COMPARISON OF CLAY TILE, STERILIZED ROCK, AND NATURAL SUBSTRATE DIATOM COMMUNITIES IN A SMALL STREAM IN SOUTHEASTERN MICHIGAN, USA

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Abstract

Periphytic diatom communities on clay tile and sterilized rock substrates were compared with those on natural substrates. Clay tiles yielded diatom communities with the least variability between replicate samples, and the greatest similarity to the natural substrate assemblage. The advantages of utilizing introduced substrate communities and problems encountered with their simulation of the natural substrate community are discussed.

Introduction

Substrates introduced into a body of water for biotic colonization have been used extensively to characterize algal communities inhabiting aquatic systems. They have been used for studying colonization, succession, productivity dynamics, and pollution assessment, as well as for strict taxonomic surveys of algae in aquatic habitats. Many types of introduced substrates have been used in the past and most of the early literature has been reviewed by Sladeckova (1962) and Wetzel (1964). In recent years, glass has been the substrate used most extensively. However, some studies have demonstrated that glass does not always give an accurate representation of the natural community (Foerster & Schlichting, 1965; Tippett, 1970; Brown, 1976; Siver, 1977; Tuchman & Blinn, 1979).

An adequate introduced substrate should possess two qualities. First, it must yield reproducible results. That is two or more substrates of the same type sampled on a given date should have similar algal composition and abundances. Second, it should accurately simulate the natural substrate community. Community composition and abundance on the introduced and natural substrates should be similar.

This study evaluated two seldom used introduced substrates, clay tiles and sterilized rocks with respect to the two characteristics. Gumtow (1955) placed cleaned rocks in a riffle of a stream in Montana, U.S.A., and found no

major difference in abundances or species composition between the treated rocks and the natural or control rocks. Blum (1954, 1957) used sterilized rocks to observe algal colonization and succession in the Saline River, Michigan. Hanic & Pringle (1978) used clay designs as a substrate involved in transporting macrophytic algae from the laboratory to the sea for culturing purposes. Adey & Vasser (1975) used ceramic tile to study marine coralline algae. This substrate type has been little-used in fresh-water systems.

Materials and methods

This study was conducted in Fleming Creek, a small agricultural watershed in Washtenaw County in southeastern Michigan, U.S.A. Sterilized rocks used as an introduced substrate type, were originally collected from the creek, and subsequently scrubbed and autoclaved to remove all attached material. The clay tiles were suspended vertically in the water column by a styrofoam flotation device. Both substrate types were placed in the stream on February 17, 1978 in a common location. They were placed in an area where many other rocks with natural periphyton communities could be collected. These natural rocks were collected randomly from the stream and served as a control. All samples were collected on March 3, 1978 after a three week incubation.

The rocks, both sterilized and untreated, were sampled with a hollow 1.6 cm diameter plexiglass tube, with three rubber washers along the edge of the tube. The plexiglass tube was placed on a flat surface of the rock in the stream. A tight seal was established by the rubber washers. The rocks were then lifted out of the stream with the periphytic algae and the associated water tightly enclosed by the plexiglass tube. A knife and a brush were then employed to scrape the algae inside the tube. The resultant algae and

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water associated with it were transferred to a collecting jar. The 2 cm^2 area enclosed by the plexiglass tube was repeatedly washed until all the periphyton was removed and placed in a collecting bottle. After a rock was used once, it was discarded and not sampled again. The 114 cm^2 clay tiles were collected as a whole unit, and placed in a plastic bag underwater to avoid disturbing attached communities by bringing them above the water surface. These were then scrubbed to remove all attached material.

Two samples for each of the three treatments were subsequently cleaned for diatom analysis. Two hyrax slides were made for each sample, and two transects were counted for each slide (Fig. 1). Diatoms were examined under 1200 X magnification using a Leitz Ortholux microscope fitted with fluorite oil immersion objectives with a nominal Numerical Aperature of 1.32. At least 2500 diatom valves were counted per slide.

Results of the enumerations were tabulated for each transect. These data were coded and entered into the computer for analysis. Data were summarized as population abundances of the mean of the two transects of a slide. Population abundances of each slide were designated as subsample communties (Fig. 1).

The population abundances of the 20 most common taxa, total assemblage abundance and species diversity (Shannon & Weaver, 1949) for each subsample community were treated as dependent variables. The abundances were transformed to the natural log scale in analysis of variance (ANOVA), Euclidian distance calculations and principal components analysis. Analysis of the following 20 taxa were chosen to represent the periphyton communities in the preceding community analyses: *Fragilaria vaucheriae, Synedra ulna, Meridion circulare, Diatoma tenue, D. vulgare, Cocconeis placentula* plus it varieties, *Achnanthes minutissima, Rhoicosphenia curvata, Navicula cryptocephala* var. *intermedia, N. cryptocephala* var. *veneta, N. gregaria, N. pelliculosa, N. radiosa* var. *tenella, N. tripunctata, Gomphonema olivaceum, Cymbella minuta, Amphora perpusilla, Nitzschia dissipata, N. linearis,* and *Nitzschia* spp. which included *N. gracilis, N. kützingiana* and *N. palea.* The cumulative abundance of these taxa accounted for about 99% of the total abundance of the subsample assemblages.

Results

General Characterization of the Communities.
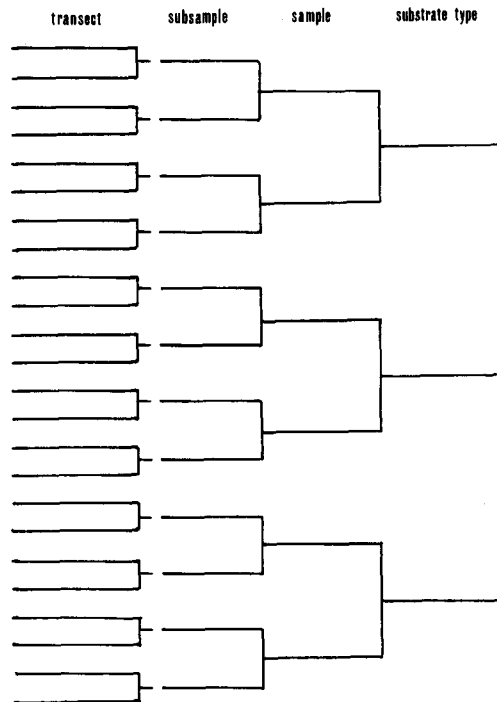In this study, 128 diatom taxa were identified, representing

Fig. 1. Schematic representation of experimental design. The use of subsample data as the lowest level in this nested design is indicated by the separation between the transect and subsample levels.

26 genera. Both introduced substrate types were dominated by *Diatoma tenue,* averaging 77.4% and 79.7% of the assemblage on the clay tiles and sterilized rocks, respectively. The other species to account for over 1% of the population on the introduced substrates were *Diatoma vulgare, Fragilaria vaucheriae, Gomphonema olivaceum, Meridion circulare, Navicula gregaria, N. pelliculosa* and *N. tripunctata.* Total densities were 5,407 and 25,728 cells/mm^2 on the two sterilized rocks, and 23,896 and 21,961 cells/mm^2 on the two clay tiles (Table 1).

The natural or control rocks were dominated by *Diatoma tenue* and *Gomphonema olivaceum.* On one of the control rocks, *G. olivaceum* was dominant averaging 27.5% of the community. On this rock, *Diatoma tenue* and *Navicula tripunctata* were secondarily important and absolute densities averaged 422,883 cells/mm^2. The second natural rock collected was dominated by *Diatoma tenue,* averaging 36.4% of the diatom assemblage and a total density of 329,872 cells/mm^2. *Diatoma vulgare, Fragilaria vaucheriae* and *Gomphonema olivaceum* consistently averaged over 10% of the community on this natural rock.

Species diversity averaged 2.278 for the natural sub-

Table 1. Diatom abundances (natural log-transformed) per mm^2 for the 20 most common taxa and for the total community. Species diversities are also included. The results of ANOVA are supplemented with Scheffé's multiple contrasts (for comparing the populations of natural rocks to the two introduced substrate types) and Tukey's pairwise contrasts (for comparing populations of the two introduced substrate types). (C.I. = Confidence Interval half-width p<0.05), (* = significant difference at p<0.05)

Taxa	Natural	Sterilized Rocks	Clay	Scheffé Multiple Contrast Contrast	C.I.	Tukey Pairwise Comparison Difference	C.I.
Achnanthes minutissima	8.480	3.612	5.335	4.006*	0.869	-1.723*	0.960
Amphora perpusilla	7.956	1.536	3.099	5.193*	1.975	-1.563*	2.183
Cocconeis placentula	7.658	1.649	2.132	5.768*	1.435	-0.483	1.587
Cymbella minuta	8.438	2.368	3.500	5.504*	3.188	-1.132	3.523
Diatoma tenue	11.402	9.145	9.782	1.939*	1.938	-0.637	2.142
Diatoma vulgare	10.474	4.951	5.194	5.402*	2.266	-0.243	2.504
Fragilaria vaucheriae	10.556	6.068	6.302	4.371*	1.754	-0.216	1.939
Gomphonema olivaceum	11.170	6.184	7.372	4.392*	2.421	-1.188	2.675
Meridion circulare	8.417	6.384	5.899	2.276*	1.404	0.485	1.552
Navicula cryptocephala var. intermedia	5.313	1.563	3.437	2.813*	1.754	-1.874	1.938
Navicula cryptocephala var. veneta	7.121	1.641	4.060	4.271*	1.223	-2.419*	1.352
Navicula gregaria	8.620	3.883	5.801	3.778*	0.908	-1.918*	1.004
Navicula pelliculosa	7.774	2.808	5.662	3.539*	0.610	-2.854*	0.674
Navicula radiosa var. tenella	7.574	1.084	2.773	5.646*	3.243	-1.689	3.585
Navicula tripunctata	10.438	4.786	6.387	4.852*	2.023	-1.601	2.236
Nitzschia dissipata	8.459	2.615	4.047	5.128*	1.929	-1.432	2.131
Nitzschia linearis	7.015	1.413	4.004	4.307*	1.204	-2.591*	1.331
Nitzschia spp.	4.466	1.453	4.380	1.550	2.461	-2.927*	2.720
Rhoicosphenia curvata	9.515	1.328	0.813	8.445*	0.973	0.515	1.075
Synedra ulna	9.911	5.565	6.003	4.082*	1.552	-0.438	1.715
Species Diversity	2.278	0.944	1.086	1.264*	0.375	-0.142	0.343
Total Density (e^n)	12.826	9.372	10.038	3.121*	1.819	-0.666	1.798
Total Density (n) (untransformed)	376,378	15,568	22,929				

strates and 1.086 and 0.944 for the clay tile and sterilized rock substrate communities (Table 1). The lower species diversities of the introduced substrate communities were attributed to their larger proportional composition of *Diatoma tenue* as compared to the natural rocks.

Reproducibility of Samples

Euclidian distances (Sneath & Sokal, 1973) were calculated to quantify the differences between subsample communities. Each subsample community was characterized by a location in a p-dimensional space, where p is the number of species. The distance between the communities, the Euclidian distance (D), was calculated as:

$$D = (x_{i1} - x_{i2})^2$$

where x_{i1} and x_{i2} are the abundances of the ith taxon in the first and second subsample communities, respectively. Euclidian distance was used as a straightforward measure of the population difference between two communities. The greater the Euclidian distance, the less similar are two communities, and the more remotely placed are the locations of the respective communities in the p-dimensional space.

Euclidian distances were also used to measure the similarity of replicate samples of each substrate type. For each substrate type, using the population abundances of the 20 most common taxa, distances were calculated between each replicate subsample community of one sample, and the two subsample communities of the other sample (Table 2). For example, the Euclidian distance between one of the two replicate subsample enumerations of the first natural substrate sample was compared with each of the two replicate subsample enumerations of the second natural substrate sample. Then the distance between the second replicate subsample enumeration of the first natural sample was also measured to the two subsample enumerations of the second natural substrate sample. This allowed for comparison of all possible independent observations.

Replicate sample communities were most different among the sterilized rocks with a distance of 45 (Table 2), natural substrate samples were intermediate with 32, and clay tile assemblages were closest at 9. A three factor nested ANOVA identified the sample communities of sterilized rocks as significantly (p < 0.05) more different from one another than clay substrate communities on the basis of Euclidian distances. The distances were evident in the following ways: there was the five-fold total abundance (un-

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Table 2. Euclidian distances between subsample communities of sample 1 (designated horizontally) and sample 2 (designated in the first column) for a specified substrate type.

Subsample No. of sample 2	Natural Subsamples of sample 1 (rock 1)		Sterilized Rock subsamples of sample 1 (rock 1)		Clay tile subsamples of sample 1 (tablet 1)	
	1	2	1	2	1	2
1	21.255	53.843	42. 851	44.632	10.043	6.880
2	17.289	35.471	53.721	39.213	14.781	2.611
$\bar{x}$	19.272	44.657	48.286	41.923	12.412	4.746
$\bar{\bar{x}}$	31.965		45.104		8.579	

transformed) difference between the two sterilized rocks; there was the moderate total abundance and substantial population composition differences between the natural substrates; and there was the negligible difference between the clay tile sample communities.

Introduced Substrate Community Representation of the Natural Community

Substantial differences existed between the natural log transformed abundances of the constituent populations of the three substrates. Analysis of variance and Scheffe's method of multiple contrasts (Scheffé, 1959) indicated that 19 of the 20 most common taxa had significantly (p < 0.05) larger population densities on the natural substrates than on the introduced substrates (Table 1), the exception being *Nitzschia* spp., a group of small species of the subgenus Lanceolatae. The largest difference occurred with *Rhoicosphenia curvata,* where abundances decreased 4651 cells/mm^2 from the natural to the introduced substrate assemblages.

The differences between sterilized rock and clay tile introduced substrates were substantially less than the contrasts of the introduced substrates with the natural substrate populations. The differences between the introduced substrate populations of the twenty major taxa were evaluated with Tukey's method of pairwise comparisons (Scheffe, 1959). Population densities were consistently smaller on the sterilized rocks than on the clay tiles. Six of the taxa demonstrated significant (p < 0.05) abundance differences: *Achnanthes minutissima, Navicula cryptocephala* var. *veneta N. gregaria, N. pelliculosa, Nitzschia linearis* and *Nitzschia* spp. The greatest difference was demonstrated by *Nitzschia* spp. with respective abundances of e$^{1.45}$ and e$^{4.38}$ cells/mm^2 on the sterilized rocks and clay tiles. Eighteen of the 20 population abundances of the clay tiles were closer to the abundances of the natural substrate community than were those of the sterilized rocks.

Euclidian distances were used to provide an integrated multiple species analysis of the introduced substrate assemblages to an average of the natural substrate community. The means of the natural substrate population abundances were obtained. The distance between the 4 subsample communities of each of the introduced substrates and the average natural substrate community was computed (Table 3). The natural substrate was a smaller mean distance, 346, from clay tiles, than the 562 from the sterilized rock communities (0.10 > p > 0.05). This substantiates the evaluation that the clay tile substrate samples were more similar to naturals than were the sterilized rock samples.

A principal components correlation matrix was used to evaluate the main sources of the community differences between substrate types, and to provide a comprehendible summary of the multidimensional spatial placement of the communities (Figs. 2 and 3). The first principal component axis correlated essentially with the total community abundance of the subsample communities. The communities of the introduced substrates were substantially closer to each other than to the natural substrate communities along this axis (Fig. 2). The axis correlated weakly, but most closely to the abundance of *Rhoicosphenia curvata* (Fig. 3), which was previously characterized with the largest abundance contrast between the natural and the artificial substrate assemblages (Table 1).

The second principal component axis was correlated to the abundance of *Nitzschia* spp. (Figs. 2 and 3). This axis isolated the clay tile substrate assemblages. This taxon was previously characterized as having the largest difference between the assemblages of the clay tile and sterilized rock substrates. The placement of the two introduced assemblages were closer to each other than to the natural assemblage. Also, the clay tile assemblages were slightly closer to the naturals than those of the sterilized rocks.

Table 3. Euclidian distances between the subsample communities of
introduced substrates, and the averaged natural substrate community.

Subsample No.	Sterilized Rocks Sample No.		Clay Tiles Sample No.	
	1	2	1	2
1	618.06	534.61	336.02	366.77
2	612.53	483.12	333.92	346.54
$\bar{x}$	615.30	508.87	334.97	356.66
$\bar{\bar{x}}$	562.08		345.81	

Discussion

The large variances between the two natural substrate dia-
tom communities of Fleming Creek resulted from a tem-
poral shift in the dominant taxa appearing at the time of
sampling. Large masses of *Gomphonema olivaceum* were
breaking off the rocks and drifting downstream. This was
apparently the reason for the difference of the *G. oliva-
ceum* abundances (untransformed) which varied three-
fold between the natural substrate samples. *Gomphonema
olivaceum* forms large colonies, of firm mucilaginous
masses, which may completely cover rocks, especially in
the winter months. Blum (1954) also noted this pheno-
monon of large *G. olivaceum* colonies becoming separated
from substrates and floating downstream about the mid-
dle of February in the Saline River, another system located
in southeastern Michigan.

Diatom tenue abundance doubled from its lower density
on the natural rock upon which *G. olivaceum* was domi-
nant as compared to the replicate natural rock upon which
it was the most abundant organism, apparently capitalizing
on the surface exposed from the missing colony of *G.
olivaceum*. That *Diatoma tenue* was the species actively
invading and reproducing is substantiated by analyzing
the sterilized rocks, where this species increased exponen-
tially over a threek week period (Tuchman, unpublished
data). This illustrates a major asset of introduced sub-
strates, their ability to provide information on the colo-
nizing taxa and the metabolically active taxa during a

Fig. 2. Subsample communities from clay (C_{ij}), sterilized rock
(R_{ij}), and natural (N_{ij}), substrates plotted according to ordina-
tion from principal components analysis of population abun-
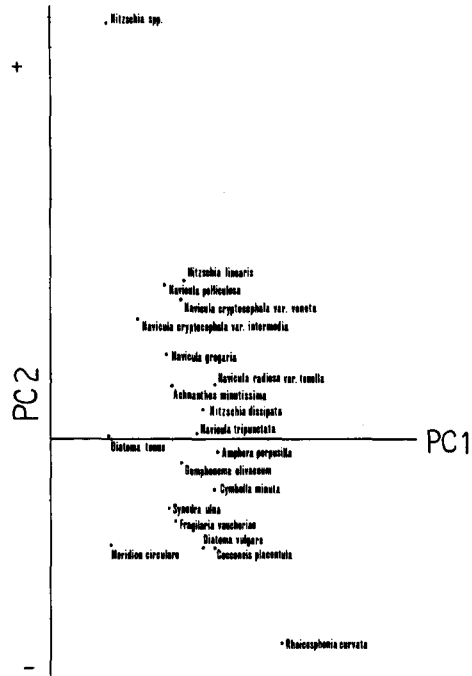dances (j^{th} subsample of the ith subsample of the ith sample).

Fig. 3. Diatom taxa plotted according to loadings from the com-
munity ordination.

certain period of time. This is information that is not
always discernible when only sampling natural substrate
communities.
natural substrate communities.

The introduced substrates were not analyzed for pro-
motion or inhibition of any specific algae. This could be
accomplished by letting the substrate incubate until 'ma-
turity' and comparing them with natural substrates and
other introduced substrates. An inhibitory effect has been
shown with glass surfaces, where lower numbers of fila-
mentous green and blue-green algae have been found when
compared to a natural substrate or other artificial sub-
strates (Brown, 1976; Tuchman & Blinn, 1979). Siver (1977)
found that glass favored the growth of *Achnanthes minutis-
sima* while it inhibited the growth of *Eunotia incisa* and
Cocconeis placentula var. *euglypta* when compared to epi-
phytic communities on plants. Further work will have to
be done to determine if such an effect exists with clay tiles
or sterilized rock.

Clay tile communities had lower variances between the
two replicate samples than did the replicate sterilized rock
samples. The quality of sample reproducibility of the nat-
ural substrates was intermediate between the two intro-
duced substrates. This seems to be due to the greater uni-

formity of the clay tile substrate compared to the rocks. All the clay tile substrates were the same size and shape, whereas the shapes of the rocks were quite variable. Of the two sterilized rocks collected, one was flat, like the clay tiles, and it possessed total diatom densities very similar to the clay tiles. The other sterilized rock, however, was slightly dome-shaped, and had total abundances a factor of five-fold lower.

The dome-shape could have caused a more laminar flow on the upstream side of the rock, where the sample was taken. A greater scouring effect on the rock surface would have resulted. The flat rock and clay tile communities were not placed under such a direct stress. On these substrates, a more turbulent type of flow would be formed along the substrate surface as the flow bounces off the front edge of the substrate.

It is assumed a laminar flow would cause greater shear force than a turbulent flow, and therefore, a greater scouring stress. Thus with less of a laminar flow, the direct shearing forces (scouring forces) on the flat substrates were less than on the domed rock. Water flow, as a function of substrate shape, could play an important role in periphyton colonization and could be the major factor for the large differences between abundances on the replicate sterilized rocks.

The area sampled on the rocks was substantially smaller than on the clay tiles. Reduced sizes of the area sampled could produce greater variability between replicate samples due to clumping of diatom populations. The greatest variability could be expected on the more mature community where growth of sessile populations (as *Gomphonema olivaceum* and *Diatoma tenue*) would tend to increase clumping. The more mature natural rock assemblages were not more different than those of sterilized rocks, suggesting the effect of population clumping on variability between substrates was probably small. We believe this possible source of difference due to the different sample sizes was minimal compared to the effect of shape of a given substrate.

Clay tile assemblages were found to be more similar to those of natural rock substrates than were the sterilized rocks. However, the communities of both artificial substrates poorly represented the natural community. Two to four week substrate incubations have often been quoted as adequate lengths of time to build a representative natural community on the introduced substrates (Patrick *et al.*, 1954; Collins & Weber, 1978). In Fleming Creek, the low winter temperatures (2°C) probably reduced the rate of development. Longer incubation times could have in-

creased the accuracy of the introduced substrate representation of the natural community. However, over the short three week time period examined, representation of the natural community may not be feasible. Gale *et al.* (1979), working on the Susquehanna River in Pennsylvania, U.S.A., monitored two types of artificial substrates on a monthly basis, and found that one month was too short a time to be able to predict changes in the algal community on natural substrates from the artificial ones.

Diatom population dynamics are markedly different between introduced and natural substrates. There are at least two inherent sources of difference between the two substrate types. First, is that while the community on the natural substrates has been developing for a few months, the introduced substrates are still in the initial colonization phase. The actively growing populations of the incubation period will be more dominant (as measured by percent composition) on the introduced than on the natural substrates. Species inhabiting, but not presently as productive as the actively growing populations, will be less important components on, or absent from the introduced substrates. Second, is that the surface characteristics and thus conditions affecting immigration of the natural and introduced substrates will be substantially different at the initial time of placing the introduced substrates into the stream. The effect of the natural substrate assemblage on subsequent periphyton immigration and growth compared to the concurrent periphyton immigration and growth on the naked introduced substrates could be important. Introduced substrate communities may never duplicate natural substrate communities, with the exception of natural substrate communities interrupted by a scouring event, or introduced substrate communities incubated for a long period of time.

Summary

Maximization of reproducibility is as practical an attribute for introduced substrates to possess as is accurate representation of the natural community. The inherent differences between natural and introduced substrate colonization makes duplication of the natural substrate assemblage by the introduced substrate assemblage difficult. Clay tiles produce more reproducible results than do the sterilized rocks. Uniform shape and size, and thus more uniform current patterns are the most probable factors contributing to the greater reproducibility of clay tiles.

Acknowledgements

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