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PRIMARY RESEARCH PAPER

Collection strategies for quantifying protist assemblages in temperate headwater streams

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Abstract The determinination of an adequate collection protocol for protists is critical in the examination of their distribution and composition in temperate headwater streams. The objective of this study was to test which sampling design/sample gear combination would yield a cost-effective, site-representative protist assemblage. Defining parameters included greatest taxa richness, abundance, morphological diversity, taxa overlap, and cell-size diversity. Two sample designs (i.e., transect and mesohabitat design) and two sample gears (i.e., benthic grab sample, and a colonizing device [polyurethane foam unit, PFU]) were tested in three 100-m reaches representing the predominant environmental conditions (i.e., fragmented woodlots and agriculture) in the study area. A two-way ANOVA was used to evaluate abundance taxa richness and abundance of the protist assemblage (fixed effects) across the three reaches (random effects). The mesohabitat sampling design had the highest mean in both taxa richness ($n = 72$, $P = 0.0012$) and abundance ($n = 72, P = 0.0004$). The highest mean was reported with the benthic grab sampler (39.89 ± 1.1) in the abundance count only ($n = 72$, $P < 0.0001$). There was no difference in the design and gear interaction. Morphological diversity, cell-size diversity and percent taxa overlap between sampling design/sample gear combinations also were examined. A higher taxa overlap of the top 10% most abundant taxa was observed with the benthic grab sampler (43–100%) versus the PFU (25–69%); however, the greatest morphological and cell-size diversity was produced by the transect design/PFU combination. We conclude a "hybrid" of the two sample designs will account for "patchy" distributional patterns of protists and use of the PFU, because of the highest yield in morphological and cell-size diversity, will provide the most cost-effective, site-representative protist assemblage in temperate headwater streams.

Keywords Headwater streams · Protist sampling methods · Polyurethane foam unit · Benthic grab sampler · Sampling design · Sampling gear

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Introduction

Methods to sample protist assemblages are well documented in a variety of aquatic systems (Finlay

et al., 1988; Lee & Soldo, 1992; Kemp et al., 1993) particularly in large river and marine environments (Caron et al., 1982; Finlay & Guhl, 1992; Sleigh et al., 1992; Lair et al., 1999; Madoni, 2005; Picard & Lair, 2005). Sampling designs and sampling gear vary from taking benthic core samples along substrate mounds (Gooday et al., 2002), to plankton net drags over lakes expanses (Liebig et al., 2006), to Niskin bottle water samples from designated stations along ocean transects (Dennett et al., 2001), to removing epilithic biofilm using a toothbrush from collected cobbles (Ledger & Hildrew, 2001), and to attaching colonizing gear to harbor posts (Xu et al., 2002). However, few studies have addressed the challenges of sampling protists in headwater streams (however see, Bott & Kaplan, 1989; Ledger & Hildrew, 1998).

Temporal and spatial variations in resources (e.g., environmental parameters, habitat, and food) occur in all the environments but particularly so in an aquatic environment where resources are constantly redistributed by stochastic and anthropogenic events (Fenchel, 1987; Biggs & Stokseth, 1996). Protist distribution is primarily a function of environmental and habitat conditions (e.g., light, dissolved oxygen, temperature, and substrate composition). In many U.S. Midwestern agroecosystems, farming to the stream's edge, stream channelization, drainage tiles, and other practices leaves the riparian corridors a mosaic of fragmented woodlots and grasslands. Abnormally deep-cut banks cause floodplain disconnection and treeless riparian areas along with roaming livestock promote bank erosion and failure. Under such conditions, increased discharge rates causes sedimentation which leads to increased homogenization of the system (Dobson et al., 1997). In addition, watersheds within an agroecosystem may span various ecoregions resulting in a variation of substrate composition (Omernik, 1987) resulting in a gradient of habitat heterogeneity (Fenchel, 1987; Cardinale et al., 2002). Ecological niches of local assemblages, particularly protists, will fluctuate accordingly with respect to growth rates, life cycle characteristics (Fenchel, 1987) and biotic interactions (Holt et al., 2004).

Headwater streams have unique characteristics that might influence protist distribution and thus sampling protocol. In these systems, water depth is often low (<40 cm) and substrates can vary greatly within the same watershed (e.g., from a boulder-cobble-gravel substrate to one dominated by sand). Because of the close interface between land and water in these narrow channels, the hydrology of headwater streams can be "flashy" in nature (Roth et al., 1996; Meyer et al., 2007). Spatial distribution of protists may be on a heterogeneous to homogeneous gradient depending primarily, but not exclusively, on substrate composition (Holt et al., 2004). Protist distribution also may be influenced by the erosional and depositional nature of headwater streams caused by variable discharge rate and water depth (Fenchel, 1987).

In our headwater study area, substrate distribution varies from a boulder–cobble–gravel–sand composition to an entirely sand substrate composition. The transect sampling design is a model for collection of protists that are homogeneously distributed, while the mesohabitat sampling design would be an appropriate collection model for a "patchy" protist distribution based an available detrital food sources in specialized areas of the reach (i.e., pools, riffles, runs) where other taxa (i.e., macroinvertebrates and fishes) populate. An increased ecological taxa interaction, because of the size differential (speculatively attributed to effects of the landuse or stochastic events on assemblage diversity), contributes significantly to the detrital or bottom-up food reserves for protists.

Evaluation of sampling design has received little attention in the literature, particularly when addressing issues of protist distribution such as "patchiness" or distributional heterogeneity (Holt et al., 2004), directly affecting community structure (Hanson & Weltzin, 2000; Tews et al., 2004) and functionality (Cardinale et al., 2002). Even less attention has been devoted to these concerns in headwater streams where conditions are relatively unique. In a river study comparing protist sampling collection gears (benthic sampling versus artificial substrate), the sampling design consisted of extracting three samples from three protist habitat categories at six stations above and below sewage treatment plants or dams (Foissner et al., 1992). Another study in a second-order stream examined the seasonal and spatial distribution of ciliates sampled from four units of a reach divided into 20 equal units (Cleven, 2004). In an effort to examine the flagellate and ciliate distribution in sediments of a sixth order lowland river, a third study extracted one core sample from four stations on each of the three transects running from the right bank to midstream (Gucker & Fischer, 2003). The U.S. EPA sampling

design for the collection of periphyton in headwater streams consists of identifying 30-m reach of thalweg, identifying all the erosional and depositional habitats within that reach, and taking one sample consisting of a 12 cm^2 surface area from six stones in one of each habitat (Fritz et al., 2006). In all of these studies, sampling designs appear to neglect heterogeneity in protist distribution patterns.

We will examine the potential use of two sampling designs in patchy conditions. A transect sampling design allows collection of samples equally across the stream channel, while a mesohabitat sampling design prescribes sample collections from habitat areas (i.e., pools, riffles, and runs) based on the stream's morphological development. The transect design may be advantageous because of its random approach, but it may not account for "patchy" protist distribution. The mesohabitat design may provide a sample where protists are more likely to be abundant because of possible increased detrital resources, but may not be representative of the reach. Consideration of sampling design (and gear choice) is important to account for distribution and inherent variation of any taxonomic group in streams (Williams et al., 2004).

Few studies have evaluated the efficacy of protist sampling gear in streams (Cairns et al., 1974; Foissner et al., 1992). Planktonic and benthic grab samplers and plankton nets are used extensively in lotic and lentic aquatic systems (Lee & Soldo, 1992). However, in headwater streams, plankton nets are not a functional tool because of the shallow stream depth and substrate variation. Direct in situ samplers (e.g., benthic grab and plankton grab samplers) are effective but become costly with respect to time and energy expended if both samplers are used to ensure a protist assemblage representation from benthic and planktonic compartments of the stream reach. Indirect in situ units, polyurethane foam units (PFU), have been used as a colonizing substrate for freshwater planktonic, periphytic, and benthic protists (Cairns et al., 1974; Shen et al., 1986; Xu & Wood, 1994; Chung et al., 1999). Protist assemblages can be collected instantaneously or established over time using colonizing samplers (Cairns et al., 1974). The pore sizes of a PFU (ca. 100–150 μm in diameter) slow the water flow through the unit allowing ample attachment space for sessile protists, which may otherwise be "sloughed off" because of the energy behind high flow rates. The PFU also provides area for crawling protists, as well as open spaces for planktonic protists (Henebry & Cairns, 1980). A disadvantage of using colonizing gear may be the inability to obtain a representative assemblage indicative of the stream condition. According to D. J. Patterson (Marine Biological Laboratory, Woods Hole, USA, personal communication), the internal structure of the colonizing sampling gear provides a unique environment and may not be comparable to the protist assemblage collected using direct sampling gear. Other disadvantages include extended time required for the placement, colonizing, and extraction of the gear, as well as potential gear loss due to flooding or vandalism (Foissner et al., 1992).

The objectives of this study were twofold: (1) to determine a sample design (transect design versus a mesohabitat design) that ensures a representative assemblage of protists, and (2) to determine which sampling gear (PFU or benthic grab sampler) provides a representative protist assemblage. We defined a site-representative protist assemblage as one obtained from a particular sampling design and gear combination, resulting in the highest compositional parameters based on structure (i.e., abundance and taxa richness) and function (i.e., morphological and cell-size diversity and taxa overlap [defined as taxa found in common between sampling design and gear combinations]). We hypothesized that the transect sample design, because of its uniform distribution across the stream channel and the ubiquitous nature of protists (Fenchel, 1987), would ensure a greater variety of sampling habitats rich with protists resulting in higher means of the defining parameters. We also hypothesized that the PFU provides a more robust protist assemblage representative of both planktonic and benthic compartments of the stream reach, because of its placement—totally submerged in the water column and in direct contact with the first few centimeters of the substrate within the stream channel. Typically, a PFU is suspended in the water column essentially to collect the planktonic assemblage. Such placement is problematic in headwater streams because of the lack of water depth, high flow rates, and high visibility making the units susceptible to vandalism. With the modified position, the PFU becomes comparable to a benthic sampler with the additional potential advantage of collecting the planktonic assemblage.

Methods

Study site

In spring 2006, three 100-m perennial reaches on a headwater tributary located in the Upper Sugar Creek subwatershed (Ohio, USA) were selected based on their representation of the range of landuse conditions found in the watershed. Reach U24D (N40°52.289, W081°49.185) is a natural primary headwater surrounded by woodlot (>100-m width forested riparian). Adjacent to the reach riparian buffer is a hog farm and row crop agriculture (conventional corn–soybean rotation). The substrate is a composition of cobble–gravel–sand. During the spring and summer months, there is ample canopy cover as well as instream cover. Water temperature stays cool (10–14°C) throughout the year because it is spring-fed. Reach U24C (N40°51.889, W081°50.456) downstream of U24D is similar in structure; however, only one side of the riparian buffer is adjacent to row crop agriculture. The substrate is predominantly cobble–gravel–sand, with boulders interspersed throughout. The riffle–run–pool sequence is more developed than in U24C, offering greater habitat variability. Reach U24B (N40°51.321, W081°50.404) is a channelized reach that empties perpendicularly into the mainstem and is surrounded by a grass riparian, high banks, and row crop agriculture. Water temperature fluctuates diurnally and seasonally because of the open canopy. The upstream portion of the reach has a cobble–gravel–sand substrate composition while the downstream portion of this reach is mainly sand–muck–silt. The substrate composition of the downstream portion most likely is a result of flooding by the mainstem.

Field methods

Two sampling designs were applied to each reach: a transect design and a mesohabitat design. In the transect design, two random sample sites were designated on each of three longitudinal transects positioned along either side and down the middle of the wetted streambed (Fig. 1A). For the mesohabitat design, pools, riffles, and runs were mapped along the length of the same reaches and numbered. Two

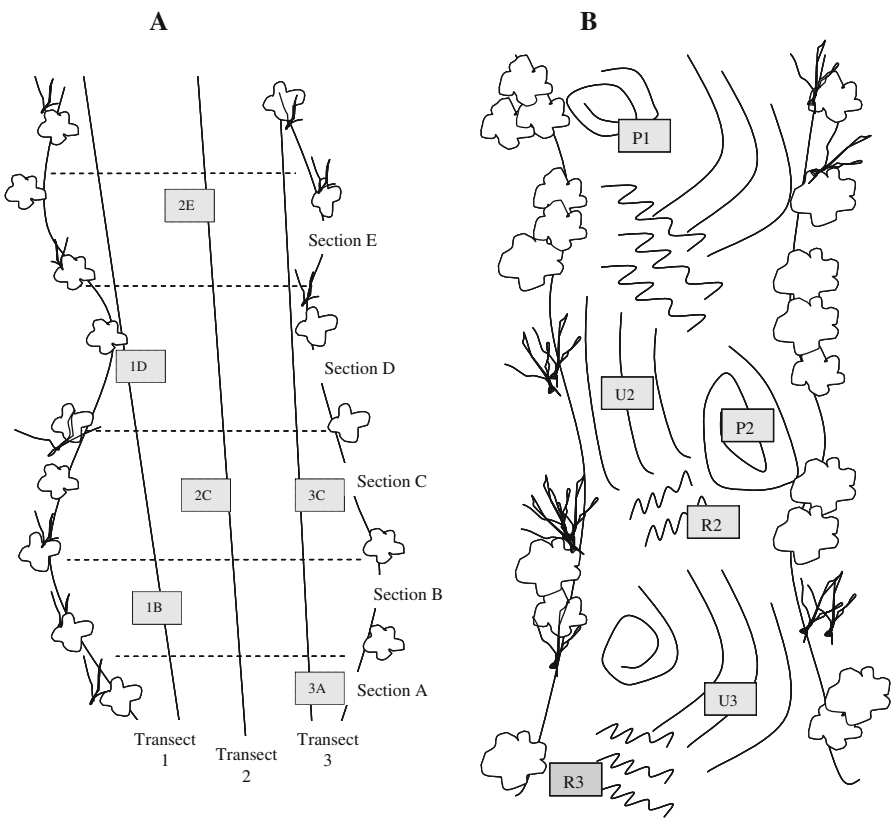

Fig. 1 The transect sampling design (**A**) consisted of three longitudinal transects with two randomly chosen sample sites (e.g., 1D, 1B) on each while the mesohabitat sampling design (**B**) were mapped out pools (P), riffles (R), and runs (U) found within the reach. Two sample sites (e.g., P1, P2) were randomly chosen per pool, riffle, and run

sample sites were randomly identified from each set of pools, riffles, and runs within each study reach (Fig. 1B). Six samples were taken for each sampling design in each reach.

Two collection gears, a benthic sampler and the PFU, were tested in both sampling designs and at each sample site in the three reaches. Benthic grab samples were processed in the field by sifting the gravel and cobble from the sample, leaving a fine to small benthic composition in the bottom of a polyethylene rectangular container. Gravel and cobble were lightly scraped with fingers to remove any materials and placed into the water–benthos mixture. Water was then decanted from the collected substrate so that only a centimeter or less was noticeable on top of the substrate. The sample was placed on ice for transportation to the laboratory and upon arrival placed into cold storage until processed (within 24 h).

The PFU was constructed from artificial polyurethane sponges (pore size diameter, 1–5 mm). Based on a unit size that allows oxygen to diffuse to the center, colonizing units were cut into 24 cm^3 cube (Yongue & Cairns, 1971). The units were covered with a 2.5-cm meshed material (nylon pond net cover) and tied off with nylon line. The units were attached to a brick with nylon line.

The PFU was positioned in the reach immediately after the grab sample was collected. The brick was submerged into the substrate allowing the colonizing unit to be in direct contact with the substrate to ensure collection of both benthic and pelagic assemblages. In the first three samples collected in U24C, ten colonizing units were placed in situ over a 12-day period in an effort to determine the most robust extraction day. Colonizing units were extracted and processed daily. Extraction of units consisted of cutting the nylon line attaching the unit to the brick while the colonizing unit was submerged, lifting the unit from the water and quickly, but gently, squeezing the water from the sponge into a funneled 50-μm polyethylene container. The sample was filtered through a 100-μm mesh to separate out meiofauna predators. The mesh containing filtered meiofauna was rinsed with in situ water into 100-ml polyethylene containers containing sugar–formalin preservative (Haney & Hall, 1973; ASTM, 2004) for examination at a later date. The filtered protist sample was transferred to a 50-ml polyethylene container and placed on ice during transport to the laboratory. At the laboratory, samples were placed in cold storage (<4°C) until processed live within 24 h.

The placement-extraction schedule was complicated by the need to process the samples alive. Because PFU were colonizing for 3 days and only 3–6 colonizing units can be processed per day, sampling occurred over a 6-week period from April to the mid-May. This procedure was repeated 14 times over the 1.5-month period to collect all the samples. All the samples were extracted on colonization day 3, and new colonizing units were put in place. Extracted colonizing and grab samples were microscopically processed on the colonizing days.

Laboratory method

Sample preparation for microscopy analysis varied with sample gear. All the samples were microscopically previewed for cell abundance per field of view (FOV). If cell abundance was scant (<10 cells per field of view), then 15 ml of water sample was filtered by gravitational force or with a hand-operated vacuum pump with pressure <5 mm of Hg. across a 0.45-μm nylon membrane backing filter (Whatman International Ltd, New Jersey, USA) to obtain 1–3 ml of water. Using a Gilson P 20 drawn, 0.15 μl of water was pipetted from a 3-ml aliquot to a slide and coverslip for observation. Samples were observed on a Zeiss Standard 16 (Carl Zeiss, Inc. Germany), equipped with Nomarski and enumerated at 300×–800× magnification. Samples were standardized by grouping flagellates, amoebas, and ciliates, followed by soft algae and diatoms and observing each group for 45 min. A target of 600–800 total cell count was obtained in most samples to ensure a representative assemblage (D. J. Patterson, Marine Biological Laboratory, Woods Hole, USA, personal communication). The total number of the FOVs observed at the various magnifications was calculated. The number of cells ml^{-1} was calculated using the total FOVs observed and volume of water under the coverslip (Sherr et al., 1993). The observed cells were identified to the genus taxonomic level and placed into five morpho-functional groups: flagellates (move and/or feed using flagella), ciliates (move and/or feed using cilia), amoebae (move and/or feed using various forms of pseudopodia), diatoms (with siliceous walls), and soft algae (without siliceous walls) (Salmaso & Padisak, 2007). In order to aid in the identification

process, all the cell taxa were interpretatively documented (line drawings) and most abundant cells were noninterpretatively documented (photographed or videotaped, Patterson, 1996) using a Canon PowerShot S3IS (Canon, Inc., Tokyo, Japan) and a Martin MM99-58 (Martin Microscope Company, South Carolina, USA) microscope adapter on the Zeiss Standard 16 or on an epifluorescence inverted Leica DM IRB (Leica, Solms, Germany) equipped with Q Imagin Retiga 2000 cooled digital camera. Microscopic samples from benthic gear were extracted by embedding a slide into the center of the benthic sample. A 15-ml syringe with the tip cut off was run through the substrate with the syringe tip placed on the slide. Protists living in the sediment were drawn into the syringe (Gasol, 1993) thus eliminating bits of substrate from getting into the microscopic sample and, ultimately, under the coverslip. A 0.15 µl of water sample was transferred to a new slide for observation. The observation protocol, described above, was then followed.

Data analysis

At the onset of this study, a one-way ANOVA with a Tukey-Kramer HSD test was conducted to determine the appropriate extraction day for colonizing PFUs regarding protist abundance and taxa richness accrual. A paired comparison was made between consecutive colonizing days. Also, an ANOVA test for protist selectivity or gear bias was conducted.

Our experimental design consisted of three reaches in which two sampling designs were used independently of one another. In each sampling design, two sample gears were tested at six sample sites within each reach (Table 1).

A two-way ANOVA was conducted to determine a difference between sampling design, gear choice, and an interaction between the two using both abundance and taxa richness counts. The analyses included a total of 481 genera (amoebae = 64 taxa, ciliates = 87 taxa, flagellates = 190 taxa, diatoms = 118 taxa, and soft algae = 22 taxa). The assumption of normality was violated in only the abundance counts. Taxa richness counts were within the normal range. The abundance counts were transformed by square root bringing them within the range of normality. Within the ANOVA model, reach was designated as a random effect while the sampling design and gear were fixed effects.

Within individual reaches, a sub-assemblage of the top 10% from the most relatively abundant taxa was identified for comparison. In the reach composites, observed taxa from all the reaches were combined, sorted according to abundance, and the top 10% selected as the sub-assemblage. Photophytes (diatoms and soft algae) were eliminated from this analysis because of the overwhelming abundance of diatoms and the relatively low abundance of soft algae. Taxa composition parameters included percent overlapping taxa and the morphological overlapping taxa, as well as morphological diversity and cell-size diversity. The percent overlapping taxa was calculating by identifying those taxa that each tested parameter had in common with other and dividing by the total taxa of that parameter. The morphological overlapping taxa identified the overlapping taxa by functional group. Similarities and differences in taxa composition were determined from this calculation. In this particular analysis, diversity refers to the presence of a morph-functional group (amoeba, ciliate, and flagellate) and cell-size classes (small, medium, and large). Because cell size in protozoa has a range of about four orders of magnitude with ciliates and amoeba, appreciably larger than most flagellates, cell size was standardized by size classes (Fenchel, 1987). Cell size based on length was compartmentalized into two classes: small (amoeba and ciliates 2–30 µm, flagellates 2–10 µm), medium (amoeba and ciliates 30–100 µm, flagellates

Table 1 Nested hierarchical experimental design

Nested hierarchy	Experiment design												*n*
Reach	24B				24C				24D				3
Sample design	M		T		M		T		M		T		2
Gear	B	PFU	B	PFU	B	PFU	B	PFU	B	PFU	B	PFU	2
Sample site	SS(6)	SS(6)	SS(6)	SS(6)	SS(6)	SS(6)	SS(6)	SS(6)	SS(6)	SS(6)	SS(6)	SS(6)	6
Total													72

10–30 μm), and large (amoeba and ciliates 100+ μm, flagellates 30+ μm). Greater diversity in protist morphology and cell size is an indicator of a representative assemblage, as well as an indicator of the trophic functionality of the protists since larger protozoa often consume smaller protozoa (Fenchel, 1987). All the analyses were conducted in JMP (Statistical Discovery, SAS Institute, Inc., version 6.0.2) and PC-ORD (McCune & Mefford, 1999, MjM Software, Gleneden Beach, Oregon, USA, version 4.20).

Results

The first task in our study was to determine the minimal extraction day for colonizing units. Regarding taxa richness, an observed difference was found over the colonization days ($n = 157$, df $= 9$, $P < 0.0001$). A difference between days 2 and 3 ($t = 4.2$, $P < 0.05$), days 5 and 6 ($t = 0.2$, $P < 0.05$), and days 8 and 9 ($t = 4.5$, $P < 0.05$) was observed (Fig. 2A). Although there was a difference found in abundance over the colonization days ($n = 157$, df $= 9$, $P = 0.153$), there was no difference in the pairwise comparison between consecutive colonization days (Fig. 2B).

Second, protist selectivity for the benthic grab sampler and the PFU were evaluated using taxa richness (Fig. 3). There was no overall difference between the gear (ANOVA: $n = 10$, df $= 4$, $P = 0.37$). However, among the five protist groups, an observed preference for the benthic grab sampler was observed in two protist groups. Algae and diatoms collected with a benthic grab sampler had a taxa richness average of 1.67 (SE ± 0.39) and 14.11 (SE ± 3.33), respectively, while algae and diatoms collected with the PFU had an average of 1.23 (SE ± 0.14) and 10.75 (SE ± 1.21), respectively.

Sample design, gear, and interaction

ANOVA tests with the random effect of reach were performed on the residuals of taxa richness (96% residuals) and abundance (27% residuals) of protists. In both ANOVAs, the whole models were significantly different (Table 2, taxa richness, $n = 72$, df $= 5$, $P < 0.0001$; abundance, $n = 72$, df $= 5$, $P = 0.0071$). We observed a difference in sampling design and the protist response variables of taxa richness ($n = 72$, $P < 0.001$) and abundance ($n = 72$, $P =$

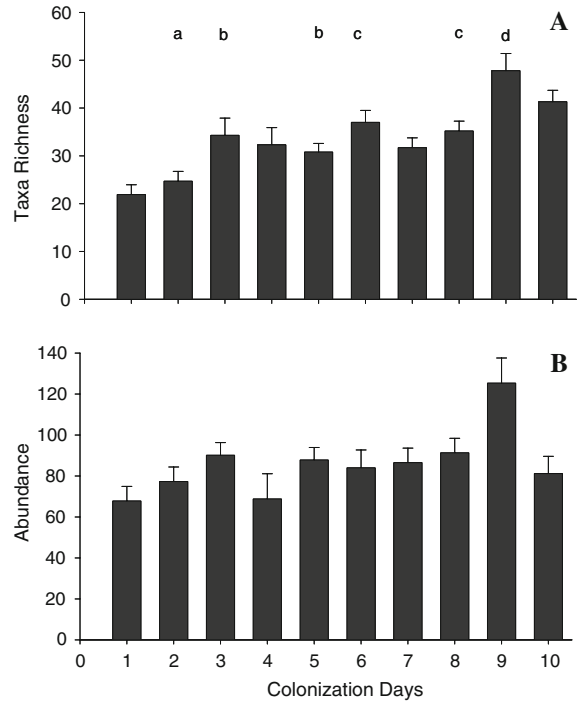

Fig. 2 One-way ANOVA analyses of mean taxa richness (**A**) and abundance (**B**) plotted over a 10-day period with a Tukey-Kramer HSD pairwise comparison of consecutive colonizing days. *Lowercase letters* indicate significant differences. *Absence of lowercase letters* indicates no significant difference between consecutive colonizing days. *Error bars* are an expression of standard error

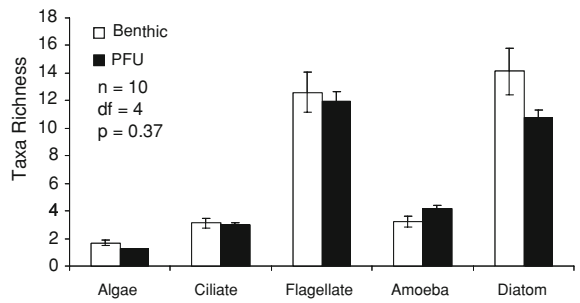

Fig. 3 Taxa richness expressed as a function of the protist compartments examined using benthic grab sampler and PFU. *Error bars* are a representation of standard error

0.0004) (Fig. 4A). The mesohabitat sampling design had the highest mean in both taxa richness (41.47 ± 1.1) and the abundance (13.3 ± 0.29) tests. Regarding gear, a difference was observed only in protist abundance ($n = 72$, $P < 0.0001$) with the benthic grab sampler having the highest mean

Table 2 Two-way ANOVA

	DF	*F* ratio	*P*-value
Taxa richness			
Design	1	11.427	0.0012*
Gear	1	2.0567	0.1563
Design * gear	1	0.2623	0.6102
Whole model	5	3.51	0.0071
Abundance			
Design	1	13.6749	0.0004*
Gear	1	39.8678	<0.0001*
Design * gear	1	1.0644	0.3060
Whole model	5	36.64	<0.0001*

* indicates a significant difference at an alpha less than 0.05

(13.87 ± 0.29) (Fig. 4B). There was no observable difference in the interaction of design and gear in either analysis. A retroactive power analysis indicated sample size was lacking to detect a significant difference in the interaction but were adequate for both sample design and gear.

Taxonomic composition

Among individual reaches in both sampling designs, the benthic grab sampler accounted for the greatest taxa overlap while the PFU provided higher morphological and cell-size diversity (Table 3). Taxa overlap of the top 10% most abundant taxa obtained was independent from sampling design; the transect design provided a 46–100% taxa overlap, similar in range with the mesohabitat design (43–100% overlap). In comparison, the PFU had lower taxa overlap whether with the transect (31–69%) or with the mesohabitat sampling designs (25–58%). The greatest morphological diversity was expressed in the transect design/PFU combination. cell-size diversity was similar between gear in both sampling designs. The highest cell-size diversity (consisting of all size compartments) was measured at reach U24B in the transect design across both sampling gear and in the mesohabitat design with only the benthic grab sampler.

Results from the reach composite analysis indicated the benthic grab sampler had a higher percent taxa overlap (transect: 73%, mesohabitat: 86%) than the PFU (transect: 50%, mesohabitat: 50%). In addition, we observed the greatest morphological and cell-size diversity in the transect design/PFU combination.

In individual reaches and the composite reach across all the sampling designs/gear combinations, the overlapping morphological group was monomorphic

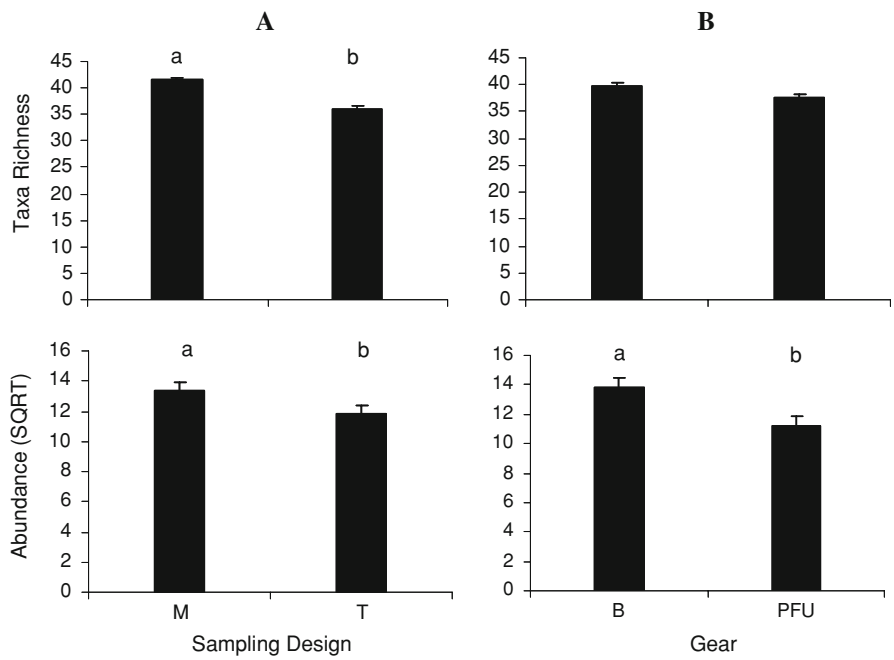

Fig. 4 Two-way ANOVA results with reach as random effect and the sampling design (**A**) and gear (**B**) as fixed effects. *Lowercase letters* (*a* and *b*) indicate significant difference between variables. *Absence of lowercase letters* indicates no difference. *Error bars* represent standard error

Table 3 Taxa compositional characteristics including percent taxa overlap, morphological taxa overlap, morphological diversity, and cell-size diversity

Sample design	Reach	Taxa overlap		Morphological taxa overlap	Morphological diversity		cell-size diversity	
		BG vs. PFU (%)	PFU vs. BG (%)		BG	PFU	BG	PFU
Transect	U24C	46	31	F	F, A	F, C	S	S, M
	U24B	100	69	F	F	F, C	S, M, L	S, M, L
	U24D	64	64	F	F	F, A	S, M	S, M
	Composite	73	50	F	F	F, C, A	S, M	S, M, L
Mesohabitat	U24C	100	58	F	F	F	S	S
	U24B	43	25	F	F	F, C, A	S, M, L	S, M
	U24D	57	33	F	F, C	F	S, M	S, M
	Composite	86	50	F	F	F	S, M	S, M

Taxa overlap represents the percentage of taxa of the first listed gear (e.g., BG = benthic grab sample) found in the second listed gear (PFU). Morphological taxa overlap refers to protist groups (amoeba [A], ciliates [C], flagellates [F], diatoms [D], and soft algae [Al]) present in the overlapped portion. Morphological diversity is a list of protist morphological compartments present in the sample site based on gear. Cell size was divided into three size compartments as defined in text: small (S), medium (M), and large (L)

(flagellates). Flagellates, specifically *Bodo* sp. and *Codonosigna* sp., were the dominant taxa in all the assemblages.

Discussion

As hypothesized, there was a difference in sample design with respect to abundance and taxa richness of protists. Higher means of these response variables were associated with the mesohabitat design, seemingly supporting the heterogeneous distribution of protists in these headwater reaches. Despite lower yields in abundance and taxa richness, the transect design provided increased functional information related to morphological and cell-size diversity. These parameters are preferential in ascertaining a site-representative assemblage because assemblage structure, in addition to trophic functionality, can be established from this information.

A great deal of literature addresses the issue of sample gear choice in protist collection; however, little has documented protist collection in headwater streams. In Foissner et al. (1992), ciliate protozoa were collected from a mesosaprobic river using the direct sample method (i.e., benthic grab sample) and artificial colonizing gear (i.e., natural sponge and leaf packets). Greater species richness was observed with the direct sample method compared to the natural sponge and the leaf packets. Results indicated colonizing gears were not appropriate for sampling protists because of low taxa accrual, excessive time and financial burdens, and potential loss of gear because of vandalism or flooding. In contrast, a study sampled 11 stations in the vicinity of a Michigan impoundment (again, an area larger than headwater streams) and found protist taxa richness much higher than those found in natural substrates concluding that the PFU was an adequate sampling gear (Pratt et al., 1987).

This study is in agreement with Pratt's findings. The PFU was the most effective sampling gear for use in the given conditions. Although abundance was slightly higher in the benthic grab sampler, compared higher morphological and cell-size diversity (functional parameters essential in assessing the ability to obtain a representative assemblage of protists) was characteristic of the PFU. In regard to structural parameters, the PFU maintained at the least a 43% taxa overlap of the benthic grab sampler, providing ample protist representation from the benthos. Planktonic taxa representation was assumed in the other 53% of the assemblage collected with a composition, at the very least, by photophytes. Thee PFUs populated with diatoms most often come from the plankton as opposed to the benthos (Stewart et al., 1985).

The taxa overlap results also address the issue that the internal structure of the PFU might create an environment different from the stream environment,

thus affecting the assemblage composition and that a benthic grab sample is a more appropriate sampling gear (D. J. Patterson, Marine Biological Laboratory, Woods Hole, USA, personal communication). Results of at least a 50% taxa overlap between stream compartments indicate minimal environmental variations between the internal structure of the PFU and in situ conditions.

Cairns et al. (1992) suggested a benthic sample may be representative of local conditions, while a planktonic-suspended PFU represents conditions across greater spatial scales. Obtaining a site-representative assemblage suggests one may need to sample both benthic and planktonic stream compartments. However, a large portion of the planktonic assemblage may be a result of the shearing effects of current on benthic organisms; dislodging the cells from the benthos, suspending them in the water column while increasing their vulnerability to the downward flow of the stream (Fenchel, 1987) implying a benthic grab sample would be sufficient. Ensuring that the planktonic portion of the reach was represented in the sampled assemblage is important. A benthic sample eliminates the planktonic assemblage representation and a PFU, if traditionally positioned (suspended in the water column), provides only a planktonic assemblage representation. Because of the deliberate placement of the PFU within the stream, both stream compartments were represented in the sampled assemblage, while simultaneously promoting economic efficiency.

Competition may be another issue of concern in choosing the PFU as the preferred sample gear. In comparison to the benthic grab sample, flagellate abundance was lower in the PFU. Spatial confinement and higher chance of interception between possible predators (e.g., ciliates and amoebae) and prey (e.g., flagellates) may be the cause. Extraction of the colonizing sample at the low end (day 3–6) of the maximal colonization curve may abate this interactive effect (Cairns et al., 1992).

With respect to gear choice, a tradeoff of efficiency for effectiveness may be well worth the effort to minimize extraneous variability to the dataset. Use of the PFU triples the time of collection but provides a uniform colonizing surface (Cairns et al., 1992), eliminating the variability introduced with inconsistent substrate compositions throughout the study area. If placed properly within the streambed, the PFU can conserve sampling effort by eliminating the need to sample in both the benthic and planktonic compartments. A PFU can be a substrate for instantaneous inhabitance and provides us with an evaluation of a system's ability to support the recolonization functionality among representative organisms (Stewart et al., 1985). Several studies have found PFU sampling gear adequate in evaluating protist biota in various aquatic systems, including rivers and streams (Chung et al., 1999; Xu et al., 2002; Liu et al., 2007; Jiang & Shen, 2007).

In conclusion, because of variable environmental conditions found in many temperate headwater streams, and particularly in the Sugar Creek watershed, a hybrid between the two tested sampling designs may be warranted to obtain a site representation of the protist assemblage. The hybrid design would consist essentially of using the transect design as the sampling infrastructure and ensuring gear placement in an equal number of mesohabitats present within the reach. The hybrid sample design, in tandem with the PFU sampling gear with modified instream position and mesohabitat placement, should result in a representative assemblage in either a heterogeneous or homogeneous habitats and in fluctuating or unstable environmental conditions, ensuring an accurate measure of protist diversity and functionality.

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