DIVERSITY AND COMMUNITY ORGANIZATION IN THE PERIPHYTON OF RECIRCULATING STREAM CHANNELS ARE DEFINED BY CURRENT VELOCITY AND NUTRIENT SUPPLY

by

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ABSTRACT

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In a series of experiments taking place in recirculating artificial stream channels, periphyton communities growing on ceramic tiles were subjected to variations in current velocity and nutrient supply. The first experiment used stream water and manipulated current velocity, subjecting periphyton communities to either high or low current velocities (30 and 10 cm $\cdot$ sec^{-1} respectively), while in experiments 2-4, modified WC media was used and periphyton communities subjected to different nutrient (low and high) and current regimes (low, high, and variable flow). Each experimental run lasted 35 days. In all experiments, greater species richness of periphyton communities was observed in the low current velocity treatments. In experiments 2-4, greater species richness was observed under conditions of high nutrient supply. Species richness and evenness also responded to changes in several structural properties of the biofilm matrix, most notably biofilm heterogeneity and thickness. The relationship between species richness and productivity, as measured with community biovolume, displayed several trends, ranging from no relationship to unimodal, depending on

experimental treatment. Multivariate analyses of periphyton communities revealed that in the experiment manipulating current velocity, communities in the two current treatments exhibited convergence, displaying greater similarity with time, while in the experiments manipulating current velocity and nutrient supply, periphyton communities exhibited divergence between nutrient treatments, displaying greater dissimilarity with time. Additionally, algal guilds responded to variations in nutrients, with greater guild diversity observed under conditions of high nutrient supply. Under conditions of high nutrients, diatom communities were dominated by 'eutrophic-motile' species, while 'non-eutrophic-adnate' species were dominant under conditions of low nutrients. These results suggest that current velocity and nutrient supply are important factors influencing periphyton communities and that community assembly is influenced by a combination of deterministic and stochastic factors.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

As humans continue to alter and modify landscapes, biodiversity is impacted, which can influence ecosystem processes such as primary productivity, stability, and resistance to disturbance (Wilson and Keddy 1988, Ehrlich and Wilson 1991, Peters and Lovejoy 1992, Folke *et al*. 1996, Chapin *et al*. 2000, Olff and Ritchie 2002). Rivers and streams represent only 0.03% of the available water supply, yet provide nearly two-thirds of the water used in the world (Perry and Vanderklein 1996). Thus, protecting this valuable resource should be a major societal concern. Maintaining 'biological integrity' has become an important component of water quality management, therefore it is important to better understand the factors influencing aquatic stream communities (Karr 1981, 1991). Impoundments, diversions, and loss of riparian buffers in rivers and streams can lead to modifications in the natural flow regime (Lytle and Poff 2004, Sweeney *et al*. 2004). Additionally, loss of riparian buffers can lead to increased inputs of nutrients from nonpoint sources (Lowrance *et al.* 1997). Therefore, flow modification and cultural eutrophication are important management issues facing rivers and streams.

Aquatic organisms in rivers and streams can be highly sensitive to changes in water quality, and the biological communities present at any given time have been influenced by many factors, most notably recent changes in flow regime and water chemistry. Attached algal or periphyton communities are particularly sensitive to these changes and modifications to water flow and chemistry can influence biofilm structure and microstructure (Battin *et al*. 2003a, Battin *et al*. 2003b), and can substantially alter species richness and abundance in these communities (Steinman and McIntire 1986). Consequently, very different biofilm communities should develop in rivers and streams impacted by flow modification and increased nutrient inputs compared with

those less affected by these influences. As the dominant primary producers in many streams, periphyton communities form the base of the food web (e.g., Minshall 1978, Lamberti 1996), provide habitat for a diversity of organisms (Dudley *et al.* 1986, Holomuzki 1989), and act as sinks and transformers of nutrients (Elwood *et al.* 1981, Lock *et al.* 1984); therefore, impacts to these communities have the potential to influence many of the important ecosystem services these communities provide (Peterson *et al.* 2001, Battin *et al.* 2003a).

1.2 Rationale

Spatial and temporal heterogeneity have long been recognized as powerful factors influencing species richness and complexity in ecological communities (Hutchinson 1961). In lotic systems, flowing water is a dominant environmental variable affecting species composition and ecological processes (Hart and Finelli 1999, Power *et al.* 1995). In naturally flowing rivers and streams, water velocity can fluctuate considerably both spatially and temporally leading to heterogeneous and highly variable environments (Passy 2001).

Aquatic organisms have developed many different behavioral, morphological, and life history strategies that adapt them to lives in highly variable environments (Lytle and Poff 2004). Attached algae and periphytic biofilms in streams are three-dimensional structures composed of multiple species of various growth habits, morphologies, and successional appearance. Periphyton communities are highly responsive to environmental factors and the collection of species in a particular site will likely be highly influenced, either directly or indirectly by the recent current regime (Peterson and Stevenson 1992, Battin *et al*. 2003b, Wellnitz and Rader 2003). For example, shear stress from frequent flooding or strong current can select for disturbance-adapted species where species sensitive to disturbance slough off their substrates. Likewise, under conditions of slow current, periphyton biomass can accumulate, thus selecting for species better adapted to competition for limiting nutrients. Additionally, photosynthetic aquatic organisms are sensitive to and differ considerably in the relative amounts of critical nutrients required for growth and reproduction (Tilman 1977, 1981, Titman 1976, Tilman and Kiesling 1984, Grover 1988, 1991). Due to the three-dimensional configuration of periphyton biofilms,

steep gradients of nutrient depletion can occur, contributing to the heterogeneous nature of periphytic biofilms (Mulholland *et al.* 1991). Increased spatial organization and heterogeneity within the benthos contributes to the observed increase in species richness compared to phytoplankton communities (Passy and Legendre 2006a, Passy and Legendre 2006b, Passy 2008).

Much of the spatial and temporal heterogeneity found in naturally flowing waters is lost in rivers and streams modified by flow regulation and the loss of riparian habitat (Dynesius and Nilsson 1994, Harding *et al.* 1998, Nilsson and Berggren 2000, Allan 2004, Sweeney *et al.* 2004). Furthermore, it is likely that nutrient concentration and physical disturbance interact to influence species richness and diversity in aquatic communities (Worm *et al*. 2002). Since current velocity and nutrient addition can be forms of disturbance in lotic ecosystems, the response of biofilm communities to nutrient addition should vary with current velocity.

1.3 Approach

Periphytic biofilms are composed of highly diverse, readily identifiable organisms with short generation times and relatively simple life cycles, making them excellent systems for testing questions of how communities are assembled in space and time, and how these processes are influenced by abiotic variables. To address questions of community assembly in periphytic biofilms in response to different flow and nutrient regimes, I initiated a series of experiments measuring community level responses in recirculating artificial streams. Since many variables can change simultaneously in the field, conducting experiments in artificial streams allowed me to control for factors that may otherwise confound results obtained in a field study. The principle questions directing this research were: would differences in flow and nutrient regimes among treatments strongly affect structure, successional patterns, species-dominance hierarchies, and species diversity in periphytic biofilms? Current velocity and nutrient supply (nitrogen and phosphorus concentrations) were the factors manipulated.

1.4 Objectives

Species interactions and diversity in periphyton communities are influenced by factors that shape the three-dimensional nature of these communities (Poff and Ward 1995, Hillebrand 2003, Passy 2008). Current velocity can influence biofilm structure, microarchitecture, and thickness (Battin *et al*. 2003a, Battin *et al*. 2003b, Passy 2007). Nutrients also influence the thickness of periphyton communities (Pringle 1990, Borchardt 1996, Passy 2007). Increasing thickness of periphyton communities can in turn, create nutrient gradients within the benthos that likely mediate species interactions (DeNicola *et al*. 2006, Passy 2008). Based on this premise, I had several objectives I wished to explore with experiments in artificial streams. In chapter two, I address the question of how current velocity and nutrient supply influence species richness and evenness of periphyton communities, and how these univariate community measures are influenced by several structural properties of the biofilm communities, i.e., biofilm heterogeneity and thickness. In chapter three, I focus on the influence of current velocity and nutrient supply on periphyton biomass accumulation and the diversity-biomass production relationship. Chapter four examines the response of periphyton community composition to variations in current velocity and nutrient supply, to observe whether communities would exhibit greater similarity or dissimilarity with time.

1.5 Experiments

Four sets of experiments were run in six artificial stream flumes on the campus of UTA. In the first experiment, water collected from the stream running through the UTA campus was placed in artificial streams and seeded with periphyton collected from several streams in the Dallas-Fort Worth area (details of streams in Chapter 2). Briefly, half the streams were subjected to a current velocity of 10 cm $\cdot$ sec^{-1}, while the remaining streams were subjected to 30 cm $\cdot$ sec^{-1}. In each artificial stream, 24 L (30% of total) was replaced with new stream water every third day for a duration of 35 days. Experiments 2-4 varied current regime (10 cm $\cdot$ sec^{-1}, 30 cm $\cdot$ sec^{-1}, and variable flow) and nutrient supply (high and low) and used modified Guillard's WC media (Guillard 1975) seeded with algae collected from the same set of streams as from experiment

one. In each artificial stream, 24 L was replaced with new WC media every third day for a duration of 35 days. Comprehensive explanations of each of the experiments are detailed in the following chapters.

CHAPTER 2

SPECIES RICHNESS AND EVENNESS OF PERIPHYTON COMMUNITIES IN ARTIFICIAL STREAMS RESPOND TO CURRENT VELOCITY AND NUTRIENT SUPPLY

2.1 Introduction

Maintaining biodiversity has become a major topic of concern as humans increasingly alter and modify landscapes in ways that impact biodiversity (Peters and Lovejoy 1992, Folke *et al*. 1996, Chapin *et al*. 2000, Olff and Ritchie 2002). As we gain knowledge of important ecosystem processes influenced directly or indirectly by species diversity, it becomes ever more important to understand the factors that impact diversity (Tilman 1999). In rivers and streams, biofilm communities support the entire food web, influence important biogeochemical processes and patterns (Battin *et al*. 2003a, b), and impacts to these communities has the potential to influence the ecosystem.

Current velocity and nutrient supply are important factors influencing dynamics in stream biofilm communities (Biggs and Smith 2002, Battin *et al*. 2003a,b). Current velocity can impact biofilm communities in various ways, from creating shear stress or drag, to stimulating metabolism (Stevenson 1996). By selecting against flow-sensitive species, increased current velocity and shear rate has been shown to hinder initial community development in bacterial biofilms (Rickard *et al*. 2004), multi-trophic biofilms (Battin *et al*. 2003a,b), and periphyton communities (McIntire 1966, Lamb and Lowe 1987, Stevenson 1996, Ghosh and Guar 1998). However, increased current velocity decreases the thickness of the diffusive boundary layers around cells and allows greater diffusion of nutrients from overlying waters (Whitford 1960, Stevenson and Glover 1993, Stevenson 1996), which may stimulate growth in later stages of community development in nutrient rich waters (McIntire 1966, Reisen and Spencer 1970, Steinman and McIntire 1986, Lamb and Lowe 1987). While current velocity has been shown to influence many aspects of periphyton communities, such as physiognomy and productivity, few

studies have examined the effect of current velocity on species richness. Results from several studies suggest the relationship between current velocity and species richness in periphyton communities is negative (Stevenson 1984, Lamb and Lowe 1987, Plenković-Moraj 2008).

Along with current velocity, nutrient supply is important in the development of periphyton communities (Francoeur *et al*. 1999, Biggs 2000, Biggs and Smith 2002, Passy 2008). Positive growth rates and the formation of thick, multi-layered periphyton communities require nutrient inputs from the interstitial spaces and overlying water column (Stevenson and Glover 1993). Fertilization has been shown to have opposing effects on species richness in terrestrial versus aquatic systems, where negative effects have been observed in terrestrial systems and positive effects in aquatic systems (Stevens *et al.* 2004, Suding *et al*. 2005, Hillebrand *et al*. 2007). While there are instances where nutrient additions have had little impact on species richness of periphyton communities (Stevenson *et al*. 1991), the majority of studies have shown an increase in species richness under high nutrient conditions (Pringle 1990, McCormick and Stevenson 1991, Hillebrand and Sommer 2000, Passy 2008). Due to the three-dimensional configuration of periphyton communities, steep gradients of nutrient depletion can occur, contributing to the heterogeneous nature of periphytic biofilms (Riber and Wetzel 1987, Mulholland *et al*. 1991, Stevenson and Glover 1993). Increased spatial organization and heterogeneity contributes to the observed increase in species richness within the benthos compared to phytoplankton communities (Passy and Legendre 2006a, Passy 2008). The mechanism leading to the observed increased species richness in streams with high nutrient conditions is thought to result from the coexistence between species in the overstory requiring high-nutrient conditions with species in the understory tolerant of low-nutrient conditions (Passy 2008).

The effect of fertilization on periphyton communities is likely to depend on the current regime in which the periphyton communities are exposed (Humphrey and Stevenson 1992, Borchardt 1996, Stevenson 1996). Additionally, while fertilization in aquatic habitats has been shown to enhance species richness, typically only a few species are favored by fertilization and an increase in dominance by these species reduces community evenness (Hillebrand *et al*.

2007). Therefore, highest accumulation of periphyton biomass would be expected to occur under conditions of high nutrient supply and low current velocity (Figure 2.1). Under these conditions, we would expect the establishment of a thick multilayered biofilm consisting of an overstory of sensitive species and an understory of tolerant species, resulting in high species richness and low evenness. Conversely, lowest biomass accumulation will occur under conditions of low nutrient abundance and high current velocity, resulting in a thin biofilm, composed of mostly tolerant species in a dense understory, leading to low species richness and high evenness. Furthermore, many rivers and streams are characterized by variations in current velocity over time; therefore an additional component of temporal variability in current velocity may be important to the structure of periphyton communities (Cardinale *et al*. 2005) by contributing to conditions where neither current-sensitive nor current-tolerant species are favored.

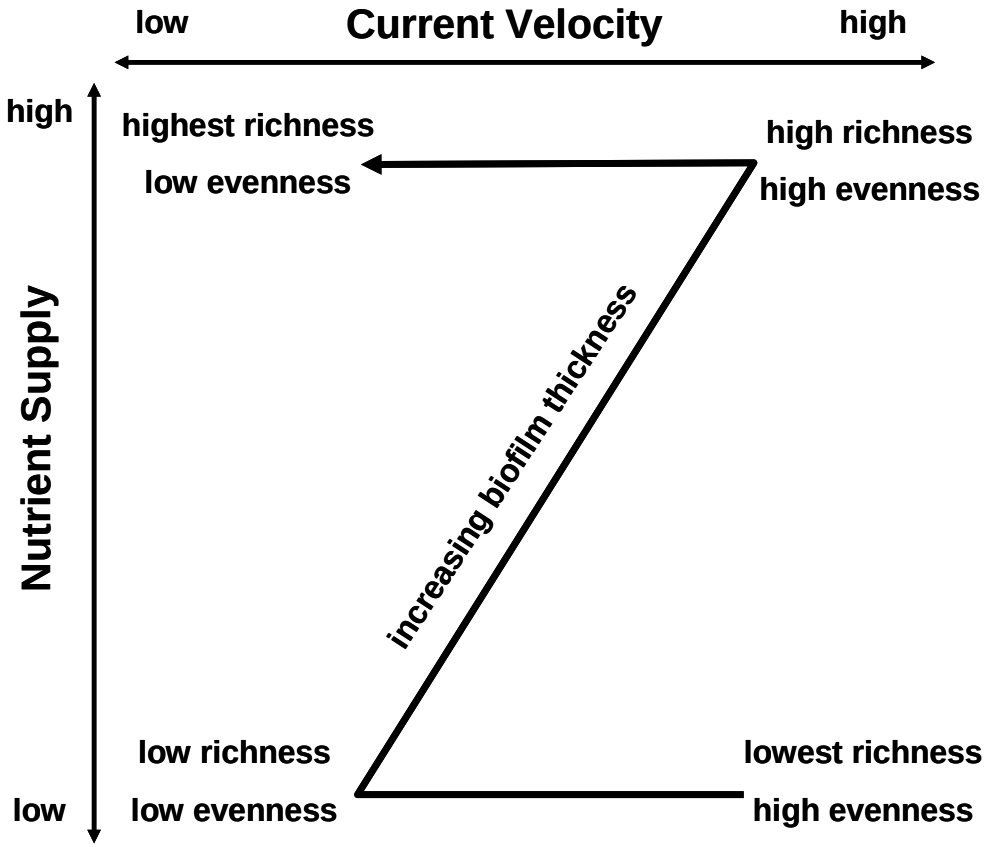


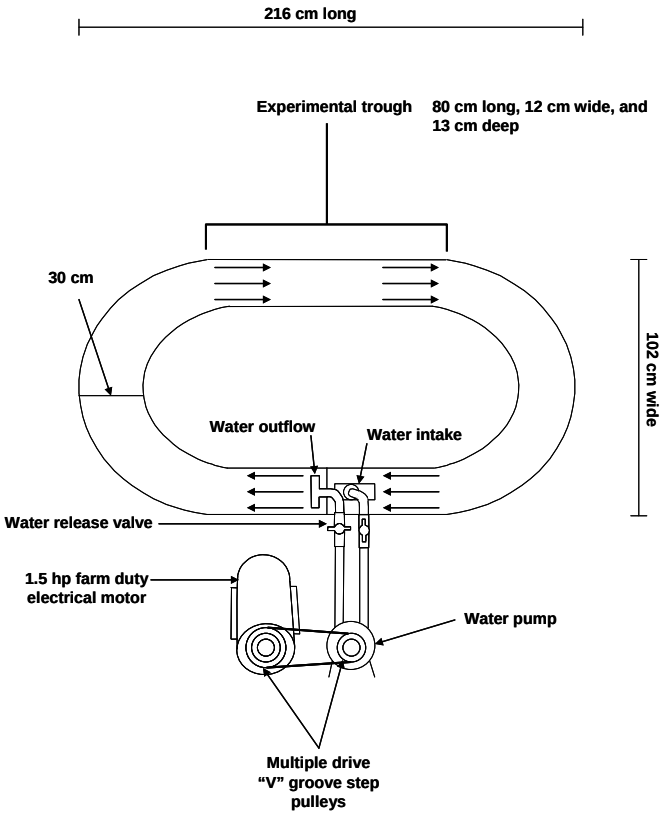
Figure 2.1 Conceptual model of the interaction between current velocity and nutrient supply on species richness and evenness

To address questions of community assembly in periphytic biofilms in response to different current and nutrient regimes, I initiated a series of experiments measuring community level responses in recirculating artificial streams. With these experiments, periphyton communities were subjected to various current × nutrient regimes and species richness and evenness examined at various points throughout succession. With these experiments, I addressed how species richness and evenness of periphyton communities respond to combinations of current × nutrient regimes, where constant and variable current regimes (*see below*) were subjected to conditions of high nutrient abundance and compared to low nutrient controls.

2.2 Methods

2.2.1 Artificial Stream Flumes

Experiments were conducted in six recirculating laboratory streams (Figure 2.2). In each artificial stream channel, an experimental trough measuring 80 cm long, 12 cm wide, and 13 cm deep maintained uniform flow (± 1cm · sec^{-1}). Eighty liters of stream water (experiment 1) or Guillard's WC media (experiments 2-4) was placed in each stream channel and modification of water flow accomplished by adjusting a belt and multiple drive step pulleys attached to a motor and water pump. More discrete modifications to flow were achieved by adjusting a water release valve. Drop-in chillers (1/5 hp, TradeWind Chillers, Escondido, CA, USA) maintained room temperature in high velocity channels, while streams with current velocities of less than 20 cm · sec^{-1} did not elevate the water temperature above room temperature (*unpublished data*). In each experimental trough, 49 × 49 mm unglazed porcelain tiles were placed equidistant from one another. One 250-watt metal halide lamp, positioned above each experimental trough, provided light at levels sufficient to saturate photosynthesis of attached algae (~200 µmol · m^2 · sec^{-1}; Hill 1996) on a 14:10 daily light: dark ratio. Every three days, 24 liters (30%) of water in each stream was removed and replaced with 24 liters of new stream water (experiment 1) or modified WC media (experiment 2). Each experimental run lasted 35 days.



(a)

(b)

Figure 2.2 Schematic diagram of artificial stream set-up (a). Artificial streams in LS 117 (b).

2.2.2 Experimental Set-up

I ran four experiments, one experiment manipulating current velocity, and three experiments manipulating current velocity and nutrient supply.

Experiment Manipulating Current Velocity

The first experiment examined various structural and successional characteristics of periphyton communities under different current regimes. In each artificial stream (n = 6), 80 L of stream water was seeded with algae scraped from substrates from a small stream in Duncanville, TX (32° 38' 03.97" N, 96° 55' 05.97" W) and a small stream in Arlington, TX (32° 41' 21.02" N, 97° 10' 21.38" W) and suspended in 2 L of water. In each artificial stream, 24 L (30% of total) was replaced with new stream water every third day for a duration of 35 days. Half the streams were subjected to a current velocity of 10 cm $\cdot$ sec^{-1}, and the other half to 30 cm $\cdot$ sec^{-1}. Current velocity was measured with a Marsh-McBirney model 2000 flowmeter (Mach-McBirney Inc., Frederick, MD, USA).

Experiments Manipulating Current Velocity and Nutrient Abundance

The second set of experiments examined succession in periphyton communities under different nutrient (low and high) and current regimes (low, high, and variable). Two streams were subjected to conditions of constant flow of 10 cm $\cdot$ sec^{-1}, another two streams subjected to constant flow of 30 cm $\cdot$ sec^{-1}, while the remaining two streams were subjected to variable flow (range 9-32 cm $\cdot$ sec^{-1}, average 20 cm $\cdot$ sec^{-1}; Figure 2.3). Additionally, nutrient concentration was varied across flow regime with either high (800 µmol $\cdot$ l^{-1}: 50 µmol $\cdot$ l^{-1} N:P) or low (20 µmol $\cdot$ l^{-1}: 1.25 µmol $\cdot$ l^{-1} N:P) modified Guillard's WC media (Guillard 1975). Modified WC media consisted of all constituents in their normal concentrations other than N and P concentrations which were varied between treatments but kept at a constant ratio consistent with Redfield values (Redfield 1958). Each artificial stream (n = 6) was seeded with algae scraped from substrates from the same streams as experiment one and suspended in 2 L of water. Every third day, 24 L of water was replaced with new media (with higher concentrations of N and P to help maintain

nutrient concentrations between replacements; 30% increase in N and P in high nutrient treatment and 120% increase for low nutrient treatment as determined from pilot study, *unpublished data*) for a duration of 35 days. Due to a lack of replication for each flow × nutrient treatment, the experiment itself was replicated 3 times and each experimental run was treated as a block.

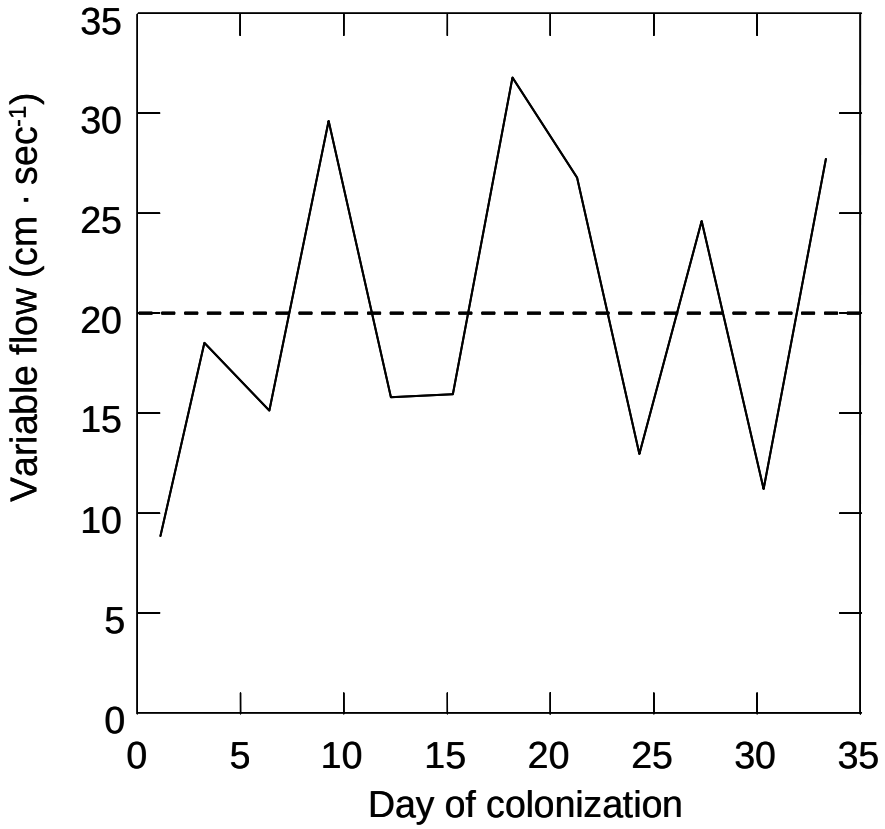
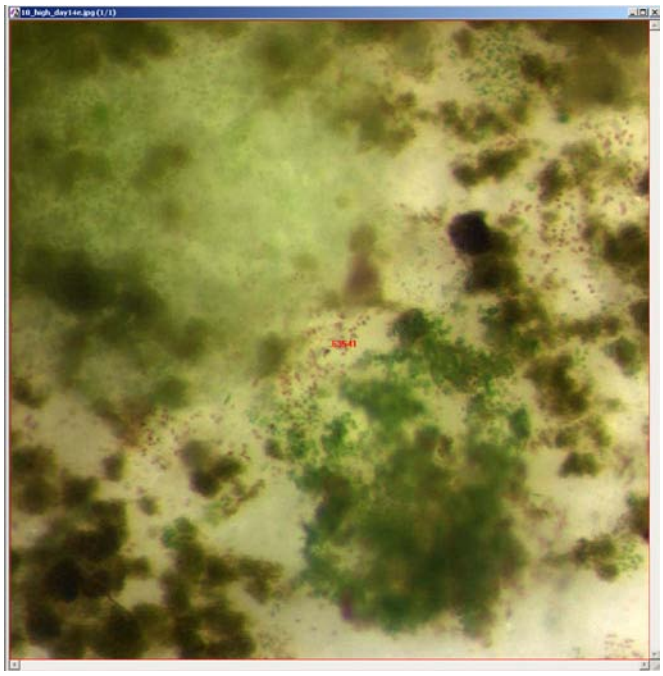


Figure 2.3 Temporal variation in current velocity for variable flow treatment (range 9-32, dotted line represents mean velocity = 20 cm · sec^{-1}).

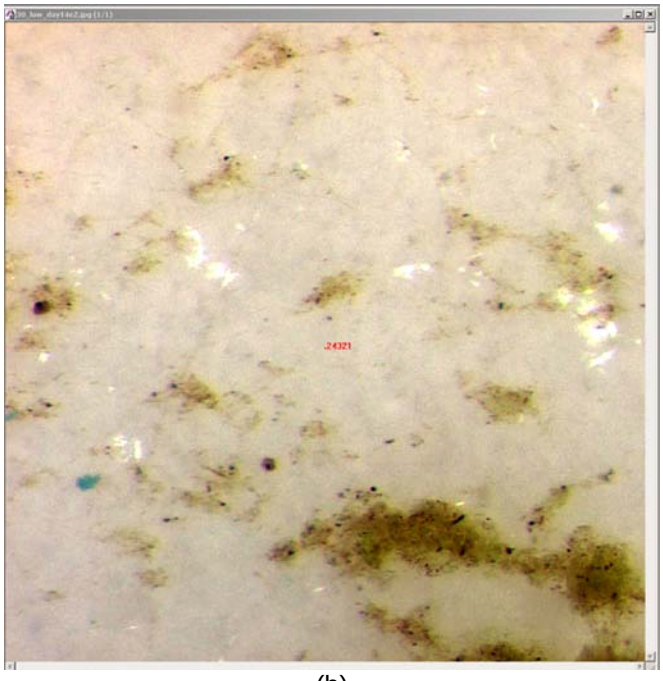
2.2.3 Sample Preparation and Analysis

An initial period of 7 days (5 days in first experiment) was allowed for biofilm colonization on tiles to occur, after which two tiles were randomly retrieved from each stream channel (tiles were taken from the same locations within each stream for each sampling period) and placed into an accompanying petri dish with enough distilled water to cover the tile. Following procedures in Larson and Passy (2005), five random fields on each tile were examined with a Zeiss Axioplan 2

LSM 510 META confocal microscope using a 40 × 0.80 NA water-immersion objective. Using confocal images, biofilm thickness was measured as the length of the z-focal plane, and mean biofilm thickness for each tile obtained by averaging biofilm thickness over the five random fields. Following observation with the confocal microscope, images of the biofilm on each tile's surface were captured with a stereo microscope at 50 × magnification and examined with Image Pro Plus, version 4.51 (Media Cybernetics, Inc., Silver Springs, MD, USA). These images were used to examine heterogeneity (fraction of pixels that vary more than 10% from average intensity of the object, values range from 0-1.0) in the whole field of view, hereafter referred as 'mosaic heterogeneity' (Figure 2.4). Following this, half the biomass on the surface of each tile was scraped with a razor blade and toothbrush. Scraped tiles were replaced back into streams and tiles were never retrieved again for the duration of the experiment. Biomass from the two tiles was consolidated and suspended in carbon filtered water and preserved in 4% buffered formalin solution. Biofilm "clumps" were separated with a pulse sonification device for 5 seconds, which was long enough to separate large clumps while avoiding cell damage (*personal observation*). After each sample was uniformly mixed, a subsample was placed into a Palmer-Maloney counting cell and observed under a light microscope at 400× magnification. To estimate periphyton abundance and composition, a minimum of 500 algal units were counted, where an algal unit was an individual cell for unicellular organisms, a 10 µm length for filaments, and 10 µm × 10 µm areas for colonies. Soft algae were identified in this count and diatoms lumped into a single taxonomic category; diatoms were identified to species later (*see below*). Average cell volume for each soft algal species was determined in every sample by measuring the dimensions of all individuals when less than twenty were encountered, and twenty when there were many individuals. Cellular biovolume was calculated by incorporating the cellular dimensions in formulae for solid geometric shapes most closely matching the shape of the cells (Hillebrand et al. 1999). In each sample cellular biomass was determined by multiplying the number of cells of each species by its mean cell volume.



(a)



(b)

Figure 2.4 Representative images of images obtained from stereoscope at 40 × magnification measuring 'mosaic' level heterogeneity. Images were captured on day 14 for 10 cm · sec^{-1} high nutrient treatment, heterogeneity = 0.63541 (a), and 30 cm · sec^{-1} low nutrient treatment, heterogeneity = 0.24321 (b).

2.2.4 Designation of diatom guilds

Diatoms exhibit well documented nutrient preferences (Van Dam *et al*. 1994), and diatoms in the experiments manipulating current velocity and nutrient supply were classified as either 'eutrophic' or 'noneutrophic' based on published accounts of tolerance for nutrients (Sladeček 1973, Van Dam *et al*. 1994). Diatoms classified as 'eutrophic' are those requiring high nutrient concentrations for growth and reproduction and 'non-eutrophic' are tolerant to low nutrient levels. Based on species descriptions in Van Dam *et al*. (1994), species were classified as 'eutrophic' if they were described as either 'meso-eutraphentic', "eutrophentic', or 'hypereutraphentic', while species described as 'oligotraphentic', 'oligo-mesotraphentic', 'mesotraphentic', or 'oligo- to eutrahentic' were classified as 'noneutrophic'. Species not described by Van Dam *et al*. (1994) were classified based on descriptions in Sladeček (1973); species classified as 'eutrophic' were species described to be 'beta- mesosaprobity' or 'alpha-mesosaprobity', while species described as 'xenosaphrobity' or 'oligosaprobity' were classified as 'noneutrophic'.

2.2.5 Statistical Analyses

For all experiments, experiment-wise error rate was $\alpha = 0.05$, corrected by a Bonferroni adjustment in case of multiple groups (Rice 1989, Legendre and Legendre 1998). All data were tested for normality and appropriate transformations made when necessary. The relationships between species richness (diatoms and soft algae) and evenness to biofilm thickness for each treatment combinations in the experiments manipulating current velocity and nutrient supply were analyzed using regression analysis with the curve-fitting software TableCurve 2D 5.01 (SYSTAT Software Inc., Richmond, CA). A parsimonious approach of selecting the simplest model with the highest r^2 was employed when determining equations best describing the relationship for each treatment. Repeated-measures analyses were performed using the General Linear Model command in SYSTAT version 11.

Experiment Manipulating Current Velocity

Using data from experiment one, a completely randomized design was used and differences in biofilm thickness, species richness and evenness resulting from treatment effects were tested with one-way repeated measures ANOVA, where 'day' was specified as a within-subjects factor, while 'current velocity' (10 cm · sec^{-1} and 30 cm · sec^{-1}) as a fixed between-subjects factor.

Experiments Manipulating Current Velocity and Nutrient Abundance

Using data from experiments 2-4, a randomized block design was used and differences in biofilm thickness, species richness and evenness resulting from treatment effects were tested with two-way repeated measures ANOVA, where 'day' was specified as a within-subjects factor, while 'current velocity' (10 cm · sec^{-1}, 30 cm · sec^{-1}, and variable flow) and 'nutrients' (high and low) as fixed between-subjects factors. Additionally, experimental run was treated as a blocking factor. During experimental run two of this set of experiments, data were lost in the variable flow, high nutrient treatment after day 14, so unequal replication occurred.

2.3 Results

2.3.1 Experiment Manipulating Current Velocity

Benthic algae from experiment manipulating current velocity included Divisions Bacillariophyta, Chlorophyta and Cyanophyta. A total of 144 taxa were encountered in this experiment, with 11 species of cyanobacteria, 27 species of green algae, and 106 species of diatoms.

The change in biofilm thickness (data pooled from all 6 study streams) differed significantly among days ($F_{8,32}$ = 48.12 $p \leq 0.001$, Table 2.1), with biofilm thickness increasing with day of colonization. The effect of current velocity on average biofilm thickness was significant, with higher biofilm thickness observed in the low current treatments ($F_{1,4}$ = 20.39, p = 0.011). The current × day interaction was not significant ($F_{8,32}$ = 0.74, p = 0.534). The change in species richness (data pooled from all 6 study streams) differed significantly among days ($F_{8,32}$ = 5.16, $p \leq 0.001$, Table 2.2). Current velocity significantly impacted species richness, with higher

average richness observed at low current velocity ($F_{1,4}$ = 9.78, p = 0.035, Figure 2.5a). The current × day interaction was not significant ($F_{8,32}$ = 0.48, p = 0.859). The change in evenness (data pooled from all 6 study streams) differed significantly among days ($F_{8,32}$ = 2.67, p = 0.037, Figure 2.5b). However, repeated measures ANOVA revealed no statistically significant difference in average evenness between current treatments ($F_{1,4}$ = 0.28, p = 0.623) and the current × day interaction was not significant ($F_{8,32}$ = 1.43, p = 0.242).

Table 2.1 Results from repeated measures ANOVA for ln-biofilm thickness, for experiment manipulating current velocity. *F*-statistic and *p*-values (in parentheses), significant values indicated with *.

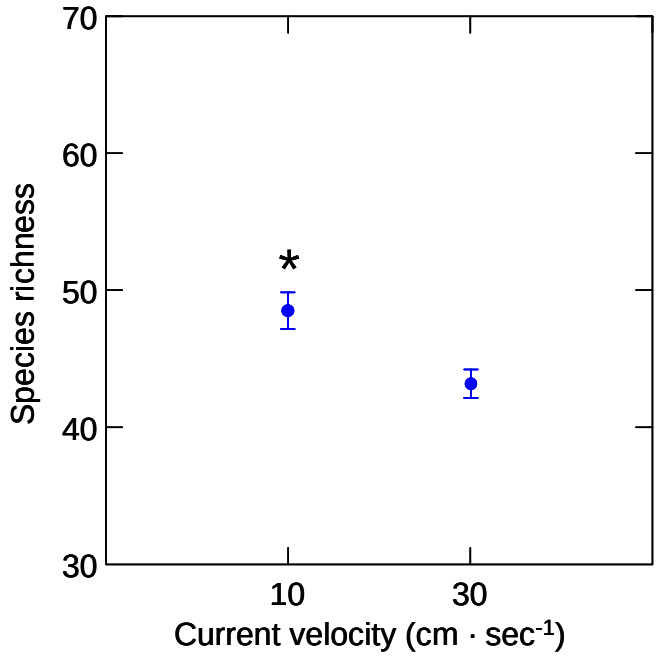
	d.f.	**ln-biovolume**
Between Subjects		
Current	1, 4	20.39 (0.011)*
Within Subjects		
Day	8, 32	48.12 (≤ 0.001)*
Day × Current	8, 32	0.74 (0.534)

Table 2.2 Results from repeated measures ANOVA for species richness and Peilou's evenness, for experiment manipulating current velocity. *F*-statistic and *p*-values (in parentheses), significant values indicated with *.

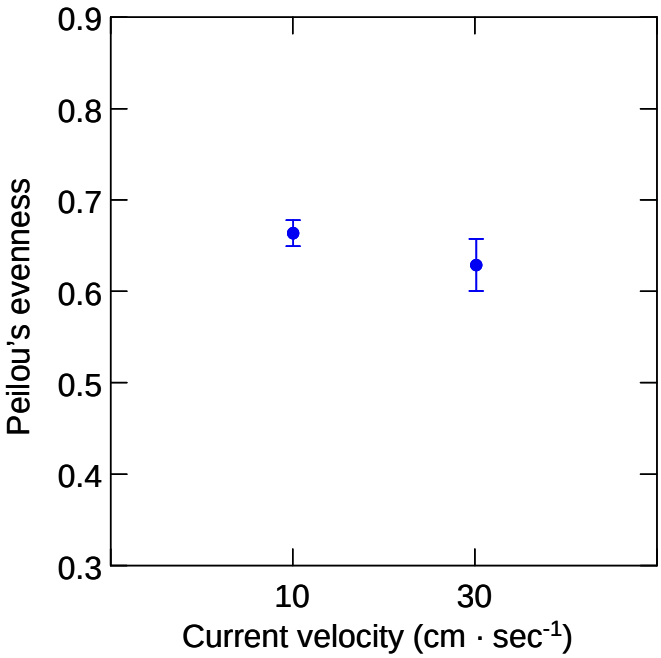
	d.f.	**Species richness**	**Peilou's evenness**
Between Subjects			
Current	1, 4	9.78 (0.035)*	0.28 (0.623)
Within Subjects			
Day	8, 32	5.16 (≤0.001)*	2.67 (0.037)*
Day × Current	8, 32	0.48 (0.859)	1.43 (0.242)

2.3.2 Experiments Manipulating Current Velocity and Nutrient Abundance

Benthic algae from experiments manipulating current velocity and nutrient abundance also included Divisions Bacillariophyta, Chlorophyta and Cyanophyta. A total of 185 taxa were encountered in this experiment, with 11 species of cyanobacteria, 38 species of green algae, and 136 species of diatoms. The highest number of species encountered over all experimental runs was in the low current, high nutrient treatment (10 High), with the lowest number of species encountered in the high current, low nutrient treatment (30 Low, Figure 2.6).



(a)



(b)

Figure 2.5 Average species richness (a) and Peilou's evenness (b) by current velocity in the experiment manipulating current velocity. Error bars represent ± 1 s.e. and significant differences indicated with *.

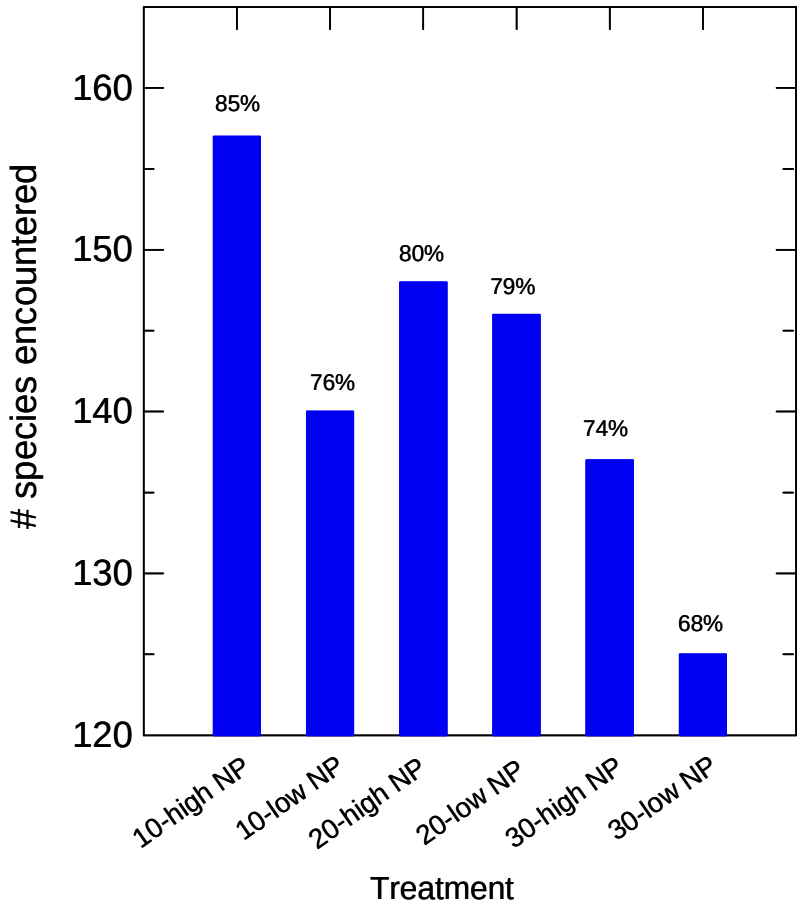


Figure 2.6 Total numbers of species encountered at least once, across replicates, for each treatment and the percentage of total number of species encountered for experiment manipulating current velocity and nutrient supply (185 total species encountered across all treatments).

Repeated measures analysis revealed the change in biofilm thickness (data pooled from all 6 study streams over three experimental runs) differed significantly among days ($F_{7,70} = 4.08$, $p = 0.002$, Table 2.3). Biofilm thickness was significantly impacted by nutrient supply, with greater average thickness observed in the high nutrient treatments across all current velocities ($F_{1,10} = 24.48$ $p \leq 0.001$, Figure 2.7). Average biofilm thickness between current velocity treatments was not significantly different from one another ($F_{2,10} = 1.46$, $p = 0.278$) and all interactions were not significant ($p > 0.05$).

Table 2.3 Results from blocked (by experimental run) repeated measures ANOVA for ln-biofilm thickness for experiment manipulating current velocity and nutrient abundance. *F*-statistic and *p*-values (in parentheses), significant values indicated with *.

	d.f.	ln-biovolume
Between Subjects		
Run	1, 10	0.15 (0.703)
Current	2, 10	1.46 (0.278)
Nutrients	1, 10	24.48 (≤0.001)*
Current × Nutrients	2, 10	0.18 (0.837)
Within Subjects		
Day	7, 70	4.08 (0.002)*
Day × Run	7, 70	0.46 (0.840)
Day × Current	14, 70	0.86 (0.588)
Day × Nutrients	7, 70	0.64 (0.701)
Day × Current × Nutrients	14, 70	0.81 (0.641)

The effects of current velocity and nutrient abundance on average species richness was significant ($F_{2,10}$ = 13.39, p = 0.001 and $F_{1,10}$ = 7.68, p = 0.013 respectively, Table 2.4, Figure 2.8a). Higher average richness was observed in the high nutrient treatments at each current velocity treatment (two-sample t-tests, $p < 0.05$), and species richness was significantly lower in the high current treatments (30 cm $\cdot$ sec^{-1}) compared to the other current treatments. Repeated measures analysis revealed the change in richness (data pooled from all 6 study streams over three experimental runs) differed significantly among days ($F_{7,70}$ = 6.91, $p \leq 0.001$). Making the assumption that the low current, high nutrient treatments were the least stressful, differences in species richness over colonization time were compared to the other treatments (Figure 2.9). The only treatments in which the difference in species richness changed through time were for the high current treatments (Figure 2.9b, c). The greatest difference in species richness between high current treatments and the low current, high nutrient control occurred around day 28 and the trend was consistent across nutrient treatments. In all of the other current × nutrient treatments, differences in average species richness did not change over colonization time and were therefore consistent throughout the duration of experiments.

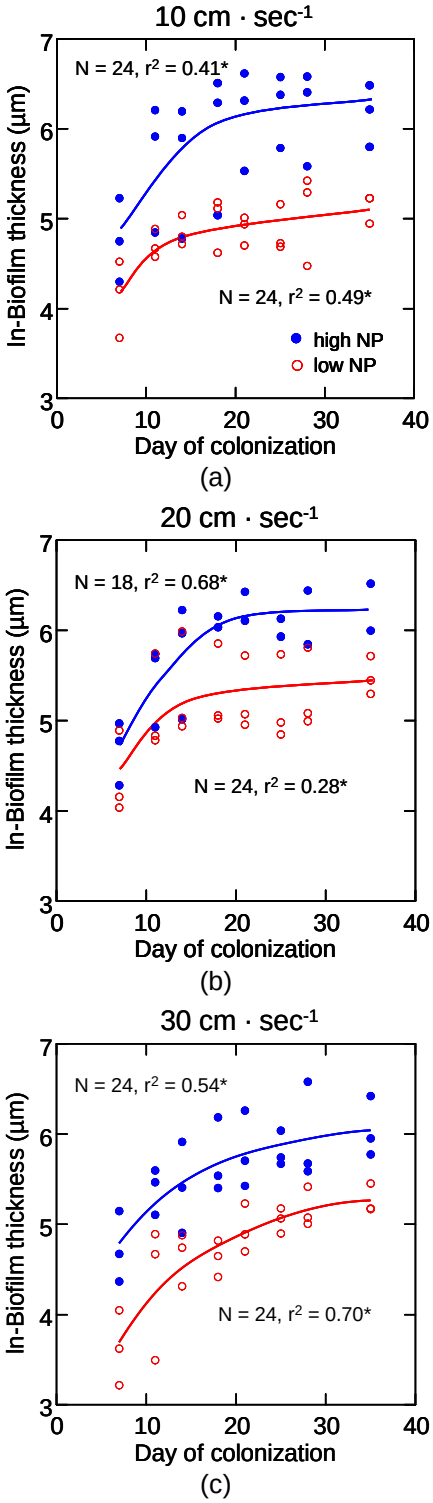


Figure 2.7 ln-biofilm thickness versus day of colonization for experiment manipulating current velocity and nutrient supply for 10 cm · sec^{-1} (a), 20 cm · sec^{-1} (b), and 30 cm · sec^{-1} (c). Trends modeled with equation: $\hat{y} = b_0 + b_1/x$, * denotes model significance

Table 2.4 Results from blocked (by experimental run) repeated measures ANOVA for species richness and Peilou's evenness, for experiment manipulating current velocity and nutrient abundance. *F*-statistic and *p*-values (in parentheses), significant values indicated with *.

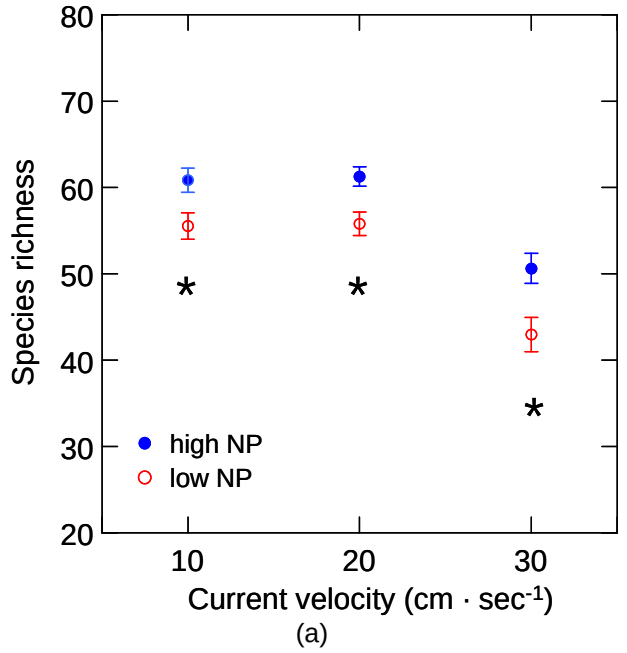
	d.f.	Species richness	Peilou's evenness
Between Subjects			
Run	1, 10	11.89 (0.006)*	0.10 (0.760)
Current	2, 10	13.39 (0.001)*	5.27 (0.027)*
Nutrients	1, 10	7.68 (0.013)*	0.14 (0.718)
Current × Nutrients	2, 10	0.19 (0.828)	2.62 (0.122)
Within Subjects			
Day	7, 70	6.91 (≤0.001)*	1.71 (0.121)
Day × Run	7, 70	4.98 (≤0.001)*	0.47 (0.857)
Day × Current	14, 70	2.91 (0.002)*	2.25 (0.014)*
Day × Nutrients	7, 70	1.17 (0.332)	1.54 (0.170)
Day × Current × Nutrients	14, 70	0.88 (0.583)	1.17 (0.320)

Different trends in species richness of diatoms and soft algae with increasing biofilm thickness were observed between treatments (Figure 2.10). In the low current, high nutrient treatments, diatom richness peaked at a biofilm thickness of around 250 µm and subsequently decreased with increasing thickness ($\hat{y} = b_0 + b_1 e^{-0.5((x-c)/d)^2}$, N = 24, $r^2 = 0.451$, $p = 0.001$, Figure 2.10a), while soft algae richness increased with biofilm thickness ($\hat{y} = b_0 + b_1 x^{2.5}$, N = 24, $r^2 = 0.388$, $p \leq 0.001$). In the low current, low nutrient treatment, diatom richness increased with biofilm thickness ($\hat{y} = b_0 + b_1 x^{0.5}$, N = 24, $r^2 = 0.144$, p = 0.021, Figure 2.10b) and there was no relationship between soft algae richness and biofilm thickness (no relationship). In the variable current, high nutrient treatment, diatom richness peaked at a biofilm thickness around 200 µm ($\hat{y} = b_0 + b_1 x + b_2 x^2 + b_3/x$; N = 19, $r^2 = 0.387$, $p = 0.009$, Figure 2.10c) and the relationship between soft algae richness and biofilm thickness was humped-shaped and peaked around 400 µm ($\hat{y} = b_0 + b_1 x + b_2 x^2$, N = 19, $r^2 = 0.456$, $p \leq 0.001$). In the variable current, low nutrient treatment, diatom richness increased with biofilm thickness ($\hat{y} = b_0 + b_1 x$, N = 24, $r^2 = 0.141$, $p = 0.022$, Figure 2.10d) and soft algae richness showed no relationship to biofilm thickness (no relationship). For the high current, high nutrient treatment, diatom richness peaked at a biofilm thickness of around 200 µm ($\hat{y} = b_0 + b_1 e^{-0.5((x-c)/d)^2}$, N = 24, $r^2 = 0.451$, $p = 0.001$, Figure 2.10e) and soft algae

richness increased with biofilm thickness ($\hat{y} = b_0 + b_1x$, N = 23, $r^2 = 0.201$, $p = 0.032$). In the high current, low nutrient treatment, diatom richness showed no relationship to biofilm thickness ($\hat{y} = b_0 + b_1x^3$, N = 23, $r^2 = 0.07$, $p = 0.197$, Figure 2.10f) and the relationship between soft algae richness and biofilm thickness was humped-shaped and peaked around 150 µm ($\hat{y} = b_0 + b_1x+b_2x^2$, N = 24, $r^2 = 0.327$, $p = 0.004$). However, it is important to note, that despite the changing trends of soft algae and diatoms within biofilm thickness, in all treatments diatom richness was always greater than richness of soft algae. Regarding species richness of diatoms, classified as either 'eutrophic' or 'non-eutrophic', greater richness of diatoms classified as 'eutrophic' was observed in the high nutrient treatments across all current velocity treatments (Figure 2.11a). Species richness of diatoms classified as 'non-eutrophic' did not differ with nutrient treatments (Figure 2.11b).

The current velocity and nutrient supply treatments had little effect on evenness of periphyton communities (Figure 2.8b). The effect of nutrient abundance on average evenness was not significant ($F_{1,10} = 0.14$, $p = 0.718$), while the effect of current velocity on average evenness was significant, with higher evenness observed in the high current, low nutrient treatment ($F_{2,10} = 5.27$, $p = 0.027$, Table 2.4, Figure 2.8b). The day × current interaction was also significant ($F_{14,70} = 2.25$, $p = 0.014$). Patterns in evenness emerged when examined against biofilm thickness (Figure 2.12). In the low current velocity treatments, evenness decreased with biofilm thickness in the low nutrient treatment ($\hat{y} = b_0 + b_1/x$, N = 24, $r^2 = 0.225$, $p = 0.019$), while in the high nutrient treatment, evenness declined initially with increasing biofilm thickness and then increased ($\hat{y} = b_0 + b_1x + b_2/x^2$, N = 24, $r^2 = 0.356$, $p = 0.0098$, Figure 2.12a). In the variable current treatments, evenness decreased with biofilm thickness in the high nutrient treatment ($\hat{y} = b_0 + b_1x^3$, N = 19, $r^2 = 0.257$, $p = 0.027$, Figure 2.12b), while in the low nutrient treatment, the change in evenness with biofilm thickness was best described with a quadratic equation, where there was an initial decrease and a slight increase at around 300 µm ($\hat{y} = b_0 + b_1x + b_2x^2$, N = 24, $r^2 = 0.511$, $p = 0.0005$). In the high current treatments, evenness decreased with biofilm thickness in the low nutrient treatments ($\hat{y} = b_0 + b_1x^2$, N = 24, $r^2 = 0.549$, $p = 0.00003$, Figure

2.12c), while in the high nutrient treatment, evenness declined initially and then increased with increasing biofilm thickness ($\hat{y} = b_0 + b_1/x + b_2/x^{1.5}$, N = 24, $r^2 = 0.434$, $p = 0.0025$).



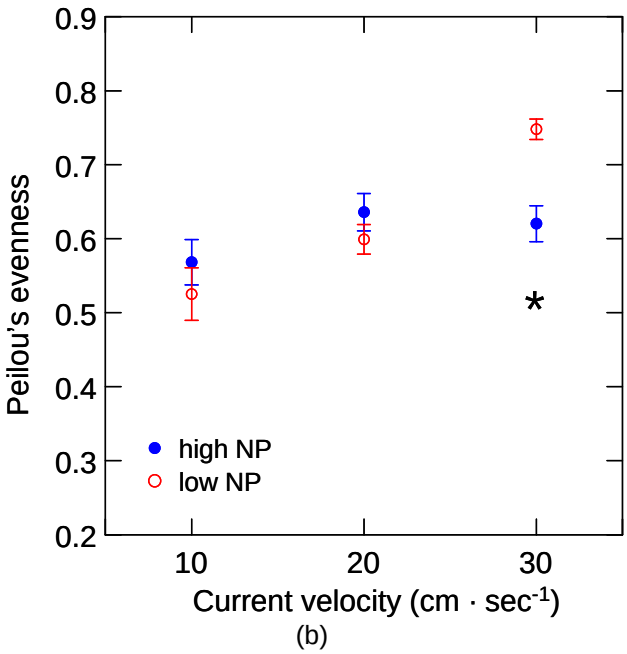


Figure 2.8 Average species richness (a) and Peilou's evenness (b) by current velocity in the experiment manipulating current velocity and nutrient supply. Error bars represent ± 1 s.e. and significant differences indicated with *.

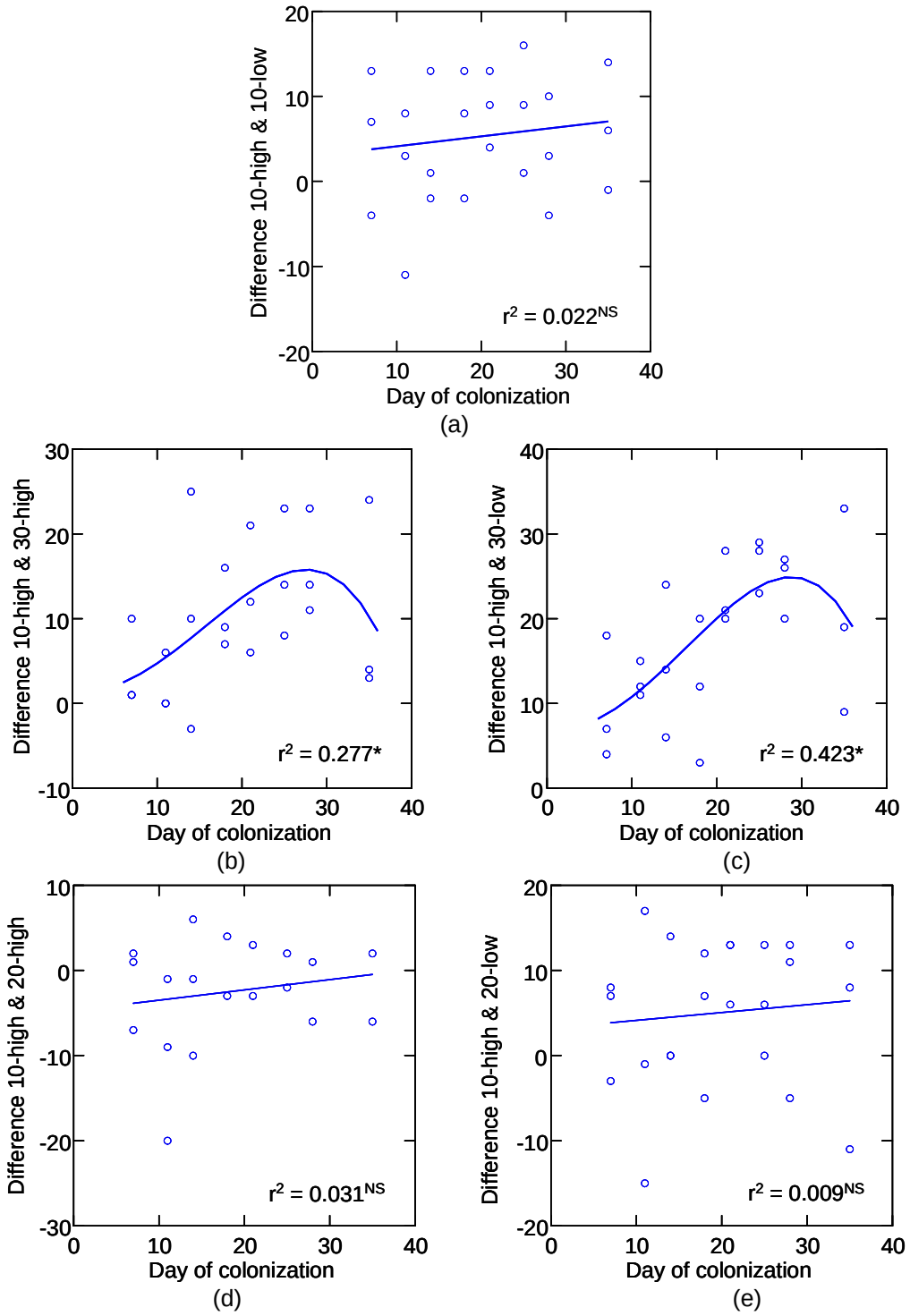


Figure 2.9 Differences in species richness with day of colonization between 10 cm· sec^{-1}, high nutrient treatment (reference) with 10-low (a) 30-high (b) 30-low (c) 20-high (d) and 20-low (e). Treatments at 30 cm· sec^{-1} modeled with $\hat{y} = b_o + b_1x^2+b_2x^4$, while other treatments modeled with linear equation. Model significance denoted by *.

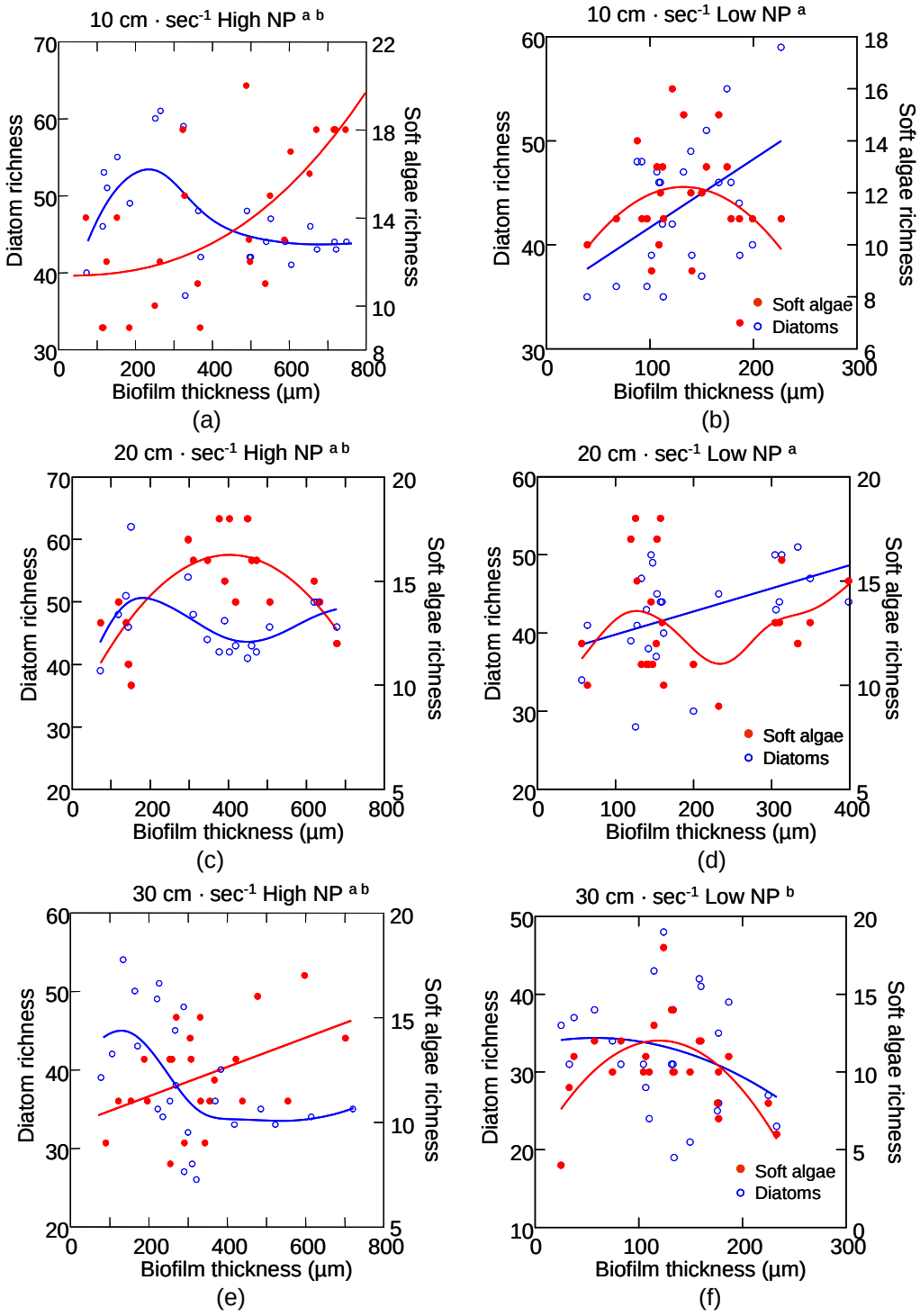


Figure 2.10 Diatom richness (left y-axis) and soft algae richness (right y-axis) versus biofilm thickness for experiments manipulating current velocity and nutrient supply. 10-high (a) 10-low (b) 20-high (c) 20-low (d) 30-high (e) and 30-low (f). Model significance for diatom richness or soft algae richness denoted by [a] and [b] respectively. Note differences in x and y axes.

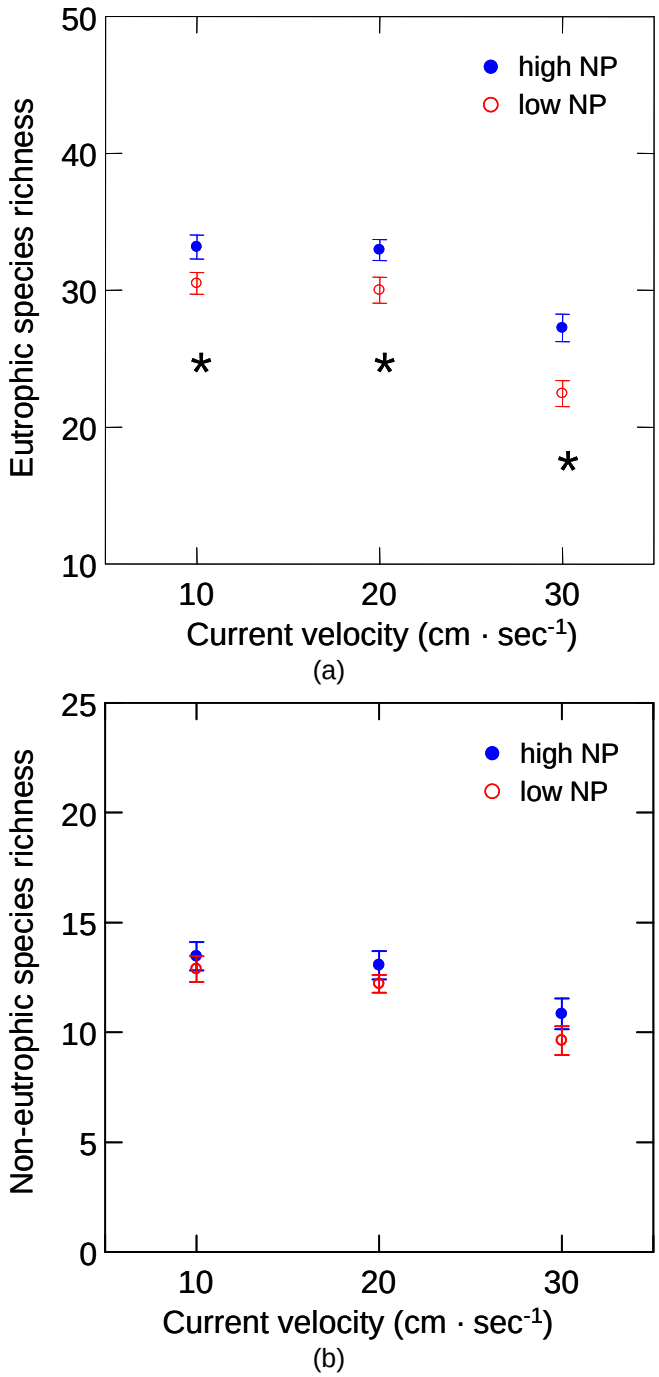


Figure 2.11 Average species richness of diatoms sensitive to low nutrient concentrations or 'eutrophic' species (a) and diatoms tolerant of low nutrient concentrations or 'non-eutrophic' species (b) in the experiment manipulating current velocity and nutrient supply. Error bars represent ± 1 s.e. and significant differences indicated with *.

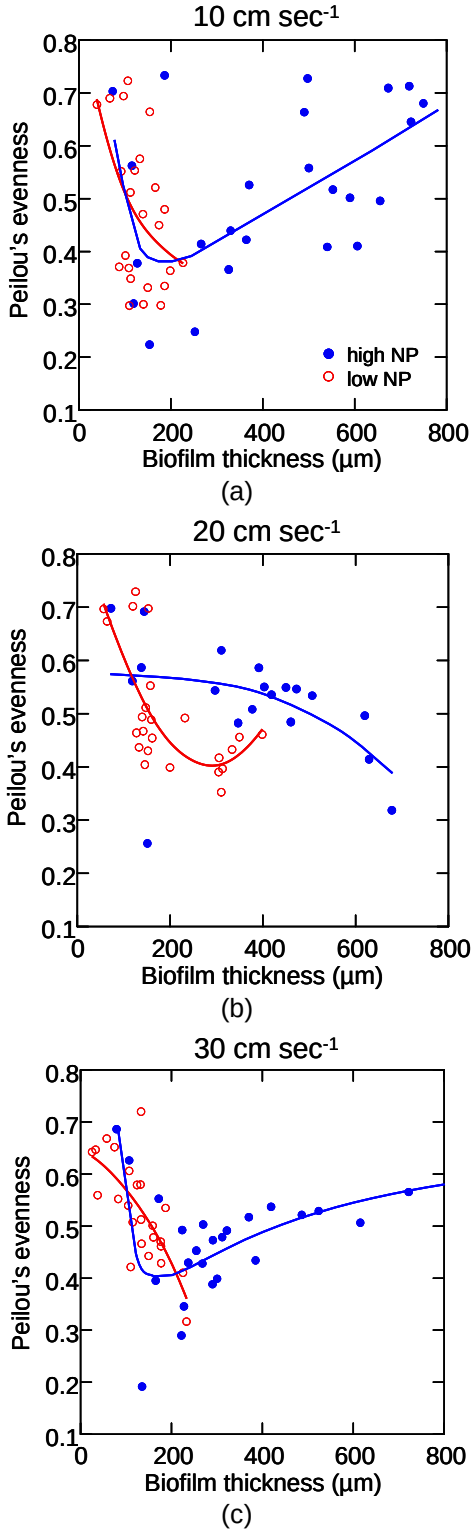


Figure 2.12 Relationship between Peilou's evenness and biofilm thickness for 10 cm · sec^{-1} current velocity (a), 20 cm · sec^{-1} current velocity (b), and 30 cm · sec^{-1} current velocity (c).

The overall relationship between species richness and evenness was negative (Figure 2.13a). When the relationship was separated by nutrient treatment, the relationship between richness and evenness was humped-shaped under high nutrient treatments and linear under low nutrient treatments (Figure 2.13b). The relationship between species richness and evenness to measured biofilm properties showed opposing trends (Figure 2.14). Species richness of periphyton communities increased with mosaic-level heterogeneity (Figure 2.14a), while evenness decreased with mosaic-level heterogeneity (Figure 2.14b).

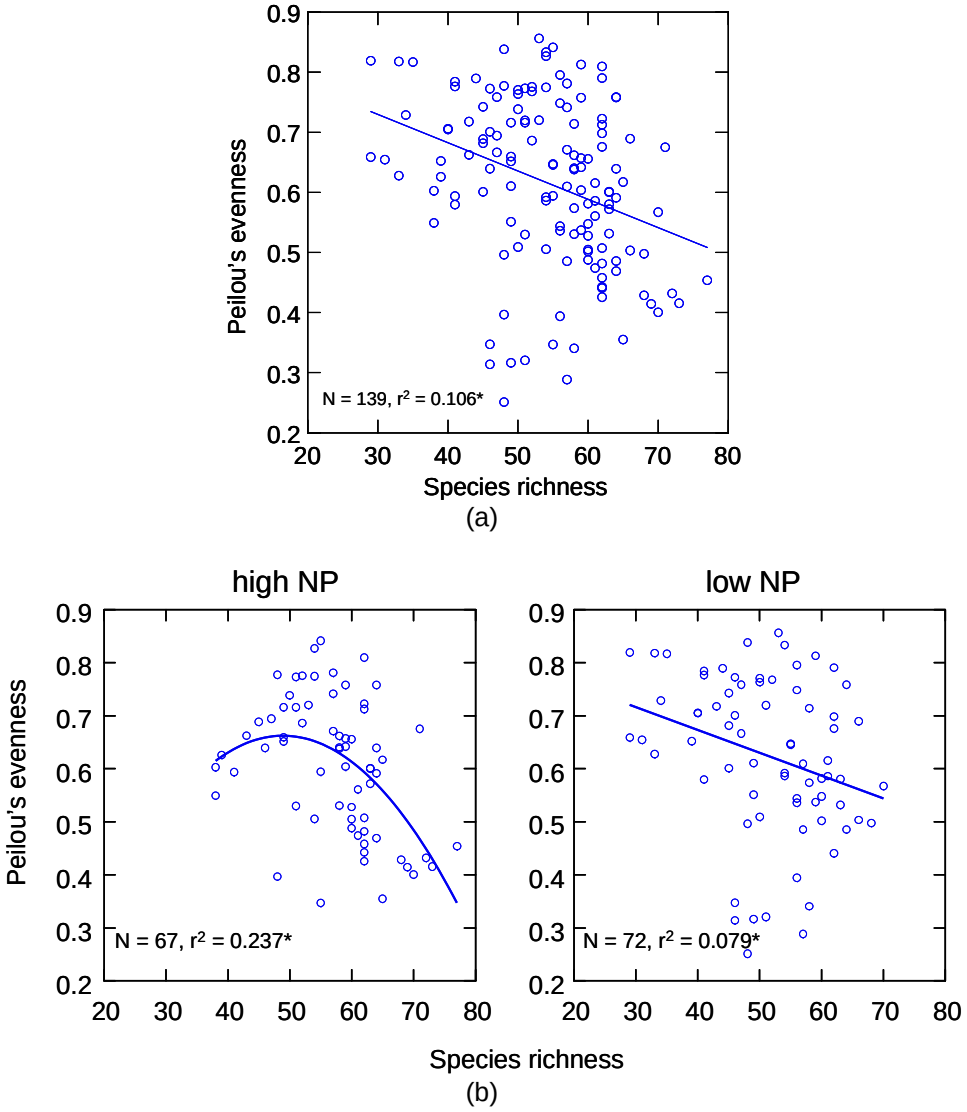


Figure 2.13 The relationship between species richness and evenness over all nutrient treatments (a) and broken-down by nutrient treatments (b).

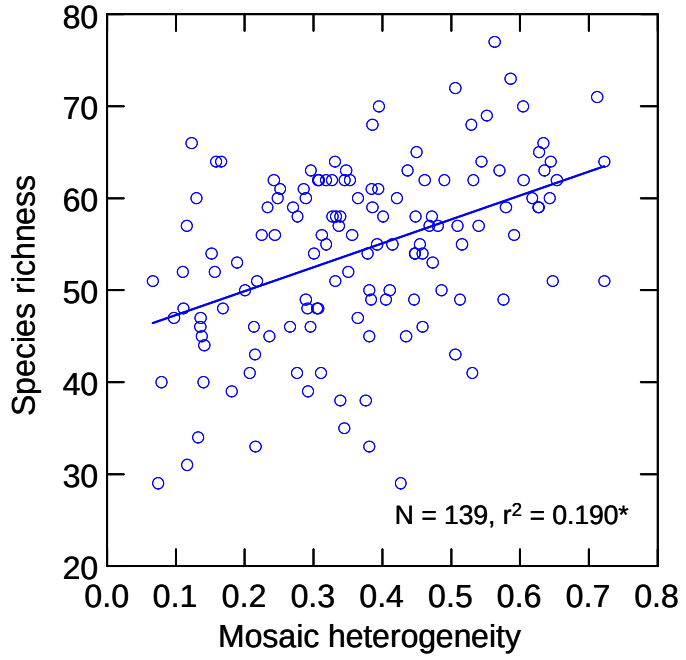


Figure 2.14 Relationship between species richness and mosaic-level heterogeneity (a) and evenness and mosaic-level heterogeneity (b).

2.4 Discussion

Species richness of periphyton communities in artificial stream flumes was significantly influenced by current velocity and nutrient supply. Under conditions of low current velocity, greater average species richness was observed in comparison to high current treatments. Greater average richness was also observed under conditions of high nutrients compared to low nutrient controls. Contrary to previous findings, nutrient addition had little influence on community evenness, as measured with Peilou's evenness, except in the high current velocity treatments.

That species richness of periphyton communities was lower under high current treatments, especially in the early stages of succession, is in line with results from previous experiments (McIntire 1966, Lamb and Lowe 1987, Ghosh and Guar 1998). An important component in the formation and succession of periphyton communities is the establishment of an organic film of detrital mucilage, bacteria, and fungi (Neu and Lawrence 1997, Sutherland 2001). The establishment of a microbial biofilm matrix, composed of extracellular polymeric substances (EPS), allows greater attachment and formation of periphyton communities on stream surfaces (Hudon and Bourget 1981, Korte and Blinn 1983). Under conditions of low current velocity, colonizing species of bacteria, fungi, and algae are allowed to settle, while under conditions of high current velocity this process is hindered. The ability of bacteria to adhere to surfaces under conditions of high current velocity can influence bacterial composition and diversity (Rickard *et al*. 2004), and in turn the growth and development of periphyton communities. Furthermore, algal immigration rates can be hampered under conditions of high current velocity (Stevenson 1983, Peterson and Stevenson 1989, Stevenson 1996). Therefore, possible explanations for the results observed in my experiments, is that perhaps in the high current treatments, the formation of a microbial matrix was delayed or the immigration of algae was hindered, thus leading to the observed differences in richness.

In the experiment manipulating current velocity, richness values converged towards the latter stages of succession, which is in agreement with several previous studies that have found that various properties of periphyton communities converge with time (McIntire 1966, Lamb and

Lowe 1987, Ghosh and Guar 1998). Species composition in the two current velocity treatments became more similar over successional time as well (Chapter 4). However, richness values were also similar in the beginning of the experiment, yet an examination of algal density early in succession (days 5 and 7) revealed significantly lower algal density in the high current treatments (two-sample t-test, $t_4 = 4.69$, $p = 0.009$ and $t_4 = 3.71$, $p = 0.021$, respectively), indicating that while there were no differences in richness values early in succession, there were differences in the density of algae between the two current treatments. Benthic algal density on bare substrata has been shown to be lower in the early stages of development in fast current regimes than in slow current regimes (McIntire 1966, Reisen and Spencer 1970, Stevenson 1984, Stevenson 1984, Steinman and McIntire 1986). In the experiments manipulating current velocity and nutrient supply, lower species richness was observed under high current velocity conditions, even in the latter stages of succession. In these experiments, there was also a divergence in species composition with time between treatments (Chapter 4). These findings are consistent with studies which have found persistent differences in algal richness, biomass, and cell densities arising from differences in current velocity (Stevenson 1984, Poff *et al*. 1990, Plenković-Moraj 2008). Initial hindrance or facilitation of the establishment of the biofilm matrix by current velocity may influence the successional trajectory of a periphyton community, such that communities exposed to different current regimes develop very differently as a result of the ability or inability of early colonizers to establish in a particular site. Furthermore, in the experiments manipulating current velocity and nutrient supply, there was a one-time supply of propagules in the beginning of the experiment, so any hindrance or facilitation in the initial establishment of biofilm matrix would be expected to influence the successional trajectories of the communities. Conversely, in the experiment manipulating current velocity new propagules were being supplied every three days during the course of the experiment, and once a biofilm matrix was able to form, the communities would have had the chance to converge over time.

With regards to the variable flow treatments, the response of species richness and evenness are difficult to interpret. Because variable flow treatments increased temporal

heterogeneity, I expected to them to have higher species richness and higher evenness relative to constant flow experiments. Richness and evenness did not differ significantly from low current treatments, but were significantly different from high current treatments. Once again, multivariate tests were more sensitive to changes in species composition (Chapter 4). However, if an increase in current velocity hinders the development of an initial biofilm matrix, then an increase in current velocity from 10 to 20 cm $\cdot$ sec^{-1} might be expected to delay the formation of a biofilm matrix and reduce richness as in the high current treatments; yet in my experiments, an examination of the current velocities for the variable flow regime revealed that velocities early in succession were more similar to low current treatments and thus not likely to have delayed the formation of a biofilm matrix. Yet, the temporal variability introduced in the variable flow treatments may have increased heterogeneity enough that neither current tolerant nor current sensitive species were favored and richness and evenness were higher than would have been observed had I run a treatment with a constant current velocity of 20 cm $\cdot$ sec^{-1}. An equally plausible explanation is that the difference in current velocity between 10 and 20 cm $\cdot$ sec^{-1} is not as great as between 10 and 30 cm $\cdot$ sec^{-1}, and 20 and 30 cm $\cdot$ sec^{-1}, and therefore there was not a difference in the univariate measures of species richness and evenness between 10 and 20 cm $\cdot$ sec^{-1}.

Under conditions of high ambient resources the establishment of thick, multi-layered periphyton communities with species coexisting in a spatially complex and heterogeneous micro-environment can occur (Stevenson and Glover 1993, Passy 2008). Under these conditions, the generation of a long resource gradient within the three-dimensional community allows a greater number of species across a broad spectrum of stress tolerance to coexist than in communities that are nutrient limited (Passy 2008). In my experiments manipulating current velocity and nutrient supply, biofilm thickness was positively related to nutrient supply and greater species richness was observed in the high versus low nutrient treatments for each of the current velocity treatments tested. However, greater richness was observed in the low current, low nutrient treatment than in the high current, high nutrient treatment, therefore richness was not only

determined by biofilm thickness. Current velocity had a distinctive influence on species richness of periphyton communities unrelated to nutrient supply, where richness was negatively influenced by high current velocity treatments. Nevertheless, richness of diatoms and soft algae were related to biofilm thickness, where the relationship varied with current velocity × nutrient supply treatment. In general, periphyton communities growing under low nutrient supply tend to be thin and composed primarily of species tolerant to low nutrient conditions and generally of smaller growth habit (Passy 2008). Periphyton communities in the low nutrient treatments tended to be composed largely of species of smaller growth habits and tolerant of low nutrients, such as the widely distributed diatom species *Achnanthidium minutissimum* (Kützing) Czarnecki (Chapter 4). Furthermore, comparison of diatoms classified as either 'eutrophic' or 'non-eutrophic' supported this observation, where greater richness of 'eutrophic' diatoms, or diatoms requiring high nutrient concentrations for growth and reproduction, were observed in the high nutrient treatments. Conversely, there was no difference in richness of 'non-eutrophic' diatoms between nutrient treatments, a result not surprising given 'non-eutrophic' diatoms were those described as being tolerant or indifferent to low nutrient concentrations.

While high nutrient supply can contribute to high richness in 3D communities, fertilization or enrichment can also lead to competitively dominant species being highly favored, resulting in lower evenness (Hillebrand *et al*. 2007). In the experiment manipulating current velocity, differences in evenness were not significant, a result not surprising given nutrient conditions were the same between treatments. In the experiments manipulating current velocity and nutrient abundance, evenness differed little between nutrient treatments, except in the high current velocity treatments. Evenness appeared to be slightly higher in high nutrient treatments relative to low nutrient controls in the low and variable current treatments, yet this difference was not statistically significant. Overall, species richness in these experiments was high (mean = 54.26, 52.65-55.87 95% CI), which may have contributed to the lack of a significant difference in evenness between treatments. Nevertheless, in the high current treatments, lower evenness was observed relative to low nutrient controls. A possible explanation for differences in evenness

seen only at high current velocity is that with low average richness in the high current treatments relative to other current treatments, fewer species were present to benefit from high nutrient supply once a biofilm matrix was formed, and the species that were there became abundant and dominated species composition (Chapter 4). Even though differences in evenness between nutrient treatments were not as great as I would have predicted (based on repeated measures analysis), multivariate tests, gauging the changes in species composition, showed treatment affects with time (Chapter 4). Furthermore, evenness appeared to be influenced by biofilm thickness, where a similar trend between evenness and biofilm thickness was observed in the low and high current treatments. In both the low and high current treatments, evenness decreased with biofilm thickness in the low nutrient treatments, yet in the high nutrient treatments, evenness decreased initially and then increased when biofilm thickness was greater than 200 µm. A similar trend was observed in the low nutrient, variable current treatment, were an initial decrease and a subsequent increase was observed at biofilm thickness of around 300 µm, yet this is speculative since thickness did not increase above 400 µm, thus making it impossible to predict how evenness would have changed beyond this point. Yet, these results suggest that at a certain point, biofilm thickness may be a reflection of greater heterogeneity or three-dimensional structure within the periphyton/biofilm matrix, which leads to greater evenness.

An examination of the relationship between species richness and evenness revealed evenness decreased with increasing richness, suggesting a more even distribution of individuals with low species richness. The trends were slightly different when examined by nutrient treatment; in the high nutrient treatments, a slight initial increased in evenness produced a humped-shaped relationship with richness, while in the low nutrient treatments, a negative relationship was observed. However, in both nutrient treatments, evenness decreased at high species richness. Little consensus has been reached on what the relationship between species richness and evenness should be, but increasing evidence suggests the relationship is negative (Gosselin 2006, Symonds and Johnson 2008). A negative relationship between species richness and evenness can result when the number of rare species increases, yet evenness declines

because of the increased mix of common and rare species (Symonds and Johnson 2008). A negative relationship can also result from increased competition at high species richness, resulting in dominant species having greater proportional abundances (Hillebrand *et al.* 2007). Evenness decreased more rapidly (greater negative slope) with richness in the high relative to low nutrient treatment, which would seem to indicate one or several dominant species having greater proportional abundance leading to lower evenness.

The relationship between species richness and evenness to mosaic-level heterogeneity revealed opposing trends. Greater species richness was observed with increasing mosaic-level heterogeneity in my experiments. Greater environmental heterogeneity can result in a higher number of available niches for species, leading to an increase in species richness with increasing heterogeneity (Tilman and Pacala 1993). However, greater mosaic-level heterogeneity was reflected in lower evenness. A potential explanation for lower evenness with increasing heterogeneity would be that while an increase in heterogeneity may favor an increase in rare species, evenness declines as a result of a few species dominating community dynamics (Symonds and Johnson 2008). In periphyton communities, the formation of three-dimensional communities results in long resource gradients within the biofilm matrix which sustains a greater number of species than in two-dimensional communities (Passy 2008), yet the high nutrient concentrations required to produce thick, multi-layered communities can lead to an increase in competitive dominance and a reduction in evenness (Hillebrand *et al.* 2007). This may be why under conditions of high nutrients the growth of certain species can sometimes reach nuisance levels in streams and rivers (i.e., *Cladophora* sp.)

In conclusion, species richness and evenness of periphyton communities growing in artificial stream flumes were sensitive to differences in current velocity and nutrient supply. Greater species richness was observed in high versus low nutrient treatments and in low versus high current treatments. Richness of diatoms and soft algae and evenness varied with biofilm thickness, as did evenness. Species richness and evenness of periphyton were negatively related to one another and species richness increased with environmental heterogeneity, while

evenness decreased with heterogeneity. Rarely have the effects of both current velocity and nutrient supply on species richness of periphyton communities been examined. My results demonstrate that the response of species richness of periphyton communities varies with the current and nutrient regime. However, as with most studies, these results raise more questions than answers and emphasize the complexity of periphyton community responses to current velocity and nutrient supply. These results also highlight the need for greater understanding of how anthropogenic fertilization and modification to the current regimes of many streams and rivers can influence the richness and diversity of these important primary producer communities that form the base of the foodweb.

CHAPTER 3

RELATIONSHIP BETWEEN ALGAL SPECIES RICHNESS AND BIOMASS ALTERED BY CHANGES IN CURRENT VELOCITY AND NUTRIENT SUPPY

3.1 Introduction

As humans modify and alter landscapes in ways that impact biodiversity, the need to better understand the consequences of changing diversity on ecosystem functioning becomes increasingly important (Dudgeon *et al*. 2006, Diaz *et al*. 2006, Balvanera *et al*. 2006, Costanza *et al*. 2007). The last several decades have witnessed renewed interest in the relationship between diversity and productivity, with examinations usually following one of two lines of inquiry. The first approach typically examines how resource availability limits the production of biomass, while simultaneously influencing the number of coexisting species (reviews by Waide *et al.* 1999 and Mittelbach *et al.* 2001). The second approach examines how the number of species in a community captures and converts resources into biomass (reviews by Tilman 1999, Loreau *et al*. 2001, and Naeem 2002). Until recently, the second approach has received less attention, but as we gain greater awareness that the relationship between diversity and productivity is bidirectional, the emphasis is shifting on how productivity responds to changes in diversity or species richness (Loreau *et al*. 2001, Schmid 2002, Worm and Duffy 2003).

The response of productivity (or any other proxy measure of productivity) to increasing diversity is hypothesized to be a saturating pattern, where an initial increase in productivity begins to level off (Hooper *et al*. 2005). It is thought that a positive response in productivity associated with increased species richness can result from facilitative actions between species, complementary resource use, or as a result of the increased probability of including more productive species in more species-rich communities ('sampling effect'). Productivity can begin to level off with increased species richness as a result of functional redundancy between species (Lawton and Brown 1993). Furthermore, in species-rich communities, increasing niche overlap

can contribute to negative species interactions and lead to a decrease in productivity (Passy and Legendre 2006).

In aquatic ecosystems, microbial biofilms are multi-layered and structurally diverse communities composed of many different species (bacterial, fungal, algal, and meiofauna) closely coexisting (Battin *et al.* 2007). Microbial biofilms growing on surfaces are important primary producers in streams, lakes, and wetlands and form the base of the food web in aquatic ecosystems (Stevenson 1996a) and have been shown to influence important biogeochemical processes and patterns in streams (Battin *et al*. 2003a,b). Therefore, factors that influence productivity and diversity in biofilm communities have the potential to influence other parts of the ecosystem.

In streams, variability in streamflow represents a primary form of disturbance (Resh *et al.* 1988, Poff *et al.* 1997), with current velocity influencing the productivity and diversity of nearly all stream organisms (Vannote 1980). Current velocity can influence the diversity of bacterial biofilms (Rickard *et al*. 2004), multi-trophic biofilms (Battin *et al*. 2003a,b), and periphyton communities (McIntire 1966, Lamb and Lowe 1987, Ghosh and Guar 1998, Passy 2007). Nutrient supply can also influence diversity in periphyton communities (Francoeur *et al*. 1999, Biggs 2000, Biggs and Smith 2002, Passy 2008), with high ambient nutrient concentrations in the overlying water column resulting in the formation of thick, multi-layered periphyton communities (Stevenson and Glover 1993). Facilitation has long been recognized as an important component to succession in periphyton communities, where preconditioning of substrate surfaces by pioneer species influences the establishment and growth of subsequent colonizing species (Hoagland *et al.* 1982, Korte and Blinn 1983, Stevenson 1983). Competition for limiting resources is also a significant factor influencing the assemblage of periphyton communities and many species exhibit distinct nutrient preferences (Sladeček 1973, Van Dam *et al*. 1994). Therefore, the establishment and growth of periphyton communities within a stream will be highly influenced by both the current regime and nutrient supply (Stevenson 1996) and the relationship between species richness and biomass production likely to be influenced by both abiotic factors.

In streams, the relationship between species richness and biomass production has shown a positive relationship in streams from frequently disturbed watersheds and no relationship in streams from watersheds with low discharge-related disturbance (Cardinale *et al*. 2005). At a larger scale, in a continental study of major benthic stream habitats, biomass production of algal communities exhibited a unimodal relationship to species richness, where biomass peaked around 45 ± 12 (SD) species in the richest-targeted habitats (Passy and Legendre 2006).

In rivers and streams, impoundments, diversions, and loss of riparian buffers can lead to modifications in the natural flow regime (Lytle and Poff 2004, Sweeney *et al*. 2004) and loss of riparian buffers can lead to increased inputs of nutrients from nonpoint sources (Lowrance *et al*. 1997). Therefore, flow modification and cultural eutrophication are important management issues facing rivers and streams. Here I present results of a study in which I measured species richness and biomass accumulation (measured as total community biovolume) of assemblages of periphyton communities in a series of experiments taking place in artificial stream flumes. Periphyton communities were subjected to treatment combinations of current × nutrient regimes, where constant and variable current regimes (see Chapter 2, Methods) were subjected to conditions of high nutrient abundance and compared to low nutrient controls. My objectives were (1) to examine the accumulation of algal biomass (measured as total community biovolume) in different current × nutrient treatments; (2) to test the prediction that the diversity-biomass production relationship would differ among streams with different current × nutrient treatments; (3) to relate the diversity-biomass relationship to competition using diatom guilds based on nutrient preferences.

3.2 Methods

Data for these analyses came from four separately run experiments; in the first experiment I used stream water and manipulated current velocity (10 and 30 cm · sec^{-1}), while for experiments 2-4, I used modified Guillard's WC media (Guillard 1975) and manipulated current velocity (10 cm · sec^{-1}, 30 cm · sec^{-1}, and variable flow) and nutrient supply (high: 800 µmol l^{-1}: 50 µmol · l^{-1} N:P or low: 20 µmol · l^{-1}: 1.25 µmol · l^{-1} N:P). Each experimental run lasted 35 days and

detailed methods for these experiments are described in chapter two, and only important differences in methods and statistical analyses are noted below.

3.2.1 Trophic Diatom Guilds and Trophic Diatom Index

As detailed in Chapter two, diatom species in the experiments manipulating current velocity and nutrient supply were classified as either 'eutrophic' or 'noneutrophic'. Additionally, diatom species were designated as one of three growth forms, i.e., low profile, high profile, and motile. Diatoms were designated as low profile if they were of short stature, which included prostrate, adnate, erect, solitary centric and slow moving diatoms. Slow moving diatoms were grouped with low profile species as they exhibit similar patterns non-motile diatoms (Hudon and Legendre 1987). High profile diatoms encompassed species of tall stature, including erect, chain-forming, tube-forming and colonial centric diatoms. Motile diatoms included comparatively fast moving species, most notably bi-raphid diatoms. Guilds were then composed of trophic-growth form combinations (e.g., eutrophic-high, eutrophic-low, etc.). Additionally, I calculated the Trophic Diatom Index (TDI, Kelly and Whitton 1995, Kelly *et al*. 2001), which has values ranging from 0 to 100, with large values indicative of highly eutrophic waters. The TDI is an index for monitoring the trophic status of rivers based on diatom composition and has been shown to be highly correlated with phosphorus concentration in rivers (Kelly and Whitton 1995).

3.2.2 Statistical Analyses

All data was tested for normality and appropriate transformations made when necessary. Repeated-measures analyses were performed using the General Linear Model command in SYSTAT version 11.

Experiment Manipulating Current Velocity

Using data from experiment one (see methods in chapter 1), a completely randomized design was used. For the analyses of total community biovolume with colonization time, quadratic regression was employed and the predictor variable centered by its mean to reduce collinearity between the first second-order monomial (Kutner *et al*. 2005). In the analysis of species richness and total community biovolume, since community biovolume changed with time,

ln-biovolume was regressed against day of colonization and the residuals (residual biovolume) plotted against species richness to be able to account for changes in biovolume only attributed to species richness.

Experiments Manipulating Current Velocity and Nutrient Abundance

Using data from experiments 2-4, a randomized block design was used and experimental run treated as a blocking factor. During experimental run two of this set of experiments, data were lost in the variable flow, high nutrient treatment after day 14, so unequal replication occurred. To examine the relationships between total community biovolume with day of colonization and the proportion of total diatom biovolume of 'eutrophic' and 'noneutrophic' species richness versus biofilm thickness for each treatment combination, relationships were analyzed using regression analysis with the curve-fitting software TableCurve 2D 5.01 (SYSTAT Software Inc., Richmond, CA). A parsimonious approach of selecting the simplest model with the highest r^2 was employed when determining equations best describing the relationship for each treatment. For the analyses of total community biovolume and species richness, regression analysis was employed using the same procedures as with experiment one.

Multivariate Analysis of Algal Communities in Experiments 2-4

Differences in species abundance of diatom guilds between treatments were examined with PRIMER software application (version 6.1; Plymouth Marine Labs, Plymouth, UK; Clarke and Warwick 2001). Compositional similarities between samples were computed with the Bray-Curtis coefficient (Bray and Curtis 1957) and a dissimilarity matrix generated by comparing algal composition of all samples using untransformed abundance all diatom species in each ecological guild. Within the PRIMER software, I used a one-way analysis of Similarities (ANOSIM) to compare rank similarities of samples between treatments (current × nutrient combinations) for each sampling date. With ANOSIM an R-statistic is calculated, which varies between 0 and 1; with high values indicative of completes separation between treatments. The value of the R-statistic reflects the observed differences between treatments, contrasted with differences among replicates within treatments and can be compared to a distribution of values expected under the

null hypothesis of no difference between treatments to obtain a test of significance (Clarke and Warwick 2001). In my case, the global ANOSIM test for overall differences between groups (each current × nutrient combination) was examined and if significant, R values for each pair-wise comparison were inspected. However, the limited number of permutations available for pair-wise comparisons in my experiments (n = 3 replicates for each treatment combination) mean it was not possible to determine the significance of R-statistic values at probabilities <10%. Yet, values of R are not unduly affected by the number of replicates in the groups being compared (Clarke and Warwick 2001), therefore, large values (close to unity) are indicative of complete separation of the groups, and small values (close to zero) evidence of little or no segregation. To provide a graphical summary of the relationship between similarity matrices, I employed ordination with nonmetric multidimensional scaling (NMDS), considered a robust ordination technique for ecological analyses (Clarke and Warwick 2001).

3.3 RESULTS

3.3.1 Experiment Manipulating Current Velocity

Regression analysis revealed community biovolume increased with day of colonization for both current treatments ($p \leq 0.001$, Figure 3.1). Slopes were not significantly different from one another, but the intercepts were, indicating differences in community biovolume over time (Table 3.1), with higher biovolume in the low current treatment. When examining the relationship between species richness and residual community biovolume, biovolume did not change with species richness for either current treatment (10: N = 27, $r^2 = 0.011$, $p > 0.05$, 30: N = 27, $r^2 = 0.02$, $p > 0.05$, Figure 3.2a, b).

3.3.2 Experiment Manipulating Current Velocity and Nutrient Supply

For all treatments, community biovolume increased with day of colonization (Figure 3.3). In all cases, a non-linear model gave the best fit, except in the 30-low treatment, where an increase in community biovolume was best described with a linear model ($p < 0.05$, Table 3.2). Greater community biovolume with time was observed in the high nutrient treatments, with all of the y-intercepts greater than the low nutrient treatments. Paired t-tests confirmed greater

average community biovolume in the high nutrient treatments at each current treatment ($p \leq 0.001$). Average community biovolume did not differ significantly between current velocities in the high nutrient treatments (one-way ANOVA $F_{2,64} = 0.098$, $p = 0.906$), however, average community biovolume differed between current velocity treatments in the low nutrient treatments (one-way ANOVA $F_{2,69} = 4.83$, $p = 0.011$). Tukey pair-wise comparisons between current treatments revealed the 30-low treatment had significantly lower total biovolume than the 20-low treatment (p = 0.010), but not the 10-low treatment (0.07). This result suggests a possible impediment of biovolume accumulation at high current velocity treatments under low nutrient conditions.

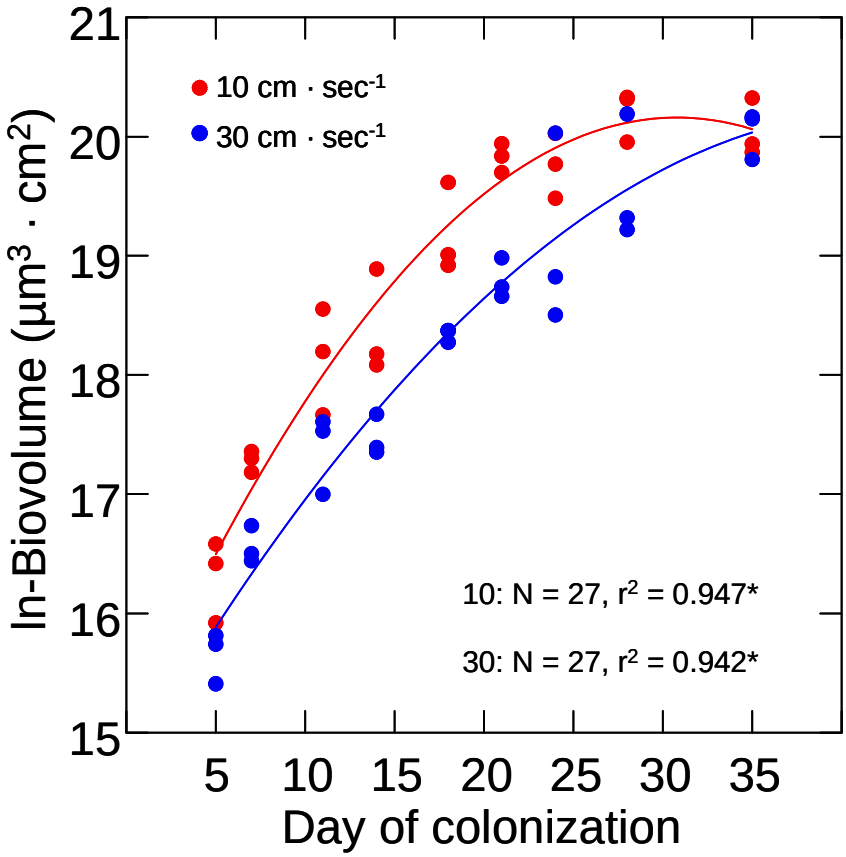


Figure 3.1 Biomass production (measured as ln-total cell biovolume) vs. day of colonization for current velocity in experiment one (manipulating current velocity). Smoother fitted by$\hat{y} = b_0 + b_1x + b_2x^2$, N = number of observations, *$p < 0.05$.

Table 3.1 Coefficients, 95% confidence intervals (CI) and coefficients of determination (r^2) of quadratic regression models of total community biovolume versus day of colonization in two current velocities. (N = number of data points).

	ln-biovolume (10 cm · sec^{-1})	**ln-biovolume (30 cm · sec^{-1})**
N	27	27
r^2	0.947	0.942
b_0	19.27	18.37
95% CI	19.09 to 19.45	18.17 to 18.57
b_1	0.139	0.149
95% CI	0.126 to 0.154	0.134 to 0.166
b_2	-0.0055	-.0055
95% CI	-0.0069 to -0.0040	-0.0047 to -0.0014

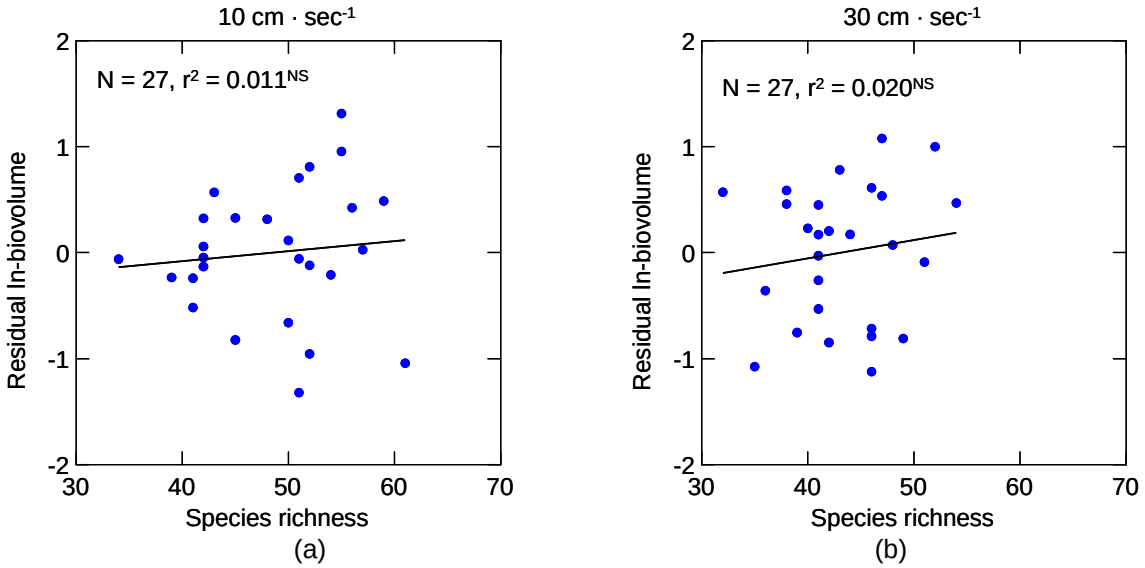


Figure 3.2 Species richness and residual ln-biovolume for 10 cm · sec^{-1} (a) and 30 cm · sec^{-1} (b) current velocity treatments in the experiment manipulating current velocity. NP = nitrogen + phosphorus, N = number of observations, *p < 0.05, NS = non-significant.

Table 3.2 Coefficients, 95% confidence intervals (CI) and coefficients of determination (r^2) of regression models of total community biovolume versus day of colonization in six treatment combinations. (N = number of data points). For all treatments except 30-low, the model $\hat{y} = b_0 + b_1/x$ was used and for 30-low, the model $\hat{y} = b_0 + b_1x$ was used.

	10-high	**10-low**	**20-high**	**20-low**	**30-high**	**30-low**
N	24	24	19	24	24	24
r^2	0.339	0.663	0.669	0.375	0.596	0.887
b_0	22.51	20.36	23.33	20.34	22.74	14.44
95% CI	21.35-23.67	19.75-20.97	22.26-24.40	19.44-21.23	21.88-23.61	13.86-15.02
b_1	-2.70	-26.61	-37.57	-21.32	-32.47	0.169
95% CI	-41.6- -9.9	-35.0- -18.2	-51.1- -24.0	-33.5- -9.14	-44.3- -20.7	0.142-0.196

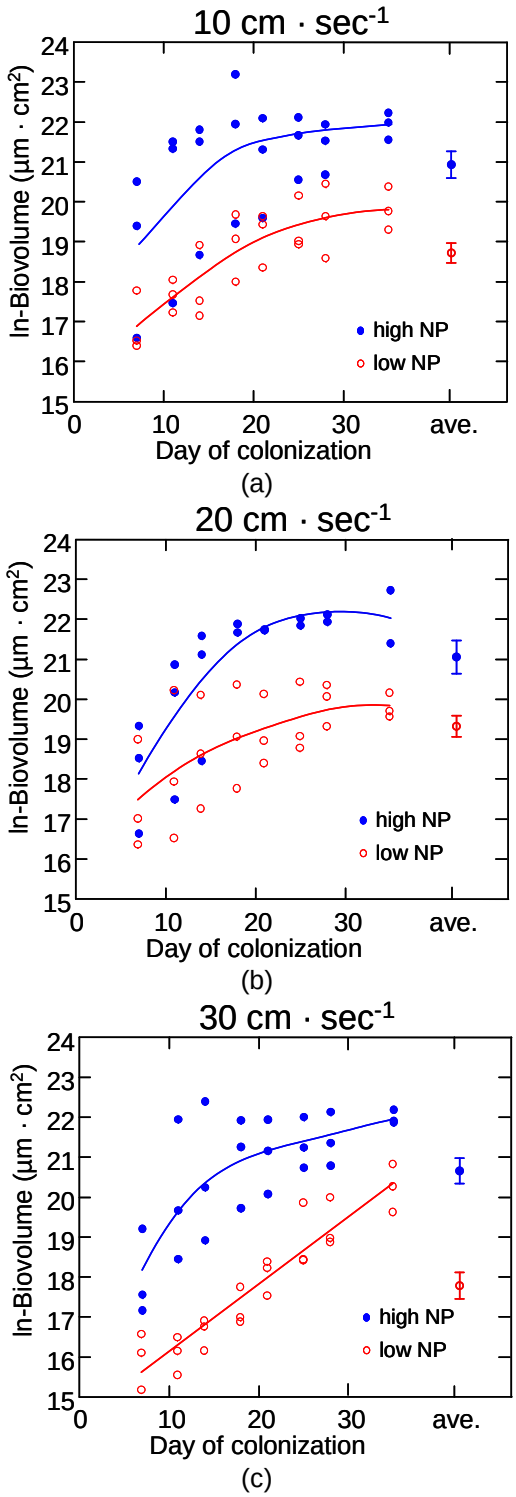


Figure 3.3 Biomass production (measured as ln-total cell biovolume) vs. day of colonization for experiments 2-4 (manipulating current velocity and nutrient supply). All treatments except 30-low fitted with model: $\hat{y} = b_0 + b_1/x$; 30-low fitted with linear model. Relationships for 10 cm · sec^{-1} (a) 20 cm · sec^{-1} (b) and 30 cm · sec^{-1} (c) current velocity treatments. Mean value ± 1 s.e. also given.

Experimental treatment combinations influenced the relationship between species richness and residual community biovolume. The relationship between species richness and residual community biovolume was significantly negative at low current velocity across nutrient levels (Fig 3.4 a, b). In the variable flow treatments, residual community biovolume increased slightly with increasing species richness in the low nutrient treatment, but the relationship was not significant ($p > 0.05$, Figure 3.4 c); in the high nutrient treatment residual community biovolume decreased slightly, but the relationship was not significant ($p > 0.05$, Figure 3.4 d). In the high current velocity treatments, richness exhibited differential relationship with biovolume depending on nutrient level. Under nutrient-limiting conditions, the relationship between species richness and biovolume was negative but not significant ($p > 0.05$, Figure 3.4 e). Under high nutrient conditions, the species richness-biovolume relationship was hump-shaped, with a quadratic model fitting the data ($p \leq 0.05$, Figure 3.4 f).

MDS ordination plots of diatom guilds revealed little separation between current or nutrient treatments early in succession (before day 14), but separation between nutrient treatments began to emerge by day 21 (Figure 3.5). By day 24, ANOSIM revealed significant differences between treatment combinations (Global R-statistic = 0.407, $p = 0.008$). Pair-wise comparisons of R-statistics revealed little difference between current velocities within the high nutrient treatments (R-statistic values all below 0.10). Within the low nutrient treatments, high R-statistics were observed between the variable and high current treatments (R-statistic = 0.63) and between the low and high current treatments (R-statistic = 1), with a fairly low R-statistic between the low and variable current treatments (0.333). Between nutrient treatments at each current velocity treatment, high R-statistics were observed between the variable flow treatments (R-statistic = 1), and moderate differences between the low current treatments (R-statistic = 0.333) and the high current treatments (R-statistic = 0.333). On day 28, significant differences between treatment combinations was again observed (Global R-statistic = 0.492, $p = 0.005$), with pair-wise comparisons of R-statistics nearly identical as those on day 24. By day 35, greater differences between treatment combinations was observed (Global R-statistic = 0.657, $p = 0.0001$), with pair-

wise comparisons revealing large differences between nutrient treatments at each current velocity (low current R-statistic = 0.963, variable current R-statistic = 1, and high current R-statistic = 1). There were no differences between current treatments at high nutrients (R-statistics all below 0.10); large differences between the low and high current treatments (R-statistic = 0.852), moderate differences between the variable and high current treatments (R-statistic = 0.593), and virtually no difference between the low and variable current treatment (R-statistic = 0.185) in the low nutrient treatments. By the end of the experiments, high nutrient treatments were dominated by eutrophic-motile diatoms, while the low nutrient treatments were dominated by low profile-non-eutrophic diatoms. Analysis of TDI index values revealed that by the end of experiments, large differences in the trophic status between nutrient treatments (mean ± 1 s.e., low nutrient: 36.65 ± 4.66; high nutrient: 87.59 ± 1.63). Differences between nutrient treatments increased with time and repeated measures ANOVA confirmed significant differences with the between subjects factor nutrient treatment on average TDI values ($F_{1,11} = 22.19$, $p \leq 0.001$) and the within subjects factor day × nutrient treatment interaction was also highly significant ($F_{7,77} = 14.23$, $p \leq 0.001$).

Plotting the relative proportion of diatoms classified as either ‘eutrophic’ or ‘non-eutrophic’ versus biofilm thickness revealed a divergence between guilds as biofilm thickness increased at all treatment combinations except low and variable flow treatments in the low nutrient treatments (Figure 3.6). In the high nutrient treatments, ‘eutrophic’ diatoms increased in dominance with increasing biofilm thickness, while ‘non-eutrophic’ diatoms decreased (Figure 3.6 b, d, f, Table 3.3). In the low nutrient treatments, an increase in biofilm thickness led to dominance by ‘non-eutrophic’ diatoms, although regression models were not significant for low and variable current treatments, while ‘eutrophic’ diatoms decreased with biofilm thickness (Figure 3.6 a, c, e, Table 3.3).

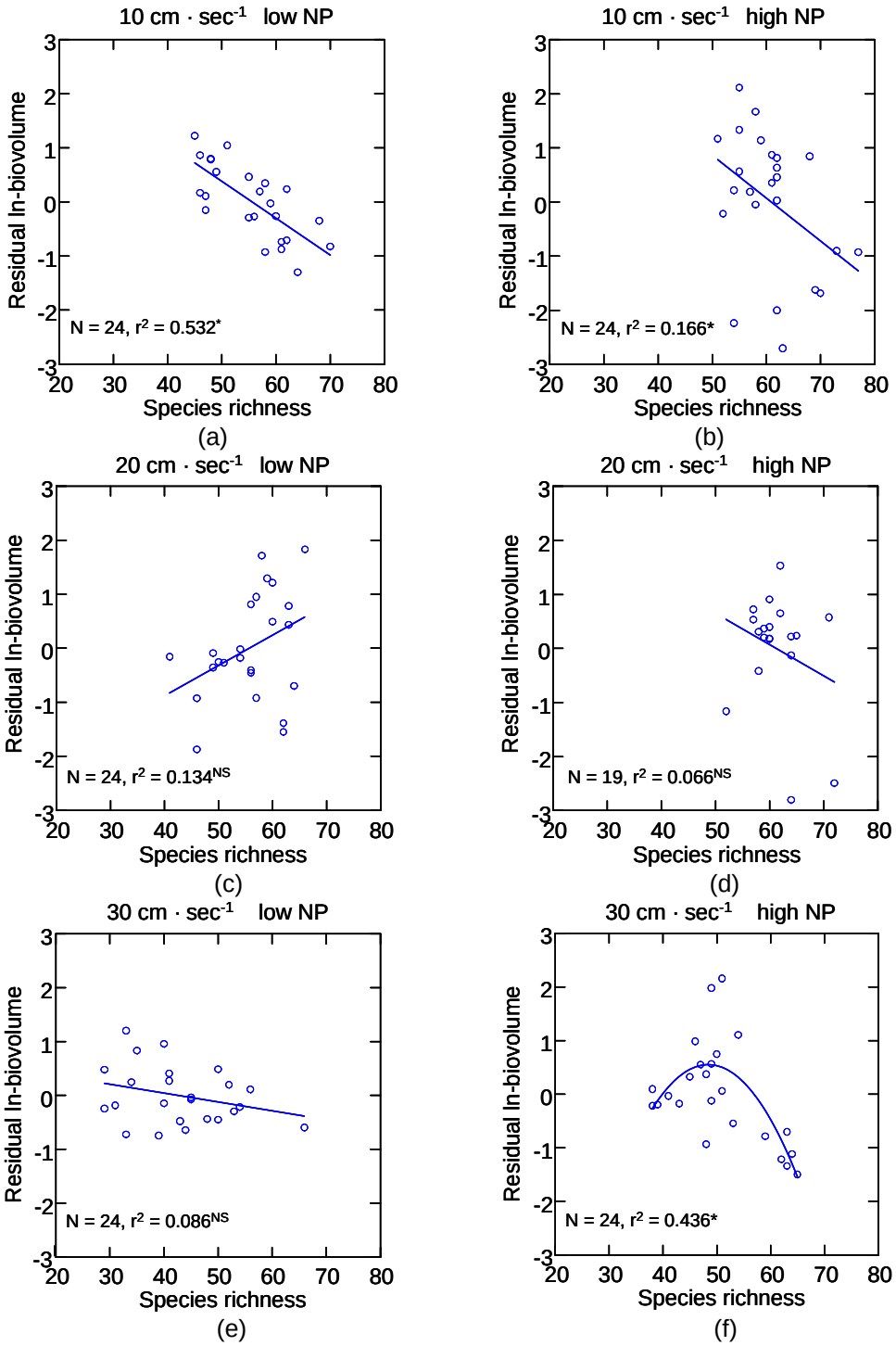


Figure 3.4 Species richness vs. residual ln-biovolume (after controlling for colonization time) for 10 cm · sec^{-1} (a-b) 20 cm · sec^{-1} (c-d) and 30 cm · sec^{-1} (e-f) current velocity treatments in the experiments manipulating current velocity and nutrient supply. NP = nitrogen + phosphorus, N = number of observations, *p < 0.05, NS = non-significant.

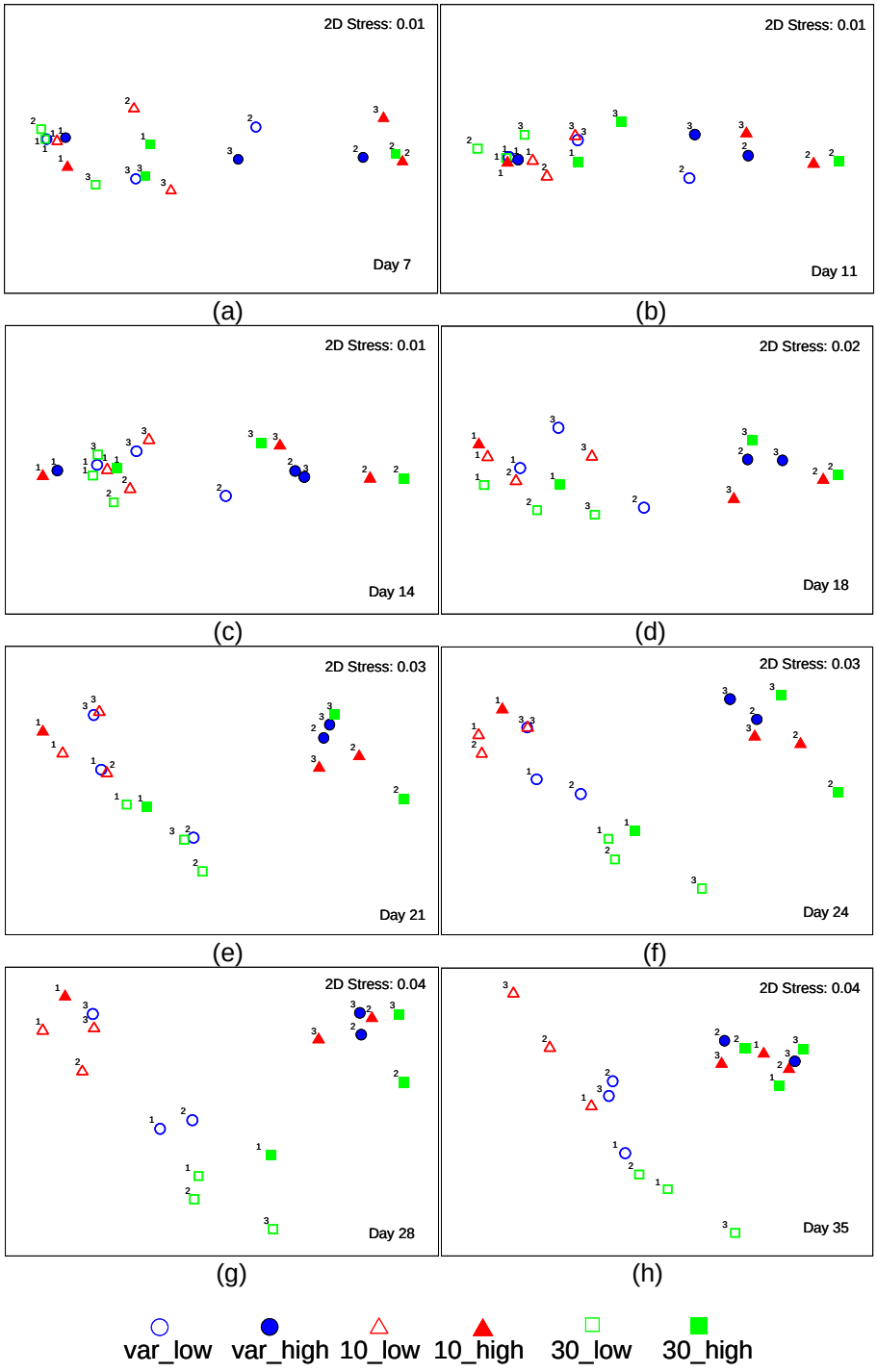


Figure 3.5 Non-metric multidimensional scaling ordinations for experiment manipulating current velocity and nutrient supply using Bray-Curtis dissimilarities and untransformed total cell densities for algal guilds. Day 7 (a) day 11 (b) day 14 (c) day 18 (d) day 21 (e) day 24 (f) day 28 (g) and day 35 (h). Numbers next to symbols represent replicate streams.

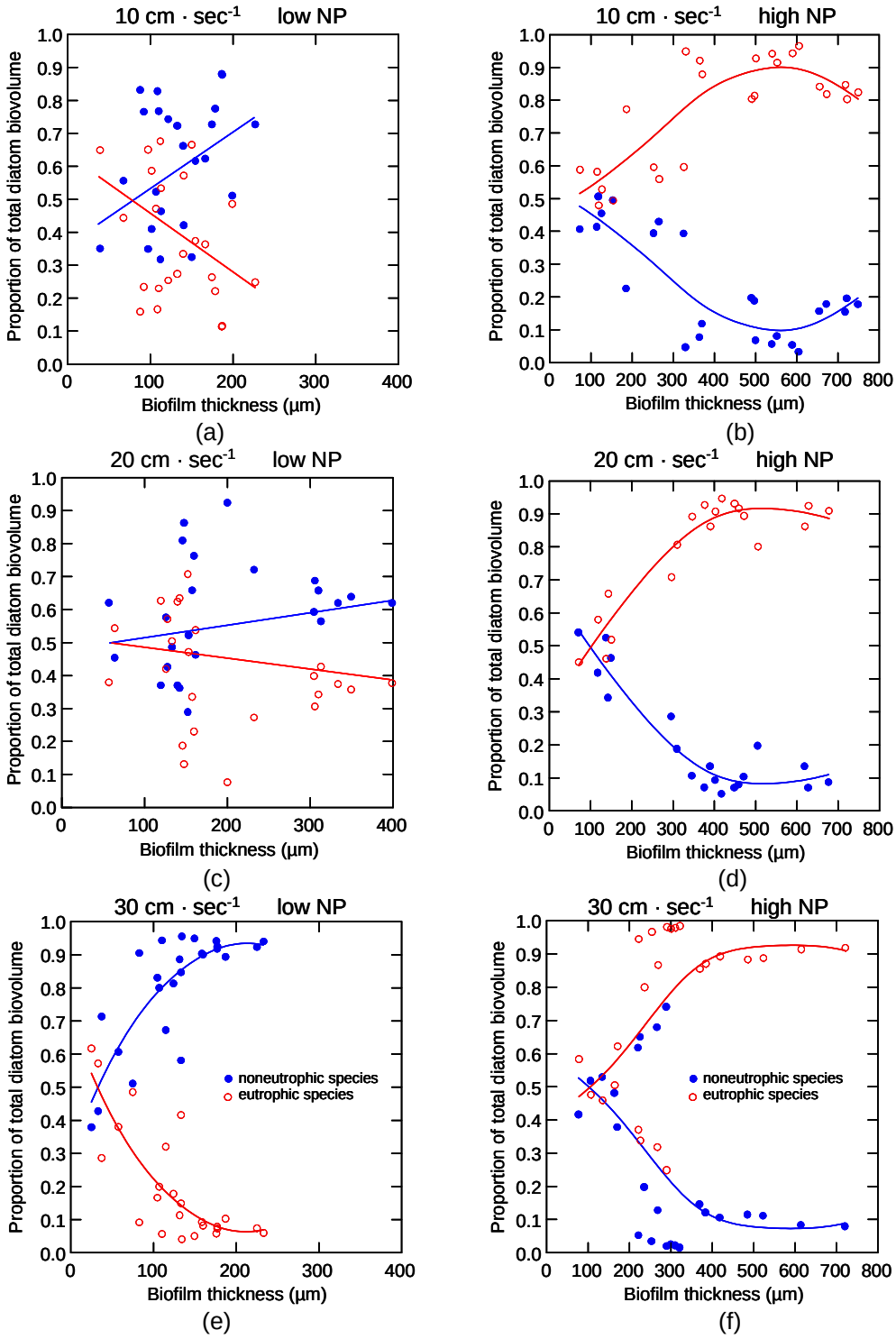


Figure 3.6 Relationship between eutrophic and noneutrophic diatoms and biovolume thickness using the proportion of total diatom biovolume for 10-low (a) 10-high (b) 20-low (c) 20-high (d) 30-low (e) and 30-high (f).

Table 3.3. Model, number of observations, coefficients of determination (r^2) and p-value for regression models of proportions (of total diatom biovolume) of 'eutrophic' and 'non-eutrophic' diatoms versus biofilm thickness.

Treatment	Diatom guild	model	N	r^2	*p*
10-low	eutrophic	$\hat{y} = b_0 + b_1x$	24	0.137	0.075
	non-eutrophic	$\hat{y} = b_0 + b_1x$	24	0.137	0.075
20-low	eutrophic	$\hat{y} = b_0 + b_1x$	24	0.080	0.182
	non-eutrophic	$\hat{y} = b_0 + b_1x$	24	0.080	0.182
30-low	eutrophic	$\hat{y} = b_0 + b_1x + b_2x^2$	24	0.652	0.00002
	non-eutrophic	$\hat{y} = b_0 + b_1x + b_2x^2$	24	0.652	0.00002
10-high	eutrophic	$\hat{y} = b_0 + b_1x + b_2x^2$	24	0.679	0.00001
	non-eutrophic	$\hat{y} = b_0 + b_1x + b_2x^2$	24	0.679	0.00001
20-high	eutrophic	$\hat{y} = b_0 + b_1x + b_2x^2$	19	0.883	0.00001
	non-eutrophic	$\hat{y} = b_0 + b_1x + b_2x^2$	19	0.883	0.00001
30-high	eutrophic	$\hat{y} = b_0 + b_1x + b_2x^2$	24	0.309	0.021
	non-eutrophic	$\hat{y} = b_0 + b_1x + b_2x^2$	24	0.309	0.021

3.4 Discussion

Current velocity and nutrient supply are predicted to influence total community biovolume (Stevenson 1996), where increased current velocity is expected to favor accumulation under high nutrient conditions. In my experiments, total community biovolume of periphyton communities growing in artificial stream flumes responded to current velocity and nutrient supply. In the experiment manipulating current velocity, community biovolume was greater in the low versus high current treatments, but this difference appeared to decrease towards the later stages of colonization. This result seems consistent with previous findings suggesting increased current velocity allows greater diffusion of nutrients and stimulates growth once a community becomes established (McIntire 1966, Reisen and Spencer 1970, Steinman and McIntire 1986, Lamb and Lowe 1987). In the experiments varying current velocity and nutrient supply, community biovolume was greater in the high versus low nutrient treatments across all current treatments. However, no differences were observed in community biovolume between current treatments under high nutrient supply. A possible explanation for no observed difference in community biovolume between current treatments in the high nutrient treatments may be that current has been shown to have varied effects on periphyton communities under different nutrient conditions (Stevenson 1996 b), and flowing water may have little effect on nutrient uptake under nutrient-

replete conditions (Borchardt *et al*. 1994). In the low nutrient treatments, community biovolume was lowest in the high current treatment, which may indicate that increased current velocity is not beneficial under low nutrient conditions and may even hinder biovolume production.

Adequate supplies of nutrients are necessary for the positive growth of thick, multi-layered periphyton communities (Stevenson and Glover 1993). For the experiments manipulating current velocity and nutrient supply, the differences in community biovolume were not as great as expected given the differences in the amounts of nutrients supplied to each nutrient treatment, suggesting some other factor was limiting biomass production. One possible factor influencing biovolume production may have been the availability of light. Shading by the overstory might have reduced light reaching the understory. Additionally, light intensity of ~200 $\mu mol \cdot m^2 \cdot sec^{-1}$ was reported to be adequate for algal growth (Hill 1996). McIntire et al. (1964) also found that light levels approximating those in my streams were sufficient for growth in artificial streams. However, community biovolume accumulation appeared to show some slight leveling-off later in colonization in high nutrient treatments; it appears that greater light intensity may have been required under conditions of high nutrient supply. Therefore, competition for light might have become severe in the high nutrient treatments. Support for this reasoning comes from the observation that growth exhibited a shell-like growth, based upon examination of images obtained with confocal microscopy (personal observation), and documented by other biofilm studies as well (Neu and Lawrence 1997). This is not completely surprising given communities were growing on inert substrates and the only source of nutrients for periphyton communities, apart from internal recycling within the biofilm, came from the water column. Furthermore, diatom communities in high nutrient treatments were composed mainly of 'eutrophic' motile diatoms which can be favored under low light conditions that can develop in the latter stages of community development (Pringle 1990, Bixby *et al*. 2009). Another possible explanation for the differences in community biovolume production between nutrient treatments not being as great was expected, is that perhaps nutrient concentrations in the low nutrient treatments weren't low enough to severely limit biovolume production. However, analysis of the average TDI values based on diatom

communities revealed large differences between nutrient treatments. Despite this, in all experiments, most notably in the high nutrient treatments, copious growth of chlorophytes, cyanobacteria, and diatoms were observed and none of the major algal groups appeared to be unfavored by the light conditions and high overall species richness was obtained across all experiments.

The relationship between species richness and biomass can vary in streams depending on disturbance regime and with the gradient in richness observed. In a regional investigation of stream algae, the response of productivity to species richness was positive under conditions of high disturbance (Cardinale *et al.* 2005) however, the sampled steams had low overall species richness, i.e. less than 40. In a continental survey on the diversity-biomass relationship in stream algal communities, a broader gradient in algal richness was captured, i.e. from 1 to about 100 species, which revealed a humped-shaped response of biomass, peaking around 45 species in the richest-targeted habitats (Passy and Legendre 2006). It was suggested that at levels of richness below the mode, i.e. less than 45, algal communities were driven by positive interactions of facilitation or complementarity, leading to an increase in biomass with richness (Passy and Legendre 2006). Facilitation or complementarity enhances the efficiency with which resources are utilized and has been cited as a mechanism behind the positive relationship between richness and productivity in other communities (Waide *et al.* 1999, Tilman *et al.* 2001, Loreau and Hector 2001, Symstad *et al.* 2003, Hooper *et al.* 2005). The establishment of a multi-level, cohesive biofilm composed of species with various profiles coexisting in a matrix of exopolymers provides some resistance to disturbance and reduces the negative effect of competition allowing for greater community biovolume (Passy and Legendre 2006). However, beyond a point, an increase in species richness can lead to stronger competition for space, nutrients and light, leading to higher mortality and a decline in community biovolume (Passy and Legendre 2006).

Examining the response of total community biovolume (after controlling for colonization time) to species richness in my experiments revealed the relationship differed among streams subjected to various current or current × nutrient treatments. In the experiment manipulating

current velocity, no relationship was observed between species richness and community biovolume, indicating weak biotic interactions. In the experiments manipulating current velocity and nutrient supply, the relationship between species richness and community biovolume varied with current × nutrient combination. At low current velocity, the relationship between species richness and community biovolume was negative across nutrient levels (Figure 3.4a, b). Species richness in these treatments was ≥45, which corresponds to communities above the mode in the continental survey where negative interspecific interactions were predicted to occur (Passy and Legendre 2006). In the high current velocity treatments, reduced species richness was observed (as low as 29), and community biovolume exhibited a differential relationship with species richness depending on nutrient level. Both richness and community biovolume were low under nutrient-limiting conditions, and the relationship between the two community properties was not significant (Figure 3.4e), suggesting the biotic interactions were weak and that abiotic stress, both nutrient limitation and physical disturbance controlled community organization. Richness and community biovolume were higher under high-nutrient conditions; the relationship between them was humped-shaped with a mode of 48 (Figure 3.4f), which is very close to the observed mode in the continental study (Passy and Legendre 2006). The gradient in species richness in this treatment was long enough to reveal both the positive and negative interspecific interactions described by Passy and Legendre (2006). Under conditions of variable current, richness values were similar to low current velocity treatments ≥41, and the relationship between community biovolume and richness exhibited no significant relationship. Under low-nutrient conditions, the trend was positive, yet the relationship was not significant (Figure 3.4c), once again suggesting weak biotic interactions. Under conditions of high nutrients, the relationship between community biovolume and richness was negative (Figure 3.4d), indicating negative interactions between species, yet the relationship was not significant. There were fewer data points in this treatment and richness was high ≥51, above the mode in the continental study (Passy and Legendre 2006). Overall, results highlight the need for further studies that examine how environmental change alters the structure and function of communities (Cardinale *et al*. 2005).

The relationship between local species richness and biomass production can be a reflection of local processes, i.e. competition, facilitation, etc., or of larger scale patterns such as dispersal and colonization (Cardinale *et al*. 2004). In the experiments manipulating current velocity and nutrient supply, propagules were only supplied once, and since each stream received the same propagules, the patterns observed in these experiments are more likely to have been generated by local forces, i.e. competition and facilitation. Large scale processes, i.e. watershed properties influencing nutrient inputs to streams, can have important influence on periphyton community structure (Passy 2009) however there is little doubt of the importance of local processes structuring periphyton communities. Competition can be inferred in these experiments as analysis of diatom trophic guilds indicated. By the end of experiments, 'eutrophic-motile' diatoms dominated the high nutrient treatments, while 'non-eutrophic-adnate' dominated in the low nutrient treatments. Motile diatoms have been observed to be superior competitors for nutrients in nutrient-rich environments (Fairchild *et al*. 1985, Van der Grinten *et al*. 1997) and can physically avoid nutrient stress within the algal mat by moving to resource-rich microhabitats (Pringle 1990, Johnson *et al.* 1997). Small adnate diatoms, on the other hand, are generally tolerant of low nutrient conditions and are favored under low nutrient conditions; in these experiments *Achnanthidium minutissimum* (Kützing) Czarnecki dominated composition at low nutrients (Chapter 4) and has frequently been observed as dominant in nutrient-poor environments (Allanson 1973, Eminson 1978, Pringle 1985, 1990)

The present results suggest the richness-biovolume relationship in algae is influenced by current regime and nutrient level. High current velocity has been shown to suppress and entire ecological guild, i.e., high profile diatoms (Passy 2007) and evidence on the effect of current velocity on species richness suggests the relationship is negative (Stevenson 1984, Lamb and Lowe 1987, Plenković-moraj 2008, Chapter 2). The effect of nutrients on species richness of periphyton communities is better understood, indicating richness increases with nutrient addition (Pringle 1990, McCormick and Stevenson 1991, Hillebrand *et al*. 2007). Yet, little information exists on the joint effects of current velocity and nutrient addition. Results from my experiments

suggest strong interaction between current velocity and nutrient supply on the relationship between species richness and community biovolume. However, high species richness was observed my experiments, indicating that nutrient limitation may not have been severe enough to produce communities with low richness as observed in the field. Therefore, studies that can obtain a wider gradient in species richness will be able to see if patterns in richness-biomass relationship are consistent with those observed at a continental level (Passy and Legendre 2006). However, my study is the first to experimentally show a negative richness-biovolume relationship using algae and link the negative trend to competition.

CHAPTER 4

CURRENT VELOCITY AND NUTRIENT SUPPLY INFLUENCE PHYSIOGONMY AND ASSEMBLY OF PERIPHYTON COMMUNITIES IN ARTIFICIAL STREAMS

4.1 Introduction

Many studies have focused on factors regulating diversity in ecosystems and have examined how one or several factors such as history, spatial and temporal heterogeneity of the environment, competition, predation, and disturbance influence diversity (Connell 1978, Sommer 1984, 1993, 1995, Reynolds *et al*. 1993, Chase 2003). In general, community ecologists have sought to answer whether communities are random collections of species assembled from a common species pool or whether they are closely connected groups of interacting species. Of interest has been whether community structure results from deterministic processes such as competition, predation, environmental requirements and physiological abilities of the different species or whether it is the result of stochastic processes such as disturbance or invasion sequences of species entering a locality (Sommer 1991, Roughgarden *et al.* 1987, Samuels and Drake 1997, Chase 2003). The search for answers to these questions has deep roots in community ecology, yet the pattern of community assembly, whether it is deterministic or historically contingent remains controversial (Samuels and Drake 1997, Belyea and Lancaster 1999, Chase 2003).

At the heart of many of the questions surrounding community assembly is whether similar communities develop in environments with comparable conditions. Drawing influence largely from the concepts of succession developed by Clements (1916), the deterministic view holds that community composition should converge towards a single configuration that is influenced by environmental conditions. The alternative view, largely developed from ideas presented by Gleason (1927), predicts community composition is the result of stochastic forces shaping the sequence and timing of species invasions. There is theoretical and experimental

evidence supporting both lines of reasoning, yet it might be more constructive to ask under what conditions is community assembly largely deterministic and when is it driven principally by stochastic events (Chase 2003)?

Benthic algal communities in rivers and streams are three-dimensional structures composed of multiple species of various growth habits, morphologies, and successional appearance. During the initial stages of community development, high current velocity can hinder the establishment of bacterial (Rickard *et al*. 2004) and algal communities (McIntire 1966, Lamb and Lowe 1987, Stevenson 1996, Ghosh and Guar 1998). High current velocity can also negatively influence algal immigration rates (McIntire 1966, Stevenson 1983, Peterson and Stevenson 1989). Additionally, the structure of the biofilm matrix can be influenced by nutrient availability (Sutherland 2001). Therefore, any delay in the establishment of a biofilm matrix combined with the inability of colonizers to establish early in community development could influence the successional trajectory of an algal community.

Throughout succession, algal communities experience temporal changes in taxonomy and distinct physiognomic shifts in response to changing environmental conditions (Hudon and Bourget 1981, Hoagland *et al*. 1982, Korte and Blinn 1983, Steinman and McIntire 1986). Many ecological adaptations (i.e., algal growth forms), have arisen in response to steep environmental gradients that develop within the benthos (Pringle 1990, Steinman *et al*. 1992, Carrick and Steinman 2001), many of which are related to resource availability and disturbance (Biggs *et al*. 1998). For example, high current velocity can lead to thin biofilms composed of species of low profile, adnate growth forms, while low current velocity may allow the formation of thick biofilms composed of high profile and filamentous growth forms (Stevenson 1983, Keithan and Lowe 1985, Lamb and Lowe 1987, Peterson and Stevenson 1992). Nutrients also affect algal physiognomy: nutrient limitation results in thin biofilms, while high supply allows for positive growth rates and the formation of thick, multi-layered algal communities (Stevenson and Glover 1993). Many algal species have well established nutrient preferences such that under conditions of low resource supply (nutrients or light), tolerant species (generally of smaller growth habit)

assemble into thin biofilms, while high resource supply results in the establishment of a three-dimensional biofilm matrix, composed of an overstory of species requiring high nutrients and an understory of species tolerant to low nutrients (Steinman and McIntire 1987, Pringle 1990, Passy 2007, 2008).

Since algal growth forms represent ecological adaptations to environmental gradients in the benthos, a large number of growth forms can be effectively reduced to a few ecological guilds to asses their potential for utilizing resources and avoiding disturbance (Passy 2007). For example, the most general physiognomic trends in benthic algal communities include the transition from low to high profile species over time with decrease in disturbance (Hoagland *et al*. 1982, Steinman 1996, Peterson 1996). Several studies have indicated filamentous growth forms represent mature algal communities as a result of their competitive superiority for light and nutrients (Lowe *et al*. 1986, Steinman and McIntire 1986, 1987), however, an increase in disturbance can also lead to a transition towards greater abundance of adnate or motile species (Lamb and Lowe 1987, Pringle 1990, Tuchman and Stevenson 1991, Fore and Grafe 2002). For this study, I segregated algal growth morphologies into three ecological guilds, i.e. low profile, high profile, and motile and assessed the change in relative proportion between guilds over time in response to variations in current velocity and nutrient supply. The low profile guild would be expected to have an advantage in resource poor or high disturbance habitats (due to their low profile within the biofilm mat and general tolerance to low nutrients conditions), while the high profile guild would have an advantage in resource-rich and low disturbance habitats (due to their advantageous spatial positioning and sensitivity to disturbance). The motile guild, due to their ability to migrate within the biofilm mat and select the most suitable habitat, are relatively less susceptible than the high and low guilds to resource limitation and disturbance stress. Additionally, the motile guild is comprised of largely eutrophic and pollution tolerant species and therefore should be favored in nutrient rich habitats. Furthermore, because greater nutrient concentrations allow the formation of three-dimensional algal communities composed of species of various growth forms, higher guild diversity would be expected in periphyton communities

growing under conditions of high nutrient concentration. These patterns were observed in the field (Passy 2007) but have not been tested experimentally.

Given the effects of current velocity on benthic algal communities are likely to vary with nutrient concentrations (Humphrey and Stevenson 1992, Biggs and Stokseth 1994, Stevenson 1996), surprisingly few studies have examined succession in benthic algal communities in response to variations in both current velocity and nutrient supply. In this experiment, I subjected periphyton communities growing in recirculating artificial stream flumes to various current and nutrient regimes. My objectives were (1) to examine succession in periphyton communities to determine whether communities subjected to nutrient and current treatments would exhibit greater similarity similar (convergence) or greater dissimilarity (divergence); (2) to assess whether the ecological guilds would display the hypothesized behavior across nutrient and disturbance gradients; (3) to test whether greater guild diversity would be observed in periphyton communities growing under high nutrient supply.

4.2 Methods

As described in greater detail in Chapter 2, four experiments were run in artificial stream flumes; the first experiment used stream water and manipulated current velocity, subjecting periphyton communities to either high or low current velocities (30 and 10 cm $\cdot$ sec^{-1} respectively), while in experiments 2-4, modified WC media was used and periphyton communities subjected to different nutrient (low and high) and current regimes (low, high, and variable). Each experimental run lasted 35 days.

4.2.1 Designation of ecological guilds

Algal growth morphologies were grouped into three ecological guilds, i.e. low profile, high profile, and motile species following Passy (2007). For species designated in the low profile guild, I included species of short stature, including prostrate, adnate, erect, solitary centric diatoms, and slow moving species. Slow moving species were included in the low profile guild since non-motile and slow moving diatoms exhibit similar patters, significantly differing from the distribution of fast moving species (Hudon and Legendre 1987). The high profile guild

encompassed species of tall stature, including erect, filamentous, branched, chain-forming, tube-forming, stalked species, and colonial centric diatoms. The motile guild included comparatively fast moving species, species with either a flagella or biraphid diatoms.

4.2.2 Statistical Analyses

The proportions of all species in each ecological guild were summed and ecological guild diversity in each sample calculated by the Shannon-Weiner index using natural log of the proportion of each guild. Guild diversity in this case is a reflection of guild richness, which has a maximum of only three, yet it shows how evenly the guilds are distributed, with high guild diversity values indicating an even distribution between all guilds. When examining the proportion of total diatom biovolume for low and motile diatom species versus biofilm thickness (obtained by confocal microscopy as outlined in the methods of Chapter 1), the curve-fitting software TableCurve 2D 5.01 (SYSTAT Software Inc., Richmond, CA) was used and a parsimonious approach of selecting the simplest model with a good fit and high r^2 was employed when determining equations best describing relationships. Repeated-measures analyses were performed using the General Linear Model command in SYSTAT version 11.

Multivariate Analysis of Algal Communities

Differences in algal community structure between treatments were analyzed with PRIMER software application (version 6.1; Plymouth Marine Labs, Plymouth, UK; Clarke and Warwick 2001). Compositional similarities between samples were computed with the Bray-Curtis coefficient (Bray and Curtis 1957) and a dissimilarity matrix generated by comparing algal composition of all samples using untransformed biovolume for all algal species and proportions of all species in each ecological guild. Within the PRIMER software, ANOSIM, SIMPER, MDS, MVDISP, and RELATE routines were performed. I used a two-way ANOSIM to compare rank similarities of samples between current types across days (experiment 1), and treatment (current × nutrient combinations) across days (experiments 2-4).

Analysis of Similarities (ANOSIM) using biovolume was used to determine differences between experimental treatments. With ANOSIM an R-statistic is calculated, which varies

between 0 and 1; which indicate no separation or complete separation respectively. The value of the R-statistic reflects the observed differences between treatments, contrasted with differences among replicates within treatments and can be compared to a distribution of values expected under the null hypothesis of no difference between treatments to obtain a test of significance (Clarke and Warwick 2001). In my case, the global ANOSIM test for overall differences between groups (current in experiment 1 and each current × nutrient combination in experiment 2) was examined and if significant, R values for each pair-wise comparison were inspected. However, the limited number of permutations available for pair-wise comparisons in my experiments (n = 3 replicates for each treatment combination) mean it was not possible to determine the significance of R-statistic values at probabilities <10%. Yet, values of R are not unduly affected by the number of replicates in the groups being compared (Clarke and Warwick 2001), therefore, large values (close to unity) are indicative of complete separation of the groups, and small values (close to zero) are evidence of little or no segregation. The nonparametric permutation test ANOSIM was employed in place of a multivariate analysis of variance (MANOVA), since MANOVAs are based on assumptions (i.e., abundances follow a multivariate normal distribution) unlikely to be satisfied for most multispecies data sets (Clarke and Warwick 2001). The percent contributions of each taxon to the overall dissimilarity between treatment types was quantified by the SIMPER routine, which indicates the importance of each taxon in discriminating treatment communities (Clarke and Warwick 2001). Ordination with nonmetric multidimensional scaling (NMDS), considered a robust ordination technique for ecological analyses (Clarke and Warwick 2001) was employed to provide a graphical summary of the relationship among communities. Additionally, to assess variability in community structure between treatment groups, an index of multivariate dispersion (IMD) was calculated. This index describes differing dispersion across groups based on similarity within groups (Clarke and Warwick 2001).

Repeated measures designs can be problematic in multivariate analyses since communities at successive time points are not independent from each other (Clarke *et al.* 2006). Therefore, data across sampling time points and at each sampling time point were examined with

MDS plots. In addition, pair-wise R-statistic values between treatments for each sampling time point were obtained from ANOSIM and plotted against day and examined with regression analysis. A positive or negative slope would be an indication of either divergence or convergence through time among treatments. Furthermore, examining interactions is problematic in a non-parametric context; yet a fully robust, rank-based concept of interaction can sometimes be tested with second-stage community analyses with MDS (Clarke *et al.* 2006). Second-stage MDS, can be thought of as an MDS plot of the pairwise similarities between MDS plots, e.g. of assemblage time trajectories. The degree to which two ordination patterns match is calculated by Spearman rank correlations between similarity matrices and is used to examine certain forms of interactions such as those found in repeated measures designs (Clarke *et al.* 2006). Unlike a first-stage MDS plot, where nearby points represent communities that are highly similar, but which may not have arrived at that point by the same 'evolutionary' path, second-stage MDS plot represents correlations of time trajectories for a series of samples, where points lying in close proximity to one another represent parallel evolution of assemblage structure through time, but in which the end points need not be the same (Clarke *et al.* 2006). A formal test of the interaction between treatments and time on community assemblage can therefore be tested for by second-stage ANOSIM and RELATE permutation tests in PRIMER (Clarke *et al.* 2006).

Experiment Manipulating Current Velocity

Using data from experiment one, a completely randomized design was used and differences in MDS axes scores resulting from treatment effects were tested with one-way repeated measures ANOVA, where 'day' was specified as a within-subjects factor, while 'current velocity' (10 cm $\cdot$ sec^{-1} and 30 cm $\cdot$ sec^{-1}) as a fixed between-subjects factor.

Experiments Manipulating Current Velocity and Nutrient Abundance

Using data from experiments 2-4, a randomized block design was used and differences in MDS axes scores resulting from treatment effects were tested with two-way repeated measures ANOVA, where 'day' was specified as a within-subjects factor, while 'current velocity'

(10 cm · sec^{-1}, 30 cm · sec^{-1}, and variable flow) and 'nutrients' (high and low) as fixed between-subjects factors. Additionally, experimental run was treated as a blocking factor.

4.3 Results

4.3.1 Experiment Manipulating Current Velocity

Benthic algae from experiment manipulating current velocity included Divisions Bacillariophyta, Chlorophyta and Cyanophyta. A total of 144 taxa were encountered in this experiment, with 11 species of cyanobacteria, 27 species of green algae, and 106 species of diatoms (Appendix A).

Species composition in the experiment manipulating current velocity varied through time and between current velocities, with MDS successfully describing the multivariate data in two dimensions (Figure 4.1). Two-way ANOSIM revealed the effects of current velocity (across days) and day (across current groups) were significant (R-statistic = 0.543, p = 0.001 and R-statistic = 0.659, p = 0.001, respectively). Additionally, repeated measures ANOVA of the MDS axes scores revealed the between subjects factor current velocity was significant for axis-1 ($F_{1,4}$ = 98.35, $p \leq 0.001$) and axis-2, ($F_{1,4}$ = 17.35, p = 0.014) and that the within subjects factor day was significant for axis-1 ($F_{8,32}$ = 98.19, $p \leq 0.001$). The current × day interaction was not significant for either MDS axis ($p > 0.05$). At each sampling date, except day 24, moderately high ANOSIM R-statistics were obtained, indicating differences in community composition between current velocities throughout the duration of the experiment (Figure 4.2). However, plotting ANOSIM R-statistics against day revealed that differences in community composition between current velocities decreased with time (N = 9, r^2 = 0.49, $p \leq 0.001$, Figure 4.3).

Early in succession, the diatom *Synedra ulna* and two species of the green alga *Oocystis* were more abundant in the low current velocity treatments and were major species discriminating current treatments from one another (Table 4.1). By day 14, the filamentous cyanobacteria *Lyngbya vandernberghenii* became abundant, most notably in the high current velocity treatment. Towards the latter stages of succession, the diatoms *Nitzschia palea* and *Cymatopleura elliptica* and the green alga *Gleocystis ampla* became abundant in the low current velocity treatment,

while the diatoms *Achnanthidium minutissimum* and *Synedra ulna* became abundant in the high current velocity treatment.

Analysis of the second-stage MDS revealed no distinct pattern between treatments, indicating similar time trajectories between current treatments over the course of the experiment (R-statistic = 0.037, p = 0.40). This suggests no interaction effect; that is time trajectories did not differ between current treatments, yet there were differences in community assemblages between current velocity treatments at each sampling time point.

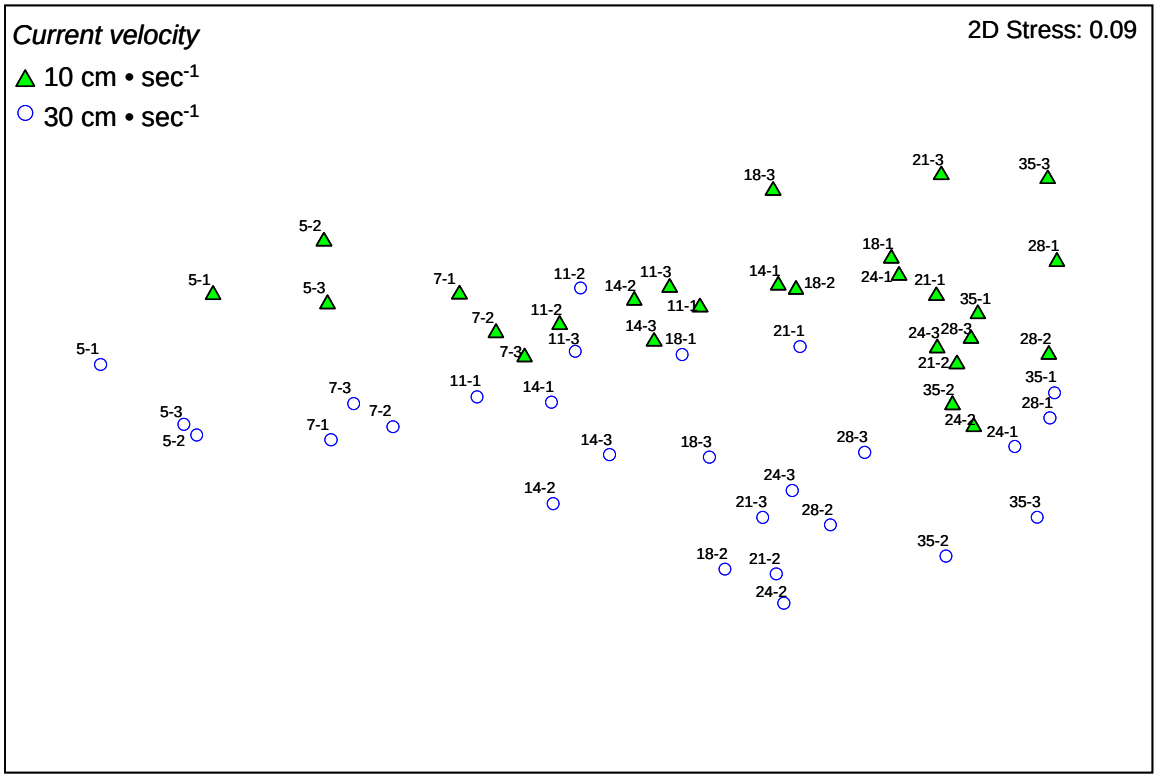


Figure 4.1 Non-metric multidimensional scaling ordination for flow only experiment using Bray-Curtis dissimilarities using untransformed species biovolume values. Numbers next to symbols represent day of colonization and stream replicate.

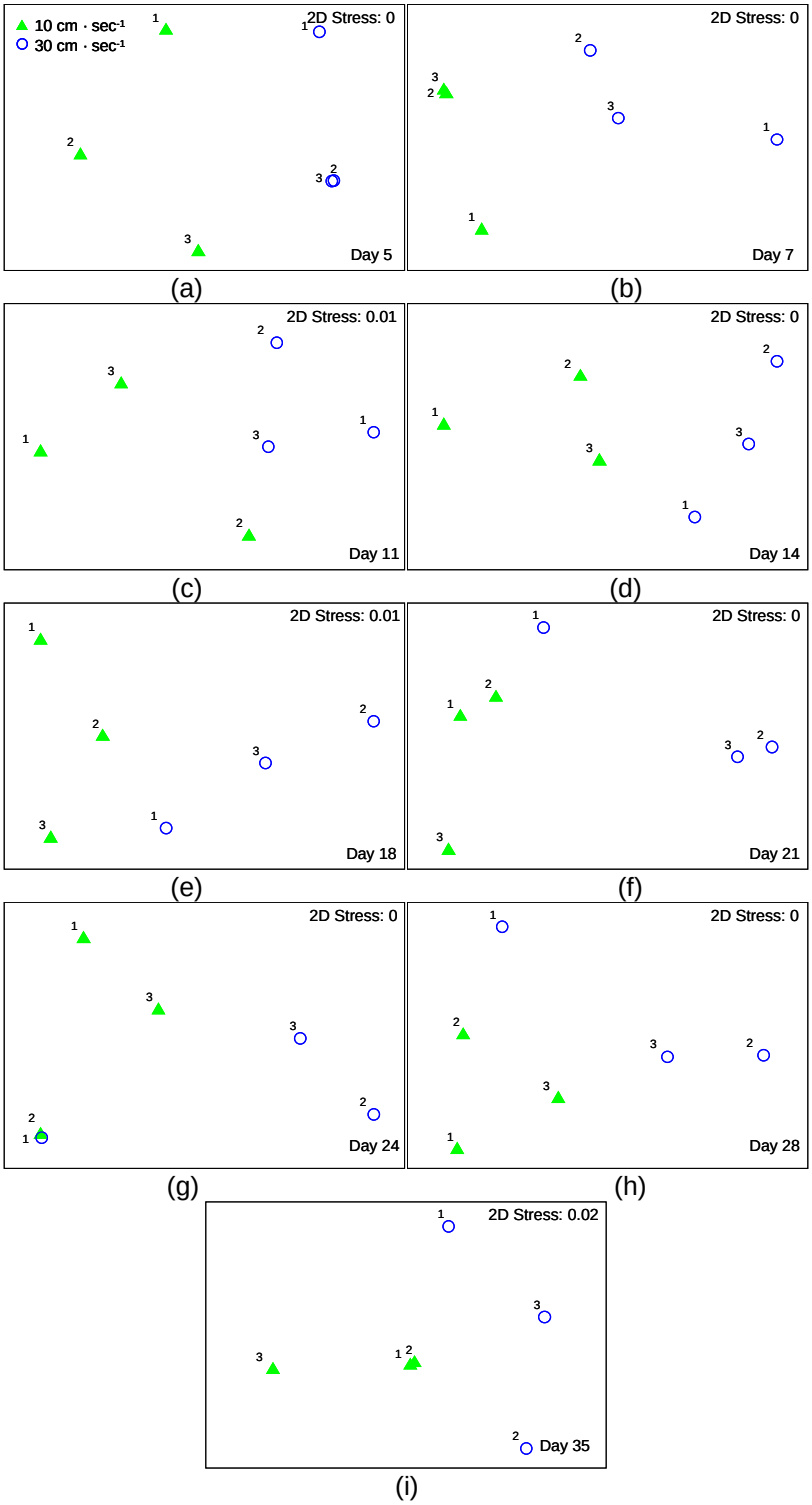


Figure 4.2 Non-metric multidimensional scaling ordinations for experiment manipulating current velocity using Bray-Curtis dissimilarities and untransformed periphyton biovolume values. Day 5 (a) day 7 (b) day 11 (c) day 14 (d) day 18 (e) day 21 (f) day 24 (g) day 28 (h) and day 35 (i). Numbers next to symbols represent replicate streams.

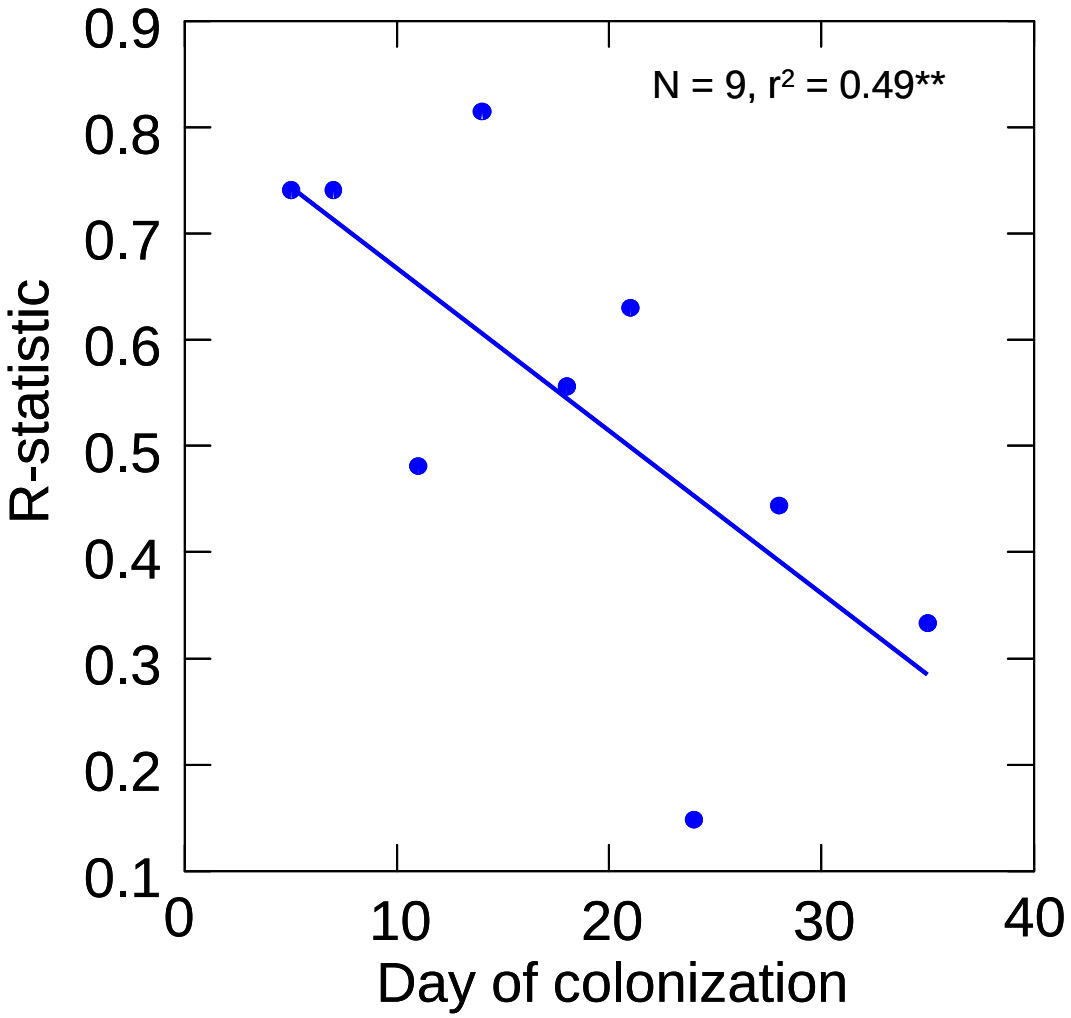


Figure 4.3 Global R-statistic at each sampling period across day of colonization for experiment 1. R-statistic values close to 1 represent complete dissimilarity while values close to 0 represent no dissimilarity.

Table 4.1. Average biovolume for discriminating species and the percent contribution to dissimilarity in the experiment manipulating current velocity. Species presented here represent those contributing to approximately 50% of the dissimilarities between current treatments for each sampling date during the experiment.

		10 cm · sec^{-1}	30 cm · sec^{-1}	
	Species	Biovolume	Biovolume	Contrib%
Day 5	*Synedra ulna*	3.2E+06	9.0E+04	26.18
	Synedra acus	9.3E+05	8.7E+04	7.9
	Zygnema sp.	6.6E+05	1.1E+06	6.86
	Stauroneis phoenicenteron	4.3E+05	6.0E+05	5.86
Day 7	*Synedra ulna*	7.7E+06	6.4E+05	27.56
	Oocystis sp. 1	6.5E+06	3.0E+06	16.69
	Oocystis sp. 2	3.4E+06	1.3E+06	9.13
Day 11	*Synedra ulna*	1.5E+07	6.5E+05	25.36
	Oocystis sp. 1	2.2E+07	1.8E+07	13.73
	Oocystis sp 2	8.4E+06	5.3E+06	9.11
	Encyonema silesiaca	5.1E+06	2.3E+06	5.69
Day 14	*Synedra ulna*	2.1E+07	8.9E+05	20.98
	Oocystis sp. 1	2.5E+07	1.1E+07	16.6
	Lyngbya vandernberghenii	5.0E+05	9.4E+06	11.07
	Oocystis sp 2	1.3E+07	5.6E+06	8.95
Day 18	*Lyngbya vandernberghenii*	3.9E+06	3.6E+07	16.4
	Oocystis sp. 1	4.6E+07	1.6E+07	15.14
	Cymatopleura elliptica	2.6E+07	0.0E+00	14.28
	Synedra ulna	2.0E+07	1.1E+06	7.98
Day 21	*Cymatopleura elliptica*	7.2E+07	0.0E+00	16.66
	Lyngbya vandernberghenii	1.8E+07	5.8E+07	12.55
	Oocystis sp. 1	5.9E+07	1.2E+07	12.02
	Synedra ulna	4.8E+07	9.3E+06	11.03
Day 24	*Synedra ulna*	4.6E+07	5.1E+07	14.37
	Nitzschia palea	5.1E+07	1.5E+07	12.82
	Oocystis sp. 1	5.0E+07	1.1E+07	11.02
	Gleocystis ampla	4.2E+07	6.5E+06	10.57
	Lyngbya vandernberghenii	3.7E+07	5.3E+07	7.85
Day 28	*Nitzschia palea*	1.2E+08	2.0E+07	17.06
	Gleocystis ampla	7.7E+07	1.0E+07	11.04
	Synedra ulna	7.8E+07	2.7E+07	10.93
	Lyngbya vandernberghenii	4.1E+07	8.9E+07	9.5
	Oocystis sp. 1	7.9E+07	3.2E+07	8.97
Day 35	*Cymatopleura elliptica*	1.0E+08	0.0E+00	13.6
	Oocystis sp. 1	1.1E+08	3.4E+07	11.54
	Synedra ulna	2.3E+07	8.7E+07	9.34
	Nitzschia palea	7.2E+07	1.7E+07	9.08
	Achnanthidium minutissimum	1.4E+07	4.5E+07	7.17
	Lyngbya vandernberghenii	5.9E+07	8.7E+07	6.77

4.3.2 Experiments Manipulating Current Velocity and Nutrient Supply

Benthic algae from experiments manipulating current velocity and nutrient abundance also included Divisions Bacillariophyta, Chlorophyta and Cyanophyta. A total of 185 taxa were encountered in this experiment, with 11 species of cyanobacteria, 38 species of green algae, and 136 species of diatoms (Appendix B).

Species composition in the experiments manipulating current velocity and nutrient supply varied through time and between current velocities. ANOSIM of average biovolume (averaged between three replicates) in different treatments across days revealed dissimilarities in algal communities (Global R-statistic = 0.567, $p \leq 0.001$, Figure 4.4). Pair-wise comparisons between treatments yielded moderately high to high R-statistics for high versus low nutrient treatments (10-high vs. 10-low = 0.973, 20-high vs. 20-low = 0.643, 30-high vs. 30-low = 0.684) and smaller values between current treatments within high nutrient treatments (10 vs. 30 = 0.412, 10 vs. 20 = 0.277, 20 vs. 30 = 0.128) and low nutrient treatments (10 vs. 30 = 0.229, 10 vs. 20 = 0.366, 20 vs. 30 = 0.457). IMD values indicated more variable community structure in the low nutrient treatments based on species biovolume (low vs. high IMD = 0.247). Variability in community structure between nutrient treatments was even more evident with the removal of day 7 (low vs. high IMD = 0.407), which was clearly separated from the other values in the high nutrient treatments, thus contributing to higher overall variability in these treatments. Furthermore, repeated measures ANOVA of MDS axes scores revealed the between subjects factor nutrient supply was significant for axis-1 and axis-2 ($F_{1,10} = 51.86$, $p \leq 0.001$ and $F_{1,10} = 14.05$, $p = 0.003$ respectively), and the within subjects factor day was significant for axis-1 ($F_{7,70} = 11.44$, $p \leq 0.001$). The day × nutrient interaction was significant for axis-1 and axis-2 ($F_{7,70} = 3.03$, $p = 0.032$ and $F_{7,70} = 10.96$, $p \leq 0.001$, respectively). All other interactions were not significant ($p > 0.05$).

Examination of algal communities at each sampling point revealed little difference between communities early in succession (i.e. days 7 and 11, Figure 4.5 a, b), with differences increasing with time in the high versus low nutrient treatments (Figure 4.5 c-h). Analysis of communities from all treatments and replicates at each sampling point time allows samples to be

properly independent and compared with Global R-statistic which increased through time (N = 8, r^2 = 0.977, $p \leq 0.001$, Figure 4.6), indicating that differences between treatments increased through time. However, an examination of pair-wise R-statistics for each treatment, which were highly positively correlated with Bray-Curtis dissimilarity values (Pearson correlation = 0.831, Bartlett Chi-square statistic = 137.6, $p \leq 0.001$), revealed differences between algal communities did not increase through time for all treatments (Figure 4.7). What becomes evident is little or no difference in algal communities between current treatments within nutrient treatments (Figure 4.7 a-f); yet large differences in algal communities between nutrient treatments increasing with time (Figure 4.7 g-o). This is not to say there was no dissimilarity in algal communities between current treatments within each nutrient treatment; for example, by day 35 in the high nutrient treatments, the centric diatom *Melosira varians* was abundant in the low current treatment and absent in the high current treatment, and the diatom *Luticola mutica* was more abundant in the high current versus the low current treatment. However, in comparison these differences were smaller and emphasis is given to results between nutrient treatments.

Early in succession (days 7 and 11) in the low current velocity treatments (10 cm $\cdot$ sec^{-1}), the diatoms *Melosira varians*, *Synedra ulna*, *Nitzschia palea*, and the green alga *Gleocystis ampla* were good discriminating species and more abundant in the high versus low nutrient treatment (Table 4.2). *Melosira varians* was always more abundant under high nutrient conditions and was a good discriminating species throughout the duration of experiments. Additionally, towards the latter stages of succession, the cyanobacterium *Lyngbya vandernberghenii* and two species of the green alga *Scenedesmus* were more abundant in the high nutrient treatments, while the green alga *Chlorolobion braunii* was more abundant in the low nutrient treatment. In the variable flow treatments (20 cm $\cdot$ sec^{-1}), few species discriminated treatments from one another early in succession (Table 4.3), but near the end of the experiments, the green alga *Scenedesmus bernardii*, the filamentous cyanobacterium *Lyngbya vandernberghenii*, and the pennate diatom *Nitzschia palea*, were discriminating species that were more abundant in the high nutrient treatment. In the high current treatments (30 cm $\cdot$ sec^{-1}), the

green alga *Gloeocystis ampla* and *Chorella* sp., the diatoms *Synedra ulna* and *Nitzshia palea*, and the filamentous cyanobacterium *Lyngbya vandernberghenii* were good discriminating species and more abundant in the high nutrient treatment early in succession (Table 4.4). Near the end of the experiments, the diatom *Achnanthidium minutissimum* was dominant in the low nutrient treatment (data not shown), but the green algae *Scendesmus bernardii*, the diatoms *Nitzschia palea* and *Luticola mutica*, and the filamentous cyanobacterium *Microcoleus* sp. were more abundant in the high nutrient treatment and were good discriminating species.

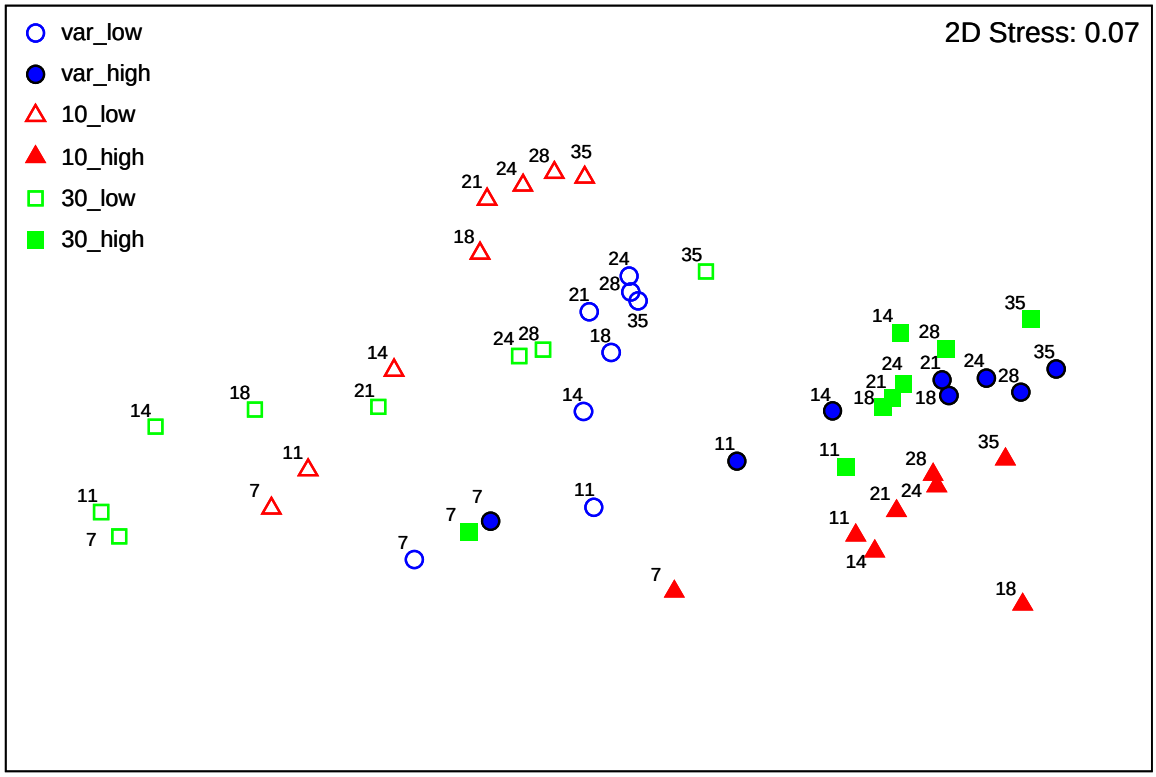


Figure 4.4 Non-metric multidimensional scaling ordination plot of periphyton communities for flow and nutrients experiments using Bray-Curtis dissimilarities and untransformed average periphyton biovolume values (average value for three replicates at each time point). Numbers next to symbols represent day of colonization.

Analysis of the second-stage MDS with ANOSIM revealed differences in time trajectories between treatments (global R-statistic = 0.226, p = 0.039, Figure 4.8). Pair-wise comparisons between treatments yielded moderate R-statistics for high versus low nutrient treatments in the low and high current treatments (10-high vs. 10-low = 0.556, 20-high vs. 20-low = 0, 30-high vs.

30-low = 0.37), with no differences between current treatments at high nutrients (all R-statistics ≈ 0) and moderate to low values between current treatments at low nutrients (10 vs. 30 = 0.593, 10 vs. 20 = 0.019, 20 vs. 30 = 0.259). These results suggest that 1) there were differences in time trajectories between nutrient treatments in the low and high current regimes, but not in the variable flow treatments; and 2) no difference in time trajectories between current treatments when nutrients are abundant, but possible differences between high and low current treatments when nutrient supply is low.

An examination on the effects of treatments on algal guilds revealed no differences early in succession, with differences emerging by day 18 (Figure 4.9 a-c). Prior to day 14, no significant difference between treatments were observed based on ANOSIM (all p-values for global R-statistics > 0.05), but after day 14, differences between treatments began to emerge (Figure 4.9 d-h). There were clear differences in growth forms between nutrient treatments and differences between the high and low current treatments in the low nutrient treatments, but there were no differences observed between current velocities in the high nutrient treatment. In general, in the low nutrient treatments, algal communities were dominated by low growth forms, while in the high nutrient treatments, composition was more diverse as high and motile growth forms dominated. Examination of average guild diversity between treatments confirmed these results, with significantly higher guild diversity (one-tailed two-sample t-tests $p < 0.05$ for each treatment) observed in the high versus low nutrient treatments (Figure 4.10). When focusing on guilds within the diatom community, composition was dominated largely by either motile or low profile guilds, depending on treatment. As biofilm thickness increased, in the low nutrient treatments, low profile diatoms dominated relative to motile diatoms in the high current velocity treatment, but did not change in the low and variable current treatments (Figure 4.11 a, c, e, Table 4.5). In the high nutrient treatments, motile diatoms dominated relative to low profile diatoms with increasing biofilm thickness (Figure 4.11, b, d, f, Table 4.5).

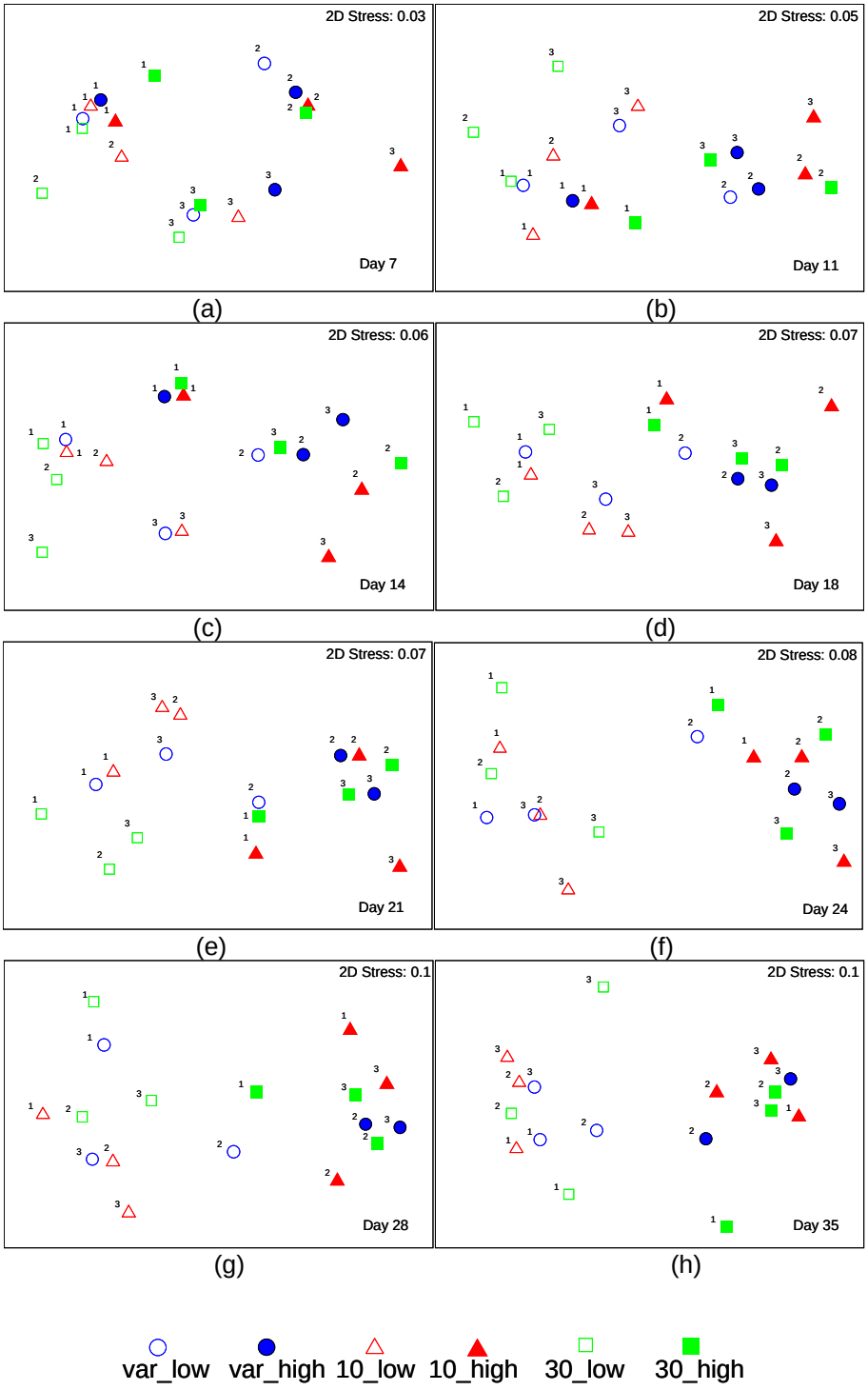


Figure 4.5 Non-metric multidimensional scaling ordinations for experiment manipulating current velocity and nutrient supply using Bray-Curtis dissimilarities and untransformed periphyton biovolume values. Day 7 (a) day 11 (b) day 14 (c) day 18 (d) day 21 (e) day 24 (f) day 28 (g) day 35 (h). Numbers next to symbols represent replicate streams.

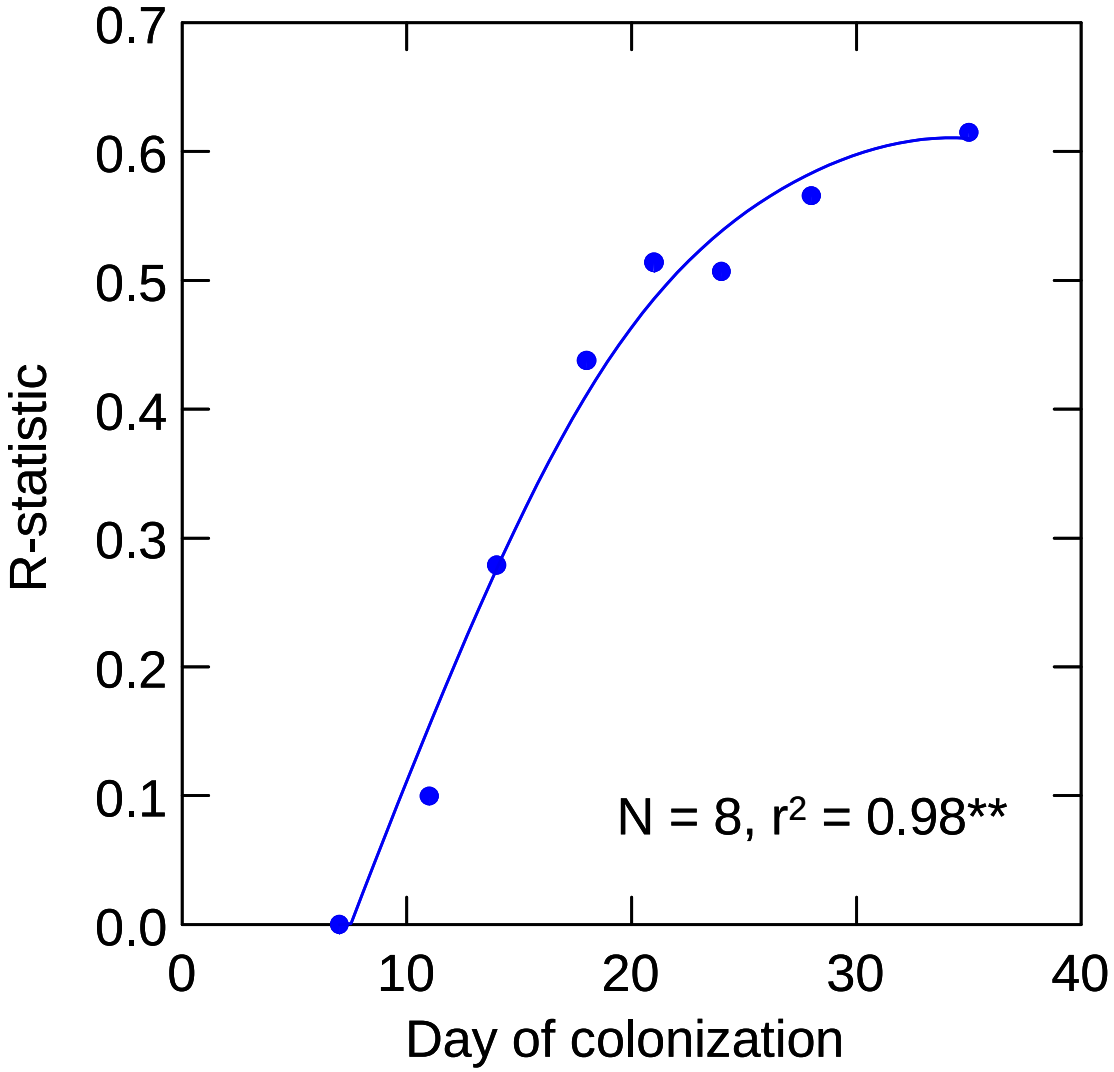


Figure 4.6 Global R-statistic at each sampling period across day of colonization for experiments 2-4; quadratic model significant.

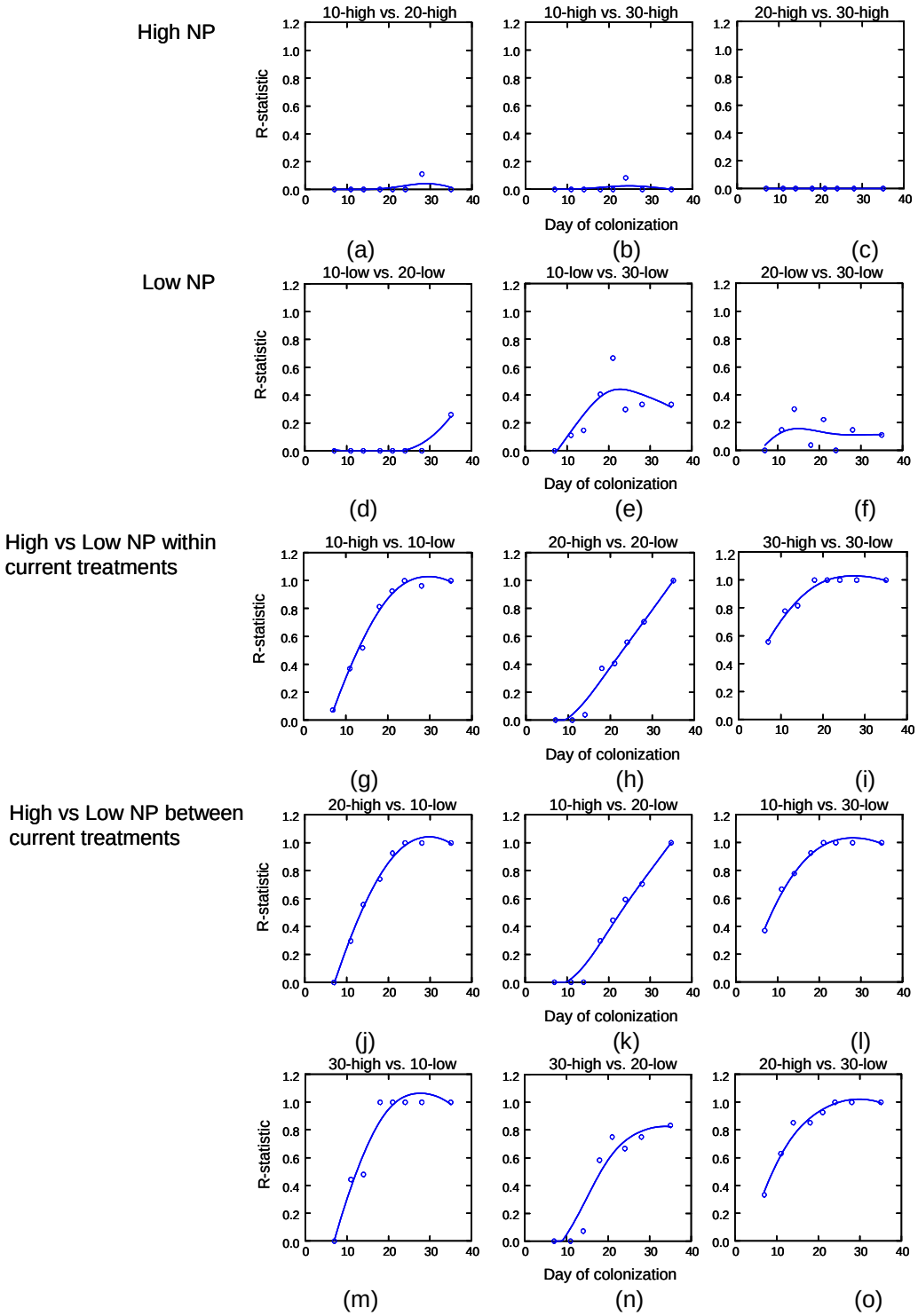


Figure 4.7 Pair-wise R-statistics from ANOSIM analyses of periphyton communities using untransformed biovolume values. High NP (a-c) low NP (d-f) high versus low NP within current treatments (g-i) and high versus low NP between current treatments (m-o). Values range from 0 to 1, with values of 1 indicative of complete separation and values of 0 indicative of no separation between communities.

Table 4.2. Average biovolume for discriminating species and the percent contribution to dissimilarity in the experiment manipulating current velocity and nutrient supply. Species presented here represent those contributing to approximately 50% of the dissimilarities between 10 cm · sec^{-1} current treatments for each sampling date during the experiment.

		10 cm · sec^{-1}	10 cm · sec^{-1}	
		Low NP	High NP	
	Species	Biovolume	Biovolume	Contrib%
Day 7	*Synedra ulna*	5.94E+06	5.93E+07	12.81
	Melosira varians	3.59E+05	6.76E+07	10.19
	Gloeocystis ampla	5.98E+05	2.19E+07	9.09
	Synedra acus	5.09E+06	2.47E+07	7.09
	Nitzschia palea	3.55E+05	1.95E+07	5.11
	Achnanthidium minutisimum var. *Affinis*	8.76E+05	7.76E+06	3.3
	Nitzschia linearis	1.14E+06	1.38E+07	3.26
Day 11	*Melosira varians*	3.40E+05	3.47E+08	17.21
	Nitzschia palea	8.71E+05	1.45E+08	8.08
	Gloeocystis ampla	1.49E+06	1.13E+08	7.05
	Synedra ulna	7.94E+06	8.01E+07	6.6
	Lyngbya vandernberghenii	2.39E+05	1.28E+07	6.37
	Surirella angusta	1.77E+05	1.06E+08	6.12
Day 14	*Melosira varians*	7.57E+05	4.74E+08	22.81
	Gloeocystis ampla	1.71E+06	2.40E+08	10.76
	Lyngbya vandernberghenii	3.52E+06	3.51E+07	10.16
	Nitzschia dubia	0.00E+00	1.75E+08	6.36
Day 18	*Cymatopleura elliptica*	0.00E+00	2.82E+09	24.69
	Melosira varians	6.31E+05	6.85E+08	19.95
	Lyngbya vandernberghenii	5.62E+06	3.60E+08	12.79
Day 21	*Melosira varians*	4.25E+05	8.69E+08	22.34
	Chlorolobion braunii	1.53E+08	2.32E+06	12.37
	Gloeocystis ampla	1.73E+06	2.20E+08	10.86
	Lyngbya vandernberghenii	8.27E+06	1.06E+08	10.54
Day 24	*Melosira varians*	2.70E+05	7.16E+08	17.96
	Scenedesmus bernardii	7.94E+06	2.48E+08	12.88
	Lyngbya vandernberghenii	1.61E+07	2.31E+08	10.5
	Scenedesmus bijugatus	0.00E+00	1.84E+08	10.42
Day 28	*Scenedesmus bernardii*	3.72E+06	3.65E+08	16.71
	Lyngbya vandernberghenii	1.49E+07	3.18E+08	12.6
	Melosira varians	9.02E+05	4.43E+08	12.49
	Chlorolobion braunii	2.52E+08	5.40E+05	11
Day 35	*Scenedesmus bernardii*	1.48E+06	1.02E+09	25.78
	Scenedesmus bijugatus	0.00E+00	4.68E+08	12.91
	Lyngbya vandernberghenii	3.45E+07	3.86E+08	12.02
	Melosira varians	1.11E+05	5.30E+08	11.66

Table 4.3. Average biovolume for discriminating species and the percent contribution to dissimilarity in the experiment manipulating current velocity and nutrient supply. Species presented here represent those contributing to approximately 50% of the dissimilarities between 20 cm · sec^{-1} (variable flow) current treatments for each sampling date during the experiment.

		20 cm · sec^{-1}	20 cm · sec^{-1}	
		Low NP	High NP	
	Species	Biovolume	Biovolume	Contrib%
Day 7	*Gloeocystis ampla*	2.58E+07	1.92E+07	17.02
	Stauroneis phoenicenteron	1.01E+07	1.06E+06	7.17
	Synedra ulna	2.52E+06	8.45E+06	5.91
	Synedra acus	2.13E+06	5.11E+06	5.17
	Chlorella sp. 1	3.36E+05	4.36E+06	3.44
	Nitzschia palea	3.71E+06	5.84E+06	3.36
	Gomphonema parvulum	1.46E+06	4.07E+06	3.22
	Nitzschia linearis	2.06E+06	4.20E+06	3.08
	Chlorella sp. 3	2.86E+06	2.25E+05	2.97
Day 11	*Gloeocystis ampla*	1.19E+08	1.77E+08	26.86
	Lyngbya vandernberghenii	1.29E+07	3.63E+07	5.84
	Scenedesmus sp.	1.67E+05	6.37E+06	5.67
	Nitzschia palea	9.02E+06	4.97E+07	5.44
	Surirella angusta	7.12E+05	3.58E+07	5.07
	Scenedesmus bernardii	2.04E+06	2.22E+07	4.33
Day 14	*Gloeocystis ampla*	8.33E+07	1.97E+08	17.24
	Lyngbya vandernberghenii	2.70E+07	1.33E+08	13.81
	Nitzschia palea	5.16E+06	1.70E+08	9.88
	Stauroneis phoenicenteron	0.00E+00	1.35E+08	6.45
	Scenedesmus bernardii	1.07E+06	1.03E+08	5.23
Day 18	*Nitzschia palea*	5.55E+06	5.43E+08	19.05
	Scenedesmus bernardii	2.45E+06	4.92E+08	17.41
	Gloeocystis ampla	4.55E+07	3.26E+08	12.06
	Lyngbya vandernberghenii	7.16E+07	3.80E+08	11.34
Day 21	*Scenedesmus bernardii*	1.29E+06	6.52E+08	24.63
	Gloeocystis ampla	2.06E+07	4.30E+08	15.62
	Nitzschia palea	3.37E+06	3.99E+08	14.89
Day 24	*Scenedesmus bernardii*	2.63E+06	9.12E+08	29.15
	Lyngbya vandernberghenii	1.17E+08	4.61E+08	11.1
	Gloeocystis ampla	1.33E+07	3.43E+08	9.47
	Nitzschia palea	2.85E+06	3.02E+08	8.99
Day 28	*Scenedesmus bernardii*	2.14E+06	1.27E+09	32.41
	Lyngbya vandernberghenii	9.31E+07	7.00E+08	16.84
	Nitzschia palea	6.58E+06	3.58E+08	9.34
Day 35	*Scenedesmus bernardii*	1.93E+06	1.92E+09	31.67
	Lyngbya vandernberghenii	1.42E+08	5.65E+08	17.35
	Nitzschia palea	4.68E+06	3.95E+08	8.72

Table 4.4. Average biovolume for discriminating species and the percent contribution to dissimilarity in the experiment manipulating current velocity and nutrient supply. Species presented here represent those contributing to approximately 50% of the dissimilarities between 30 cm · sec^{-1} current treatments for each sampling date during the experiment.

		30 cm · sec^{-1}	30 cm · sec^{-1}	
		Low NP	High NP	
	Species	Biovolume	Biovolume	Contrib%
Day 7	*Gloeocystis ampla*	2.69E+05	1.33E+07	8.87
	Synedra ulna	8.42E+05	7.37E+06	7.44
	Chlorella sp. 1	4.40E+05	2.87E+06	6.94
	Nitzschia palea	2.29E+05	8.40E+06	6.65
	Stauroneis phoenicenteron	0.00E+00	2.81E+06	6.61
	Synedra acus	1.67E+06	3.18E+06	5.84
	Scenedesmus bijugatus	0.00E+00	2.80E+06	4.58
	Achnanthidium minutisimum var. *Affinis*	3.82E+05	5.84E+06	3.61
Day 11	*Lyngbya vandernberghenii*	5.10E+04	4.67E+07	18.2
	Nitzschia palea	2.26E+05	3.22E+08	10.87
	Gloeocystis ampla	3.21E+05	6.75E+07	10.1
	Chlorella sp. 1	4.18E+05	1.13E+07	8.33
	Nitzschia flexa	0.00E+00	1.42E+08	4.34
Day 14	*Lyngbya vandernberghenii*	1.91E+05	8.84E+07	27.68
	Nitzschia palea	2.18E+05	7.41E+08	23.1
	Surirella angusta	8.50E+04	2.32E+08	7.74
Day 18	*Nitzschia palea*	1.35E+05	6.52E+08	25.17
	Lyngbya vandernberghenii	3.28E+06	1.83E+08	22.25
	Gloeocystis ampla	9.63E+05	1.52E+08	6.1
Day 21	*Nitzschia palea*	3.06E+05	4.84E+08	21.21
	Lyngbya vandernberghenii	8.77E+06	2.27E+08	20.96
	Microcoleus sp.	1.39E+06	1.47E+08	10.09
Day 24	*Nitzschia palea*	2.91E+05	5.63E+08	22.86
	Microcoleus sp.	4.18E+06	2.14E+08	11.74
	Lyngbya vandernberghenii	1.82E+07	1.69E+08	9.88
	Scenedesmus bernardii	0.00E+00	1.37E+08	7.26
Day 28	*Scenedesmus bernardii*	0.00E+00	4.07E+08	17
	Microcoleus sp.	7.89E+06	3.45E+08	15.95
	Nitzschia palea	1.28E+06	3.61E+08	14.99
	Zygnema sp.	0.00E+00	2.39E+08	6.09
Day 35	*Scenedesmus bernardii*	0.00E+00	1.31E+09	30.78
	Luticola mutica	0.00E+00	7.15E+08	19.79
	Microcoleus sp.	2.95E+07	3.05E+08	7.12

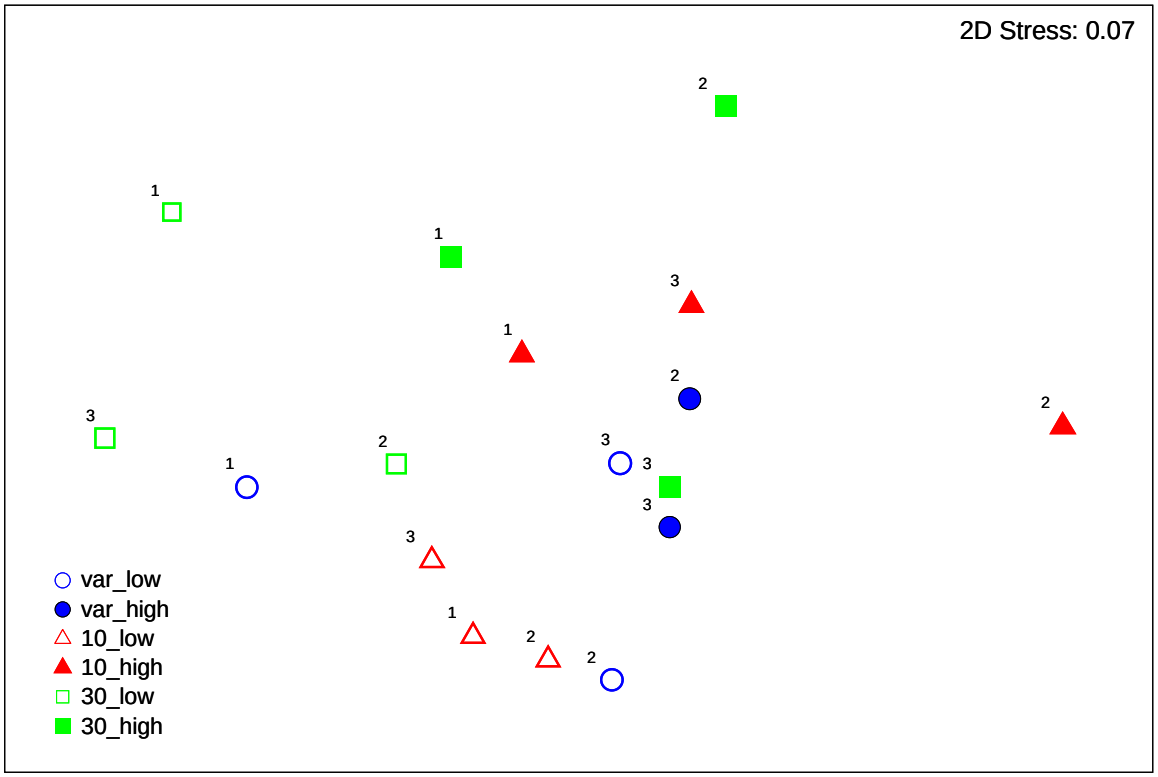


Figure 4.8 'Second-stage' multidimensional scaling ordination for experiment manipulating current velocity and nutrient supply using Bray-Curtis dissimilarities of periphyton biovolume values. Each symbol represents the pattern of community change for each stream through time. Numbers next to symbols represent stream replicate.

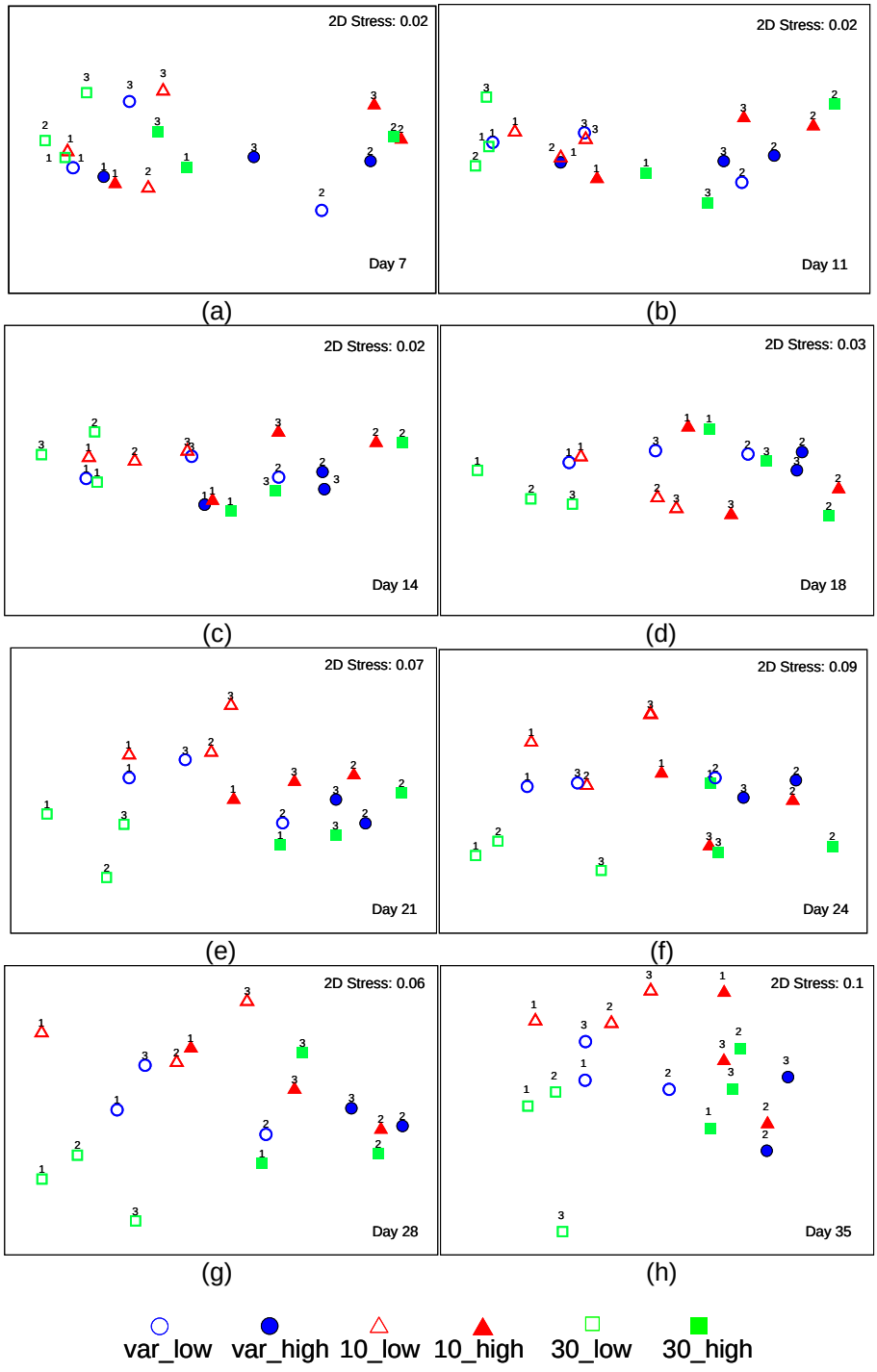


Figure 4.9 Non-metric multidimensional scaling ordinations for experiment manipulating current velocity and nutrient supply using Bray-Curtis dissimilarities and untransformed biovolume values for algal guilds. Day 7 (a) day 11 (b) day 14 (c) day 18 (d) day 21 (e) day 24 (f) day 28 (g) day 35 (h). Numbers next to symbols represent replicate streams.

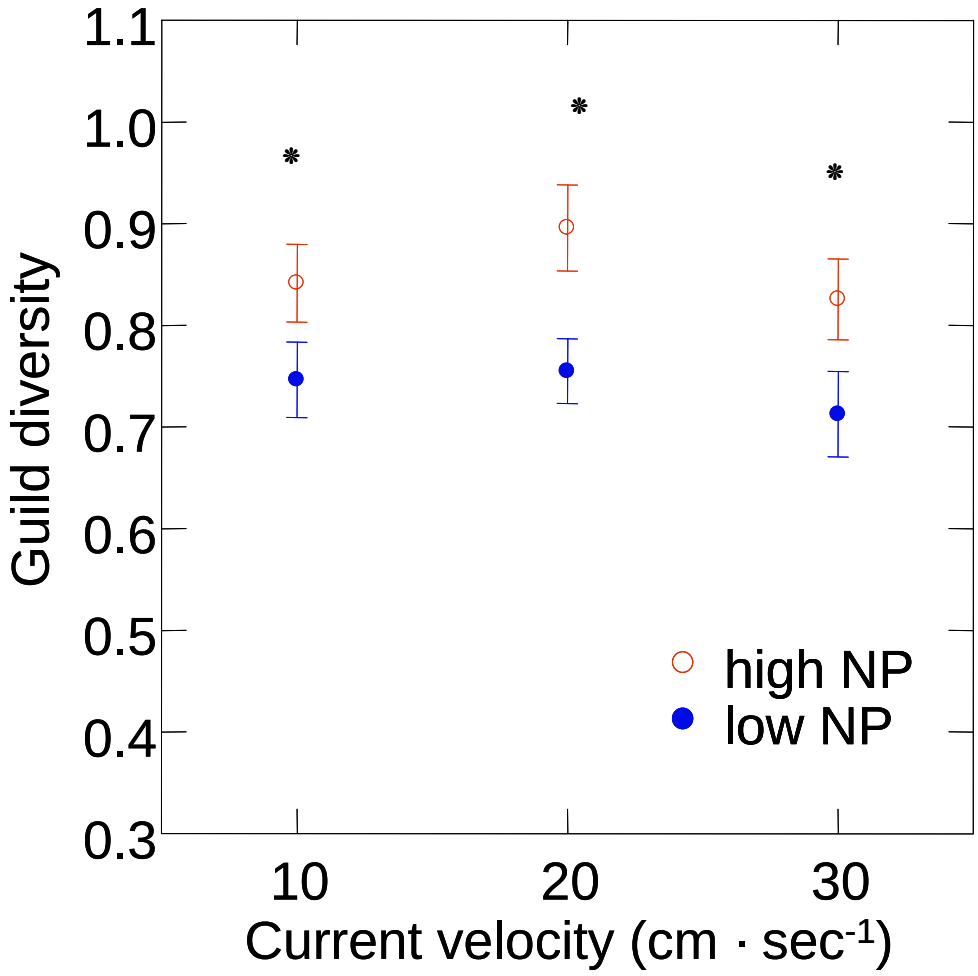


Figure 4.10 Comparison of average guild diversity between nutrient treatments across current velocities in periphyton in artificial streams. Error bars represent ± 1 standard error.

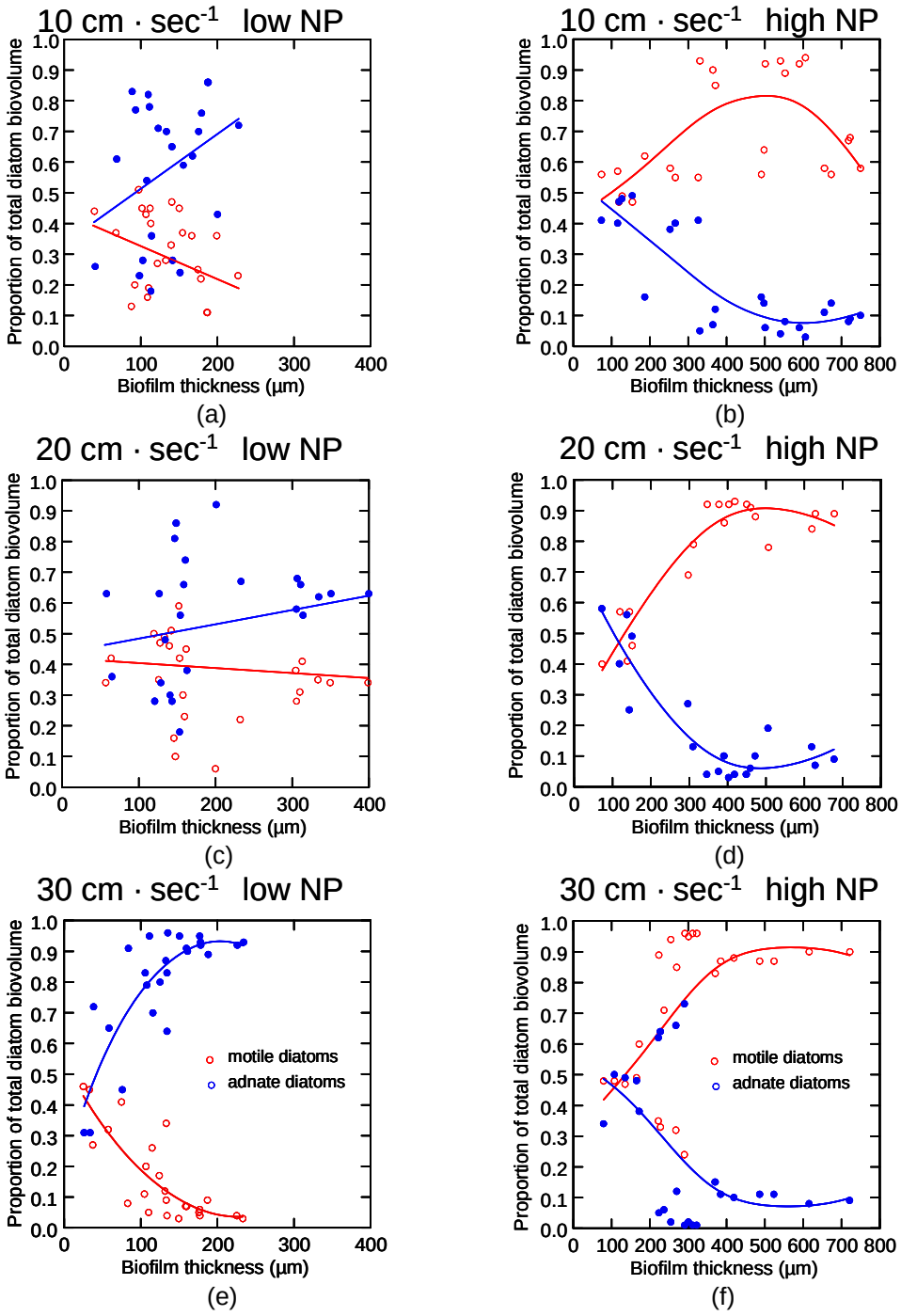


Figure 4.11 Proportion of total diatom biovolume of motile diatoms and adnate diatoms versus biofilm thickness for 10-low (a) 10-high (b) 20-low (c) 20-high (d) 30-low (e) and 30-high (f). Regression models described in Table 4.5

Table 4.5. Model, number of observations, coefficients of determination (r^2) and p-value for regression models of proportions (of total diatom biovolume) of 'motile' and 'adnate' diatoms versus biofilm thickness.

Treatment	Diatom guild	model	N	r^2	*p*
10-low	motile	$\hat{y} = b_0 + b_1x$	24	0.099	0.132
	adnate	$\hat{y} = b_0 + b_1x$	24	0.093	0.147
20-low	motile	$\hat{y} = b_0 + b_1x$	24	0.030	0.417
	adnate	$\hat{y} = b_0 + b_1x$	24	0.098	0.136
30-low	motile	$\hat{y} = b_0 + b_1/x^{0.5}$	24	0.514	0.00008
	adnate	$\hat{y} = b_0 + b_1/x^{0.5}$	24	0.687	0.00001
10-high	motile	$\hat{y} = b_0 + b_1x^2 + b_2x^3$	24	0.474	0.0012
	adnate	$\hat{y} = b_0 + b_1x + b_2x^2$	24	0.705	0.00001
20-high	motile	$\hat{y} = b_0 + b_1x^2 + b_2x^{1.5}$	19	0.876	0.00001
	adnate	$\hat{y} = b_0 + b_1x + b_2x^2$	19	0.821	0.00001
30-high	motile	$\hat{y} = b_0 + b_1/x^{0.5}$	24	0.313	0.0045
	adnate	$\hat{y} = b_0 + b_1x^{0.5}$	24	0.238	0.0155

4.4 Discussion

Current velocity and nutrient supply influenced algal community succession, where trends of greater community similarity (convergence) and dissimilarity (divergence) were observed. In the experiment manipulating only current velocity, multivariate analyses and ANOSIM revealed differences in community structure in the high current versus the low current treatments. These differences remained through the duration of the experiment, yet algal communities in the two current treatments appeared to become more similar as the experiment progressed. This might be explained, in part, by the fact that in that particular experiment, new propagules were being supplied regularly throughout the duration of the experiment with the addition of new stream water. For benthic algae, the optimum current velocity for accumulation on substrata can vary during community development (Stevenson 1996) and substratum conditioning has been shown to enhance colonization in fast currents (Peterson and Stevenson 1989). During early stages of colonization on bare substrata, high current velocity can hinder bacterial attachment (Rickard *et al*. 2004) and algal immigration (McIntire 1966), which may delay the initial development of a biofilm matrix (Lamb and Lowe 1987, Stevenson 1996, Ghosh and Guar 1998, Battin *et al*. 2003a). Possible evidence supporting this claim was the observation that early in this experiment algal density was significantly lower in the high current treatments (Chapter 2), a result consistent

with previous studies (McIntire 1966, Reisen and Spencer 1970, Stevenson 1984, Steinman and McIntire 1986). However, once a matrix was formed and the substrate conditioned for the arrival of later colonizers, differences in initial communities between treatments were observed to decrease (McIntire 1966, Lamb and Lowe 1987) and propagules supplied to all stream flumes were essentially the same, since they came from the same stream water supplied every three days. Additionally, once a community becomes established, increased current velocity can stimulate algal metabolism by increasing nutrient transport to cells, thus increasing growth compared to communities in lower current velocities (Whitford 1960, Stevenson and Glover 1993, Stevenson 1996); so the observation that in this experiment communities in the two current treatments were becoming more similar with time is not completely unexpected. Greater similarity in community composition between treatments was largely driven by similarities in the abundance of *Lyngbya vandernberghenii*, which was abundant in both current treatments in the latter stages of the experiment.

In the experiments manipulating current velocity and nutrient supply, there was evidence for increasing dissimilarity with time or divergence between algal communities exposed to high and low nutrient concentrations. While community similarity between current treatments changed little with time within each nutrient treatment, especially within the high nutrient treatments, large differences were observed between nutrient treatments, with algal communities becoming more dissimilar with time. Further evidence of this was revealed in a second-stage MDS, which suggests the successional trajectories of algal communities high nutrient treatments were different than communities in the low nutrient treatments. It is likely that in the high nutrient treatments, the rapid formation and development of a microbial matrix is favored (Costerton *et al.* 1995). In these experiments, propagules were supplied only once at the beginning of each experiment. Therefore, any delay in establishment of an initial microbial biofilm matrix might have hindered community development and influenced the successional trajectories of the periphyton communities. Additionally, preferential settlement of species with distinct nutrient preferences might have also contributed to successional divergence. Under conditions of limited dispersal,

species establishment may be driven by reproduction of early colonists rather than by immigration (Young *et al*. 2001). With no further propagules added to supplement immigration, communities would be reliant only upon those species already present for subsequent colonization. Furthermore, greater richness was observed in the high nutrient treatments (Chapter 2) meaning the number of species available for colonization later in succession were further reduced in the low nutrient treatments. Therefore, it is possible that periphyton communities in these experiments may have been limited in later stages of succession by the species pool, where species requiring an existing mat for successful immigration (mid- and late-successional colonizers) may have not been present in later stages of the experiments. However, species richness in nutrient-current experiments was high (mean = 54.26, 52.65-55.87 95% CI, Chapter 2), with more species than in the experiment receiving a continual supply of propagules, therefore, the supply of propagules alone cannot explain the observed differences in species composition between high and low nutrient treatments. These results suggest that periphyton community composition is not exclusively influenced by historical factors and that other factors such as competition are also likely important determinants of community assembly.

Nutrient addition is predicted to reduce evenness in periphyton communities by increasing dominance by one or several species (Hillebrand 2007). However, in these experiments, there was little difference in average evenness between treatments, only between nutrient treatments under conditions of high current velocity (Chapter 2). Therefore, high nutrient supply did not appear to alter patterns in average dominance as measured by evenness; however nutrient concentration did determine which species were favored in each nutrient treatment. For example, in the high nutrient treatments, species composition was dominated in the latter stages of succession by several *Scenedesmus* species, the motile diatom *Nitzshia palea*, and by several species of filamentous cyanobacteria (e.g., *Microcoleus* sp. and *Lyngbya vanderberghenii*). *Lyngbya vanderberghenii* was also abundant in the low nutrient treatments, which were dominated by species of smaller growth forms, such as the green alga *Chlorolobion braunii*, the diatom *Achnanthidium minutissimum*, and the cyanobacterium *Chamaesiphon fucus*. Within high

nutrient treatments, a dramatic change in composition was observed between early and late succession. In the low nutrient treatments, dramatic changes in composition between early and late succession were not observed, yet differences in composition between sampling points were greater. Multivariate dispersion analysis confirmed this, as greater variability in species composition was observed in the low nutrient treatments relative to high nutrient treatments. Low nutrient supply likely represents a form of stress, and greater variability or an increase in multivariate dispersion has been observed in stressed versus unstressed marine macrobenthic communities (Warwick and Clarke 1993).

While there were clear separations in species composition between high and low nutrients, there was little clear separation between current treatments within nutrient treatments. This does not mean there were no differences in algal communities in response to current velocity. For example, in the high nutrient treatments, a species sensitive to current velocity was more abundant in the low current treatment (e.g., *Melosira varians*), while in the high current treatment the small motile species *Luticola mutica* was more abundant. The lack of difference in composition between current treatments at high nutrients might have been due to abundant growth of the filamentous cyanobacteria *Lyngbya vanderberghenii* in each current treatment at high nutrients which formed tightly intertwined mats that may have provided some protection against current velocity. This, combined with the observation that the diatoms were composed of mainly motile forms, able to move to positions in the mat and avoid current stress may have contributed to the lack of difference at high nutrients between current velocity treatments. Furthermore, current can have varied effects in periphyton communities (Stevenson 1996), and current velocity may have little effects on nutrient uptake when benthic algae are nutrient replete (Borchardt *et al*. 1994). Additionally, when nutrients were low, *Achnanthidium minutissimum* was more abundant at high current velocity than in the low and variable current velocities. Therefore, there does appear to be an influence of current velocity on species composition, but the inability to detect differences based on ANOSIM may have been have resulted from variation between replicates within each treatment, therefore making it difficult to detect these differences in a

multivariate context. This variation among replicates is attributable, in part, to initial differences in seed algae for each experimental run (*data not shown*), yet despite these initial differences, consistent patterns were observed, especially between nutrient treatments. Once again, this suggests that deterministic factors, such as competition for limiting resources or environmental requirements of the species in the species pool as being important in community assembly of algal communities.

In my experiments, algal growth forms grouped into ecological guilds followed predicted patterns in response to nutrient supply. Periphyton communities growing under low nutrient conditions were thinner than communities growing under high nutrient conditions (Chapter 3) and were generally composed of species from the low profile guild (e.g, *Achnanthidium minutissimum*). These results are consistent with other studies showing resources at low supply typically support only thin biofilms, composed of species of smaller growth habit (Steinman and McIntire 1987 Pringle 1990, Passy 2007). Motile species, mostly comprised of bi-raphid diatoms (e.g., *Navicula* and *Nitzshia* spp.), became abundant in the high nutrient treatments, which is consistent with results showing motile species are superior competitors for nutrients in nutrient-rich environments (Fairchild *et al*. 1985, Van der Grinten *et al*. 1997). Algal species that have a relatively fixed position within the algal mat will have a greater likelihood for nutrients or light to become limiting, therefore, motile species can overcome this limitation and physically avoid nutrient stress within the algal mat by moving to resource-rich microhabitats (Pringle 1990, Johnson *et al.* 1997), and motility can also be advantageous in latter stages of community development when light becomes limiting (Pringle 1990). In my experiments, this was most evident in the diatoms, where the relative abundance of motile species increased wit biofilm thickness, while the relative abundance of adnate species decreased with biofilm thickness.

Nutrients in high supply support a greater number of species and contribute to greater structural complexity in periphyton communities (Hillebrand 2007, Passy 2008). In the experiments manipulating current velocity and nutrient supply, guild diversity of algal communities increased in the high nutrient treatments, which is consistent with results observed in stream

diatom communities, where guild diversity increased with nutrient availability (Passy 2007). Under low nutrient conditions, periphyton communities are more likely to be two dimensional in structure, supporting fewer niches which can be occupied by species of similar growth habit, while under conditions of high nutrients, the formation of a thick, three dimensional periphyton community can lead to strong gradients of light attenuation and nutrient depletion, creating many more niches for species of diverse growth morphologies (Passy 2007). Greater species richness in these experiments was observed in the high nutrient treatments relative to the low nutrient treatments (Chapter 3).

In microbial ecology, there is a long standing notion that most microbes (single celled eukaryotes included) are not dispersal limited, have cosmopolitan distributions, and that the environmental conditions of a particular site filter or determine which species are found there (Baas Becking 1934). However, increasing evidence suggests that not all microbial species have cosmopolitan distributions and unlimited dispersal abilities (Whitaker *et al*. 2003, Whitfield 2005). Results from my experiments suggest that immigration and the continual supply of new propagules are likely to be important components influencing the assembly and successional trajectories of algal communities. My results also suggest that competition is an important component influencing the assembly of algal communities.

Overall, in artificial stream experiments manipulating current velocity and nutrient supply, algal physiognomy and community structure clearly responded to differences in nutrient supply, while the effects of current velocity were less dramatic. Multivariate analyses revealed broad community separation between high and low nutrient treatment streams for both species composition and algal growth forms. These results indicate that in these experimental streams, nutrient concentration of the water had more of an impact on algal communities than did current velocity. Human alterations of the nitrogen and phosphorus cycles continue to increase nutrient supply and productivity to many ecosystems worldwide (Vitousek *et al*. 1997). As my experiments clearly demonstrate, nutrient enrichment of streams and rivers has the potential to impact species composition and diversity of periphyton communities, which may also have

important implications for the species that depend on them and the important ecosystem functions these communities provide (Battin *et al.* 2003 b).

APPENDIX A

LIST OF ALGAL TAXA SAMPLED FROM ARTIFICIAL STREAMS FOR THE EXPERIMENT MANIPULATING CURRENT VELOCITY

Taxon
Anabaena sp.
Aphanocapsa sp.
Calothrix parietina [Thuret 1875] Bornet *et* Flahault
Chamaesiphon focus (Rostafinski) Hansgirg
Chrococcus sp.
Lyngbya sp.
Lyngbya vandernberghenii Symoens *et* van der Werff
Merismopedia glauca (Ehrenberg) Nägeli 1845
Microcoleus sp.
Oscillatoria sp.
Pseudanabaena catenata Lauterborn 1914-17
Ankistrodesmus fusiformis Corda ex Korshikov 1953
Chlorella sp. 1
Closterium sp.
Coelastrum microporum Nägeli in A. Braun 1855
Cosmarium sp.
Cylindrocystis sp.
Gleocystis ampla (Kütz.) Rabenh.
Gongrosira papuasica (Borzí) Tupa 1974
Lagerheimia genevensis (Chodat) Chodat 1895
Microspora tumidula Hazen
Monoraphidum irregulare (G. M. Smith) Komárková-Legnerová 1969
Oocystis naegelii
Oocystis sp. 1
Oocystis sp. 2
Phacus spp.
Scenedesmus arcuatus (Lemmermann) Lemmermann 1899
Scenedesmus bernardii G.M. Smith 1916
Scenedesmus bijugatus Kützing
Scenedesmus sp.
Selenastrum bibraianum Reinsch 1867
Spirogyra sp.
Stichococcus bacillaris Nägeli 1849
Stigeoclonium sp.
Tetraedron muticum (A. Braun) Hansgirg
Unknown green 1
Unknown green 2
Zygnema sp.
Achnanthes coarctata (Brébisson) Grunow
Achnanthidium exiguum (Grunow) Czarnecki
Achnanthidium minutissimum (Kützing) Czarnecki
Amphora inariensis Krammer
Amphora libyca Ehrnberg 1840
Amphora ovalis (Kützing) Kützing
Anomoeoneis sphaerophora (Kützing) Pfitzer
Aulacoseira distans var. *nivalis* (Smith) Haworth
Caloneis alpestris (Grunow) Cleve 1894
Caloneis bacillum (Grunow) Cleve
Caloneis schumanniana (Grunow) Cleve
Caloneis silicula (Ehrenberg) Cleve
Cocconeis pediculus Ehrenberg

Cocconeis placentula Ehrenberg
Craticula ambigua (Ehrenberg) D. G., Mann ex Round et al. (1990)
Craticula cuspidata (Kützing) Mann
Cyclotella meneghiniana Kützing
Cyclotella radiosa (Grunow) Lemmermann 1900
Cymatopleura elliptica (Brébisson) Smith
Cymatopleura solea var. *apiculata* (Smith) Ralfs
Cymbella affinis Kützing
Cymbella cistula (Ehrenberg) Kirchner
Cymbella subleptoceros
Denticula thermalis Kützing 1844
Diadesmis confervacea Kützing
Diatoma hiemale var. Mesodon
Diploneis ovalis (Hilse ex Rabenhorst) Cleve
Encyonema silesiaca (Bleisch) Mann
Epithemia sp.
Eunotia bilunaris (Ehrenberg) Mills
Eunotia minor (Kützing) Grunow
Eunotia pectinalis (Müller) Rabenhorst
Eunotia praerupta Ehrenberg
Eunotia robusta Ralfs
Fistulifera saprophila (Lange-Bertalot et Bonik) Lange-Bertalot
Fragilaria brevistriata Grunow in Van Heurck 1885
Fragilaria capucina Desmazières
Fragilaria crotonensis Kitton
Fragilaria pinnata Ehrenberg 1843
Fragilaria vaucheriae (Kützing) Petersen
Fragilariforma bicapitata (Mayer) Round et Williams
Fragilariforma constricta (Ehrenberg) Williams et Round
Fragilariforma virescens (Ralfs) Williams et Round
Frustulia vulgaris (Thwaites) DeT.
Gomphonema acuminatum Ehrenberg
Gomphonema angustatum (Kütz.) Rabh.
Gomphonema augur var*. Sphaerophorum*
Gomphonema clavatum Ehrenberg
Gomphonema contaturris
Gomphonema gracile Ehr. emend. V. H.
Gomphonema grovei var*. Lingulatum*
Gomphonema intricatum var. *vibrio* (Ehr.) Cl.
Gomphonema kobayasii Kociolek & Kingston
Gomphonema parvulum (Kütz.) Kütz.
Gomphonema rhombicum Fricke
Gomphonema truncatum Ehrenberg
Gyrosigma acuminatum (Kütz.) Rabh.
Hantzschia amphioxys (Ehr.) Grun.
Luticola goeppertiana (Bleisch) Mann
Luticola mutica (Kutz.) Mann
Mastogloia smithii Thw.
Meridion circulare (Grev.) Ag.
Navicula digitoradiata (Gregory) Ralfs in Pritchard 1861
Navicula gastrum var*. Gastrum*
Navicula halophila (Grunow) Cleve 1894
Navicula jaernefeltii Hustedt 1942

Navicula levanderi Hustedt
Navicula menisculus Schum.
Navicula trivialis Lange-Bertalot
Neidium densestriatum (Oestrup) Krammer
Nedium iridis (Ehrenberg) Cleve 1894
Nitzschia acicularis (Kützing) Smith
Nitzschia amphibia Grunow
Nitzschia angustatula Lange-Bert.
Nitzschia clausii Hantz.
Nitzschia communis Rabenhorst
Nitzschia filiformis (W. Sm.) V. H.
Nitzschia hungarica Grunow 1862
Nitzschia linearis (Ag. ex W. Sm.) W. Sm.
Nitzschia littoralis Grunow in Cleve & Grunow 1880
Nitzschia microcephala Grunow
Nitzschia palea (Kützing) Smith
Nitzschia sinuata var. *tabellaria* (Grun.) Grun. in V.H.
Pinnularia biceps Greg.
Pinnularia bogotensis var*. Undulata*
Pinnularia gibba Ehrenberg
Pinnularia legumen (Ehr.) Ehr.
Pinnularia viridis (Nitz.) Ehr.
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot
Reimeria sinuata (Greg.) Kociolek & Stoermer
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot 1980
Rhopalodia gibba (Ehr.) O. Müll.
Rhopalodia gibberula (Ehr.) O. Müll.
Sellaphora pupula var. *capitata* Skvortzvow et Mayer
Sellaphora pupula fo. *rostrata* (Hustedt) Bukhtiyarova
Stauroneis kriegeri Patr.
Stauroneis phoenicenteron (Nitz.) Ehr.
Stauroneis smithii Grunow
Staurosira construens var. *subsalina* (Hust.) Andresen et al.
Surirella angusta Kützing
Surirella vata var. *Pinnata*
Synedra acus Kützing
Synedra tabulate (C. Agardh) Kützing 1844
Synedra ulna (Nitz.) Ehr.
Tabellaria flocculosa (Roth) Kütz.
Tabellaria quadriseptata Knud.

APPENDIX B

LIST OF ALGAL TAXA SAMPLED FROM ARTIFICIAL STREAMS FOR THE EXPERIMENTS MANIPULATING CURRENT VELOCITY AND NUTRIENT SUPPLY

Taxon
Anabaena sp.
Aphanocapsa sp.
Calothrix parietina [Thuret 1875] Bornet *et* Flahault
Chamaesiphon focus (Rostafinski) Hansgirg
Chrococcus sp.
Lyngbya sp.
Lyngbya vandernberghenii Symoens *et* van der Werff
Merismopedia glauca (Ehrenberg) Nägeli 1845
Microcoleus sp.
Oscillatoria sp.
Pseudanabaena catenata Lauterborn 1914-17
Ankistrodesmus fusiformis Corda ex Korshikov 1953
Chlorella sp. 1
Chlorella sp. 2
Chlorella sp. 3
Chlorolobion braunii (Nägeli) Komárek 1979
Coelastrum microporum Nägeli in A. Braun 1855
Coenochloris fotti (Hindák) Tsarenko 1990
Coleochaete irregularis E.G. Pringsheim 1860
Cosmarium sp.
Cylindrocystis sp.
Didymogenes palatina Schmidle 1905
Eudorina elegans Ehrenberg 1831
Gleocystis ampla (Kütz.) Rabenh.
Gongrosira papuasica (Borzí) Tupa 1974
Microspora tumidula Hazen
Monoraphidum irregulare (G. M. Smith) Komárková-Legnerová 1969
Monoraphidum minutum (Nägeli) Komárková-Legnerová 1969
Nannochloris bacillaris Naumann
Oocystis naegelii A. Braun 1855
Oocystis sp. 1
Oocystis sp. 2
Oonephris obesa (West) Fott 1964
Pediastrum sp.
Phacus sp.
Scenedesmus abundans (Kirchner) Chodat 1913
Scenedesmus arcuatus (Lemmermann) Lemmermann 1899
Scenedesmus bernardii G.M. Smith 1916
Scenedesmus bijugatus Kützing
Scenedesmus sp.
Sphaerobotrys fluviatilis Butcher 1932
Sphaerocystis schroeteri Chodat 1897
Spirogyra sp.
Stichococcus bacillaris Nägeli 1849
Stigeoclonium sp.
Unknown flagellate
Unknown green 1
Unknown green 2
Zygnema sp.
Achnanthes conspicua Mayer
Achnanthidium exiguum (Grunow) Czarnecki

Achnanthes marginulata Grunow in Cleve & Grunow 1880
Achnanthidium minutissimum (Kützing) Czarnecki
Amphipleura pellucida (Kützing) Kützing
Amphora inariensis Krammer
Amphora libyca Ehrnberg 1840
Amphora montana Krasske
Amphora ovalis (Kützing) Kützing
Amphora pediculus (Kützing) Grun.
Anomoeoneis sphaerophora (Kützing) Pfitzer
Aulacoseira distans var. *nivalis* (Smith) Haworth
Bacillaria paradoxa Gmelin
Brachysira vitrea (Grunow) Ross
Caloneis bacillum (Grunow) Cleve
Caloneis schumanniana var. *biconstricta* (Grunow) Reichelt
Caloneis silicula (Ehrenberg) Cleve
Cocconeis pediculus Ehrenberg
Cocconeis placentula Ehrenberg
Craticula ambigua (Ehrenberg) D. G., Mann ex Round et al. (1990)
Craticula cuspidata (Kützing) Mann
Cyclotella meneghiniana Kützing
Cymatopleura elliptica (Brébisson) Smith
Cymatopleura solea var. *apiculata* (Smith) Ralfs
Cymbella affinis Kützing
Cymbella caespitosa Brun
Cymbella cistula (Ehrenberg) Kirchner
Cymbella laevis Naegeli ex Kützing
Cymbella microcephala Grunow in Van Heurck 1880
Cymbella subleptoceros
Denticula tenuis Kützing 1844
Diadesmis confervacea Kützing
Diploneis ovalis (Hilse ex Rabenhorst) Cleve
Encyonema silesiacum (Bleisch) Mann
Eunotia bilunaris (Ehrenberg) Mills
Eunotia circumborealis Lange-Bertalot et Nörpel
Eunotia minor (Kützing) Grunow
Eunotia pectinalis (Müller) Rabenhorst
Eunotia praerupta Ehrenberg
Fallacia monoculata (Hustedt) Mann
Fallacia omissa (Hustedt) Mann
Fallacia pygmaea (Kützing) Stickle et Mann
Fallacia subhamulata (Grunow) Mann
Fistulifera pelliculosa (Brébisson ex Kützing) Lange-Bertalot
Fistulifera saprophila (Lange-Bertalot et Bonik) Lange-Bertalot
Fragilaria capucina Desmazières
Fragilaria capucina var. *mesolepta* Rabenhorst
Fragilaria crotonensis Kitton
Fragilaria intermedia (Grunow) Grunow
Fragilaria intermedia var. *Littoralis*
Fragilaria virescens Ralfs 1843
Frustulia vulgaris (Thwaites) DeT.
Gomphonema acuminatum Ehrenberg
Gomphonema angustatum (Kütz.) Rabh.
Gomphonema augur var. *Sphaerophorum*

Gomphonema clavatum Ehrenberg
Gomphonema gracile Ehr. emend. V. H.
Gomphonema grovei var*. Lingulatum*
Gomphonema kobayasii Kociolek & Kingston
Gomphonema parvulum (Kütz.) Kütz.
Gomphonema rhombicum Fricke
Gomphonema truncatum Ehrenberg
Gyrosigma acuminatum (Kütz.) Rabh.
Hantzschia amphioxys (Ehr.) Grun.
Luticola goeppertiana (Bleisch) Mann
Luticola mutica (Kutz.) Mann
Melosira varians Ag.
Meridion circulare (Grev.) Ag.
Navicula accomoda (Hustedt) Mann
Navicula cryptocephala Kützing
Navicula digitoradiata (Gregory) Ralfs in Pritchard 1861
Navicula gastrum var*. gastrum* (Ehrenberg) Kützing 1844 var. *gastrum*
Navicula halophila (Grunow) Cleve 1894
Navicula jaernefeltii Hustedt 1942
Navicula menisculus Schum.
Navicula molestiformis Hustedt 1949
Navicula placenta Ehrenberg 1854
Navicula radiosa Kützing
Navicula schroeterii Meist.
Navicula trivialis Lange-Bertalot
Navicula viridula (Kütz.) Kütz. emend. V. H.
Nedium iridis (Ehrenberg) Cleve 1894
Neidium productum (W. Smith) Cleve 1894
Nitzschia acicularis (Kützing) Smith
Nitzschia amphibia Grunow
Nitzschia angustatula Lange-Bert.
Nitzschia cf. calida Grun. in Cl. et Grun.
Nitzschia clausii Hantz.
Nitzschia communis Rabenhorst
Nitzschia dissipata (Kützing) Grunow
Nitzschia dubia W. Sm.
Nitzschia flexa Schum.
Nitzschia gracilis Hantz. ex Rabh.
Nitzschia hungarica Grunow 1862
Nitzschia inconspicua Grunow
Nitzschia intermedia Hantz. ex Cl. et Grun.
Nitzschia linearis (Ag. ex W. Sm.) W. Sm.
Nitzschia littoralis Grunow in Cleve & Grunow 1880
Nitzschia microcephala Grunow
Nitzschia nana Grun. in V. H.
Nitzschia palea (Kützing) Smith
Nitzschia perminuta (Grun.) Peragallo
Nitzschia recta Hantz. ex Rabh.
Nitzschia sigma (Kütz.) W. Sm.
Nitzschia sinuata var. *tabellaria* (Grun.) Grun. in V.H.
Nitzschia sociabilis Hustedt
Nitzschia sp*.*
Nitzschia umbonata Lange-Bert.

Pinnularia biceps Greg.
Pinnularia bogotensis var*. Undulata*
Pinnularia braunii var. *amphicephala* (A. Mayer) Hust.
Pinnularia gibba Ehrenberg
Pinnularia legumen (Ehr.) Ehr.
Pinnularia microstauron (Ehr.) Cl.
Pinnularia subcapitata Greg.
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot
Reimeria sinuata (Greg.) Kociolek & Stoermer
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot 1980
Rhopalodia gibba (Ehr.) O. Müll.
Rhopalodia gibberula (Ehr.) O. Müll.
Sellaphora pupula var. *capitata* Skvortzvow et Mayer
Stauroneis anceps Ehrenberg
Stauroneis phoenicenteron (Nitz.) Ehr.
Stauroneis smithii Grunow
Surirella angusta Kützing
Surirella lapponica A. Cleve 1895
Surirella ovalis Bréb.
Surirella ovata var. *pinnata* (W. Sm.) Brun
Synedra acus Kützing
Synedra tabulate (C. Agardh) Kützing 1844
Synedra ulna (Nitz.) Ehr.
Synedra ulna var. *oxyrhynchus* (Kütz.) V. H.
Tabellaria flocculosa (Roth) Kütz.
Tabellaria quadriseptata Knud.
Thalassiosira weissflogii (Grun.) Fryxell & Hasle

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