:algal activity scaled on the basis of cell density (bacteria and chlorophyll-*a* (algae) was highest on week 3 and declined thereafter with no perceptible difference between cobble and tile (Figure 7).

Algal community structure

Algal taxon richness in stream 3 versus 6 was very consistent on both tile and cobble (Figure 8). Richness was fairly stable throughout the study, especially on tile. By week 11, richness on cobble increased from week 9.

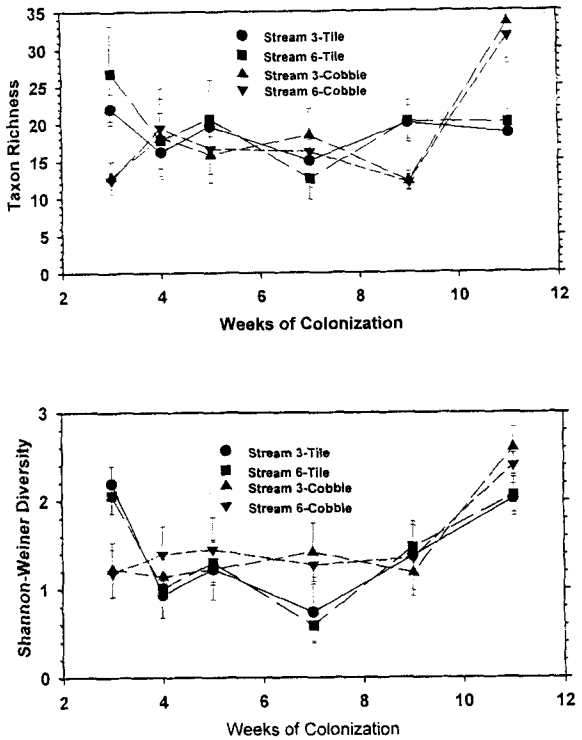

Figure 8. Algal species richness (upper graph) and diversity (lower graph) based on cell density on tile and cobble.

The increase may reflect additional habitat complexity associated with increased bacterial density noted in the same time period. Algal diversity was evaluated based on cell density and biovolume (Figure 8, cell density only). Trends were similar for both: diversity was initially higher, declined through the early stages of the study, and increased again towards the end of the experiment. Tile and cobble communities were similar and highly replicable in both streams.

Algal populations

Biddulphia (= Pleurosira) laevis, Cocconeis placentula, Navicula tripunctata var. *schizonemoides, N. symmetrica*, and *N. cryptocephala* were biovolume dominants. Of these, *Biddulphia* was numerically the least common (lowest in cell density), but due to its large biovolume of 10 000 μm^3, this taxon became a biovolume dominant. *Biddulphia* increased throughout the study, particularly in the last two weeks (Figure 9) and was highly variable due to the low cell density (see Figure 2 and discussion therein). *Cocconeis placentula* was a numerical and biovolume dominant (Figure 9).

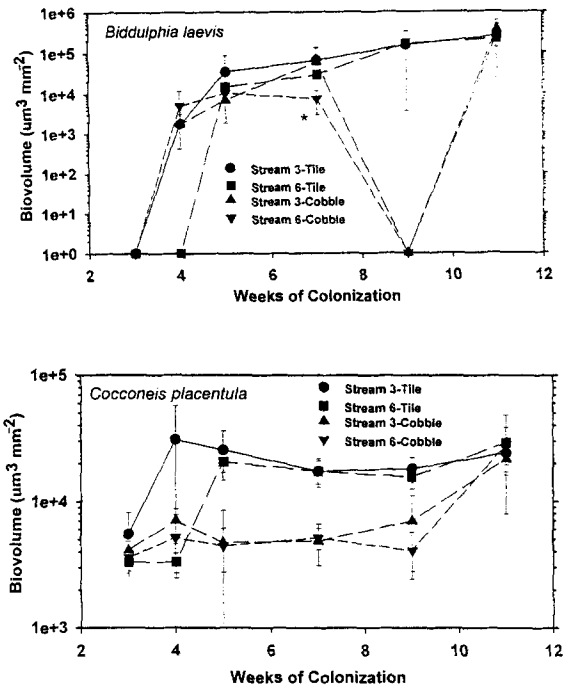

Figure 9. Biovolume density on tile and cobble for *Biddulphia (= Pleurosira) laevis* (upper graph) and *Cocconeis placentula* (lower graph).

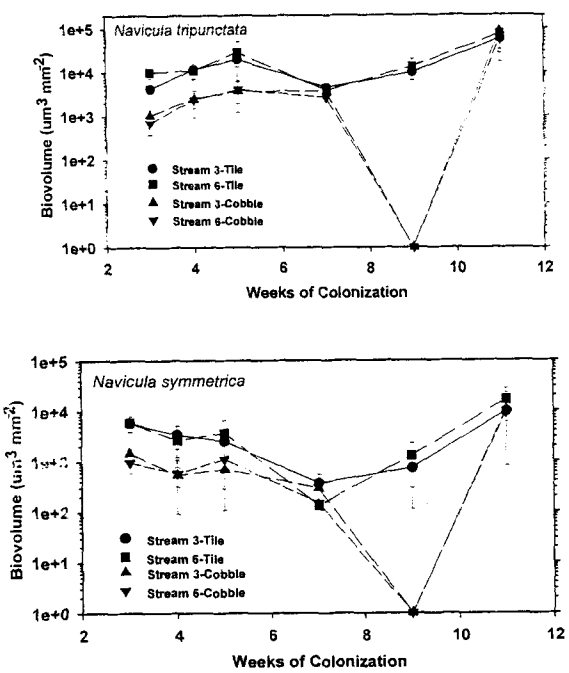

Figure 10. Biovolume density on tile and cobble for *Navicula tripunctata* v. *schizonemoides* (upper graph) and *Navicula symmetrica* (lower graph).

Table 5. Lipid biomass (total, neutral, polar and glycolipids) of bacteria and algae from week 11 tile samples at the Experimental Stream Facility. Sample = 5 for each group. Means ± 1 SD are given for each group. All comparisons used a *t*-test.

Endpoint (units)	Stream 3	Stream 6	*p*-value
Bacterial total lipid (^{3}H dpm/min/mm^2) × 10^{-3}	37.12±12.30	46.34±11.15	0.250 (T)
Bacterial neutral lipid (^{3}H dpm/min/mm^2) × 10^{-3}	1.92±1.06	2.08±0.94	0.806 (T)
Bacterial glycolipid (^{3}H dpm/min/mm^2) × 10^{-3}	17.80±8.73	19.26±7.79	0.787 (T)
Bacterial polar lipid (^{3}H dpm/min/mm^2) × 10^{-3}	5.50±3.41	4.82±1.66	0.699 (T)
Bacterial membrane: storage lipid ratio	0.29±0.05	0.25±0.01	0.421 (W)
Algal total lipid (^{14}C dpm/min/mm^2) × 10^{-3}	73.40±24.70	77.70±28.50	0.805 (T)
Algal neutral lipid (^{14}C dpm/min/mm^2) × 10^{-3}	8.40±3.97	10.00±4.21	0.554 (T)
Algal glycolipd (^{14}C dpm/min/mm^2) × 10^{-3}	55.84±17.82	60.98±22.27	0.698 (T)
Algal polar lipid (^{14}C dpm/min/mm^2) × 10^{-3}	3.48±1.55	2.80±5.00	0.841 (W)
Algal membrane: storage lipid ratio	7.41±1.46	6.91±2.32	0.699 (T)

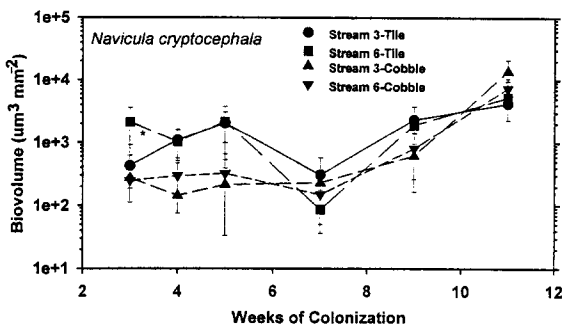

Figure 11. Biovolume density on tile and cobble for *Navicula cryptocephala.*

The density of this taxon was generally greater on tile, although by week 11 there were no density differences compared to cobble. *N. tripunctata* and *N. symmetrica* (Figure 10) were also relatively stable in biovolume density with greater densities on tile throughout the study except on week 11 where cobble and tile were similar. *N. cryptocephala* was initially greater in density on tiles, but by week 11 was greater in density on cobble (Figure 11). With few exceptions, dominant algal populations were highly similar in both streams regardless of substratum type leading to the conclusion that streams were replicable at the level of populations. Recruitment into the stream mesocosms was evaluated by algal drift evaluations (Belanger, Rupe, McCormick & Cairns, unpublished) conducted during protozoan investigations. In that study, algal drift was evenly distributed across all streams. Combined with the large volume of river water containing microbial propagules delivered to each stream, differences are minimized.

Discussion

Autotrophic and heterotrophic periphytic community structure and function were highly similar in replicate stream mesocosms on both tile and cobble substrata. Forty significant differences were found in 480 different endpoint evaluations (all endpoints, all times). Of these, most were on week 3, the first sampling week, and associated with population-level differences. Significant differences at the population level were most often associated with small algal populations that exert little influence on density and diversity, and these differences may be an artifact of a chance encounter of a rare population during algal enumeration. Coefficients of variation were lowest for large populations. Dominant populations in stream 3 versus 6 were rarely significantly different. It is likely that these population-level differences are associated with the stochasticity of initial colonization events. This stochasticity is more intense on tile substrata than on cobble. As periphyton communities mature they are more strongly influenced by endogenous processes than by exogenous colonization influences. By week four and for the remainder of the experiment there was less than a 6% difference in periphyton endpoints between replicate streams; the mesocosms are near the 95% confidence level for replicability.

The information used to describe control stream mesocosms substantially supports the conclusion that vulnerabilities associated with using non-replicated treatment streams are relatively small. Developing dose-response relationships to toxicants has been the objective of numerous stream microcosm and mesocosm studies. In the study of anionic surfactant expo-

sure to stream communities at the ESF, only control streams were replicated while treatments were not. The power of exposure-response experimental designs more than offsets concerns about the use of unreplicated doses (Guckert, 1994; Graney et al., 1989). Replication, which is the standard in typical ANOVA experimental designs, necessarily sacrifices the range of potential exposure concentrations available to the researcher (Kosinsky, 1989).

Other researchers have investigated variability in microbial communities in microcosms and stream mesocosms. Pratt & Bowers (1992) reported that taxon richness of protistan communities in flow-through laboratory microcosms had the lowest CV of 15 different community structure and function measures. Richness had the greatest statistical sensitivity for all endpoints given equal sample sizes and is believed to generally be a sensitive ecological indicator of effects. McCormick et al. (1991) evaluated protozoan communities replicated in six streams at the ESF and found similar results.

Genter & Amyot (1994) and Kosinsky (1984) used highly replicated microcosms to assess variability during toxicant-induced stress. Microcosms were partially or completely re-circulating with no additional colonization. To counter-act the potential spurious findings associated with system divergence, test system and sample replication were increased at the expense of understanding dose-response relationships. The driving forces behind structuring aquatic population and community responses are likely to be test chemical exposures and not spuriously developed stream ecosystems with individual trajectories. The reason for this is probably because the test system remains open during the entire study, with substantial levels of recruitment that are evenly distributed across the stream mesocosms, eliminating structural and functional drift of communities that might occur in closed systems.

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