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BENTHIC BACTERIAL-ALGAL RELATIONSHIPS IN A STREAM EPILITHON

By

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ABSTRACT

BENTHIC BACTERIAL-ALGAL RELATIONSHIPS IN A STREAM EPILITHON

By

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My research on the benthic bacterial and algal communities in a stream epilithon attempted to determine: 1.) the extent to which bacterial and algal biomass correlate in various stream habitats, 2.) the extent that bacterial biomass and productivity respond to changes in algal biomass and changes in stream DOC throughout epilithon colonization. The first question was investigated in a study in which benthic bacterial and algal biomass were compared between a stream run, riffle, and pool throughout colonization time. A correlation was not found between benthic bacterial and algal biomass. The second question was investigated in a controlled, replicated experiment in which a series of experimental stream channels was employed. This study addressed the relative importance of allochthonous and autochthonous inputs to the benthic bacterial community during different stages in epilithon colonization. Bacterial productivity responded to increased DOC early in epilithon colonization and both increased DOC and algal biomass in late colonization.

Dedicated to the sense of wonder I gained exploring my favorite stream as a young boy with Geoff and Buzz.

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INTRODUCTION

In the last decade the stream epilithon (benthic biofilm) has received a great deal of attention due to its major role in stream primary productivity, secondary productivity, decomposition, nutrient retention, and metal adsorption. The epilithon consists of a polysaccharide matrix which contains a diverse assemblage of algae (mostly diatoms), cyanobacteria, heterotrophic bacteria, fungi, and protozoa which have varying degrees of direct contact with the stream's water column (Lock 1984). This complex three-dimensional matrix readily develops on available substrate through a series of successional stages (Geesey et al. 1977, Stock and Ward 1989). Slime-producing bacteria are usually the initial colonizers and conditioners of bare substrates, followed by small diatoms and fungal spores which require a bacterial conditioning for successful adhesion (Winterbourn 1986). Algal exudates and continued bacterial exudates further the development of the epilithon's matrix. Larger diatoms, filamentous algae, and autotrophic bacteria are more likely to be found in the later stages of the epilithon's development and further the vertical component of the polysaccharide matrix. Hence, the polysaccharide matrix and numerous exoenzymes found throughout it are produced by the epilithon's various living constituents.

Much of the earlier work on the epilithon concentrated on describing its structure by defining its constituents (Geesey et al. 1977, Geesey et al. 1978, Lock and Williams 1980, Rounick and Winterbourn 1983, Korte and Blinn 1983, Lock et al. 1984, Hamilton and Duthie 1984). As the structural framework of the epilithon was elucidated, research began to focus on how various physical and chemical parameters impacted specific components of the epilithon (Horner and Welch 1981, Krewer and Holm 1982, Maurice et al. 1987, Goulder 1988, Paul and Duthie 1988, Corning et al. 1989, Poff et al.1990). Numerous studies have dealt with the role of grazing and herbivory on the epilithon in recent years as well (Winterbourn et al. 1985, Fuller et al. 1986, Hershey et al. 1988, Winterbourn and Fegley 1989, Winterbourn 1989, 1990, Collier and Winterbourn 1990, Mullholand et al. 1991, Gelwick and Matthews 1992, Power 1992).

Although our knowledge on the epilithon's structure, function, and role in stream ecosystem dynamics has increased during the last decade, much is still unknown (Winterbourn 1986, Meyer et al. 1988). Much of our knowledge about the epilithon is derived from studies which investigated only one of its components (e.g. diatoms, bacteria, fungi). Few studies have attempted to look at the epilithon as a whole and few studies have adequately addressed the relationship between the epilithon's components. In particular, there has not been an adequate attempt to determine if the bacterial community of the epilithon obtains its carbon from the autochthonous community within the epilithon or from allochthonous organic material in the water column. Although aquatic bacteria are generally thought to rely on algal biomass in many aquatic ecosystems (Cole 1982, Cole et al. 1988, White et al. 1991), such a bacterial-algal relationship has not been well documented in the epilithon of stream ecosystems.

The extent in which streams are autochthonously driven by energy acquired through photosynthesis within the stream or allochthonously driven by energy derived from organic matter from the stream's watershed has been a long standing debate among stream ecologists. Minshall (1978) argued that many studies over-emphasized "dependency" on allochthonous organic matter and underestimated the importance of autotrophy. Minshall argued that too many studies focused on algal standing biomass and failed to consider the algae's turnover rate. The polysaccharide matrix of the epilithon which houses this algal component is embedded with a diverse bacterial community as well. This bacterial community is capable of deriving energy from algal exudates and senescencing algal cells as well as gaining energy from allochthonously produced organic matter in the water column.

Some researchers have suggested that the bacterial component of the epilithon is capable of utilizing exudates from healthy algal cells (Bauld and Brock 1974, McFeters et al. 1978, Haack and McFeters 1982a, 1982b, Arias Morales 1987, Goulder 1988, Kaplan and Bott 1989, Blenkinsopp et al.

1991). Hence, there is some evidence that algae within the epilithon may support heterotrophic bacteria in some systems. In oligotrophic streams which are void of a substantial amount of allochthonous organic matter, there appears to be little utilization of the dissolved organic matter from the water column according to several researchers (McFeters et al. 1978, Haack and McFeters 1982b). Haack and McFeters (1982b) found that bacterial utilization of algal extracellular products was greatest during high primary productivity. In a related study, Haack and McFeters (1982a) found that bacterial activity and turnover was greatest as the phototrophic community declined due to a possible increase in algal lysis products occurring during algal cell mortality. Interestingly, this study did not find an increase in bacterial biomass associated with the increased activity. Arias Morales (1987) found that *E. coli* uptake of labeled algal extracellular products was greatest at the maximum algal growth response in a tropical stream in Puerto Rico. Similarly, Kaplan and Bott (1989) found increases in bacterial productivity during peak photosynthesis hours, however, their conclusions were confounded with an increased water temperature relationship during the mid-day. Blenkinsopp (1991) found that biofilm storage products (e.g glycogen) were greater in light grown epilithons, possibly due to the presence of algae or possibly due to the thickness of the light grown epilithons. Lock and Williams (1981) suggest that the bacterial community facilitates the disintegration of the epilithon during low DOM conditions by utilizing algal exudates that are needed to maintain the polysaccharide matrix that supports the epilithon's constituents.

These studies suggest that bacteria can adhere and survive in the nutrient-rich microzone or phycosphere that surrounds autotrophs following the production and excretion of organic exudates. This nutrient-rich microzone could support bacteria in a nutrient poor environment that might otherwise be unfavorable (Cole 1982). The availability and labile nature of these exudates may enable them to support a much greater bacterial community than the various refractory allochthonous organic compounds in the water column (McDowell and Likens 1988). It is also conceivable that a thick, tightly packed epilithon may function as a diffusional barrier (Lock and Williams 1980, Peterson and Grimm 1992), thus preventing heterotrophs within the polysaccharide matrix from readily utilizing the organic matter from the water column. Ladd et al. (1979) found that uptake and mineralization of glutamic acid was significantly slower in an undisturbed epilithon matrix compared to a homogenized epilithon matrix. Lock et al. (1984) contend that even organic compounds as small and labile as glucose may have significant difficulty diffusing through the epilithon's matrices. They argue that the rate of diffusion, which is greatly affected by concentration gradients, would be especially retarded in pristine streams which may have low concentrations of labile products compared to that present within the epilithon. The polysaccharide matrix could also exclude large refractory macromolecules which might make up a large portion of the allochthonous organic matter in many headwater and lower order streams.

While several studies have suggested an algal dependence by the epilithon's bacterial community, others (Kaplan and Bott 1983, Bott et al. 1984, Ford and Lock 1987, Findlay 1993) have suggested that benthic bacterial biomass can be supported by dissolved organic matter. Bott et al. (1984) concluded that dissolved organic carbon in the water column supported 55% of the benthic bacterial biomass and that particulate matter attached to the benthic bacterial layer served as a potential nutrient pool for the bacteria, which may have limited the bacterial community's size. Ford and Lock (1987) developed a competitive access model for allochthonous organic carbon adsorption. They found that high-molecular-weight compounds saturated adsorption sites on the epilithon's surface and slowly diffused into the epilithon matrix. When these larger refractory organic compounds were removed from the water column low-molecular-weight compounds could more readily adsorb into the matrix. Interestingly, Ford and Lock suggest that a stream that is organically-rich, but has a great deal of large refractory organic compounds, may slow epilithon metabolism by filling all the potential adsorption sites on the epilithon matrix surface. However, in some cases metabolism may be increased if the adsorbed macromolecules provide a limiting nutrient to the epilithon.

Findlay et al. (1993) compared a closed canopy stream to a stream in an adjacent clear-cut watershed in the Hubbard Brook Experimental Forest and found that neither bacterial biomass nor bacterial productivity were even weakly coupled to either algal biomass or algal productivity in either stream. Subsequent manipulation of these epilithon communities in the field (shading) and laboratory (increased light and inorganic nutrients) failed to elucidate a coupling between the benthic bacterial and algal communities. They attribute the absence of a detectable bacterial-algal relationship to the oligotrophic nature (very low algal standing crop and productivity) of the Hubbard Brook streams, and propose that more eutrophic streams may be more suited for benthic bacterial-algal coupling.

In an earlier model, Lock et al. (1984) propose that the thick matrix may actually support the bacterial community's utilization of allochthonous organic matter by encouraging adsorption of organic material and providing a dense matrix that is embedded with exoenzymes that enable heterotrophs to be "on the spot" to incorporate labile products from refractory compounds that are readily being broken down. Kaplan and Bott (1983) contend that the benthic bacterial community selectively removes low-molecular-weight compounds from the water column since high-molecular-weight organic matter requires the production of exoenzymes to degrade the lower quality material. However, it may be advantageous to produce exoenzymes if they are not readily lost to the water column and will probably remain close to the cells which release them. Sinsabaugh and Linkins (1988) found that exoenzyme activity closely correlated with biomass in four epilithon communities exposed to different light and water velocity regimes. Freeman et al. (1990) found that high molecular weight organic matter actually inhibited several metabolic processes within the river epilithon, in particular the activity of several extracellular enzymes was inhibited.

Another aspect to consider when discussing bacterial activity within the epilithon is substrate availability. Some researchers have suggested that benthic bacteria may be substrate limited in environments that lack a well developed epilithon (Haack and McFeters 1982, McEldowney and Fletcher 1986, Morikawa 1988). McEldowney and Fletcher (1986) concluded that the conditioning of the solid surface, type of bacterial species, and complexity of organic molecules can influence the amount of bacterial attachment. An epilithon with a thin polysaccharide matrix (due to nutrient limitation, lack of algal growth, high shear stress, etc.) may lack the necessary surface area to maintain a large bacterial biomass.

Although there have been some studies which have attempted to determine relationships between autotrophic and heterotrophic communities within the stream epilithon (Haack and McFeters 1982a, Haack and McFeters 1982b, Arias Morales

1987, King and Cummins 1989a, King and Cummins 1989b, King and Cummins 1989c, Kaplan and Bott 1989, Hudson et al. 1992, Findlay 1993), few have adequately considered or properly manipulated the key environmental parameters that support the epilithon's biomass and metabolic activity (Findlay 1993). King and Cummins (1989abc) conducted several general studies in which they analyzed community productivity and respiration for multiple stream orders and measured several environmental parameters including light, temperature, and detritus biomass, but their methodology and sampling regime limit their conclusions to generalizations concerning P/R ratios. Several researchers have documented the importance of light availability in structuring the autotrophic community (Hudon and Bourget 1983, Lock et al. 1984, Behmer and Hawkins 1986, Duncan and Blinn 1989) and numerous papers have discussed the possible importance of allochthonous organic matter in structuring the bacterial community of the epilithon (Kaplan and Bott 1983, Bott et al. 1984, Ford and Lock 1987). These two variables are particularly important since they are the two main carbon vectors to the epilithon. Studies which have attempted to look at how the benthic bacterial and algal communities in a stream epilithon are affected by variations in both the epilithon's light availability and allochthonous organic matter availability are rare (Kaplan and Bott 1989, Hudson et al. 1992, Findlay et al. 1993). I am interested in elucidating the extent in which a stream epilithon's bacterial community relies on the epilithon's algal community to support its biomass and productivity.

I would argue that the epilithon's bacterial community can utilize both autochthonous energy derived from algal exudates and lysed algal cells within the epilithon, as well as allochthonous energy derived from organic matter processed in the water column. However, I would argue that the autochthonous component should be more important in supporting the bacterial community's biomass and productivity then the allochthonous component, regardless of the stream's degree of nutrient richness. Hence, I contend that the role of autotrophy in supporting bacterial biomass and productivity within a stream epilithon has been underestimated in the literature, while the importance of allochthonous organic matter has been overestimated. Appropriate manipulative studies which attempt to tease apart the relative importance of each possible carbon source in regulating the bacterial community's biomass and productivity for a wide array of streams are needed.

My attempt to determine the extent that benthic bacteria rely on benthic algae in a stream epilithon is presented in the following two chapters, which are each organized in the form a manuscript. Each chapter (i.e. manuscript) summarizes a separate study. The first chapter focuses on work conducted on the Ford River in 1991 and the second chapter focuses on work conducted at the Kellogg Biological Station's Experimental Stream Facility in 1992. In the 1991 Ford River study I attempted to determine the extent that bacterial biomass correlated with algal biomass between an adjacent stream run, riffle, and pool throughout colonization time. In the 1992 Experimental Stream Facility study I attempted to experimentally determine the extent to which bacterial biomass and productivity were impacted by increased algal productivity and increased DOC in the water column throughout colonization time.

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CHAPTER 1

TEMPORAL AND SPATIAL VARIATION IN THE BENTHIC BACTERIAL AND BENTHIC ALGAL ASSEMBLAGES BETWEEN A STREAM RUN, RIFFLE, AND POOL

ABSTRACT

Although bacterial and algal biomass frequently correlate in most aquatic ecosystems, there has been little evidence for such a correlation between stream benthic bacteria and algae. Benthic algal biomass and benthic bacterial biomass were compared between a run (depth 23 cm, velocity .08 m/s), riffle (depth 10 cm, velocity .50 m/s), and pool (depth 50 cm, velocity .02 m/s) along a ten m section of an open canopy, third order stream in Michigan's Upper Peninsula during the summer of 1991. Three spatially close, yet contrasting habitats (locations) were selected for study in an attempt to vary algal succession and biomass accumulation. I hypothesized that benthic bacterial biomass would correlate with benthic algal biomass within a summer epilithon assemblage, regardless of habitat. Epilithon colonization occurred for 3, 7, 14, 21, 28, and 35 days on unglazed ceramic tiles (2.54 cm^{-2}) which were attached to inverted trays and anchored to partially buried cinder blocks (n=3) across the three locations. Standing crop organic matter (expressed as ash-free dry weight or AFDW), chlorophyll a, and bacterial biomass (with epifluorescence

microscopy) (DAPI) were measured. Scanning electron micrographs and light microscope counts revealed diverse differences between the algal assemblages throughout colonization. Chlorophyll a and AFDW increased in the run and pool throughout the colonization period. In the riffle, chlorophyll a and AFDW increased at a greater rate than the other habitats early in colonization, yet decreased in late colonization. Repeated measures nested ANOVAs revealed significant differences between locations and between trays within each location for both chlorophyll a and AFDW. Benthic bacterial biomass increased rapidly and remained similar between the three habitats throughout colonization. A repeated measures nested ANOVA detected no significant differences between location or trays within each location for bacterial biomass. Bacterial biomass did not correlate with either Chlorophyll a or AFDW in any of the three habitats or on any of the sampling days throughout colonization.

INTRODUCTION

Relationships between bacterial and algal biomass have been well documented in aquatic ecosystems (Cole 1982, Cole et al. 1988, White et al. 1991), but evidence for such a relationship between benthic bacteria and benthic algae in a stream epilithon is sparse (McFeters et al. 1982, Haack and McFeters 1982a, 1982b, Goulder 1988). Two traditional mechanisms postulated for such a relationship are that aquatic bacteria: 1.) rely on nutrient-rich algal exudates from live cells (Cole 1982) and 2.) rely on nutrients released from lysed or senescing algal cells (Haack and McFeters 1982).

A stream epilithon is a diverse assemblage of algae (mostly diatoms), heterotrophic bacteria, cyanobacteria, fungi, and protozoa incorporated into a polysaccharide and glyco-protein matrix on stream substrates (Lock et al. 1984). This complex three-dimensional matrix readily develops and thickens on available substrate through a series of successional stages (Korte and Blinn 1983, Oemke and Burton 1986). The epilithon's colonization dynamics and composition can vary considerably between habitats in which physical (e.g. light, velocity) (Oemke and Burton 1986, Stevenson and Peterson 1989), chemical (e.g. DOC, PO_4) (Pringle 1990), and biological (e.g. invertebrate grazing) (McCormick and Stevenson 1989) parameters vary. The stream epilithon frequently plays a major role in stream primary production, secondary production, decomposition, and nutrient retention (Lock 1981, Winterbourn 1986).

Two particularly important physical parameters in structuring a stream epilithon community during colonization time are light availability and water velocity. Generally, decreases in light availability will decrease algal biomass (Fuller et al. 1986) and alter algal composition within the epilithon (Hudon and Bourget 1983). Differenes in water velocity above the epilithon's boundary layer can also dramatically alter algal biomass and composition (Hudon et al. 1987; Peterson and Stevenson 1989), but the relationships are not as clear. Decreases in water velocity can lead to nutient limitations for some benthic algal communities and increases in water velocity can erode the epilithon's biomass or trigger sloughing of the epilithon (Horner and Welch 1981). Hence, habitats which have dramatically different light and water velocity regimes should have differences in algal biomass and composition and follow different colonizational trajectories.

Unlike a planktonic environment, diffusion of exudates from live algal cells and released nutrients from lysed cells is thought to be inhibited by the physical nature of the epilithon's matrix. The proximity of benthic bacteria and benthic algae within the epilithon's matrix would intuitively suggest that the bacterial community would have both algal exudates from live cells and lysed nutrients from senescing cells available. Hence, the correlation frequently found between bacteria and algae in other aquatic ecosystems should also exist between benthic bacteria and algae in stream ecosystems. Although some studies have demonstrated such a correlation (Haack and McFeters 1982a, 1982b, and Goulder 1988), others have not (Findlay 1993).

Unlike past studies which have attempted to find a relationship between benthic bacterial and algal biomass in stream epilithon communities, this study looks at bacterial biomass and algal biomass accumulation and their relationship in three contrasting habitats (locations) throughout colonizational time. A sampling regime which accounts for variation within each location, between each location, and between sampling days was employed. This study also considers the role that algal community composition may play in a bacterial and algal relationship. I hypothesized that benthic bacterial and algal biomass in a stream epilithon would correlate regardless of habitat throughout colonizational time.

METHODS

Field research was conducted in July, 1991 on a third order, open canopy section of the Ford River in Michigan's Upper Peninsula. Clay tiles (2.54 cm^{-2}) attached to plastic cafeteria trays with silicon served as the substrate for epilithon colonization. Epilithon communities which are representative of communities colonized on natural substrate have been shown to colonize clay tiles (Tuchman and Stevenson 1980, Lamberti and Resh 1985). Trays were inverted and fastened to partially buried cinder blocks in order to prevent movement, minimize siltation, and minimize macroinvertebrate grazing. Trays containing tiles were arranged latitudinally across an adjacent run, riffle, and pool within a ten meter stretch of the river such that three trays were placed across each location (n=3) (Fig. 1). The three locations had contrasting depths and flow velocities throughout the study (Table 1). Replicate trays within each location were at similar depths and water velocities (Table 1), hence well matched in regards to physical variables.

Sampling Regime

Tiles were removed from each of the nine trays after 3, 7, 14, 21, 28, and 35 days of colonization. On a given sampling day, tiles from each tray were carefully processed in the field to minimize damage to the epilithon structure. Three tiles (from each tray) were placed in individual whirl-paks and iced for standing crop organic matter (AFDW) analysis. Similarly, three tiles were placed in whirl-paks, wrapped in aluminum foil, and iced for chlorophyll a analysis via fluorometry. Three tiles were fixed in a 5% filtered formalin solution to preserve bacterial cells for epifluorescence microscopy. Bacterial biomass as ug C/cm^{-2} was estimated based on direct microscopic counts (DMC)

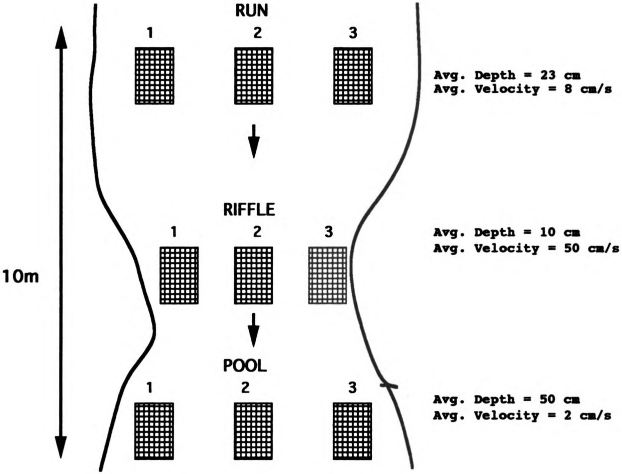

Fig. 1. Schematic diagram of the study site on the Ford River (MI) which highlights the spatial proximity of the three locations (run, riffle, and pool) and the placement of trays within each location. Average depth and velocity for each location is provided. Values represent the pooled mean from each tray within the location on each sampling day (n=18).

Table 1. Depth of each tray (cm) (top #) and water velocity (cm/s) (bottom #) 5cm above each tray at each location on each sampling day. 0 denotes a velocity less than 1 cm/s. Note: Water velocity was not recorded on Day 3.

COLONIZATION TIME	RUN			RIFFLE			POOL		
	TRAYS			TRAYS			TRAYS		
	1	2	3	1	2	3	1	2	3
Day 3	32	34	29	13	18	15	50	48	63
Day 7	21	25	19	9	9	9	40	51	53
	8	9	9	55	51	47	2	0	1
Day 14	20	20	18	6	10	10	41	53	57
	6	9	10	68	60	52	4	7	3
Day 21	19	22	17	6	7	9	37	52	56
	5	9	11	48	62	34	4	0	2
Day 28	18	21	16	7	9	8	38	53	55
	5	6	8	54	46	28	5	0	1
Day 35	24	25	22	12	12	13	55	56	50
	8	9	8	35	70	36	4	3	2
mean (tray)	22	25	23	9	11	11	44	52	56
	6	8	9	52	58	40	4	2	2
mean (loc) (±SE)	23 ±1.2			10 ±1.4			50 ±3.6		
	8 ±0.8			50 ±5.4			2 ±0.6		

(Bratbak 1985). Three tiles were placed in whirl-pak bags and allowed to air dry for diatom counts and energy dispersive x-ray microanalysis (see Laboratory Protocol). Diatom counts were limited to days 7, 21, and 35. Energy dispersive x-ray microanalysis was limited to day 35. Lastly, one tile was fixed in 5% glutaraldehyde for possible scanning electron microscope viewing. Current velocity (using a Marsh-McBirney Model 2000 portable flowmeter), depth, and temperature were measured at each tray on each sampling day.

Laboratory Protocol

Chlorophyll a - Upon returning to the laboratory whirl-paks containing tiles were removed from the dark, ice bath and frozen for 24 hrs to lyse cells. A known volume of buffered 90% acetone was added to each individual whirl-pak bag to extract pigments and freezer stored until filtered for fluorometric analysis. All samples were analyzed within a month of collection. Chlorophyll a was then determined by the procedures outlined in Methods 1003C and 1002G in Standard Methods (APHA 1985) and converted to ug/cm^{-2}.

Standing Organic Matter (AFDW) - Samples within whirl-pak bags were stored frozen until determination. The difference between a sample's oven dried weight (after 24 hrs in 60^{0}C drying oven) and ashed weight (1 hr in 450^{0}C ashing oven) was determined as an estimate of the weight of the standing organic matter as expressed as ash-free dry weight (AFDW). This weight includes all organic matter found in the epilithon. Hence, it is not an estimate of simply the algal and bacterial components of the epilithon, but rather it includes all organisms, detritus, and flocculated organic materials.

Diatom Relative Abundance - Field preserved samples were scraped from the tile with a razor blade and returned to whirl-pak bags. One ml sub-samples were pipetted onto 22 mm^2 glass coverslips. The coverslips were air dried and permanently mounted on glass slides using Hyrax medium. Counting to determine diatom relative abundance was done at 1,250x magnification on a Leitz Laborlux 11 microscope equipped with phase contrast illumination. Tiles from each tray were pooled and >250 valves per tray were counted for colonization days 7, 21, and 35.

Epilithic Bacterial Standing Crop (Biomass)- Field preserved samples were scraped with a sterile razor blade, homogenized with a tissue grinder, and returned to whirl-pak bags. Sub-samples were then stained for 20 min. in the dark on ice with DAPI (4'6-diamidino-2-phenylindole, 20 ug/ml, Sigma; to yield a final concentration of 2 ug/ml), a DNA specific stain which fluoresces when exposed to UV light. This concentration has been found to maximize direct cell counts, but avoid overstaining in stream benthic samples (Haack et al. 1988). Portions (0.25-3.0 ml) of stained sub-samples were filtered onto pre-stained black, 25 mm diameter, 0.2 um pore size, polycarbonate membrane filters (Poretics Corporation) which were backed with 25 mm, 0.45 um Millipore filters. Blanks were routinely prepared to assure that only the samples were contributing to the bacteria being counted. Bacteria was observed with a Leitz Laborlux II microscope, equipped with an HBO 50W mercury light source, Leitz wide-band UV filter set A (excitation filter 340-380, mirror 400, and barrier filter 430 nm), a 100/1.25 oil immersion objective, and 15x oculars. For days 3 and 7, two sub-samples from each tile were counted and on subsequent days (14, 21, 28, and 35), one sample was counted per tile. At least 20 cells were counted/field; 10 fields were counted for each sample. Past work has shown that 80% of the technique's variance is caused by variance in microscope fields as opposed to variance in filters or sub-samples (Kirchman et al. 1982), hence emphasis was placed on multiple field counts per filter per sample. Dilute sub-samples are necessary for stream benthic samples due to autofluorescence emitted from frustules and sand and light inhibition from detritus and silt. Biomass estimates were made by dividing cells into ten size classes (2 cocci, 4 narrow rods, and 4 wide rods) (Haack et al. 1988). Cell volumes were calculated using simple geometric shapes. Volumes of cocci were calculated as spheres, and rods were assumed to be cylinders capped with half spheres. Bacterial biomass as ug C/cm^{-2} was estimated by multiplying mean number of cells /cm^{-2} x mean cell volume (MCV) of all cells of that sample x 5.6 x 10^{-13} g C/um^{-3} (Bratbak 1985, Haack et al. 1988).

Scanning Electron Microscopy (SEM) - Tiles were removed from the glutaraldehyde solution, dried in sequential concentrations of ethanol (10, 25, 50, 75, 90, 95, 100%), and stored in 100% ethanol. Splintered pieces of tiles were critical-point dried under CO_2, mounted on stubs, and sputter-coated with gold. A JEOL JSM-35C electron microscope was used. Scanning electron microscopy was limited to observing one sample from each location on each day. Quantitative data was not collected, however obvious differences in algal composition were noted (e.g. diatoms vs filamentous green algae vs. cyanobacteria).

Energy Dispersive X-ray Microanalysis (EDAX) - An attempt to determine spatial variation in biologically relevant elemental concentrations within the epilithon of the three habitats using energy dispersive x-ray microanalysis proved unsuccessful due to a dilution effect from dead frustules and silt and sand deposits within the epilithon and elemental movement out of living cells during the epilithon dehydration procedure. I had hoped to detect differences in Si, P, and Fe related to diatom abundance, however sediment, detritus, silt, and dead frustules appear to dilute any significant biological findings. The technique fails to indicate any biological importance, merely the presence of certain atoms. I feel that it is only useful in epilithon studies when streams or sites with radically different water chemistries are being compared (Rounick and Winterbourn 1983) or manipulations with metal or nutrient concentrations are being used. This type of use somewhat defeats the purpose of having spatial resolution of several microns.

Statistical Analysis

Repeated measures nested ANOVAs were conducted to test the main effects of location and trays within location throughout colonization time for three separate dependent variables; AFDW, chlorophyll a, and bacterial biomass. For the repeated measures nested ANOVAs tiles were "nested" into trays and trays were "nested" into location. Post-hoc tests on pairs of means using Tukey's HSD procedure were conducted when significant ($p < .05$) main effects for location were detected in the repeated measures nested ANOVAs. When analyzing the correlation and covariance between bacterial biomass and chlorophyll a, as well as between bacterial biomass and AFDW, the trays served as the "experimental units" because separate tiles were used for each parameter estimated. In other words, bacterial biomass, chlorophyll a, and AFDW were not determined for each individual tile. Since the trays are the experimental units, the means of samples from each tray were used in the analyses. Regression analyses were conducted on the experimental units to determine if bacterial biomass correlated with either chlorophyll a or AFDW throughout the study. Individual sampling days and locations were analyzed as well. ANCOVAs for each sampling day were performed in which bacterial biomass served as the dependent variable, location was the independent variable, and chlorophyll a and AFDW served as covariates. ANCOVAs help determine whether variation in bacterial biomass is being masked by an interaction with a covariate. Significant variation between bacterial biomass and an independent variable (location) on individual sampling days may be masked by covariance with either chlorophyll a or AFDW. Repeated measures ANCOVAs cannot be performed because the covariates vary through time. The assumption of homogeneity of slopes was met for all ANCOVAs. Multiple ANCOVA and correlation p-values were adjusted or corrected with a sequential Bonferroni technique (Rice 1989) to ensure that results remain true to the desired pre-set alpha levels. This procedure was performed due to the large number of ANCOVAs and correlations. Diagnostics were performed on the residuals of the models of all the analyses to verify that errors were normally distributed, had a constant variance, and were independent. In the repeated measures nested ANOVAs multivariate analysis was interpreted when different from univariate analysis since compound symmetry is not an assumption for the multivariate analysis. All statistical analyses were performed with SYSTAT 5.1 (Wilkinson 1989).

RESULTS

Sets of trays within each of the three locations (run, riffle, and pool) remained well matched throughout the study regarding water depth and water velocity (Table 1). Dramatic water depth and velocity differences between locations were evident throughout the course of the study (Table 1).

Chlorophyll a increased significantly ($p < .05$) throughout colonization time in all three locations (Table 2, Fig. 2). Chlorophyll a increased steadily through colonization time in both the run and pool locations, however in the riffle location chlorophyll a increased rapidly and then oscillated (Fig. 2). Significant differences ($p < .05$) were found between trays in all three locations (Table 2). A significant ($p < .05$) location main effect was detected as well (Table 2). Post-hoc tests on pairs of means from the locations using Tukey's HSD did not reveal significant ($p < .05$) differences (Table 3).

Standing crop organic matter (AFDW) increased significantly ($p < .05$) throughout colonization time in the run and pool locations (Table 2, Fig. 3). Dramatic increases occurred on colonization day 35 in both locations. In the riffle location, AFDW increased rapidly in early epilithon

Table 2: Results of the Repeated Measures Nested ANOVAs. Tiles (n=3) are nested into tray. Trays (n=3) are nested into location (n=3). The three locations are "run", "riffle", and "pool". Repeated measures combine sampling days (n=6). A decomposition of the source of variance "trays" is provided in order to illustrate variance within each location. Dependent variables include chlorophyll a, standing crop organic matter (AFDW), and bacterial biomass.

Source of variation	(df)	MS	F	P
CHLOROPHYLL a:				
Between Subjects				
Tray(run)	2	11.94	5.69	0.012
Tray(riffle)	2	6.67	3.18	0.007
Tray(pool)	2	17.91	8.53	0.002
Location	2	8.89	4.24	0.031
Error	18	2.10		
Within Subjects				
Time	5	93.31	48.30	<0.000
Tray(run) x Time	10	5.72	2.96	0.022
Tray(riffle) x Time	10	3.07	1.59	0.001
Tray(pool) x Time	10	7.01	3.63	<0.000
Location x Time	10	33.34	17.26	<0.000
Error	90	1.93		
AFDW:				
Between Subjects				
Tray(run)	2	1.82	9.71	0.001
Tray(riffle)	2	1.34	7.15	0.005
Tray(pool)	2	1.05	5.58	0.013
Location	2	15.83	84.41	<0.000
Error	18	0.19		
Within Subjects				
Time	5	44.69	177.36	<0.000
Tray(run) x Time	10	2.69	10.68	0.005
Tray(riffle) x Time	10	0.39	1.55	0.010
Tray(pool) x Time	10	1.20	4.78	<0.000
Location x Time	10	11.98	47.56	<0.000
Error	90	0.82		

Table 2 (cont.):

Source of variation	(df)	MS	F	P
BACTERIAL BIOMASS:				
Between Subjects				
Tray(run)	2	0.00	0.01	0.998
Tray(riffle)	2	0.01	0.74	0.489
Tray(pool)	2	0.02	2.69	0.095
Location	2	0.06	0.78	0.471
Error	18	0.01		
Within Subjects				
Time	5	0.02	3.96	0.003
Tray(run) x Time	10	0.00	0.25	0.956
Tray(riffle) x Time	10	0.01	1.95	0.202
Tray(pool) x Time	10	0.00	0.55	<0.000
Location x Time	10	0.01	2.73	0.006
Error	90	0.01		

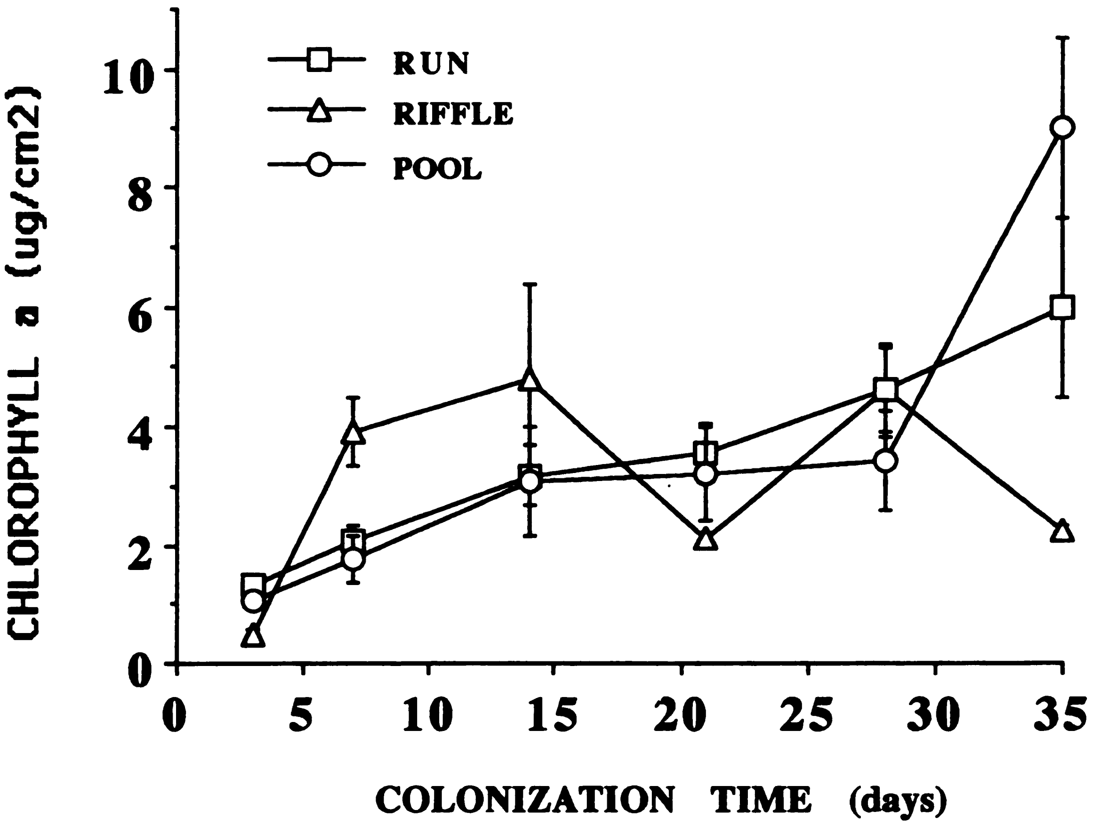

Figure 2. Chlorophyll a for the three locations throughout colonization time.

Table 3. Post-hoc tests on pairs of means using Tukey's HSD procedure for locations from Repeated Measures Nested ANOVAs in which a significant ($p < .05$) location main effect was detected. NS denotes $p > .05$.

Dependent Variable	Comparison	p-value
Chlorophyll a	run vs. riffle	NS
	run vs. pool	NS
	riffle vs. pool	NS
AFDW	run vs. riffle	NS
	run vs. pool	NS
	riffle vs. pool	NS

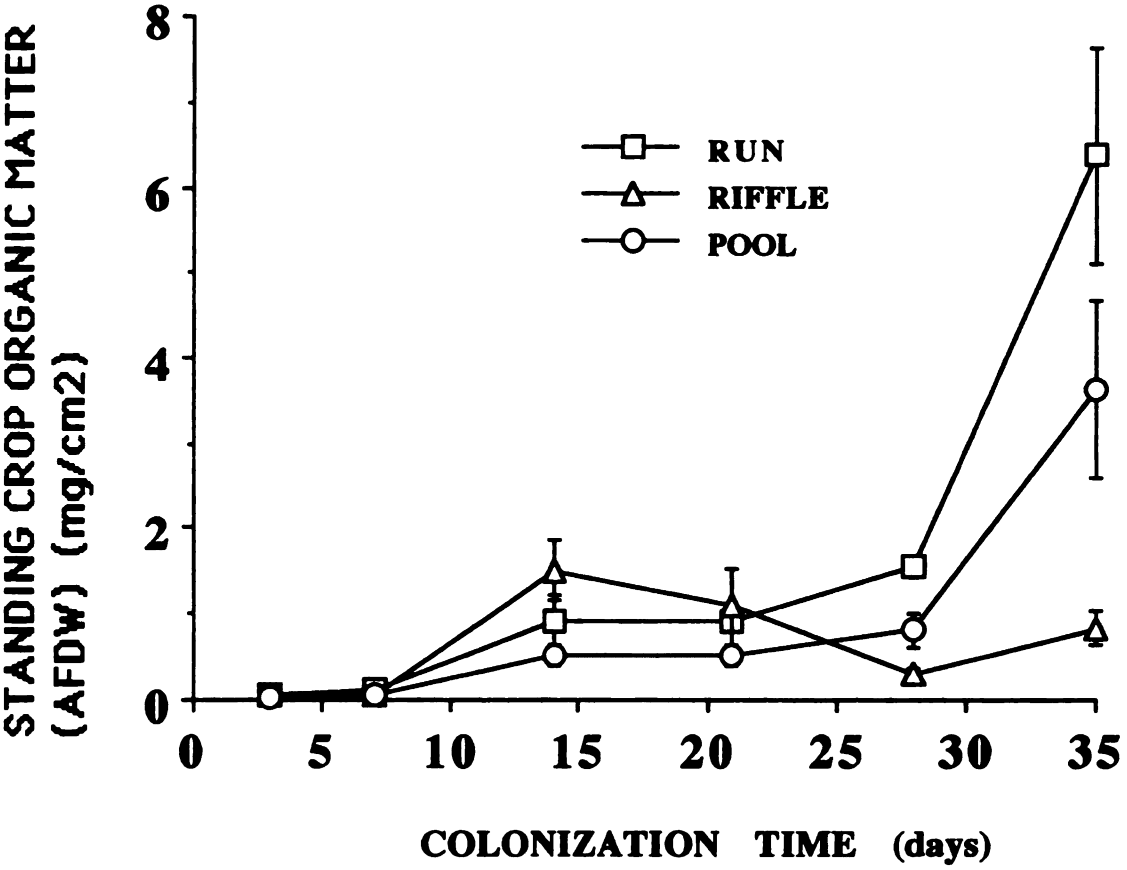

Figure 3. Standing crop organic matter (AFDW) for the three locations throughout colonization time.

colonization and decreased in late colonization (Fig. 3). Significant differences ($p < .05$) were found between trays in all three locations (Table 2). A significant ($p < .05$) location main effect was detected as well (Table 2). Post-hoc tests on pairs of means from the locations using Tukey's HSD did not reveal significant ($p < .05$) differences (Table 3).

Bacterial biomass increased rapidly in early epilithon colonization in all three environments and generally decreased in late epilithon colonization (Fig. 4), however a significant ($p < .05$) change throughout colonization time was only found for the pool environment (Table 2). Bacterial biomass did not significantly ($p > .05$) differ between locations or between trays within locations (Table 2).

The epilithon's diatom composition varied between locations and within locations through colonization time (Fig. 5). Achnanthes minutissima and Cocconeis placentula dominated community relative abundance in all locations throughout colonization time (Fig. 5). It appears that Achnanthes minutissima facilitates the colonization of Cocconeis placentula in the riffle location. Scanning electron micrographs showed that the riffle location's epilithon was dominated by tightly packed small diatom species throughout colonization. The pool and run locations appeared to contain more inorganic matter and detritus and had epilithons which appeared less cohesive, although this may be an artifact of the SEM dehydration procedure. The

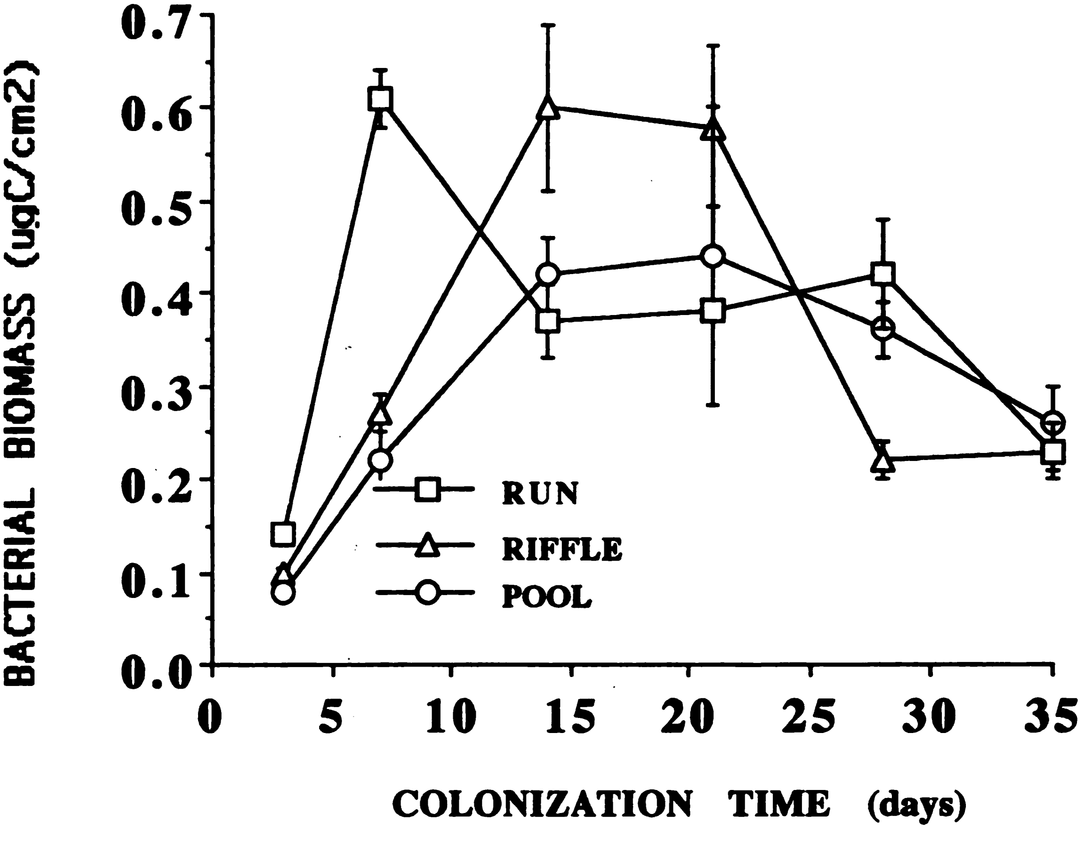

Figure 4. Bacterial biomass (epilithic standing crop biomass) for the three locations throughout colonization time.

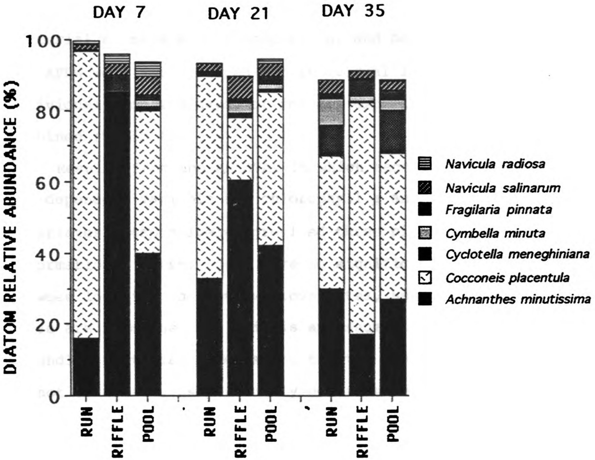

Figure 5. Diatom relative abundance for the three locations on colonization days 7, 21, and 35.

pool and run locations also contained cyanobacteria, especially in late colonization in which blooms of Anabaena sp? were observed.

Significant ($p < .05$) correlations between both bacterial biomass and chlorophyll a, and bacterial biomass and AFDW were not detected for individual locations, individual sampling days, or for all experimental units combined (Table 4).

Results from the ANCOVAs in which bacterial biomass was the dependent variable and chlorophyll a and AFDW the covariates, respectively, provided three pieces of information. First, there were no significant interactions between location and the two covariates, hence slopes for these are homogeneous. This is an assumption of ANCOVA. Secondly, bacterial biomass and the two possible covariates do not correlate, as previously demonstrated in the regression analyses. Thirdly, only on epilithon colonization day 7 is the location main effect significant ($p < .05$) when adjusting for the covariate (Table 5). Since bacterial biomass, chlorophyll a, and AFDW were estimated from separate tiles removed from a given tray, a nested ANCOVA could not be performed. Hence, how bacterial biomass varies between trays when adjusting for possible covariates could not be analyzed.

Table 4: Correlations between bacterial biomass and chlorophyll *a* and between bacterial biomass and AFDW for each location (3 trays x 6 sampling days), each sampling date (nine experimental units), and all experimental units on all sampling days ("All"). Note: Probabilities have *not* been adjusted with a Bonnferoni procedure since none of the correlations are significant.

		Chl. *a*		AFDW	
Data Set	n	r^2	p-value	r^2	p-value
Run	18	.04	.415	.08	.249
Riffle	18	.15	.111	.17	.089
Pool	18	.14	.126	.01	.722
Day 3	9	.02	.696	.05	.573
Day 7	9	.06	.538	.23	.197
Day 14	9	.01	.798	.08	.471
Day 21	9	.17	.272	.16	.288
Day 28	9	.06	.533	.23	.197
Day 35	9	.17	.272	.20	.225
All	54	.01	.636	.00	.969

Table 5. Results of the ANCOVAs for each sampling day in which bacterial biomass is the dependent variable, location is the independent variable, and either chlorophyll a or AFDW is the covariate. Trays serve as the "experimental units" for the ANCOVA analysis since separate tiles from each tray were used to estimate the three parameters. Two repeated measures ANCOVAs cannot be conducted because the covariates vary between days, hence individual ANCOVAs for each sampling day were performed. Since multiple tests are being conducted on the same parameter, p-values have been corrected with a sequential Bonferroni procedure (see methods). Hence, for the following p-values one (*) means p < .05 and two (**) means p < .01 as corrected by the sequential Bonferroni procedure. For all ANCOVAs location df=2, covariate df=1, and error df=5.

		COVARIATE			
		Chlorophyll a		AFDW	
DAYS	Source	F-ratio	P	F-ratio	p
Day 3	Location	10.8	.015	11.6	.013
	Covariate	0.2	.651	0.6	.616
Day 7	Location	46.3	.001**	16.0	.007*
	Covariate	5.5	.067	0.8	.759
Day 14	Location	6.80	.037	3.8	.100
	Covariate	2.5	.177	0.5	.527
Day 21	Location	2.2	.213	0.2	.209
	Covariate	<0.1	.998	0.7	.666
Day 28	Location	4.6	.073	0.5	.508
	Covariate	0.7	.434	0.5	.490
Day 35	Location	0.7	.539	0.5	.520
	Covariate	0.1	.850	0.8	.809

DISCUSSION

Although bacterial biomass frequently correlates with algal biomass in many aquatic ecosystems (Cole et al. 1988, White et al. 1991), such a relationship between benthic bacteria and benthic algae in stream ecosystems has been debated. This study attempted to address such a bacteria-algae relationship in a stream epilithon by investigating bacterial and algal biomass variation through colonization time within and between three contrasting stream locations. An adjacent run, riffle, and pool were selected as the three locations of interest due to their spatial proximity and dramatic differences in depth (light availability) and water velocity. Both of these physical parameters are known to be very important in structuring the epilithon's algal community (Hudon and Bourget 1983, Hudon et al. 1987, Peterson and Stevenson 1989), hence it was postulated that the three locations would have different colonization dynamics and algal biomass accumulation. The anticipated range of algal biomass at the three locations and throughout the 35 day colonization period was intended to provide ample algal biomass variation for looking at bacterial biomass covariation.

Algal Biomass and Standing Crop Organic Matter

Repeated measures nested ANOVAs demonstrated significant differences in chlorophyll a and AFDW between the three locations, yet also indicated that there were significant differences between trays within each location (Table 2). Although significant differences were expected between the three locations and throughout colonization time, dramatic differences between trays within the locations were not. Throughout the study trays within each location appeared well matched as far as water depth and water velocity were concerned (Table 1). In fact, one of the main considerations for initially selecting this study site was the apparent homogeneity of the water depth and velocity latitudinally across the adjacent run, riffle, and pool. The significant differences in the chlorophyll a and AFDW between trays within locations provides some evidence to support the idea that a stream habitat (i.e. run, riffle, pool) contains a "mosaic" of microhabitats (Pringle et al. 1988), hence it is inherently heterogeneous. Besides being surprised by the differences in chlorophyll a and AFDW observed between trays within habitats, I was surprised that the differences between locations were not more dramatic considering the contrasting physical features between the three locations (Table 3 and Fig. 2 and 3). The lack of dramatic differences may suggest that the increased algal biomass anticipated at shallow depths due to increased light availability may be counterbalanced by the deleterious effect of increased shear stress due to an increase in water velocity. This was most evident on colonization day 35 where the riffle environment, which received the greatest amount of light and had the greatest water velocity, had less chlorophyll _a_ and AFDW than the deeper pool and run locations (Figs. 2 and 3). Decreases in chlorophyll _a_ and AFDW late in colonization in the riffle location may be related to sloughing events in which a portion of the epilithon is physically removed by shear stress.

Algal Community Composition

Diatom composition varied considerably between the three locations in early colonization (day 7), and was more similar in the three locations late in colonization (day 35) (Fig. 5). Diatoms appeared to dominate the algal community in all three environments early in colonization and the riffle location throughout colonization, however cyanobacteria, _Anabaena_ _sp_??, was observed on the surface of tiles in the run and pool late in colonization and may have been responsible for the sudden increases in chlorophyll _a_ in those locations late in colonization. Although cyanobacteria is a type of bacteria, it is ecologically more similar to algae due to its ability to produce chlorophyll and conduct photosynthesis. Had heterotrophic bacterial biomass been found to correlate with chlorophyll _a_ such information on algal community composition and cyanobacteria may have been quite useful in looking for relationships between bacteria and specific guilds of diatom species or different sources of chlorophyll a. It is important that future work which investigates relationships between benthic bacteria and benthic algae consider variation in algal community composition between locations and successional stages and not describe the algal community based on biomass estimates alone.

Bacterial Biomass

While significant differences were detected between and within locations for both chlorophyll a and AFDW, there were no significant differences in bacterial biomass either between locations or trays within locations (Table 2). A lack of significant differences in bacterial biomass was not predicted, especially considering the differences in chlorophyll a and AFDW. Between colonization day 3 and day 14 bacterial biomass increased rapidly in all three environments as expected, yet generally decreased in late colonization (Fig. 4). Since the estimates for bacterial biomass are based on DMCs which do not distinguish between viable and non-viable bacterial cells, the initial increase in bacterial biomass would be predicted based on the accumulation of bacterial cells from the water column during epilithon formation. However, the lack of significant variation between the run, riffle, and pool and slight overall decrease in biomass in late colonization are difficult to explain convincingly. Although, the bacterial biomass results are difficult to interpret, they are based on a large and comprehensive number of counts considering the time limitations of the technique, hence I am confident that the trends reported are indeed real. Bacterial biomass was estimated from >200 cells from each tile and determined for a minimum of three separate tiles for each tray within each location on each sampling day, hence on any one sampling day a minimum of 1800 bacterial cells were counted at each location.

Bacterial-Algal Relationship

I initially hypothesized that bacterial biomass would correlate with algal biomass throughout colonization time regardless of habitat. This hypothesis was based on the fact that benthic bacteria and benthic algae are embedded within the polysachharide matrix of the epilithon, hence bacteria should be able to rely on algal exudates from live algal cells and degrading material from lysed algal cells within the epilithon. Since conducting this study I have refined my ideas on bacterial-algal relationships in stream epilithons, and would predict that if bacterial biomass is responding to the presence of algal biomass it should be most evident in late epilithon colonization when a thick, mature epilithon has developed. A thick, late successional epilithon should have less interaction with the DOC in the water column, hence available carbon to support bacterial production should come from either live algal exudates or lysed algal cells within the epilithon. The sampling scheme employed in this study enabled epilithon development to be tracked over a 35 day colonization period, hence changes in a possible bacterial-algal relationship could be looked at through colonization time.

Although bacterial biomass was predicted to covary with algal biomass throughout epilithon colonization, or at least in late epilithon colonization, the results of this study suggest that such a relationship did not occur in the epilithon communities studied. Significant correlations were not found between either bacterial biomass and chlorophyll a, or between bacterial biomass and AFDW at any of the three sites (when data from all six sampling days was pooled) or on any of the six sampling days (when data from all three locations was pooled) (Table 4). Results from separate ANCOVA's conducted for each sampling day in which bacterial biomass was the dependent variable, location was the independent variable, and either chlorophyll a or AFDW was used as a covariate concurred with the results from earlier analyses (Table 5). Only on colonization day 7 is there a main effect or location effect when chlorophyll a and AFDW are used as individual covariates. Unfortunately, nested ANCOVAs, in which tiles would be nested into tray, could not be utilized because chlorophyll a, AFDW, and bacterial biomass were estimated from separate tiles to ensure precision in estimates. In retrospect, a subsampling regime should have been employed in which these parameters were measured from the same tile. Degrees of freedom are dramatically reduced in the analyses when the experimental unit is changed from "tile" to "tray".

Several reasons can be postulated for why bacterial biomass did not correlate with either chlorophyll a or AFDW in this study: 1.) a mature, late successional epilithon community was not sampled, 2.) grazing by microorganisms within the epilithon reduced bacterial numbers disproportionately between trays, 3.) the percentage of viable cells within the various epilithon communities sampled varied considerably, 4.) benthic bacteria rely primarily on DOC in the water column.

Oemke and Burton (1986) found that mature diatom communities were established after a 28 day colonization period in both pools and riffles in a fourth order section of the Ford River, hence it is likely that mature epilithon communities were established during this study's 35 day colonization period. However, chlorophyll a and AFDW increased throughout the course of the study in both the run and pool locations suggesting that epilithon communities in these locations may not have reached a climax successional stage. Regardless, major 'reset' events to epilithon colonization such as scouring events (e.g. dislodgement of a debris dam or increased sediment in the water column following a storm), macroinvertebrate grazing, and sudden increases in discharge were not observed during the course of the study, hence it is likely that mature epilithon communities were capable of developing.

Grazing by microorganisms within the epilithon may have reduced bacterial numbers disproportionately, but I have no evidence to suggest patchy grazing activity. Recent work by Kaplan and Bott (19??) (Note: Kaplan/Bott's scheduled NABS '93 talks deal with this issue and I have written them to better address this issue.) suggests that ciliates, flagellates, protozoa, and other meiofauna within the epilithon can remove a significant portion (>80%) of the bacterial biomass within an epilithon and can keep biomass cropped at a set density. Truly little is known about the roles that bactivory and algivory by microorganisms within the epilithon play in structuring the epilithon community. Much more research is necessary on the grazing microbial community within the epilithon.

As addressed earlier, bacterial biomass based on DMCs does not estimate the percentage of the bacterial community that is viable. Hence, if only a small percentage (5-30%) of the bacterial community is viable and a large portion of non-viable bacteria are continually accumulating within the epilithon through colonization, then it may be very difficult to detect if the viable bacteria are covarying with algal biomass. Recent advances in microscopy may make accurate viable vs. non-viable counts possible with stains which detect dividing cells (NEED REFERENCE). Biomass estimates of viable cells and non-viable cells coupled with estimates of bacterial productivity (e.g. ^{3}H-thymidine incorporation into bacterial DNA) would greatly enhance our ability to detect bacterial-algal associations.

Although I have attempted to provide a series of 'strawmen' to explain why this study failed to detect a benthic bacterial reliance on benthic algae, it is quite possible that such a relationship truly does not exist in the epilithon communities studied. A much more comprehensive study by Findlay et al. (1993) failed to find a bacterial-algal linkage in epilithon communities in streams within the Hubbard Brook Experimental Forest even though such a relationship was postulated. They concluded that even if there was a weak bacterial-algal linkage, it was not readily detectable due to the relative large allochthonous input in the oligotrophic streams they studied. They suggested that such a relationship is more likely in more eutrophic streams. Although the Ford River has much greater chlorophyll a levels than the streams studied by Findlay et al. (1993), it appears to fall within the middle of the range of chlorophyll a estimates for temperate streams provided by Lock (1981), hence the Ford River would probably be classified as meso-eutrophic.

The quantity and quality of the DOC in the Ford River may be ample to support the benthic bacterial community and the epilithon's polysachharide matrix may be such that a large influx of stream water penetrates the epilithon. This may explain why significant differences in bacterial biomass were not detected between the locations and between trays within the locations. Much of the Ford River has a sandy bottom, hence epilithic algal communities on the surface of the sandy bottom may be frequently scoured and disturbed releasing exudates into the water column. The labile nature of these exudates coupled with the rapid turnover time of epilithic algae, may mean that such labile exudates could make up a significant portion of the streamwater's labile DOC. Hence, even though the Ford River's $P/R < 1$ and frequently little algal standing stock biomass is observed on the river's sandy bottom (personal observation), benthic algae could still be increasing the river's DOC quality (Minshall 1978). Although this is completely speculative, it highlights the need to better understand the quantity, quality, and source of DOC in the water column in such a study. During this study, regrettably, no attempt to characterize the Ford River's DOC was made.

Conclusion

The results of this study suggest that bacterial biomass does not correlate with algal biomass in a meso-eutrophic stream epilithon throughout epilithon colonization. Such a correlation may have been very difficult to detect considering the variable nature of stream benthic communities, inherent difficulty in manipulating epilithon communities, limited characterization of the bacterial and algal communities, and lack of characterization of the stream's DOC. Future studies which pursue bacterial-algal relationships in stream epilithons need to utilize a more controlled and manipulative experimental approach and expand the characterization of the benthic algal and bacterial communities.

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CHAPTER 2

BENTHIC BACTERIAL-ALGAL RELATIONSHIPS IN A STREAM EPILITHON

ABSTRACT

This study examined the extent to which the benthic bacterial community in a stream epilithon responds to autochthonous energy inputs (benthic algae in the epilithon) and labile allochthonous inputs (DOC found in the water column) throughout colonization. I hypothesized that the benthic bacterial community would readily respond to labile allochthonous inputs early in colonization, when the epilithon in which it resides is poorly developed, and respond to increased algal productivity as a mature epilithon develops. Twelve, non-circulating, stream side experimental channels were constructed along Augusta Creek in southwest Michigan. Over six thousand gallons of stream water an hour were pumped from the stream into a reservoir and gravity-fed across ceramic tiles which served as the artificial substrate in the 3 m experimental channels. The experimental channels ensured the development of a mature epilithon community, enabled the removal of macroscopic grazers, minimized sedimentation problems, and permitted proper replication of treatments. A 2x2 factorial was employed with 3 replicate blocks in which light (shading, 90% removal of PAR) and DOC (glucose addition)

were manipulated. Tiles were sampled throughout a 25 day colonization period. Standing crop organic matter was determined with ash-free dry weight (AFDW) measurements. The benthic algal community was characterized with chlorophyll a estimates and live diatom biovolume estimates for both individual species and the community as a whole. The benthic bacterial community's biomass was estimated by direct microscopic counts (DMC) with epifluorescence microscopy (DAPI) and productivity was estimated by measuring the rate at which [^{3}H]thymidine was incorporated into bacterial DNA. Shading significantly reduced chlorophyll a, live diatom biovolume, and AFDW throughout colonization. Live diatom community composition between treatments was surprisingly similar throughout colonization time. Bacterial biomass based on DMCs increased significantly through colonization time, but revealed little variation between treatments. Bacterial productivity was found to be significantly greater in the treatments receiving glucose additions throughout colonization, however bacterial productivity increased in the unshaded, non-glucose treatment in late colonization. These results suggest that bacterial productivity may respond to DOC in the water column throughout epilithon colonization, but may also rely on the presence of algae late in colonization when a mature epilithon has developed. These results also suggest that differences in bacterial productivity may not result in detectable differences in bacterial biomass in the epilithon as measured with DMCs.

INTRODUCTION

Interactions between bacteria and algae have been well documented in many aquatic ecosystems (Cole 1982, Cole et al. 1988, White et al. 1991), however there has been considerable debate regarding such a relationship between the benthic bacterial and benthic algal communities in a stream epilithon (Haack and McFeters 1982a, Goulder 1988, Kaplan and Bott 1989, Findlay et al. 1993).

The stream epilithon (biofilm) is a diverse assemblage of algae (mostly diatoms), heterotrophic bacteria, fungi, cyanobacteria, and protozoa embedded into a three-dimensional polysaccharide and glyco-protein matrix on stream substrates (Lock 1981, Rounick and Winterbourn 1983, Lock et al. 1984). This complex three-dimensional matrix readily develops and thickens on available substrate through a series of successional stages (Korte and Blinn 1983, Oemke and Burton 1986). The stream epilithon has received a great deal of attention due to its importance in stream ecosystem function. It has been shown to play an important role in primary production (Minshall 1978, Lock 1981), decomposition (Ladd et al. 1979, Ford and Lock 1987), nutrient retention (Paul and Duthie 1988, Mullholand et al. 1991), and the sequestering of DOC from the water column (Lock 1981), as well as in supporting a diverse grazing community (Fuller et al. 1986, Mayer and Likens 1987, Winterbourn 1990, Power 1992).

Some studies have suggested that the epilithic bacterial community may be supported by the benthic algal community within the stream epilithon (Haack and McFeters 1982a, 1982b, Goulder 1988). Mechanisms frequently postulated for such a relationship include: 1.) the presence of a nutrient rich microzone or phycosphere surrounding live algal cells (Cole 1982), 2.) algal senescence within the epilithon (Haack and McFeters 1982), 3.) the formation of a diffusional barrier which excludes organic matter from the water column (Lock 1981, Peterson and Grimm 1992).

However, while several studies have suggested an algal dependence by the bacterial community, others (Bott et al. 1984, Ford and Lock 1987, Findlay et al. 1993) have demonstrated that benthic bacteria can be supported by allochthonous inputs or dissolved organic matter in streams. Lock et al.(1984) proposed that the epilithon matrix could actually support the heterotrophic community's utilization of allochthonous organic matter by encouraging adsorption of organic material and providing a dense matrix that is embedded with exoenzymes. Recent studies (Sinsabaugh and Linkins 1988, Freeman et al. 1990) have suggested that biofilm enzyme activity varies considerably in response to changes in the epilithon's composition.

While several past studies have attempted to determine the extent to which benthic bacterial biomass and productivity in stream epilithons rely on benthic algae, few have attempted to use an adequate experimental approach in the field (Bott and Kaplan 1989, Findlay et al. 1993), none have fully considered the role algal community composition might play, and few have considered the role of colonization time or the successional state of the epilithon (Blenkinsopp et al. 1991). Blenkinsopp et al. (1991) suggested that epilithon thickness and density could influence storage product deposition within the epilithon regardless of the stream's water chemistry. In a study that investigated the role of nutrient cycling and grazing in regulating periphyton communities, Mulholland et al. (1991) proposed a related model which suggested that a thin, disturbed epilithon should rely primarily on nutrients from the water column, while a thick, mature community should rely primarily on nutrients recycled within the epilithon.

I hypothesized that the benthic bacterial community would readily respond, as measured by increased bacterial biomass and productivity, to labile allochthonous inputs (DOC in the water column) early in colonization, when the epilithon in which it resides is poorly developed, and respond to increased algal productivity as a thick, mature epilithon develops. I would argue that the epilithon's bacterial community can utilize both autochthonous energy derived from the benthic algal community within the epilithon, as well as DOC in the water column. However, I would argue that the autochthonous component is more important in supporting bacterial biomass and productivity than the allochthonous component in a well developed, late successional epilithon.

METHODS

Study Site and Experimental Channel Facility

Twelve non-circulating, stream-side experimental channels were designed and constructed beside a third order section of Augusta Creek in the Kellogg Biological Station's Experimental Forest in Southwest Michigan. Augusta Creek is a warm water trout stream and a tributary of the Kalamazoo River. Its watershed contains a combination of natural wetlands, deciduous forests, and agricultural fields.

Over six thousand gallons of stream water an hour were pumped with a high volume, centrifugal pump (Teel Industrial Series, model 1P551A, 2HP) from Augusta Creek through PVC piping into a 450 gallon opaque, fiberglass reservoir. An industrial suction strainer was attached to the PVC intake pipe (5.08 cm diameter), which was secured within a protective ring of submerged cinder blocks to prevent intake obstruction. The reservoir enabled water to be gravity fed from a constant head into the experimental channels via PVC pipes mounted to the base of the reservoir (Fig. 1). The initially translucent reservoir was darkened with black spray paint to minimize the internal growth of benthic algae and then painted with an additional coat of white spray paint to

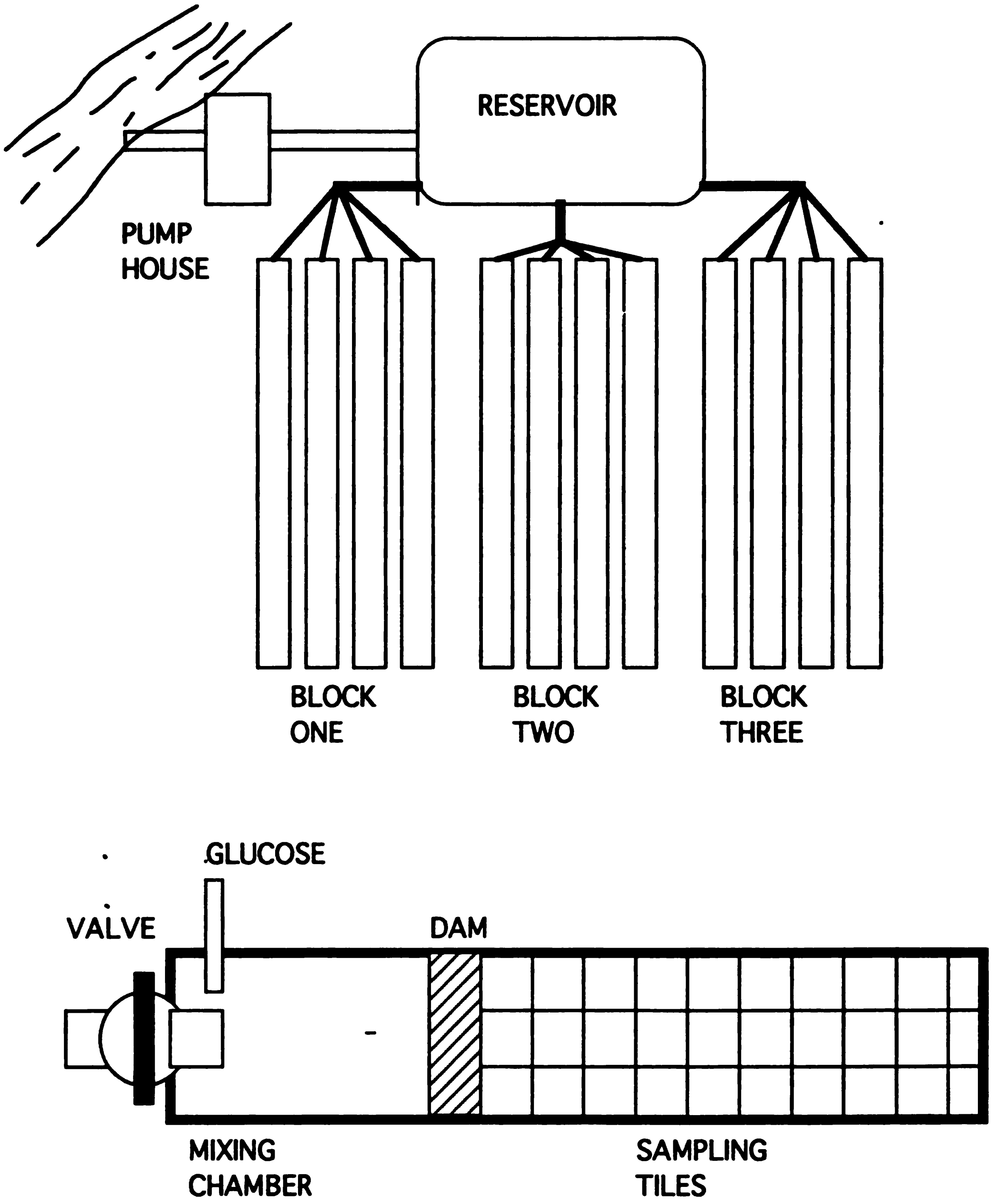


Figure 1. (Above) Schematic diagram of stream-side, experimental channels and their attachment to a central reservoir. (Below) Schematic diagram of the upstream section of one of the non-shaded experimental channels.

minimize heat absorption. PVC pipe diameter was maximized and length minimized in order to minimize drag. High pressure, adjustable plastic ball valves regulated flow in PVC pipes entering individual experimental channels. White vinyl rain gutters (3 m length x 10 cm width x 10 cm height) mounted on elevated supports served as experimental channels. Long channels were used to assure homogeneous flow in working (sampling) sections. Channels were lined with attached white (non-glazed) ceramic tiles (2.54 cm x 2.54 cm each) which served as the artificial substrate for epilithon colonization. Epilithon communities which colonize clay tiles have been shown to be representative of communities colonized on natural substrate and clay tiles aid in quantifying the epilithon community (Tuchman and Stevenson 1980, Lamberti and Resh 1985). The channel floor was completely covered with attached tiles to minimize variation in the channel's boundary layer. Sampled tiles were immediately replaced to prevent boundary layer disturbance (Stevenson 1983). Fluorescent inert dye injections prior to experimentation were performed to assure that there was adequate mixing in the water column, even flow across tiles, and standardized water velocity between channels. Flow rates were nearly 1 m/sec. Discharge between channels was standardized throughout the experiment. Discharge and flow velocity were monitored at least twice daily throughout the course of the experiment. Flow rate and discharge could be adjusted throughout the experiment by adjusting the high pressure ball valves connected to each channel's intake. White channels and white tiles were used to prevent the absorption of excess heat during the initial stages of epilithon colonization. Water returned to the stream from the experimental channels entered downstream of the intake via a large trench to prevent re-circulation of water. Because of the rapid flow through time, water temperature was not noticeably altered by the experimental stream facility.

Experimental channels were employed in this experiment in order to provide proper replication of treatments, to control and manipulate epilithon colonization, to assure the development of a mature epilithon community, to enable the removal of macroscopic grazers, and to minimize the adverse impact of sedimentation.

Experimental Design

A completely randomized block design (3 blocks), with a 2x2 factorial (twelve channels total) in which light and DOC (dissolved organic carbon) were experimentally manipulated was employed during two separate colonization periods. The first colonization period is referred to as the "preliminary experiment" and the second colonization period is referred to as the "primary experiment". The experimental channels served as the "experimental units" or interdispersed replicates, and individual tiles within channels served as samples. Light was experimentally manipulated by shading experimental channels throughout epilithon colonization (90% of photosynthetically active radiation (PAR) was removed). The shading treatment was intended to reduce algal biomass within the epilithon and simulate the light removal of a closed canopy stream section. DOC was experimentally manipulated with periodic glucose additions throughout epilithon colonization. Stream water DOC was experimentally elevated from approximately 3 mg/l to 10 mg/l. Glucose was dripped into designated experimental channels for 72 hours prior to sampling dates. In the primary experiment (Aug. 21 through Sept. 14, 1992) tiles were sampled on days 3, 10, and 25, hence glucose additions occurred on days 0-3, 7-10, and 22-25. In the preliminary experiment (July 24 through Aug. 17, 1992) sampling occurred on days 10, 20, and 25, hence glucose additions occurred on days 7-10, 17-20, and 22-25 (see Preliminary Experiment). A mixing chamber created by the placement of a secured 3cm tall dam in each experimental channel assured mixing prior to the channel's working or sampling section (Fig. 1). Drip rates were monitored every six hours.

Although naturally present in very low concentrations, glucose was used as the additional DOC source because it is extremely labile and would ensure contrasting treatments. The recalcitrance of the 3 mg/l DOC found naturally in the stream water used in the experiment would have been extremely difficult to simulate and control and may not have been appropriate for such a controlled experiment in which distinct environments were desired. Glucose was added only 72 hours prior to epilithon sampling and not throughout colonization so that the epilithon's response at a given colonization time was monitored and to assure that comparable, diverse microbial communities were present throughout the study. Sampling days were spaced such that there was ample recovery time between glucose additions.

Characterization of the Epilithon

Total organic standing crop biomass was characterized with ash-free dry weight (AFDW) estimates. The epilithon's benthic algal community was characterized with estimates of chlorophyll a and diatom biovolume. Diatoms constituted nearly all the benthic algal biomass. Live algal biovolume (LABV) was determined by conducting live/dead cell ratio and cell biovolume estimates on a per species basis. The benthic bacterial community's standing biomass was estimated with direct microscopic counts (DMC) using epifluorescence microscopy. Bacterial productivity was determined by measuring the rate at which [^{3}H]thymidine was incorporated into bacterial DNA.

Sampling Protocol

On each sampling day, twenty tiles were carefully removed with a razor blade from each experimental channel within the designated working section of each channel. The sampling days in the primary experiment were selected to represent early, intermediate, and late stages of epilithon colonization and were based on observations and data from the preliminary study. Sampling was conducted between 12:00 pm and 3:00 pm on relatively cloudless days. The sampled tiles were immediately replaced to avoid prolonged disturbance to the channel's flow dynamics. Sampled tiles were randomly placed in individual, pre-labelled whirl-paks and placed in the dark and on ice to prevent degradation of chlorophyll and unnatural cellular activity. For each of the twelve channels, five tiles were sampled for AFDW estimates, five tiles were sampled for chlorophyll a estimates, three tiles were sampled for live diatom biovolume estimates (primary experiment only), three tiles were sampled for direct microscopic counts of bacteria, and four tiles were sampled for measuring bacterial production ([^{3}H]thymidine uptake experimentation). Tiles sampled for AFDW and chlorophyll a estimates remained in the dark on ice until returning to the laboratory, however tiles intended for direct microscopic counts were field-fixed with filtered 5% formalin and tiles intended for the [^{3}H]thymidine uptake procedure were bathed in 20 ml of water from the sampled channel and then placed on ice in the dark. Tiles intended for live algal biovolume determination were bathed in 48 ml of channel water and preserved with 2 ml of M3 solution (0.05% KI, 1.0% Iodine, 5% glacial acetic acid, 25% formalin). This preservation method does not disturb the protoplasm of live cells, hence live and dead diatom frustules can be discriminated from one another. This is particularly important in this study since diatoms make up nearly all of the benthic algal community and are frequently over-estimated on the basis of frustule counts alone.

Preliminary Experiment

A preliminary experiment was conducted immediately prior to the primary experiment in order to monitor epilithon colonization in the manipulated experimental channels, gain estimates of sample variation within experimental units, and assure that field and laboratory protocol was sound. The preliminary and primary experiments were identical, except that the preliminary experiment's epilithon community was sampled on days 10, 20, and 25. This time frame was judged to be inadequate and was appropriately modified. The preliminary experiment ensured that the appropriate sampling regime and sampling protocol was being utilized to accurately address the study's primary questions. Diatom biovolume was not estimated throughout this experiment and bacterial productivity was not estimated on colonization day 20.

Laboratory Protocol

Chlorophyll _a_ - Upon returning to the laboratory, whirl-paks containing tiles were removed from the dark, ice bath and frozen for 24 hrs. to lyse cells. A known volume of buffered 90% acetone was added to each whirl-pak to extract pigments and freezer stored until filtered for fluorometric analysis. All samples were analyzed within a month of collection. Chlorophyll _a_ was then determined by the procedures outlined in Methods 1003C and 1002G in _Standard Methods_ (APHA 1985) and converted to ug/cm^{-2}.

Standing Organic Matter (AFDW) - Samples within whirl-paks were stored frozen until determination. The difference between a sample's oven dried weight (after 24 hrs. in 60°C drying oven) and ashed weight (1 hr. in 450°C ashing oven) was determined as an estimate of the weight of the standing organic matter. This weight includes all organic matter found in the epilithon. Hence, it is not an estimate of simply the algal and bacterial components of the epilithon, but rather it includes all organisms, detritus, and flocculated organic materials.

Live Algal Biovolume - Field preserved samples were scraped from the tile with a razor blade and returned to whirl-pak bags. Each bag was sonicated for 5 min. to further disrupt aggregates of cells. One ml sub-samples were pipetted onto

22 mm^2 glass coverslips. The coverslips were air dried and permanently mounted on glass slides using Hyrax medium. Counting to determine live and total diatom density on a per species basis were done at 1,250x magnification on a Zeiss microscope equipped with phase contrast illumination. Frustules in which internal protoplasm was readily apparent were designated as "live". Relatively dilute sub-samples were used to assure an accurate determination of live cells. Transects were taken moving across the coverslip until 100-200 frustules were counted. Estimates of diatom densities were then calculated from these quantified samples via the equation:

$$\text{cells cm}^2 = \frac{\text{(valves counted) (area coverslip) (volume conc.)}}{\text{2 (area transect) (volume subsample) (area sampled)}}$$

Estimates of live diatom densities were then calculated with the same equation, except "valves counted" was replaced with "live valves counted". Cell volumes were estimated for the ten most common species based on the "shoebox" method in which volume is based on the (length x width x height) of representative frustules. Total live algal biovolume (LABV) was quantified by summing each species' live density multiplied by its species specific cell volume. Total frustule biovolume (TFBV), which includes both live and dead frustules, was analyzed in order to compare with LABV.

Epilithic Bacterial Standing Crop (Biomass)- Field preserved samples were scraped with a sterile razor blade and returned to whirl-pak bags. Each bag was sonicated to further disrupt aggregated microbes. Sub-samples were then stained for 20 minutes in the dark on ice with DAPI (4'6-diamidino-2-phenylindole, 20 ug/ml, Sigma; to yield a final concentration of 2 ug/ml), a DNA specific stain which fluoresces when exposed to UV light. This concentration has been found to maximize direct cell counts, but avoid overstaining in stream benthic samples (Haack et al. 1988). Portions (0.25-3.0 ml) of stained sub-samples were filtered onto pre-stained black, 25 mm diameter, 0.2 um pore size, polycarbonate membrane filters (Poretics Corporation) which were backed with 25 mm, 0.45 um Millipore filters. Blanks were routinely prepared to assure that only the samples were contributing to the bacteria being counted. Bacteria was observed with a Leitz Laborlux II microscope, equipped with an HBO 50 W mercury light source, Leitz wide-band UV filter set A (excitation filter 340-380, mirror 400, and barrier filter 430 nm), a 100/1.25 oil immersion objective, and 15x oculars. At least 20 cells were counted/field; 10 fields were counted for each sample. Past work has shown that 80% of the technique's variance is caused by variance in microscope fields as opposed to variance in filters or sub-samples (Kirchman et al. 1982), hence emphasis was placed on multiple field counts per filter per sample. Dilute sub-samples are necessary for stream benthic samples due to autofluorescence emitted from frustules and sand and light inhibition from detritus and silt. Biomass estimates were made by dividing cells into ten size classes (2 cocci, 4 narrow rods, and 4 wide rods) (Haack et al. 1988). Cell volumes were calculated using simple geometric shapes. Volumes of cocci were calculated as spheres, and rods were assumed to be cylinders capped with half spheres. Bacterial biomass as ug C/cm^{-2} was estimated by multiplying mean number of cells /cm^{-2} x mean cell volume (MCV) of all cells of that sample x 5.6 x 10^{-13} g C/um^{3} (Bratbak 1985, Haack et al. 1988).

Bacterial Production- Epilithic bacterial production was estimated by measuring the rate at which [^{3}H]thymidine (TdR) is incorporated into bacterial DNA (Findlay et al. 1984, Hudson et al. 1990). The laboratory protocol outlined by Findlay et al. (1984) was closely followed. On each sampling day, four tiles from each experimental channel were returned on ice, in the dark, to the laboratory immediately following sampling. Each whirl-pak bag contained 20 ml of channel water, as well as the sample tile.

Incubation- One nmole (20 uCi/ nmole) which was diluted from a stock solution of 84 uCi/nmole (supplied from ICN (Cat # 2403905), was added sequentially to each sample to initiate incubation. Although this is a relatively low specific activity, it was determined to be adequate due to the low amount of indigenous thymidine (effective pool size) found naturally in the water column. The specific activity used was based on the results of the isotope dilution experiment in which the thymidine "effective pool size" was estimated (see Isotope Dilution) (Findlay et al. 1984). Two of the four samples from each channel were "killed" immediately after the addition of [^{3}H]TdR with 5% filtered formalin and then incubated for an hour. The remaining two samples were "killed" after a one hr. incubation. All samples were incubated at stream temperature and mechanically mixed. Following incubation, all samples were filtered onto a 0.45um filter. Each sample was rinsed three times with 5 ml of 5% filtered formalin to remove unincorporated [^{3}H]TdR. Filters were returned to the representative whirl-pak bag and frozen along with the tile until extraction.

Extraction of Bacterial DNA- Alkaline extract (20 ml of 0.6N NaOH + 1% SDS + 25mM EDTA) was added to frozen samples which were then mechanically shaken at 4^{0}C for 18 hrs. The supernatant in the whirl-pak bags contains hydrolyzed RNA, intact DNA, and protein. 5ml of the supernatant was chilled to 0^{0}C, neutralized with 1ml of 3N HCL, and acidified with TCA to a final concentration of 5%. Carrier DNA (0.1 mg) in the form of salmon sperm (Kodak, Cat# 135-9892) was added to aid the precipitation of DNA and protein during centrifugation (15,000 x g, 15 min, 4^{0}C). The supernatant, which contained the hydrolyzed RNA, was carefully aspirated. Additional cold 5% TCA was added to the pellet, the sample was re-centrifuged, and the supernatant was again aspirated to assure that the majority of the hydrolyzed RNA was removed. DNA was hydrolyzed from the pellet in 5% TCA for 30 min at 95^{0}C. A one ml aliquot of the supernatant was radioassayed (dpm in DNA). Differences in dpm between the samples that were immediately "killed" and samples that incubated for an hour prior to the formalin addition in each channel were used as the dpm (corrected dpm) in the DNA. Productivity (P) (measured in ugC/cm^{-2} x hr^{-1}) was then estimated (Findlay et al. 1984, Hudson et al. 1990):

P = (DPM in DNA/S.A.) x (cells/TdR) x (C/cell).

Where:

DPM in DNA = DPM incorporated into DNA x cm^{-2} x h^{-1}

S.A. = specific activity (uCi), DPM of added [^{3}H]TdR corrected for effective pool (determined from isotope dilution)

cells/TDR = cells produced per mole of TdR incorporated (2 x 10^{18}) (Moriarty 1986)

C/cell = average C content per cell as estimated from DMCs [(0.2 um^{-3}/cell) x (5.6 x 10^{-7} ugC/um^{-3})]

Isotope Dilution- The labelled [^{3}H]TdR experimentally added to samples can be diluted by pre-existing cellular and extracellular sources of thymidine which vary between locations. Hence, it is imperative that this pre-existing source or "effective pool size" of thymidine is estimated to assure an accurate estimate of productivity. The isotope dilution also facilitates safe, economical use of the [^{3}H]TdR stock, since the minimal specific activity necessary for exceptable detection can be estimated. Twenty-four tiles which had been in an untreated experimental channel were removed after 10 days of colonization and returned to the laboratory as outlined above. The isotope dilution series consisted of five treatments (0, 0.5, 1.0, 5.0, and 10.0 nmole of non-radioactive thymidine) (Sigma Cat#T-5108), and a killed control which were each replicated four times. A low specific activity of [^{3}H]TdR, 20 uCi per sample, was initially used. Incubation and extraction was conducted as outlined above. Effective pool size was estimated by determining the x-axis intercept of the plot of the corrected dpm for the five treatments as outlined in Findlay et al (1984). The effective pool size was estimated to be 4.7 nmoles per tile (Fig. 2), which is a relatively small pool size (Stuart Findlay, personal communication).

Statistical Analysis

Analysis of variance with repeated measures (ANOVAR) was conducted to test main effects of two levels of light (shade vs. non-shade), main effects of two levels of glucose (addition vs. no addition), change over time (three selective sampling dates), and interactions between the three for each of the five main dependent variables of interest; chlorophyll a, AFDW, live algal biovolume, bacterial biomass, and

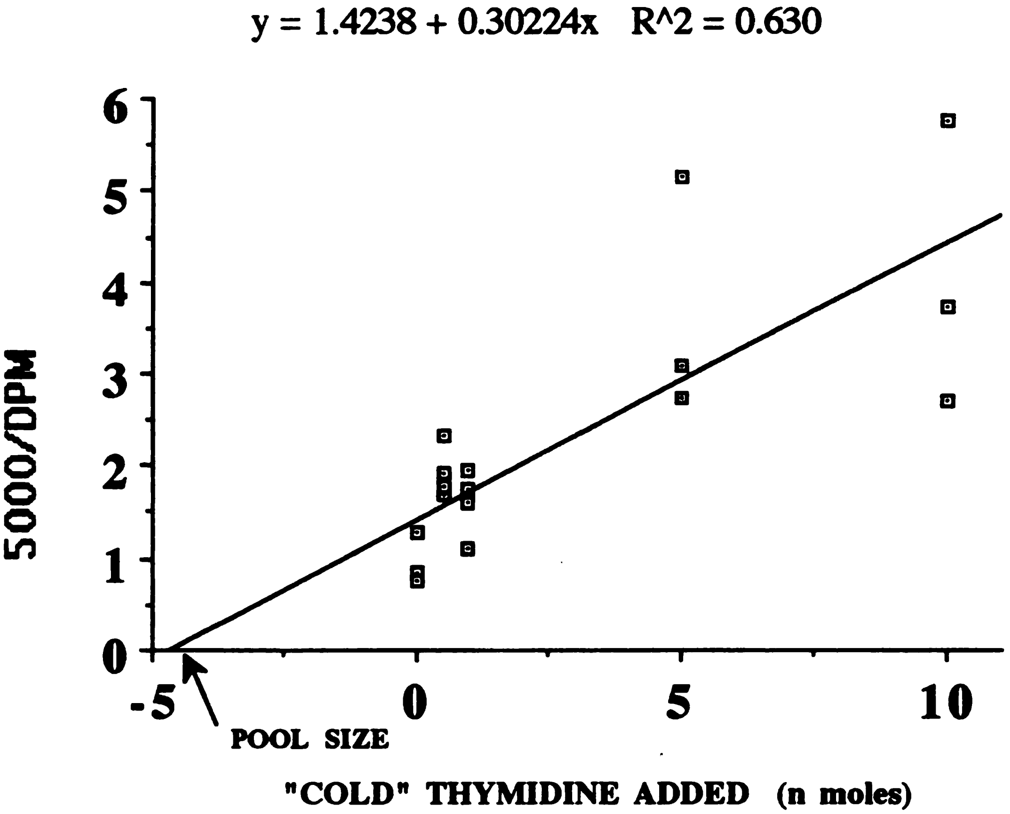


Figure 2. Isotope dilution plot for ten day epilithon in control channels. Each point represents an individual tile sampled. Intercept with horizontal axis is the pool size which dilutes [3H]Tdr.

bacterial production. All variables were natural log transformed to eliminate correlations of means and variances associated with comparing samples from different colonization times. Since the experimental channels are the experimental units, the means of samples within a channel were used in the analyses. Diagnostics were performed on the residuals of the models to verify that errors were normally distributed, had a constant variance, and were independent. Multivariate analysis was interpreted when different from univariate analysis since compound symmetry is not an assumption for the multivariate analysis. Power analyses were conducted for each repeated measure (i.e. each sampling day) for ANOVARs in which no significant main effects were detected. Power is a function of the pre-set alpha value, effect size (magnitude of differences between means), sample size, and sample variance. If power is low, then the beta error is high. Thus, when power is low, negative results (non-rejection of null hypothesis) are ambiguous. All statistical analyses were performed with SYSTAT 5.1 (Wilkinson 1989).

RESULTS

Primary Experiment

AFDW - Standing crop organic matter as measured by AFDW was found to be significantly reduced ($p < .001$) in the shaded vs. non-shaded treatments overall (Table 1, Fig. 3). A

Table 1. Results of the ANOVARs for the primary experiment. Dependent variables include standing crop organic matter (AFDW), chlorophyll a, "live" diatom biovolume, frustule biovolume, bacterial biomass, and bacterial productivity.

Source of variation	(df)	MS	F	P
ASH-FREE DRY WEIGHT:				
Between Subjects				
Block	2	0.26	2.55	0.158
Glucose	1	0.44	4.29	0.084
Shade	1	13.08	126.66	0.000
Shade x Glucose	1	0.38	3.65	0.105
Error	6	0.10		
Within Subjects				
Time	1	6.03	124.54	0.000
Block x Time	1	0.01	0.29	0.756
Glucose x Time	1	0.01	0.10	0.758
Shade x Time	1	2.09	43.23	0.001
Shade x Glucose x Time	1	0.03	0.58	0.474
Error	6	0.05		
CHLOROPHYLL a:				
Between Subjects				
Block	2	0.04	1.08	0.398
Glucose	1	0.05	1.48	0.274
Shade	1	24.00	629.72	0.000
Shade x Glucose	1	0.01	0.25	0.636
Error	6	0.04		
Within Subjects				
Time	2	4.45	123.68	0.000
Block x Time	4	0.03	0.81	0.543
Glucose x Time	2	0.12	3.43	0.067
Shade x Time	2	1.58	44.07	0.000
Shade x Glucose x Time	2	0.07	1.96	0.183
Error	12	0.04		

Table #1 (cont.):

Source of variation	(df)	MS	F	P
LIVE DIATOM BIOVOLUME:				
Between Subjects				
Block	2	0.15	1.25	0.352
Glucose	1	0.02	1.38	0.723
Shade	1	27.40	236.96	0.000
Shade x Glucose	1	0.01	0.09	0.777
Error	6	0.116		
Within Subjects				
Time	2	0.27	2.94	0.092
Block x Time	4	0.20	2.25	0.124
Glucose x Time	2	0.01	0.04	0.960
Shade x Time	2	0.57	6.31	0.013
Shade x Glucose x Time	2	0.00	0.01	0.989
Error	12	0.09		
DIATOM FRUSTULE BIOVOLUME:				
Between Subjects				
Block	2	0.04	2.34	0.178
Glucose	1	0.01	0.80	0.406
Shade	1	26.76	1665.43	0.000
Shade x Glucose	1	0.00	0.01	0.924
Error	6	0.02		
Within Subjects				
Time	2	1.97	15.39	0.000
Block x Time	4	0.20	1.55	0.250
Glucose x Time	2	0.01	0.06	0.938
Shade x Time	2	0.51	4.00	0.047
Shade x Glucose x Time	2	0.02	0.15	0.863
Error	12	0.13		

Table #1 (cont.):

Source	(df)	MS	F	P
BACTERIAL BIOMASS:				
Between Subjects				
Block	2	0.68	7.00	0.027
Glucose	1	0.05	0.54	0.491
Shade	1	0.00	0.01	0.930
Shade x Glucose	1	0.03	0.32	0.593
Error	6	0.10		
Within Subjects				
Time	2	19.53	379.27	0.000
Block x Time	4	0.28	5.51	0.009
Glucose x Time	2	0.51	0.99	0.399
Shade x Time	2	0.04	0.69	0.522
Shade x Glucose x Time	2	0.12	2.32	0.140
Error	12	0.05		
BACTERIAL PRODUCTIVITY:				
Between Subjects				
Block	2	1.32	4.58	0.062
Glucose	1	14.53	50.30	0.000
Shade	1	0.20	0.69	0.437
Shade x Glucose	1	1.79	6.21	0.047
Error	6	0.29		
Within Subjects				
Time	2	12.84	48.67	0.000
Block x Time	4	0.16	3.72	0.034
Glucose x Time	2	2.78	10.53	0.002
Shade x Time	2	0.87	3.29	0.072
Shade x Glucose x Time	2	0.43	1.16	0.240
Error	12	0.26		

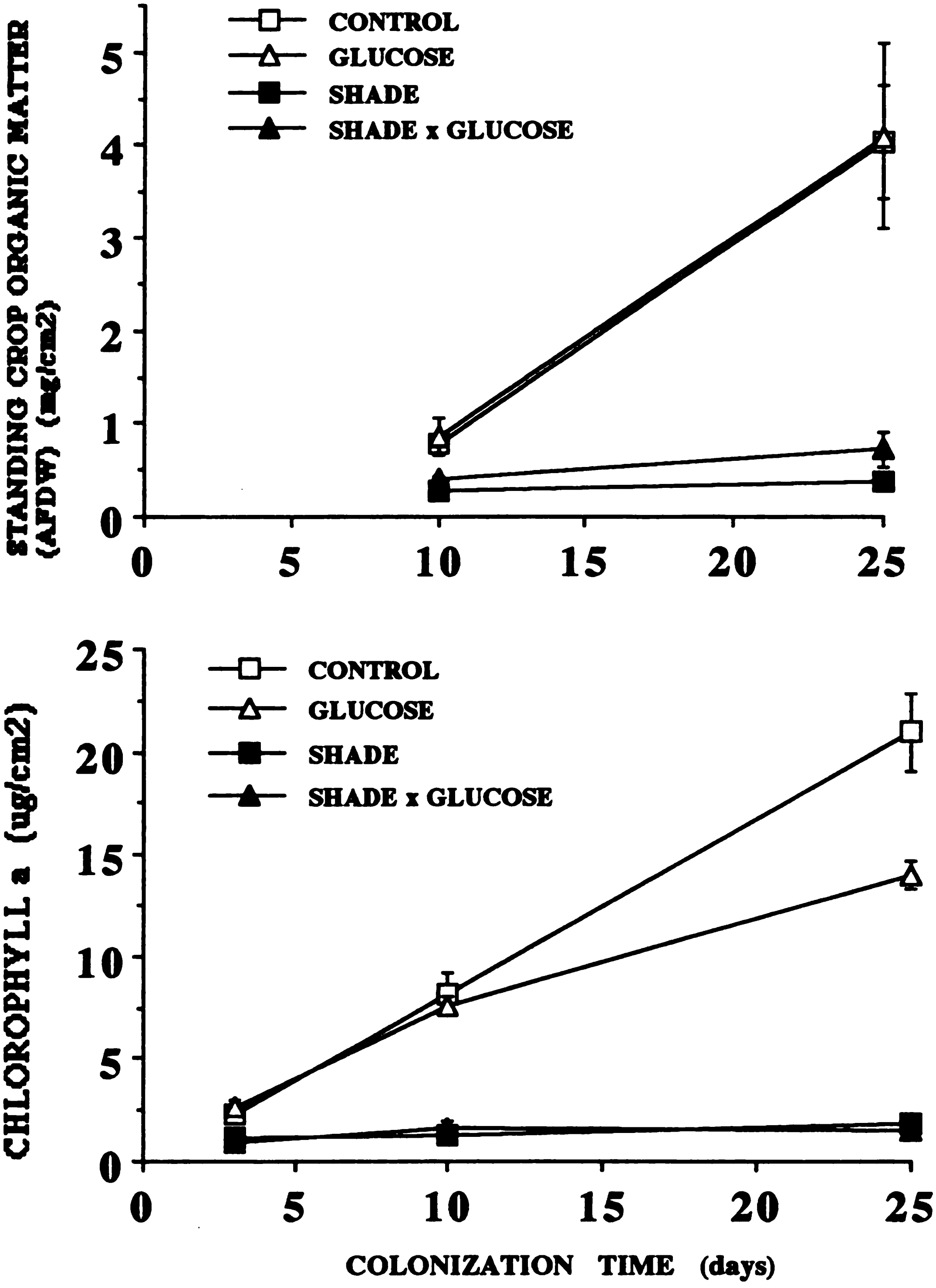


Figure 3. Standing crop organic matter (AFDW) (above) and chlorophyll a (below) through colonization time for the primary experiment. Each point is the mean from three replicate channels (n=3). Error bars represent +/- 1 SE.

significant ($p < .001$) shade x time effect occurred as well. Overall, AFDW was not significantly affected ($p = .084$) by the glucose treatment (Table 1).

Chlorophyll a - Throughout the primary experiment chlorophyll a was found to be significantly reduced ($p < .001$) in the shaded vs. non-shaded treatments (Table 1, Fig. 3). A significant shade x time effect ($p < .001$) occurred as well. Dramatic differences occurred on all sampling days, regardless of colonization time (Fig. 3). Chlorophyll a was not significantly affected ($p = .274$) by the glucose treatment (Table 1). Overall, chlorophyll a levels increased dramatically throughout colonization time in the non-shaded channels (Fig. 3). These results are consistent with those of standing crop organic matter.

Live Algal Biovolume (LABV) - LABV was significantly reduced ($p < .001$) in the shaded treatments (Table 1, Fig. 4). LABV was not affected by the glucose treatment ($p = .723$) (Table 1). Results are consistent with those of chlorophyll a, however LABV does not increase significantly through colonization time ($p = .092$) (Table 1, Fig.4) like chlorophyll a does. Analysis of algal biovolume based on total live and dead frustules counted or "frustule biovolume" (TFBV) revealed similar treatment effects as the LABV analysis (Table 1), however its change through colonization time is highly significant ($p < .001$) (Fig. 4). TFBV

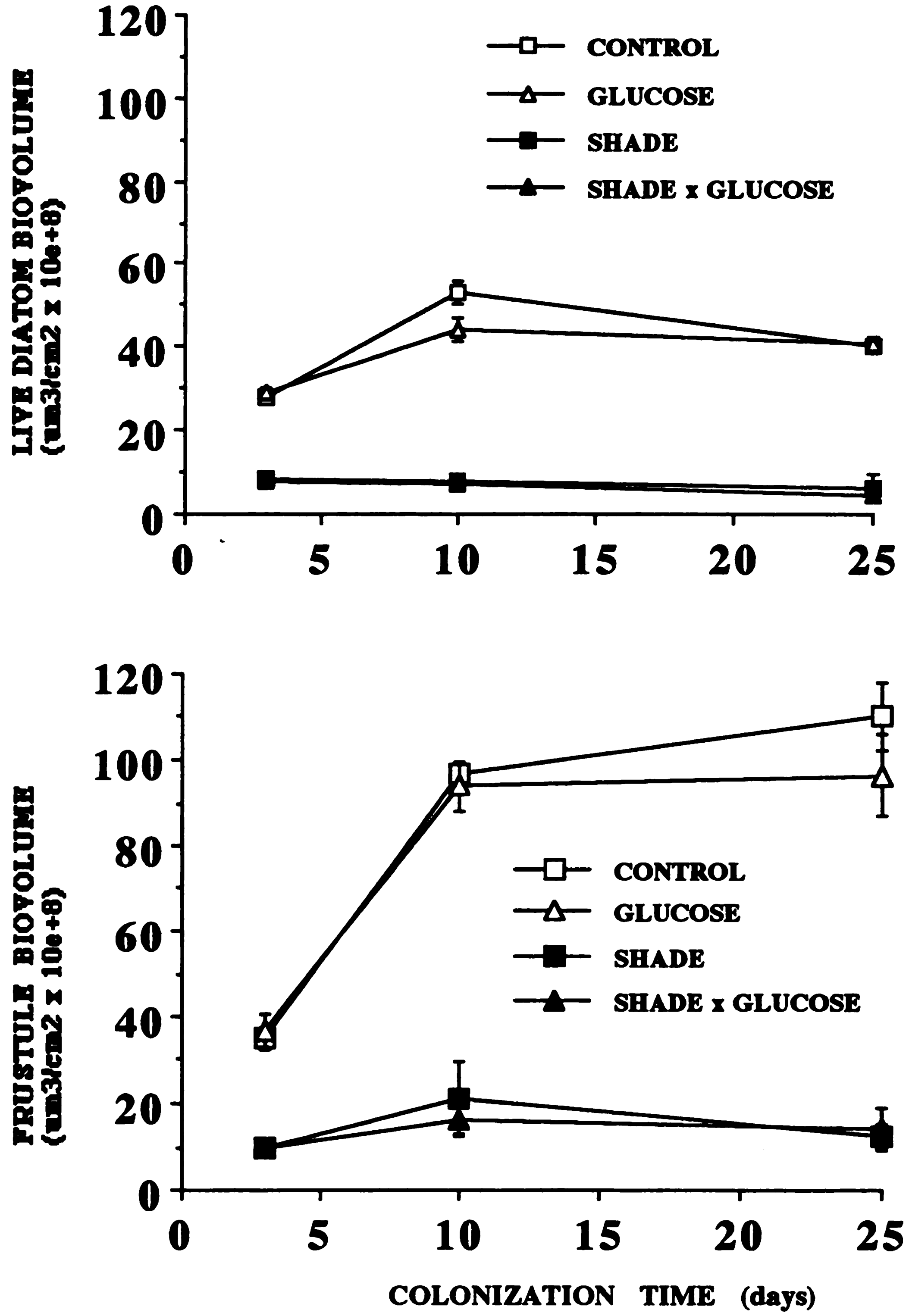


Figure 4. Live diatom biovolume (above) and frustule biovolume (below) through colonization time for the primary experiment. Each point is the mean from three replicate channels (n=3). Error bars represent +/- 1 SE.

increases throughout colonization time similar to chlorophyll a.

Diatom community composition - Surprisingly, live diatom communities were very similar between treatments and through colonization time (Fig. 5, Fig. 6). Synedra ulna and Nitzschia linearis, both large diatoms, dominated live biovolume estimates in all treatments throughout colonization. This similarity between treatments is desirable for this experiment since differences in community composition may have confounded differences detected in algal biomass.

Bacterial Biomass - Estimates of epilithic bacterial standing crop based on direct microscopic counts did not significantly differ between treatments (Table 1, Fig. 7). Overall, bacterial biomass increased significantly ($p < .001$) throughout colonization time (Fig. 7). Since no significant main effects were detected for the ANOVAR, power analyses were conducted for each repeated measure (i.e. for each sampling day). The power of the actual analyses was low (power ~0.14 - 0.28) primarily because the effect sizes (standard deviation of standardized means) were low compared to the standard deviation within each mean (Cohen 1988). However, if there had been larger differences between means (effect sizes > 0.80), then power would have been

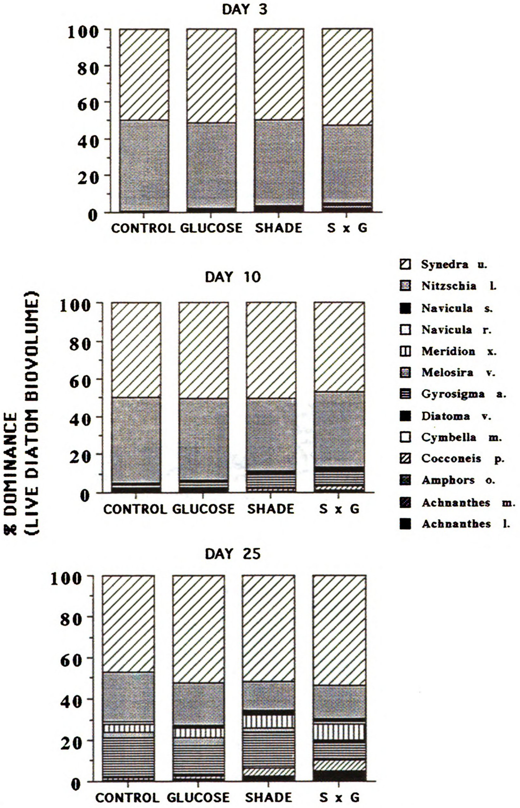
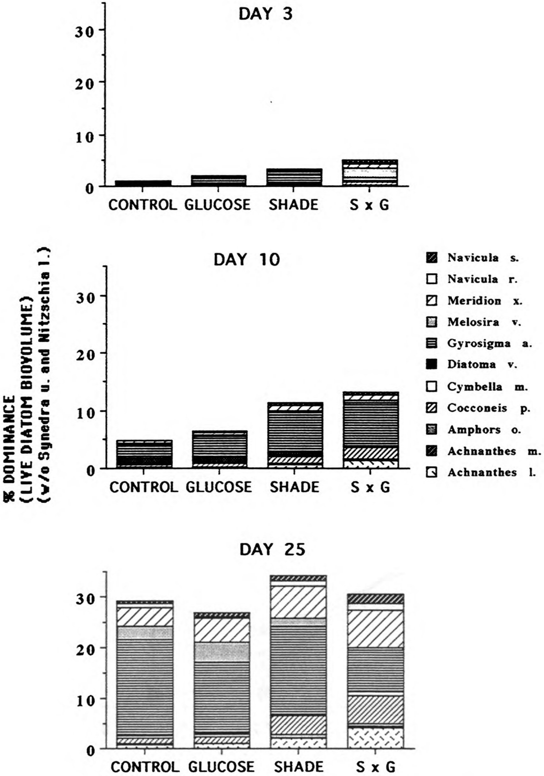


Figure 5. Percent dominance in terms of live diatom biovolume for dominant diatom species for each sampling day during the primary experiment. Each bar graph represents the mean from three replicate channels (n=3).



DAY 3

DAY 10

DAY 25

Figure 6. Percent dominance in terms of live diatom biovolume for dominant diatom species, excluding Synedra and Nitzschia, for each sampling day during the primary experiment. Each bar graph represents the mean from three replicate channels (n=3).

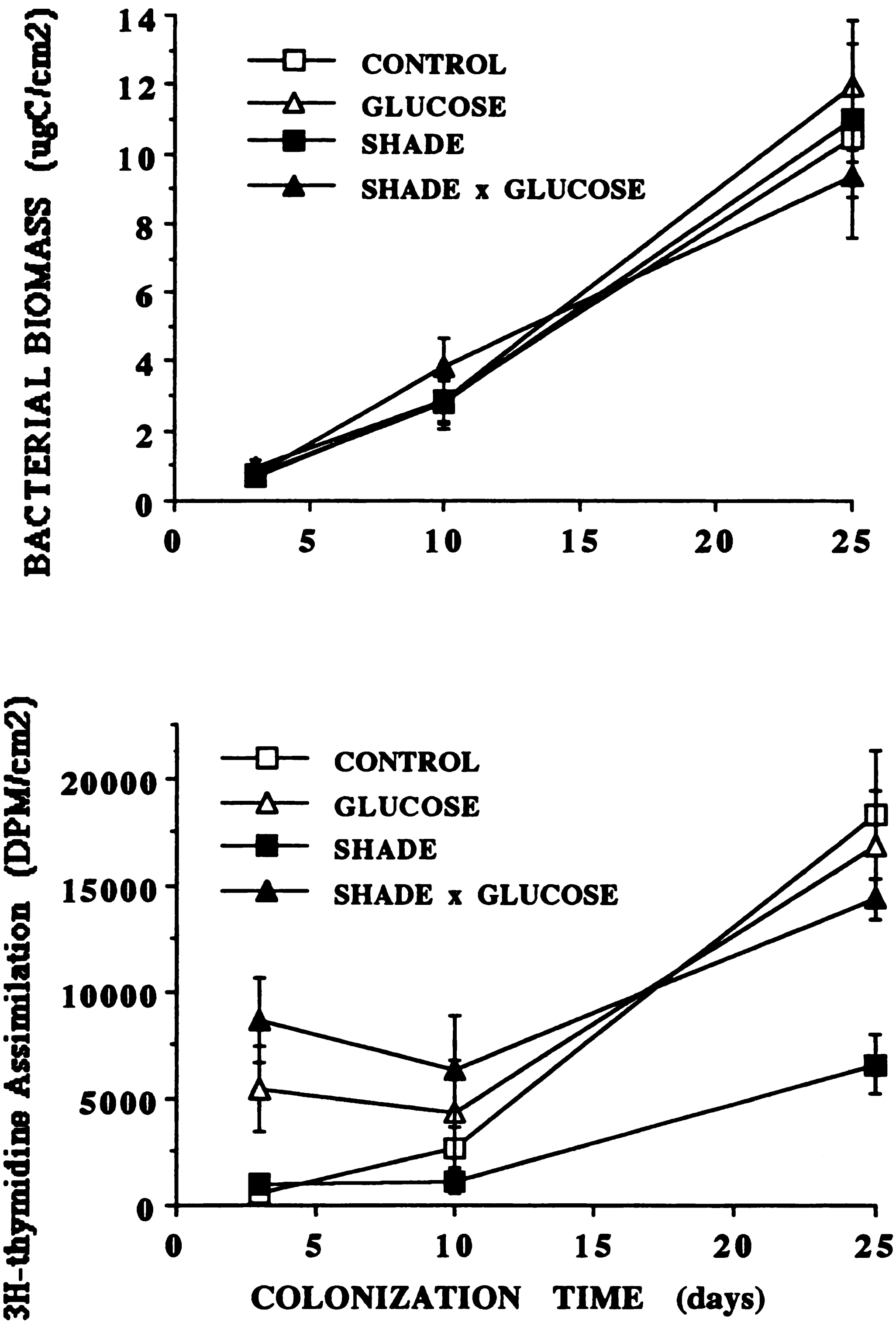


Figure 7. Bacterial biomass (above) and bacterial productivity (below) through colonization time for the primary experiment. Each point is the mean from three replicate channels (n=3). Error bars represent +/- 1 SE.

considerably higher ($p > 0.48$), even with the reported variance within replicates.

Bacterial productivity ([^{3}H]TdR assimilation) - Overall, estimates of bacterial productivity in the primary experiment revealed a significant glucose x time interaction ($p < .01$) (Table 1, Fig. 7). A glucose effect is most evident early in colonization; most dramatically on day 3 (Fig. 7). On day 25 the control treatment, which receives ambient light and no glucose additions, has a bacterial productivity similar to the glucose treatments, while the shade only treatment is reduced relative to the other treatments (Fig. 7).

Preliminary Experiment

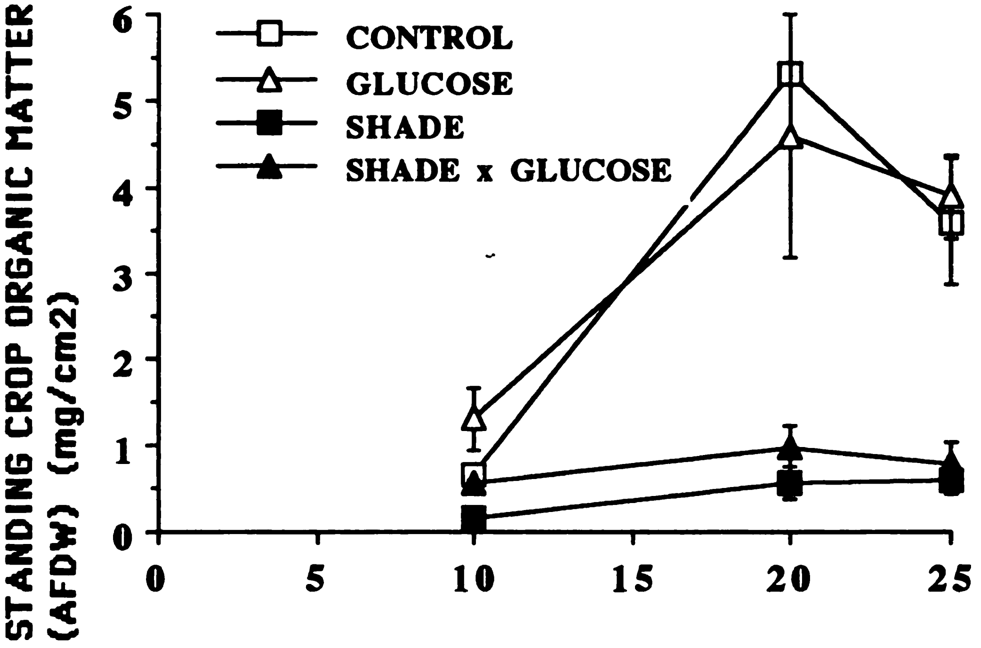
Overall, results from the preliminary experiment were similar to those found in the primary experiment. Similar trends were detected for both standing crop organic matter and chlorophyll _a_ (Fig. 8). Results from the ANOVARs reveal a significant shade response for both ($p < .001$), however a significant glucose response was detected for AFDW ($p < .05$) (Table 2). Quantitative diatom counts were not conducted for the preliminary experiment, however _Synedra_ _ulna_ and _Nitzschia_ _linearis_ appeared to dominate live biovolume in all treatments (personal observation). Bacterial biomass generally increased throughout colonization (Fig. 9), but significant differences were neither detected between nor

Table 2. Results of the ANOVARs for the preliminary experiment. Dependent variables include standing crop organic matter (AFDW), chlorophyll *a*, bacterial biomass, and bacterial productivity.

Source of variation	(df)	MS	F	P
ASH-FREE DRY WEIGHT:				
Between Subjects				
Block	2	0.18	1.35	0.327
Glucose	1	1.68	12.48	0.012
Shade	1	20.44	152.36	0.000
Shade x Glucose	1	0.33	2.48	0.166
Error	6	0.13		
Within Subjects				
Time	1	4.28	26.29	0.000
Block x Time	1	0.96	5.91	0.007
Glucose x Time	1	0.32	1.99	0.179
Shade x Time	1	0.64	3.91	0.048
Shade x Glucose x Time	1	0.22	1.35	0.295
Error	6	0.16		
CHLOROPHYLL *a*:				
Between Subjects				
Block	2	0.05	0.79	0.497
Glucose	1	0.26	4.29	0.084
Shade	1	27.64	453.91	0.000
Shade x Glucose	1	0.00	0.01	0.919
Error	6	0.06		
Within Subjects				
Time	2	3.11	34.32	0.000
Block x Time	4	0.10	1.10	0.401
Glucose x Time	2	0.38	0.42	0.667
Shade x Time	2	0.22	2.39	0.133
Shade x Glucose x Time	2	0.19	2.14	0.161
Error	12	0.09		

Table 2 (cont.):

Source of variation	(df)	MS	F	P
BACTERIAL BIOMASS:				
Between Subjects				
Block	2	0.15	1.30	0.340
Glucose	1	0.00	0.00	0.972
Shade	1	0.01	0.12	0.746
Shade x Glucose	1	0.31	2.75	0.148
Error	6	0.11		
Within Subjects				
Time	2	0.13	2.00	0.178
Block x Time	4	0.09	1.39	0.296
Glucose x Time	2	0.02	0.33	0.725
Shade x Time	2	0.01	0.10	0.904
Shade x Glucose x Time	2	0.05	0.75	0.493
Error	12	0.06		
BACTERIAL PRODUCTIVITY:				
Between Subjects				
Block	2	1.05	8.51	0.018
Glucose	1	2.19	17.73	0.006
Shade	1	0.57	4.64	0.075
Shade x Glucose	1	1.65	1.34	0.291
Error	6	0.12		
Within Subjects				
Time	1	44.80	604.05	0.000
Block x Time	2	0.89	11.95	0.008
Glucose x Time	1	0.31	4.13	0.088
Shade x Time	1	0.53	7.16	0.037
Shade x Glucose x Time	1	0.02	0.28	0.614
Error	6	0.07		



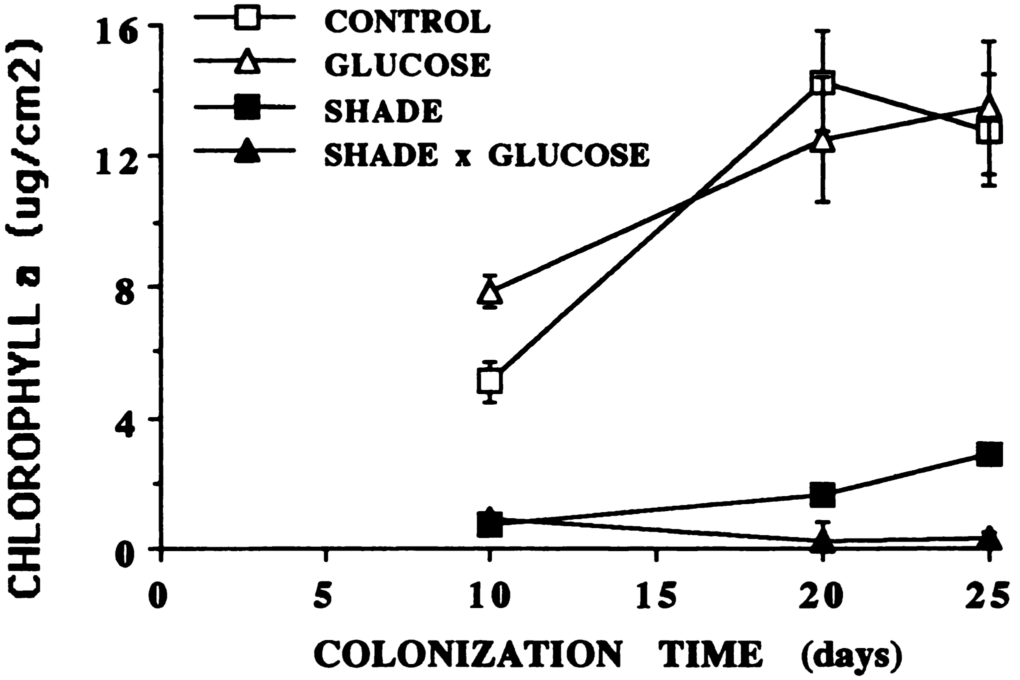


Figure 8. Standing crop organic matter (AFDW) (above) and chlorophyll a (below) through colonization time for the preliminary experiment. Each point is the mean from three replicate channels (n=3). Error bars represent +/- 1 SE.

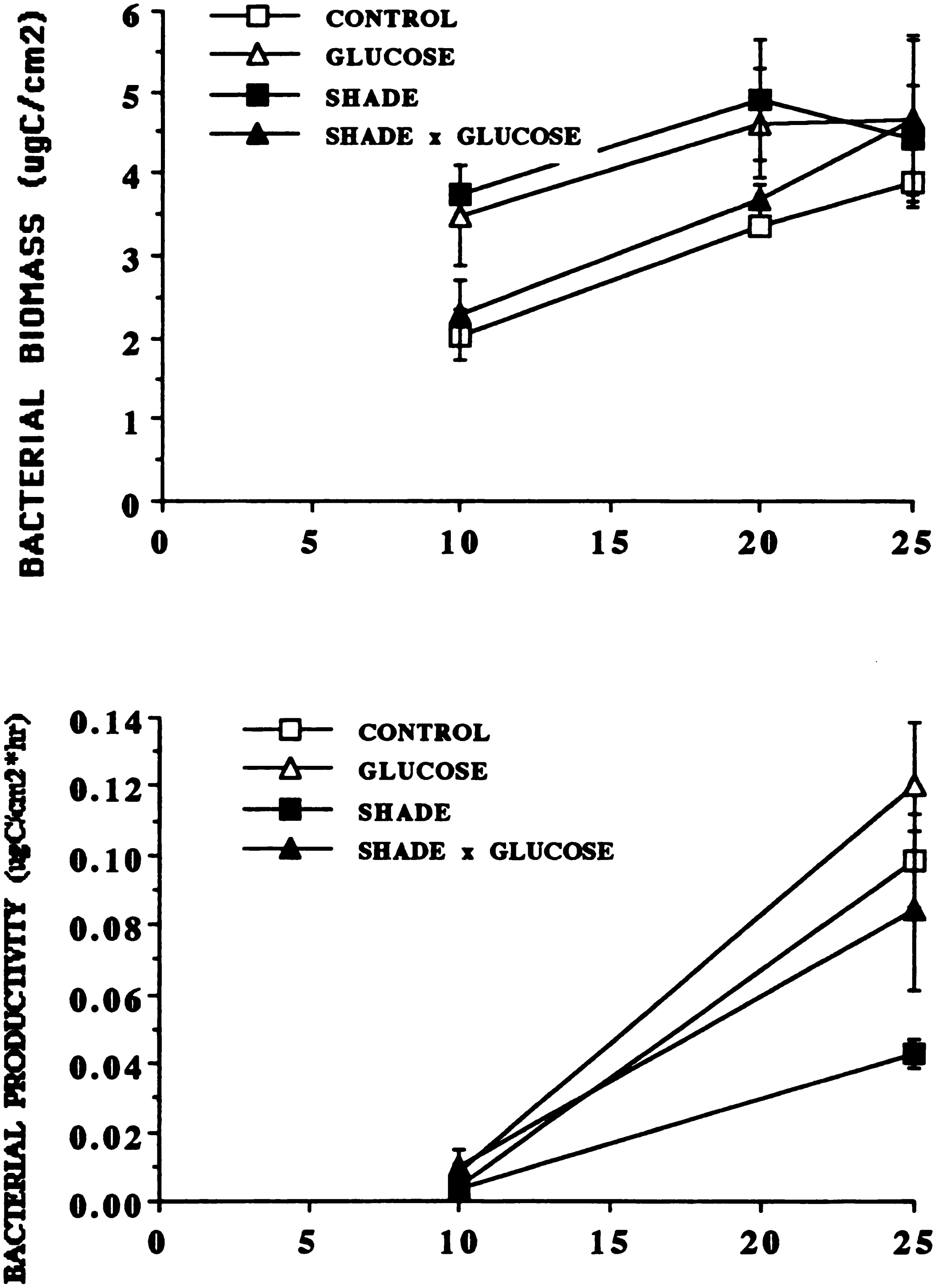


Figure 9. Bacterial biomass (above) and bacterial productivity (below) through colonization time for the preliminary experiment (Note: There are no productivity estimates for day 20.). Each point is the mean from three replicate channels (n=3).

within subjects (Table 2). Bacterial productivity was estimated in the preliminary experiment on colonization days 10 and 25 only. A significant glucose x time response was detected ($p < .01$)(Table 2), however on day 25 the control treatment has a productivity comparable to the glucose treatments. Bacterial productivity results for both the primary and preliminary experiment reveal strikingly similar trends for day 25 (Fig. 7 and 9).

DISCUSSION

While several past studies have attempted to determine the extent to which benthic bacteria in a stream epilithon rely on benthic algae as predicted from bacterial-algal interactions in other aquatic ecosystems (Cole 1982, Cole et al. 1988, White et al. 1991), few have used an adequate experimental approach in the field (Kaplan and Bott 1989, Findlay et al. 1993) and few have fully considered the role of colonization time or successional state of the epilithon (Blenkinsopp et al. 1991). This study utilized stream-side experimental channels to provide proper replication of experimental units and to ensure controlled treatments in which the epilithon community could be sampled throughout colonization time. Unpredicted catastrophic events to epilithon succession such as increased discharge following a storm, scouring from dislodged debris, and grazing from macroscopic invertebrates and fish were avoided to ensure comparisons between early and late successional epilithon communities.

Frequently, studies which attempt to document a relationship or linkage between an aquatic bacterial community and algal community rely on correlating chlorophyll a estimates with bacterial biomass. This approach can be informative for generating a testable hypothesis, but may lead to spurious correlations or fail to consider the limitations inherent to each measure. This study attempted to go beyond simply characterizing algal biomass based on chlorophyll a and considered changes in diatom community composition and live diatom biovolume. These measures coupled with estimates of standing crop organic matter can provide a more thorough understanding of an epilithon's structure throughout colonization time. Although this study does not investigate changes in bacterial community composition, it does attempt to consider both standing crop bacterial biomass and bacterial productivity independently. This approach is necessary since DMCs do not discriminate between viable and non-viable individuals, hence only a small portion of the individual bacteria counted may be active. Also, grazing on bacteria within the epilithon and accumulation of non-viable bacteria from the water column throughout epilithon colonization can lead to interpretation difficulties when only DMCs are used.

Algal Community Response

The experimental design employed established four very different environments for epilithon colonization. The shading treatment dramatically reduced the algal community as planned, but did not prevent formation of an epilithon. This is evident from both the chlorophyll _a_ (Fig. 3) and live diatom biovolume estimates (Fig. 4), as well as AFDW estimates in the primary experiment (Fig. 3). It is important to note that the glucose treatment did not significantly alter any of these parameters in the primary experiment. Such a glucose response by the algal community would have confounded treatments within the factorial design.

Besides being concerned about the glucose manipulation altering algal biomass, there was also concern that algal community composition would be dramatically different between treatments. Anticipating different diatom assemblages, I initially planned to investigate the possibility of species' specific responses, however live diatom community composition was surprisingly similar between treatments throughout colonization time in the primary experiment (Figs. 5 and 6). Live diatom biovolume in all treatments was dominated by _Synedra_ _ulna_ and _Nitzschia_ _linearis_, both very large diatoms, throughout colonization time. Their large size may have made them drop into the boundary layer and embed into the epilithon's matrix more readily then smaller diatom species (Jan Stevenson, personal communication). Once embedded into the epilithon's matrix they may have been responsible for out-competing other diatom species for light due to their size, and motility in the case of *Nitzschia linearis*. Although they constitute the majority of the live diatom biovolume and most likely are responsible for a large portion of the chlorophyll *a*, the large individual size of these dominant species may not be conducive to a bacterial response due to their small surface/volume (s/v) for a given volume. For example, an epilithon community dominated by smaller diatom species may have similar chlorophyll *a* and live biovolume estimates, but much greater s/v per given volume. Although a relationship between diatom s/v and bacteria biomass in aquatic ecosystems has been postulated, to my knowledge it has not been tested for a stream epilithon community. Although such a question is beyond the scope of this study, especially considering community similarity between treatments, it does highlight the need to investigate beyond the algal biomass level and consider algal community composition.

Bacterial Community Response

The combined results concerning the algal community's biomass and composition infer that the experimental design was sufficient at establishing the desired conditions necessary to test the experiment's hypothesis. Had this not been the case, subsequent analysis of treatment differences in the bacterial community's biomass and productivity would have been severely limited.

Although specific treatment differences in the standing crop bacterial biomass were hypothesized, in particular, increased biomass was predicted in the DOC (glucose) treatments early in colonization and in the non-shaded (increased algae) treatments late in colonization, no significant treatment differences were found in either the preliminary or primary experiment throughout colonization time. These findings were surprising, especially in light of the bacterial productivity results.

Bacterial productivity, as estimated from [^{3}H]thymidine incorporation into bacterial DNA, responded to increased DOC (glucose addition) early in colonization, but responded to both the presence of algae (non-shade treatment) and increased DOC in late colonization in the primary experiment. Results for the two days sampled in the preliminary experiment generally agree with results from the primary experiment. The combined results from both experiments are in general agreement with the experiment's hypothesis. Interestingly, on day 25 in both experiments the productivity in the DOC/non-shade treatment was not dramatically larger than the non-DOC/non-shade and DOC/shade treatments (Figs. 7 and 9). Similar productivity estimates for the non-shaded control and DOC manipulated channels in late colonization may suggest that a maximum productivity ("ceiling") was reached for the given environments. It is possible that available carbon is not limiting the bacterial community's production in these treatments. Differential change between treatments throughout colonization time makes interpretation of the ANOVAR difficult for bacterial productivity.

Obvious discrepancies between the bacterial community's biomass and productivity were not anticipated. Other study's have demonstrated relationships between the two (White et al. 1991, Findlay et al. 1988, Findlay et al. 1993). Why are differences in bacterial productivity not resulting in noticeable differences in bacterial biomass? Two possible reasons can be postulated: 1.) accumulation of non-viable cells from the water column throughout colonization, and 2.) density-dependent grazing of bacteria by microorganisms within the epilithon.

Bacterial biomass increases throughout colonization time similarly in all treatments in the primary experiment, hence non-viable bacteria may be accumulating in the epilithon's matrix during epilithon development. As noted earlier, biomass estimates based on DMCs do not differentiate between viable and non-viable cells, hence only a small portion of the bacteria counted may actually be contributing to the community's productivity. If only a small percentage of the total bacteria counted (e.g. 10%) were viable then even large differences in productivity may not result in discernible differences in biomass.

Although bacterial biomass increases throughout colonization time on a per area basis, it is unknown whether its relative density within the epilithon remains similar throughout colonization time. Density-dependent grazing by microorganisms within the epilithon may act to crop bacterial biomass such that differences between treatments are not different when viewed as a series of "snapshots" in colonization time. Our current knowledge lacks a clear understanding of the possible role internal grazers play in epilithon structure and function (Kaplan and Bott ???).

Possible Mechanism for Bacterial Reliance on Algae

While traditional views on bacterial dependence of algae in aquatic ecosystems has stressed the role of the nutrient-rich phycosphere, less emphasis has been placed on the role algal senescence may play. Algal senescence may be particularly important in a stream epilithon since lysed or degrading algal material may not readily escape the epilithon's matrix and may remain available to the bacterial community.

In this study live diatom biovolume remains similar throughout colonization time in the shade and non-shade treatments (Fig. 4), respectively, yet chlorophyll a increases dramatically through colonization time (Fig. 4). This discrepancy can be explained by one of three probable mechanisms: 1.) chlorophyll a is being produced by cyanobacteria or filamentous green algae in late colonization 2.) late colonizing diatoms contain more chlorophyll a per biovolume, or 3.) chlorophyll a lysed from early colonizing diatoms remains undegraded within the epilithon's matrix. Since cyanobacteria and filamentous algae were not prevalent during this study (personal observation) and diatom species composition remained similar between treatments throughout colonization time (Fig. 5) the first and second explanations appear unlikely, however a significant increase ($p < .001$) in frustule (live and dead diatoms) biovolume throughout colonization time (Table 1) (Fig. 4) may provide some support for the third explanation.

Although the extent in which exudates from live algae opposed to polysachharides released from senescent algal cells support a bacterial community were not explicitly tested in this experiment, there is some weak evidence that bacteria productivity may be stimulated by algal cell senescence within the stream epilithon. Bacterial biomass does not differ significantly between treatments in late colonization where the chlorophyll a/live diatom biovolume ratio is greatest, however bacterial productivity in the non-shade treatment increases dramatically.

Role in Stream Ecosystems

While past studies have both supported and dismissed benthic bacterial reliance on benthic algae in stream epilithon communities, few have adequately addressed the role that the epilithon's successional stage may play in such a relationship. This study suggests that bacteria residing in epilithon communities that are not fully developed and in an early successional stage may readily respond to labile DOC in the water column, yet in late successional stages respond to both labile DOC and the presence of benthic algae. Hence, stream epilithons which have colonization trajectories continually disturbed or "re-set" by scouring, grazing, or increased discharge may rely primarily on allochthonous inputs, while communities within a well developed epilithon which supports a large algal biomass may rely on both allochthonous and autochthonous inputs.

It is important to note that this study was conducted in a productive system in which a thick, algal rich epilithon readily develops. Hence, in oligotrophic streams in which relatively thin, algal poor epilithon communities develop, bacterial productivity may not be significantly stimulated by the presence of algae (Findlay et al. 1993). An appreciation of successional changes in epilithon structure, limitations to algal productivity, and natural disturbance events will lead to a greater understanding of temporal and spatial variation in benthic bacterial activity and the degree in which the benthic bacterial community relies on benthic algae. Further work which examines how differences in both DOC quality and quantity and subtle changes in light quality and quantity impact the benthic bacteria community across a range of streams with varying productivity and stability is necessary to properly develop an accurate paradigm of bacterial reliance on algae in stream epilithons. Such work may be particularly timely as ecologists strive to understand how important ecosystem processes are altered by subtle changes in the biosphere.

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SUMMARY

Although much is known about bacterial-algal relationships in most aquatic ecosystems (Cole 1982, Cole et al. 1988, White et al. 1991), a clear paradigm for a possible bacterial-algal relationship in the epilithon of stream ecosystems has not been established. While it is thought that bacteria in many aquatic ecosystems rely on algal exudates or lysed algal cells as a primary carbon source, debate continues over the extent that benthic bacteria in stream ecosystems utilize benthic algae, as opposed to DOC in the water column, as a primary carbon source. My research on the benthic bacterial and algal communities in a stream epilithon attempted to determine: 1.) the extent to which bacterial biomass and algal biomass correlate in various habitats in a productive stream throughout colonization time, 2.) the extent that the bacterial community responds (as measured in biomass and productivity) to changes in algal biomass in the epilithon and changes to DOC in the water column.

The first question was investigated in a descriptive study conducted in 1991 on the Ford River in Michigan's Upper Peninsula in which benthic bacterial biomass and algal biomass were compared between an adjacent stream run, riffle, and pool throughout colonization time. Although a relationship was predicted, no correlation was found between benthic bacterial biomass and algal biomass throughout

colonization time in any of the habitats studied. The scope of this study was limited since the bacterial and algal communities were only characterized in terms of their biomass. Limitations inherent to this field approach were discussed.

The second question was investigated in a controlled experiment conducted in 1992 at the Kellogg Biological Station's Experimental Stream Facility in which the bacterial community's response (as measured in bacterial biomass and productivity) to increased DOC in the water column and increased algal biomass throughout colonization time was determined. This study addressed the relative importance of allochthonous and autochthonous inputs throughout epilithon colonization to the benthic bacterial community. The use of experimental streams permitted a controlled, replicated study to be conducted throughout the course of epilithon colonization. This study characterized the epilithon's algal community and bacterial community in a more comprehensive manner and addressed the need for future studies in this area to do the same. Bacterial productivity, as estimated from ^{3}H-thymidine incorporation into bacterial DNA, responded to increased DOC (glucose addition) early in colonization, but responded to both the presence of algae (non-shade treatment) and increased DOC in late colonization. Similar productivity estimates for the non-shaded control and DOC manipulated channels in late colonization may suggest that a maximum productivity ("ceiling") was reached for the given environments. Bacterial biomass estimates based on direct microscopic counts did not correspond with estimates of bacterial productivity. Bacterial biomass increased throughout colonization time, but did not vary between treatments as predicted. Possible reasons for these findings include density dependent grazing by micoorganisms within the epilithon and accumulation of non-viable cells from the water column throughout colonization. Benthic bacterial communities in stream epilithons which have colonization trajectories continually disturbed or "re-set" by scouring, grazing, or increased discharge may rely primarily on allochthonous inputs, while communities within a well developed epilithon which supports a large algal biomass may rely on both allochthonous and autochthonous inputs.

This work coupled with the recent work of others (Kaplan and Bott 1989, Hudson et al. 1992, Findlay et al. 1993), suggests that a coupling between benthic bacteria and benthic algae in a stream epilithon may be more pronounced in eutrophic streams which develop a thick, mature epilithon, and nearly undetectable in more oligotrophic streams. This coupling between benthic bacteria and benthic algae may not be routinely detected with bacterial biomass estimates based on direct microscopic counts, but may be detected with estimates of bacterial productivity. An accurate and comprehensive paradigm of bacterial and algal relationships in the epilithon of stream ecosystems is still lacking. In order for such a paradigm to be developed, studies on how changes in DOC quality and quantity, and changes in algal biomass and productivity impact bacterial biomass and productivity across a wide range of stream types are necessary.