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Colonization History And Alternative Community States In Experimental Microcosms

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Craig Zimmermann
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Dedication

I would like to dedicate this dissertation to my wife for her patience in this long endeavor and my parents for instilling a love of learning.

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Abstract

Using s suite of disparate experimental systems, three tests of the effect of variation in community history on community states were performed. The first test explored the effect of species invasion order on the structure and invasibility in soil microbial communities. Microcosm communities were assembled by augmenting an existing soil community with sequential introductions of three bacterial strains under three alternative sequences. Assembled communities were then probed with a genetically engineered bioremediative bacterium to test the relative vulnerability of these communities to this strain. Results indicated that variation in invasion order resulted in the production of alternative community states with distinct vulnerabilities to invasion. The second test explored the effect of the interaction of variation in invasion sequence with varying productivity level on community composition and media chemistry properties. Detritus-based aquatic communities consisting of bacteria and protists were assembled under two alternative sequences on a gradient of five nutrient concentrations. In total, five unique community states were found to emerge from the interaction of species order and productivity level. Alternative states arising from sequence effects were found at two of the five nutrient levels tested. In addition, sequence effects were found to produce unique biologically-mediated changes to media chemistry. Notably, such effects were not necessarily reflected by observable changes at the species compositional level. The third test evaluated trends in species turnover data for evidence of convergence in community composition among a suite of 15 artificial pond microcosms established at the same location. Ponds were arranged in five clusters of three. Here, an explicit manipulation of

invasion sequence was not performed; rather, microcosms are assumed to possess similar histories and similar environments based on spatial proximity. Evidence of convergence was sought using five alternative compositional classification schemes. No evidence for convergence for the overall study site was found. Results of multiple analyses indicated a weak degree of convergence at the cluster level. Disturbance arising from multiple heavy rainfall events, however, had a strong disruptive effect on this convergence. Stronger evidence was found for a divergence in composition between two sets of microcosms that were independent of spatial proximity.

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Chapter 1: Overview

Early views held ecological succession to be a deterministic process in which a relatively orderly and predictable turnover of species directs the community towards a single, well defined state (e.g. Cowles 1901; Clements 1916; Odum 1969). Such views, however, have been challenged over the years by a number of ecologists (e.g. Gleason 1926; Elton 1930; Engler 1954; Chesson and Case 1986; Wu and Loucks 1995). Evidence has accumulated over the years, for example, that suggest that variation in the timing or sequence of species invasions can have lasting impacts on patterns of species composition (Law and Morton 1996; Drake et al. 1999; Morin 1999a). Theoretical investigations of two species Lotka-Volterra competition models have long demonstrated that the outcome of competitive exclusion can depend on initial species densities (Lotka 1932). These predictions have been substantiated by a number of classic laboratory experiments wherein manipulations of introduction sequence or initial densities of competing species were found to alter the competitive outcomes (Gause 1934; Park et al. 1948; Tilman 1977; Gilpin et al. 1986). Controlled field experiments under somewhat more natural conditions have also found evidence for *priority effects*, wherein species that colonize a community earlier can have differential impacts on subsequently arriving species (Harper 1961; Benke 1978; Wilbur and Alford 1985; Blaustein and Margalit 1996). The influence of priority effects has been implicated in long term successional studies wherein differences in initial species composition have been found to have lasting effects on later assemblages (Engler 1954). Case studies of alternative stable states have

also implicated variation in the order of species arrival as causal mechanisms for the observed divergence in community structure (e.g. Sale 1977; Sutherland and Karlson 1977; Wood and del Moral 1987; Fastie 1995).

Recently, theoretical and experimental investigations into the processes of community assembly have addressed the potential influence of colonization history by constructing trophically complex communities through iterated species invasions. Theoretically based studies undertaking a variety of modeling approaches have shown that permuting the sequence of invasion can produce invasion resistant states of variable species composition (Drake 1990b; Law and Morton 1993, 1996; Luh and Pimm 1993; Kokkoris et al. 1999). Microcosm experiments in community assembly have also demonstrated that manipulating the sequence and timing of invasions can have strong impacts on resulting community structure and invasibility (Robinson and Dickerson 1987; Robinson and Edgemon 1988; Drake 1991; Huxel 1995). The goal of my study is to contribute to the area of empirical community assembly studies. Using s suite of disparate experimental systems, three tests of the hypothesis that the effects of differences in community history can affect community structure and properties (e.g. invasibility) were carried out. In that regard, each of the following chapters were written as stand-alone papers that have either been published or will be shortly submitted for review. Thus, while the chapters may be abrupt, the conceptual development is not. A synopsis of each chapter follows with an outline of specific questions I addressed associated with each experiment.

Chapter 2: Historical Contingency and Alternative Community States

Here, I discuss theoretical and empirical evidence in support of alternative community states that has accumulated over the last two decades and how historical effects have been implicated as causal mechanisms. I also discuss and summarize recent research in community assembly processes that have shown the alternative states can emerge from differences in order in which species invade a community. Finally, I offer potential avenues of exploration that future studies could undertake to contribute to an overall theory of community assembly.

Chapter 3: A Test of Invasion Sequence Effects on Community Structure and Invasibility in Soil Microbial Microcosms

To date, laboratory investigation into community assembly processes have been essentially limited to aquatic systems (e.g. Robinson and Dickerson 1987; Robinson and Edgemon 1988; Drake 1991; Huxel 1995; Flum 1997). Consequently, the degree to which experimental findings are system-dependent is unknown. Demonstrations of assembly effects in other system types, could, therefore, contribute to a general theory of community assembly. Here, I explore the effect of species invasion order on the structure and invasibility of soil microbial communities. This is, to the best of my knowledge, the first test of community assembly effects in such systems. This experiment was performed within a larger context of environmental bioremediation. Current research in bioremediation is exploring the potential role that introduced microorganisms can play in the amelioration of environmental contaminants (Bouwer 1992; Forsythe et al. 1995; Sayler et al. 1997). While successful colonization of deployed organisms is a tacit

assumption of such an approach, the influence of colonization history in altering invasion vulnerabilities could have potential ramifications for effective field implementation (Sayler et al. 1997). Here, I assemble soil microbial communities by augmenting a chemically contaminated soil community with sequential introductions of three microbial strains – known to catabolize hydrocarbons – under three sequence permutations. Assembled communities are then probed with a genetically engineered bioremediative bacterium to test the relative vulnerability of these communities to this strain. Community dynamics are tracked for a 136-day period following introductions and differences in community structure and invasibility are evaluated. I ask: 1) Does changing the order of invasion of competing microbial species result in alternative community states? and 2) Is the capacity of a genetically engineered microorganism to successfully colonize a competitive soil environment can be affected the history of invasion associated with the recipient community?

Chapter 4: The Interaction of Invasion Sequence Effects with a Productivity Gradient in Assembled Protist Communities

Experimental studies in community assembly have demonstrated that invasion sequence effects can interact with environmental factors – such as light availability or nutrient richness – to influence resulting community structure (e.g. Dickerson and Robinson 1985; Drake 1991; Huxel 1995). Conversely, Flum (1997) found that alternative invasion sequences, in turn, could differentially alter physical conditions. The degree of variation in environment factors considered in these studies, however, has generally been limited to two levels. A better understanding of how the effects of

colonization history play out under varying environmental regimes could be gained by assembling communities across broader gradients of one or more environmental variables. Here, I assemble detritus based protist communities under two sequence permutations on a productivity gradient consisting of five discrete levels of nutrient richness. Population abundance by species was quantified for a 64-day period following completion of introductions. Media chemical properties were also quantified for each community upon completion of the experiment. The following questions are asked: 1) Does change in the order of species invasion order produce alternative community states? 2) Does a given invasion sequence produce alternative communities with changing productivity level? 3) What is the interaction of sequence and productivity effects? and 4) Does changing the order of invasion have differential effects on abiotic parameters?

Chapter 5: Do Similar Communities Develop on Similar Sites? – A Test of Community Convergence in a Long Term Field Microcosm Study

Supporters of community convergence hold that – for given set of species and a given set of environmental conditions – community trajectories will converge in a more or less predictable fashion to the same community composition (Cowles 1901; Clements 1916). That convergence is a necessarily inevitable result of community development, however, has been become increasingly challenged over time (e.g. Gleason 1926; Elton 1930). In this study, I experimentally test for community convergence by establishing a suite of 15 artificial pond communities at the same physical location. Given similar settings, do similar communities emerge? Here, an explicit manipulation of invasion sequence is not performed. Rather, systems are assumed to possess similar histories and similar

environments based on spatial proximity. Given census data collected over the course of the eighth growing season, I examine patterns of species composition for evidence of convergence. I assess evidence of convergence in composition among microcosms communities for the following situations: 1) the overall study site, 2) among experimental clusters, and 3) among systems independent of spatial location. I also evaluate convergence based on alternative approaches to community classification.

Chapter 6: Conclusions

Evidence has accumulated over the last two decades from both theoretical and empirical sources that suggest that invasion history can alter community structure. Many questions, however, remain unanswered. Here, I address what I perceive to be the three key questions:

1. *Are some ecosystem types more prone to historical effects than others?*

Previous experimental demonstrations of sequence effects have been limited to aquatic systems. I have found evidence in this study that sequence effects can also play a role in influencing soil communities. The potential for assembly effects in other systems (i.e. marine, terrestrial) are also addressed.

2. *What conditions or circumstances are likely to promote historical effects?*

I discuss the effects of a) productivity level, b) invasion characteristics, and c) disturbance. I have found, for example, that the effects of variation in species order on community structure can be contingent on productivity. I have also found that regimes of intense and frequent disturbance can readily disrupt trends in community convergence.

3. *What are the mechanisms underlying the effects of invasion history?*

I discuss a) priority effects, b) deterministic versus indeterministic community trajectories, and c) keystep species. In this study, I have found that strong priority effects emerging within some invasion sequences can alter resulting community structure. I have also found evidence that a varying degree of determinism that can arise between community trajectories as a function of alternative colonization history. The variation thus produced by such indeterminism could provide the conditions onto which further variation could be created. I also discuss the theoretical possibility that the timing of particular species or sets of species could play a fundamental role in the generation of alternative community states.

I conclude this discussion with suggestions on how the findings of community assembly research could find application in ecological engineering projects such as ecological restoration and environmental remediation.

Chapter 2:
Historical Contingency and Alternative Community States

2.1 Introduction

A balance of nature philosophy is common among many cultures. While it has taken various forms, most generally view nature as possessing a natural order that is best understood as a balance of creative and destructive processes (see Egerton 1973 for review). Such a philosophy has a long history in ecology and has been a long standing assumption that – either implicitly or explicitly – has underlain much of the modern ecological paradigm (Egerton 1973; Wu and Loucks 1995). Ideas concerning equilibrium, stability, and homeostasis in populations and communities are central ecological concepts that convey these assumptions. While the balance of nature paradigm has been subject to periodic criticism since the early part of the last century (e.g. Gleason 1926; Elton 1930), only within the last three decades has the weight of opposing evidence accumulated to the point where the prevailing paradigm can no longer be held tenable. In turn, the conceptual framework for new nonequilibrium-based paradigm is emerging (Chesson and Case 1986; Jørgensen et al. 1992; Wu and Loucks 1995). Within this framework, the rich and complex role that history can play in structuring communities is becoming evident (Chesson and Case 1986). Here, I discuss evidence for alternative community configurations arising from the same species pool and how theoretical and experimental studies in community assembly demonstrate how manipulations of colonization history (e.g. invasion sequence, invasion rate, and founder

abundance) can bring about their formation (Robinson and Dickerson 1987; Drake 1988, 1990a; Robinson and Edgemon 1988, Law and Morton 1993, 1996; Huxel 1995).

2.2 Changing Viewpoints on the Effect of History

Lewontin (1969) proposed two opposing viewpoints for addressing the potential influence of historical factors on community structure. One viewpoint offers that – for a fixed species pool and a fixed set of environmental constraints – a single globally stable equilibrium point exists. Such communities when perturbed are assumed to return to this same configuration. As convergence is considered to be the ultimate outcome of deterministic processes, historical information is unnecessary for an explanation of final community structure. Historical events are simply detours from an inevitable outcome. This viewpoint is consistent with the basic assumptions of the balance of nature paradigm that prevailed during the early days of modern ecology. Local diversity is assumed to be the outcome of largely deterministic processes operating within the community and historical influences were often ignored or minimized – either because such information was unavailable or because prevailing paradigm subverted its role (Chesson and Case 1986; Ricklefs 1987; Tanner and Hughes 1996).

Lewontin's (1969) opposing viewpoint offers that multiple locally stable equilibria are possible and an explanation as to why a particular state emerges may require reference to historical events. Theoretical and empirical evidence in support of this latter view has steadily accumulated for the last two decades and has posed a serious challenge to the single equilibrium paradigm (Wu and Loucks 1995). In particular, species assemblages of variable composition have been observed to arise from the same species pool seemingly under the same environmental conditions (Sutherland 1974; McCune and

Allen 1985; Barkai and Branch 1988). Long-term successional studies have also found evidence of alternative developmental pathways that diverge toward different community endpoints (Wilson and Agnew 1992; Fastie 1995). Furthermore, experimental studies have shown that the mechanisms and processes responsible for maintaining a given community can be distinguished from those that created it (Drake 1991; see also Petraitis and Latham 1999). Community structure, as a consequence, is becoming increasing viewed as a dynamic product of real time and historical processes (Ricklefs 1987; Cody 1989; Drake et al 1999)

2.3 Alternative Stable States: Theory and Evidence

Alternative stable states[1] are defined as communities that can assume two or more alternative species configurations that persist under the same environmental regime (May 1977; Knowlton 1992). Lewontin (1969) distinguished two classes of these states. The first class addresses alternative states in which all species are represented in each case, but at different relative abundance. Species that are dominant in one state could be correspondingly rare in another. The second class addresses alternative states that are characterized by different combinations of species wherein one or more species are missing in each state. Knowlton (1992) provides a theoretical demonstration of how these two classes can emerge in simple two species systems. A given scenario, however, could potentially contain states of both classes (Law and Morton 1993). That is, a species pool capable of producing alternative states under a given environmental regime may result in states that both incorporate the same species composition at relative

[1] Also termed as alternative permanent states, alternative communities, alternative assemblages, alternative equilibria, multiple equilibria, multiple stable states, multiple stable points, and multiple domains of attraction.

abundance as well as states that exclude some species from membership.

The theoretical possibility of alternative stable states has been demonstrated in a variety of mathematical models (Noy-Meir 1975; Gilpin and Case 1974; May 1977; Case and Casten 1979; Scheffer 1990; Drake 1990a; Knowlton 1992; Scheffer et al. 1993; Namba and Takahashi 1993; Janse 1993; Rietkerk and Van de Koppel 1997; Ward and Thornton 1998; Chase 1999; Van de Koppel et al. 2001) and laboratory microcosms studies (Robinson and Dickerson 1987; Robinson and Edgemon 1988; Drake 1991). Evidence for the existence of alternative stable states in natural systems has been also offered from a variety of ecosystem types. Table 2.1[2] offers some examples of case studies reported in the literature. While early empirical observations of multiple states were largely anecdotal (May 1977), more recent field studies are taking a controlled experimental approach in order to expose the underlying mechanisms responsible for the observed divergence of community structure (Barkai and Branch 1988; Dublin 1990; Berlow 1997; Petraitis and Dudgeon 1999). Additional evidence for alternative states has come from long-term studies of successional processes (e.g. Fastie 1995). As the convergence of successional pathways in a constant environment is implicit in theories that assume a single equilibrium state, evidence for divergent pathways in such situations is consistent with a theory of alternative states.

Theoretical and empirical studies have shown that alternative states can arise from: 1) differences in initial conditions, 2) perturbations that push the system from one community states to another, or 3) historically related factors (Lewontin 1969; Gilpin and Case 1974; May 1977). Key to the their formation are threshold effects – i.e. nonlinear

[2] All tables are located in Appendix B.

or discontinuous responses to one or more factors (May 1977; Friedel 1991; Laycock 1991; Knowlton 1992; Scheffer et al. 2001). Threshold effects can cause systems to exhibit *hysterisis* or an abrupt change of state when critical thresholds are crossed (Scheffer 1990; Scheffer et al. 2001). Once crossed, positive feedback can accelerate divergence through amplification of environmental factors that promote the new community (Wilson and Agnew 1992). Cause and effect, therefore, need not be proportional and small perturbations can produce large and potentially irreversible consequences. Moreover, theory holds that key ecological interactions can be altered when such changes occur (Petraitis and Dudgeon 1999).

2.4 Community Assembly and Tests of Historical Contingency

Historical effects have been increasingly shown to play a major role in influencing population and community structure (Connell 1980; Chesson and Case 1986; Ricklefs 1987; Hughes 1989; Drake 1991; Ricklefs and Schluter 1993; Tanner and Hughes 1996). Community level studies of historical effects have primarily focused on how variation in initial conditions (e.g. Engler 1954; Gilpin et al. 1986; Myster and Pickett 1990) or in the timing and sequence of colonization (e.g. Sale 1977; Sutherland and Karlson 1977; Alford and Wilbur 1985; Robinson and Dickerson 1987; Wood and del Moral 1987; Drake 1991) can affect subsequent community properties. In particular, a causal relationship between variation in the order of species arrivals and the formation of alternative stable states has been implicated in some of these studies (Sutherland and Karlson 1977; Fastie 1995). Here, I discuss concepts of *community assembly* and how associated research is exploring the influence of colonization history in the production of alternative community states.

2.4.1 Communities as Assembled Entities

Communities do not spontaneously appear as intact entities; rather they assemble in time through a complex dynamic of species turnover driven by colonization and extinction processes. Investigations into the processes of *community assembly* seek the general principles or mechanisms by which communities form from species pools (see Drake 1990a; Belyea and Lancaster 1999; Keddy and Weiher 1999; Samuels and Drake 1999). Most authors attribute the concept of community assembly to Jared Diamond (see however Booth and Larson 1999). Diamond (1975) analyzed a data set of 147 species of birds distributed in various combinations across 50 islands off the coast of New Guinea. Combining elements of island biogeography, niche theory, and interspecific competition, Diamond proposed a number of general principles – which he termed *assembly rules* – as mechanisms responsible for the patterns of coexistence he observed. While Diamond's results were controversial (see Connor and Simberloff 1979; Diamond and Gilpin 1982; Gilpin and Diamond 1984), his paper influenced the generation of a new area of research in community ecology.

Currently, investigation into community assembly comprises a broad – some would say disorganized – collection of research that generally falls into one of two types (Belyea and Lancaster 1999). The first type seeks assembly rules for natural communities through statistical analysis of distributional patterns of species and/or functional traits (e.g. Weiher et al. 1998; Wilson and Whittaker. 1995; Cody 1999). This is essentially the same approach used by Diamond (1975). What exactly constitutes an assembly rule, however, has been a source of ambiguity resulting in a common confusion between cause and effect (Morin 1999a; Belyea and Lancaster 1999; Booth and Larson

1999). In an attempt to clarify this ambiguity, Belyea and Lancaster (1999) offer the following defintion:

> *Assembly rules are general and mechanistic, and operate within the case-specific constraints imposed by colonization sequence and environment.*

That is, assembly rules should addresses community processes that possess a generality that offers some degree of predictability across ecological systems of different types. The authors stress that the mechanisms underlying assembly rules should arise from dynamics internal to the community – i.e. through direct species interactions or through species-mediated changes to the environment. Their ultimate expression, however, could be potentially modified by the boundary conditions established by such parameters as invasion history and environmental conditions. By far, the majority of research attempting to elucidate rules of assembly has been limited to a single taxonomic or functional (i.e. guild) group. This research has been reviewed elsewhere (Weiher and Keddy 1995; Belyea and Lancaster 1999). Potential problems, however, arise with this type of approach. First, extant communities are presumed to be at equilibrium and don't represent some transient states. Second, restricting the focus to a few trophic levels can obscure processes occurring at the community level. Third, is its necessarily *a posteriori* nature. That is, it is the product of assembly that is observed rather than the assembly process itself. Where multiple assembly rules are operating, the interaction among rules could potentially modify their effect on community properties (Drake et al. 1996a; Belyea and Lancaster 1999). Unraveling cause and effect could, therefore, be elusive.

The second type of research has explored the role of historical contingency by investigating how variation in colonization history can affect community structure and

properties (i.e. invasibility). Controlled experiments involving the manipulation of introduction sequence and/or initial species densities have a long history in ecological research (e.g. Gause 1934; Park et al. 1941; Paine 1977; Alford and Wilbur 1985). Such experiments, however, typically have been limited to only two or three species. Recent community assembly research in this area has investigated the influence of colonization history through the construction of more trophically complex communities. Given the obvious logistical problems associated with an investigation of this scope, research to date has been largely conducted with either computer simulations or laboratory microcosms. In the following sections, I will provide a description of some of the research in community assembly that has used these two approaches. As the nature of basic assumptions employed affected experimental outcomes, this research is described in detail. I follow this with a synthesis of findings and suggestions for possible future research.

2.4.2 Community Assembly *In Silico*: Theoretical Studies

Post and Pimm (1983) used a Lotka-Volterra model to simulate a community assembly process that included both species colonization and extinction. Assembly proceeded by an initial invasion of all producers followed by sequential invasions of single consumer species randomly generated under specified food web constraints of species connectance and interaction strength. Alternative simulations were conducted to test the effect of variation in these parameters. They found that species richness approached an equilibrium value in all simulations with the richness values negatively correlated with connectance. They also found invasion resistance increased approximately linearly with time, i.e. "mature" food webs were more resistant to invasion

than "immature" webs. As complete invasion resistance was not attained, alternative equilibria were not addressed.

Drake (1988, 1990a) modeled a community assembly process using a similar model as Post and Pimm (1983), except that colonists were drawn from predetermined pools consisting of species with explicitly defined interaction coefficients. Two species pools with and without omnivory were considered. When modeled as discrete communities, the assembly process always culminated in an invasion resistant state. A single exception was the occurrence of a stable community cycle in which a community continuously looped between three distinct states. Communities assembled from small species pools required fewer number of time steps, on average, to reach invasion resistance. Notably, different invasion sequences were found to produce alternative community states that differed in species richness, composition and food web topology. While Drake concluded that an increase in species pool size would likely produce an increase in the number of alternative states, the effect of the addition of omnivores was not specifically addressed. Drake (1990a) also modeled the assembly process as an interconnected patch landscape in which species entered through a single patch and diffused through adjacent patches. Species that failed to invade the entry patch, therefore, were prohibited from the landscape. He found that the similarity in composition among landscape patches was correlated with the time to fixation for the entry patch, i.e. the quicker this patch became invasion resistant, the more similar was the remaining landscape. Invasion vulnerable patches could become "protected" from invasion when surrounded by invasion resistant patches.

The algorithms used in Drake (1988, 1990a) and Post and Pimm (1983) employed a

number of computation shortcuts to avoid the long processing time of numerical integration. Morton et al. (1996) addressed a number of key assumptions introduced by these shortcuts that potentially limit the generality of results (see also Case 1991). A comparison of results produced by suspect algorithm with those obtained by integrating the solutions revealed that the former method overestimated the prevalence of alternative states. Morton et al. (1996) argue that these results suggest a need for an alternative computationally efficient method for modeling assembly processes. They argue that the traditional criterion of asymptotic stability – local or global – is inappropriate as species can coexist without tending to an equilibrium point (see also Anderson et al. 1992). A measure of dynamic boundedness might, therefore, be more appropriate (Lewontin 1969; Holling 1973). Morton et al. (1996) propose a measure of community stability known as *permanence* (see also Law and Morton 1993, 1996; Sigmund 1995; Law 1997). Permanence is a global criterion that simply identifies species capable of long-term coexistence without any constraints of required equilibrium (Hofbauer and Sigmund 1988; Jansen 1987). Specifically, a set of species is said to be *permanent* if no solution, with all species initially at positive densities, tends toward a zero density for one or more species. As such, it is likely a more realistic measure of community stability. A flaw with the permanence criterion, however, is its inability to distinguish between alternative states that possess the same species composition at different relative abundance.

Using Lotka-Volterra dynamics and the permanence criterion, Law and Morton (1993, 1996) performed a number of simulation studies to explore the assembly process (see also Law and Blackford 1992; Morton and Law 1997; Law 1997). Invaders were randomly drawn from fixed species pools of variable size and were assumed to occur

singularly and at intervals longs enough to allow a return to an asymptotic state. In addition, energy constraints were implemented to mimic a realistic conversion of prey into new consumers. They found: 1) the vast majority of species pools analyzed contained only a single permanent community endpoint. Pools with two or three alternative permanent states were also found, but were rare; 2) permanent endcycles were also observed in which communities continuously cycled through two or more states (c.f. Drake 1988, 1990a); 3) the number of time steps required to reach an invasion resistant state was positively correlated with the size of the species pool, and 4) communities at endpoints may lack reassembly pathways, i.e. attempts to reconstruct the community with only species present in the endpoint is impossible (see also Law and Blackford 1992). These results echo the so-called "humpty-dumpty" effects of Drake (1985, 1990a; Luh and Pimm 1991) wherein in communities often cannot be reconstructed with only species extant in the final state. Here, intermediate states containing one ore more transient species are required to “catalyze” the outcome.

Kokkoris et al. (1999) evaluated the change in interaction strength in competition communities assembled through random sequential invasions drawn from a pool of 40 competing species. They used a standard Lotka-Volterra model and solutions were integrated. Interaction values were constrained such that competitive exclusion in two species systems was not permitted. They found that multiple alternative states arose from differences in invasion sequence consistent with previous studies. Moreover, mean interaction strength declined as assembly proceeded with invasion resistant states characterized by having a higher proportion of weaker competitors than the species pool.

The preceding studies make three key assumptions concerning the nature of invasions:

1) invasions occur as single species at discrete time steps, 2) invader colonize at a low relative abundance, and 3) the community returns to an equilibrium before the next invasion. Huxel (1995), Lockwood et al. (1997), and Büssenschütt and Pahl-Wostl (1999) performed simulation studies in which these assumptions were tested. Huxel (1995) relaxed the first two assumptions by first, allowing two species invasions per time step, and second, by setting founder abundance near the equilibrium value. Complete invasion resistance failed to emerge in either simulation and colonization success was notably higher when founder abundance was high. Lockwood et al. (1997) tested the third assumption by comparing communities assembled at low and high frequencies relative to equilibration time. They found that two thirds of all species were successful at some point. Here, long periods of dominance by early colonists led to persistent alternative states. When invasions were frequent, on the other hand, species residence time was considerably reduced such that communities moved through a complex cycle of states in which nearly all species were successful at some time.

Büssenschütt and Pahl-Wostl (1999) explored the long-term behavior of dynamic food webs under constant pressure from waves of invaders. A highly abstracted trophic structure, similar to that Law and Morton (1996), was used in which physiological parameters and feeding relationship were allometrically defined. Assembly proceeded by an initial invasion of all primary producers followed by "waves" of 20 random generated consumers at intervals that allowed a return to equilibrium. Nutrient pools were refreshed at different input rates to assess the effect of nutrient richness on diversity values. While they found that species richness and diversity approached an asymptotic value, invasion resistance did not occur. Rather the probability of success among

invaders remained relatively constant and equilibrium values were maintained through constant species turnover. Alternative states, as a consequence, were not found. They also found, consistent with some predictions (e.g. Tilman 1982), that diversity near the climax state peaked at an intermediate nutrient levels.

Luh and Pimm (1993) took an alternative approach to modeling community assembly using a simplified model without population dynamics called *community transition graphs* (see also Pimm 1991). Community transition graphs are N-dimensional Boolean networks in which each community state is represented as species presence/absence. Transition pathways between each state are represented as movement toward one of the two states. Using species pools of varying size, the authors explored the statistical properties of such graphs in which transition values were randomly assigned. Where more than one transition was possible from a given state, each transition occurred with equal probability. The large majority of random graphs were found to contain only a single persistent state. Graphs with more than one state were also found, but their frequency declined exponentially with the number of states present. The total number of steps to a persistent state could be long with one or more transient loops en route. When graphs containing simple implausible cycles were removed from analysis, most graphs were found to contain multiple states with the number of states positively correlated with species pool size. Here, while paths to a persistent state were shortened, they often contained more complex transient loops.

2.4.3 Community Assembly *In Vitro*: Microcosm Studies

Dickerson and Robinson (1985) assembled microcosm communities to validate predictions of the island biogeography equilibrium theory (MacArthur and Wilson 1963,

1967.) Freshwater communities were assembled through controlled introductions consisting of alternative container size and invasion rates to simulate differences in island size and insularity. The species pool consisted of 19 protistan species of mixed trophic levels. The time allowed between invasions was stochastically determined with mean interarrival times of 2 and 4 weeks to represent "near" and "far" islands. As invasion schedules were independently derived for each species, a given invasion event could potentially consist of a variable combination of species. They found species richness accumulated until a dynamic equilibrium was reached consistent with theoretical predictions. While richness values also declined with insularity, examination revealed that both sets of communities possessed an identical number of core species with the higher richness of less remote islands was due entirely to a greater number of transient species. This finding is more consistent with alternative theories that view island communities as more permanent entities in which the observed turnover is ascribed to invaders denied establishment by species already present (Lack 1973, 1976; Gilbert 1980). Also contrary to expectations, the smaller microcosms sustained a greater number of species despite nearly an order of magnitude difference in size. Evidence of alternative states of relative dominance was also found. A mixotrophic green algae (*Ochromonas*) was found to dominate roughly half of the low invasion rate communities. The remaining communities were dominated by a protozoan (*Paramecium bursaria*). No coexistence between these two species was found. Robinson and Dickerson (1985) tested the invasibility of these communities with sequential invasions by three species unencountered in the assembly process. Colonization success was highly variable among invaders. To test Elton's (1958) theory that vulnerability to invasion is inversely related

to community complexity, the dependence of invader persistence on species richness was evaluated. No significant correlation was found. Rather, persistence seemed better correlated with the presence or absence of specific species in the recipient community.

Dickerson and Robinson (1985) hypothesized that their paradoxical finding regarding island size and species richness was the result of subtle habitat differences generated by the containers used. Dickerson and Robinson (1986), therefore, repeated their experiment with modifications to reduce the confounding effects of container geometry. Mean interarrival times were increased to 6 and 8 weeks to reduce the influence of transient species on species richness. Here, findings were more consistent with theoretical predictions: richness values in the large microcosms were equal to or greater than those of the smaller systems. The seemingly contradictory findings emerging from these separate experiments indicate that habitat diversity is likely a more important determinant of species richness than simply habitat size alone.

Of more interest to the current study, incidental observations made during the above experiments indicated that differences in the sequence of introduction – though not a formal part of the experimental design – seemed to influence the resulting community structure. To more explicitly test these effects, Robinson and Dickerson (1987) performed a subsequent experiment in which communities were assembled under two alternative invasion sequences at two invasion rates. Here, the species pool consisted of both protozoans and green algae. They found: 1) differences in species richness arising form sequence effects only occurred at the high invasion rate, and 2) differences in community composition were influenced by both invasion rate and sequence with sequence effects greatest at the high rate.

The invasion schedule used in Robinson and Dickerson (1987) prevented a unambiguous assessment of the relative influence of rate and sequence on richness values. Robinson and Edgemon (1988), therefore, performed a similar experiment with design modifications to remove this ambiguity. Nine sets of communities were assembled under three sequence permutations conducted at three invasion rates with a fixed pool consisted of 28 phytoplankton species. Each sequence was generated by random assignment of each species to one of four species groups. Groups were then introduced in fixed order at regular intervals of one to three weeks to simulate differences in invasion rate. This order was continuously cycled until the experiment was terminated. This scheduling differed from Robinson and Dickerson (1987) in two fundamental ways: 1) time intervals between invasion were fixed rather than stochastic, and 2) the number and composition of species in each invasion step were fixed rather than variable. They found that, while both invasion sequence and rate affected richness values, invasion rate accounted for most of the observed variation. A significant interaction between these factors, however, was also found. This indicates that both the *order* and *timing* of invasions can be critical. In addition, a high degree of similarity in composition was found between communities assembled under the same sequence at the low and median rates. Community sets assembled under different sequences at the high invasion rate, on the other hand, showed a greater similarity to each other than to those corresponding systems assembled at lower rates. High rates of invasion, therefore, can have a mitigating effect on the differences in invasion history. Such results are consistent with theoretical predictions (e.g. Lockwood et al. 1997).

Drake (1991) explored the influence of sequence effects by constructing freshwater

communities in laboratory microcosms of two sizes – a small 250 mL system and a large 40 L system. Using a small 250 ml system, the effect of invasion sequence on competitive interactions was explored among three phytoplankton species – *Ankistrodesmus, Scenedesmus,* and *Selenastrum*. All possible sequence permutations were tested with 10-day invasion intervals. In pure culture trials, *Ankistrodesmus* and *Selenastrum* were found to produce cell densities at least an order of magnitude greater than that of *Scenedesmus*. In all sequence treatments, however, *Scenedesmus* was clearly dominant. Two alternative states of different relative abundance were found. When *Scenedesmus* was introduced early, both competitors either became rare or went extinct. When *Scenedesmus* was introduced last, however, coexistence of all species was found. These communities were then probed with a suite of three invertebrate consumers to test their invasibility. All consumers were found to successfully colonize in each treatment with no change in producer dominance. Addition experiments were conducted in which a 15-day invasion interval was tried on selected sequences, but no change was found.

Using a large 40 L system, Drake (1991) explored the influence of sequence effects with a more trophically complex pool of species that included bacteria, protozoans, phytoplankton, and invertebrates. Ten sequence permutations of single species introductions were tested with 15-day invasion intervals. Initial introductions consisted of a single producer species and all bacteria to establish basal resources and nutrient recycling pathways. Otherwise, producers and consumers were mixed throughout each sequence. This scheduling protocol differed from that of Robinson and Dickerson (1987) and Robinson and Edgemon (1988) in two ways: 1) invasion events consisted of single species, and 2) each species was only allowed a single invasion attempt except in cases of

immediate failure where it was reintroduced on the following day. Drake observed that, in contrast to the small systems, sequence effects played a significant role in influencing both community composition and invasion vulnerability. Here, no single producer was found to dominate the large system. Strong priority effects were found in many sequences such that the initial producer introduced remained dominant throughout the experiment. In other sequences, though, the initial producer had no such advantage and was quickly displaced by the next producer introduced. Consistent with the findings of Robinson and Edgemon (1988), the timing as well as the sequence of invasions played a key role in influencing community structure. For example, *Ankistrodesmus* and *Selenastrum* were the first and second producers introduced, respectively, in two of the sequence permutations. A*nkistrodesmus* lost dominance to *Selenastrum* when the introductions were separated by 15 days, but remained dominant when separated by 30 days. Vulnerability to invasions by consumers was highly variable among assembled communities – some were readily invaded, while others were resistant to all consumers. Colonization success among consumers was also highly variable with only a single species successfully invading all treatments. Additional experiments were conducted with 10-day intervals on selected treatments with no significant change in results.

Drake et al. (1993) constructed replicate landscapes of interconnected aquatic microcosms to test the influence of sequence effects and dispersion on landscape properties (see also Zimmermann et al. 2002). Each landscape consisted of a triangular array of eleven patch microcosms with a single corner patch serving as the point of entry for all new species. Into each entry patch, communities were assembled according to a single sequence permutation found in previous experiments to reliably reproduce a single

community state. Concomitant with such introductions, aliquots of suspended organisms were transferred between adjacent patches along pathways emanating from the entry patch. They found that all entry patch communities converged to the same state. Species composition amongst the remainder of the landscape, however, converged to one of several alternative states with variance in composition between landscapes increasing with distance from the entry patch (but see also Grover and Lawton 1994; Drake et al. 1994).

Huxel (1995) explored the interaction of sequence effects with nutrient supply on the competitive interactions among four species of green algae species – A*nkistrodesmus, Scenedesmus, Selenastrum,* and *Chlamydomonas*. Three invasion factors were considered: 1) invasion order was tested with four sequence permutations with each species as an initial invader, 2) invasion rate was tested with simultaneous introductions and invasion intervals of 10 and 15 days, and 3) invader founder abundance was tested at low and high densities. At low founder abundance, *Scenedesmus* quickly become dominant among all simultaneous and 10 day interval treatments. All competing species became rare or went extinct. When the invasion interval was increased to 15 days, however, the initial invader always became dominant with the exception of *Chlamydomonas* (c.f. Drake 1991). At high founder abundance, no change was observed in either simultaneous or 10 day interval invasion rates. With 15 day intervals, however, all species became dominant when the initial invader. These results were obtained in microcosms without media replenishment. To test the effect of continuous nutrient levels on competitive interactions, two sequences – *Chlamydomonas*-first and *Ankistrodesmus*-first – were repeated in a continuous flow scenario with 10-day invasion intervals.

Scenedesmus had been found to always dominate under these invasion scenarios without media replenishment. This was again observed in the *Chlamydomonas*-first sequence. In contrast, codominance among *Scenedesmus, Selenastrum,* and *Chlamydomonas* was observed *in* the *Ankistrodesmus*-first sequence. Huxel (1995) was, therefore, able to demonstrate how variation in invasion parameters and abiotic conditions can interact to alter competitive dominance hierarchies.

Flum (1997) assembled lotic periphyton communities in artificial streams to examine the role of invasion history on species composition, biomass accumulation, and water chemistry. The species pool consisted of eight species of green algae and four species each of cyanobacteria and diatoms. For purposes of introduction, species were arranged in groups of four by taxa. Three sequence permutations were then designed such that each taxa constituted an initial introduction – i.e. diatoms first, green algae first, cyanobacteria first. Divergent trajectories in community composition with attendant changes in biomass and water chemistry were found midway through the experiment. By the conclusion of the experiment, however, communities assembled under all sequence were observed to converge to nearly identical species compositions. Notably, however, biomass and water chemistry properties remained divergent over the complete experiment. This suggests that the influence of sequence effects on ecosystem properties can persist beyond the point at which similar effects on species composition have faded (Patten and Witkamp 1967). Subsequent experiments to test the effect of differences emergent at the ecosystem level on subsequent community invasibility were not explored.

2.5 Synthesis: Colonization History and Community Phenomenon

Collectively, the theoretical and experimental studies in community assembly revealed

a number of related community phenomena arising from manipulations of colonization history. Here, I synthesize and contrast relevant findings. Specifically, I address 1) invasion resistance, 2) alternative stable states, 3) community cycles, 4) humpty-dumpty effects, and 5) deterministic and indeterministic assembly trajectories. Figure 2.2[3] provides a graphical representation of these various phenomena.

2.5.1 Invasion Resistance

Elton (1958) predicted that complex communities are more resistance to invasion. His predictions were based in part on anecdotal observations that species-poor systems such as islands or crop monocultures are more vulnerable to invasion than species-rich systems. Theoretical support for his hypothesis arose in a number of theoretical assembly studies (e.g. Post and Pimm 1983; Drake 1990a; Law and Morton 1993, 1996; Luh and Pimm 1993; Kokkoris et al. 1999). Post and Pimm (1983) found that sequential invasions led to a linear increase in resistance with time As novel species were randomly generated with each invasion step, however, complete invasion resistance was not attained When communities were assembled from finite species pools on the other hand, the assembly process culminated in community states that were resistant to invasion by some proportion of the remaining species pool (Drake 1990a; Law and Morton 1993, 1996; Morton and Law 1997; Kokkoris et al. 1999). Among these studies, the following trends can be drawn: 1) richness of final community state increases with species pool size but approaches saturation (Law and Morton 1997), 2) the proportion of the excluded species pool members therefore increases with species pool size (Law and Morton 1993, 1996; Morton and Law 1997), 3) communities assembled from smaller

[3] All figures are located in Appendix A.

species pools reached invasion resistance quicker than larger pools (Drake 1990a; Law and Morton 1993, 1996; Morton and Law 1997), and 4) mature communities – as defined by the total number of invasion attempts – are more resistant than younger communities of the same species richness (Post and Pimm 1983; Law and Morton 1993, 1996; Morton and Law 1997). The development of invasion resistance can, therefore, be viewed as an iterative process of trial and error in which community structures are continuously tested by invasions until a stable invasion resistant solution is attained. This process is characterized by an asymptotic accrual of species and decreasing turnover. The time required to an invasion resistant solution is shorter in species poor systems, but the degree of invasion resistance – in terms of proportion of the excluded pool members – is small. Consequently, invasion resistance is tenuous and more readily invaded by exotic species. In contrast, the time to invasion resistance is longer in species-rich systems, but the subset of excluded pool members increases with species pool size. Such systems should, therefore, display resistance to broader range of exotic invaders. From this it can be seen that invasion resistance is not a simple correlate of community richness, but rather a complex function of regional species pool richness and time. The more species and time that assembly has to play with, the more likely an invasion resistant state can be found.

The development of invasion resistance in the preceding studies was contingent on a number of strict assumptions concerning the nature of invasions. Invaders are assumed to arrive singularly, at low relative abundance, and at time intervals slow relative to the time for a return to an asymptotic state. Relaxing any of these assumptions, however, can subvert the development of invasion resistance (Huxel 1995; Lockwood 1997;

Büssenschütt and Pahl-Wostl 1999). Elton (1958) recognized that communities likely fall on a gradient of invasion resistance contingent on the their relative openness to invasion. Viewed collectively, the various assumptions used address a varying degree of community openness. The assumptions of Post and Pimm (1983), Drake (1988, 1990a), and Kokkoris et al. (1999) are consistent with the assembly in relatively closed systems in which invaders arrive at a trickle such as remote islands or highly insular habitats. Relaxing these assumptions open the community to a greater influence by invading species. Perhaps the most extreme of the assumptions made are that *all* species either arrive in continuous waves of many species (Büssenschütt and Pahl-Wostl 1999) or at high founder abundance (Huxel 1995). These assumptions would seem unrealistic for most natural systems. They may, however, be a somewhat fairer reflection of cases of human mediated introductions in marine communities in which entire assemblages are often transported such as wooden ship hullfouling or ballast water release in estuarine ecosystems (Carlton 1989; Carlton and Geller 1993).

Evidence of invasion resistance arising in assembled communities was also found in experimental studies. Robinson and Dickerson (1985) found that invader success was variable between communities, but did not find support for Elton's (1958) prediction that resistance was correlated with community complexity as measured by species richness. Rather, they attributed colonizer success to the presence of particular species in the recipient communities. The use of species richness as a surrogate measure of community complexity would seem problematic. An examination of their results also reveal tentative evidence that the mean persistence time of successful invaders was greater among communities assembled under high invasion rates. Such findings, therefore,

would not be inconsistent with theoretical predictions that invasibility increases with invasion frequency (Lockwood et al. 1997). Moreover, Elton's (1958) hypothesis does not address the probability of success for any single invader, but rather the mean probability of many invasions. As Robinson and Dickerson (1984) only considered three invaders, this would seem a weak test. Drake (1991) also found varying degrees of resistance to consumer invasion resulting from manipulations of invasion sequence. Some communities were readily invasible, while others were completely resistant. Correlations of resistance with measures of community complexity, however, were not made and changes in invasion rate had no apparent effect in these systems.

2.5.2 Alternative Stable States

The emergence of alternative community states as a consequence of variation in colonization history was shown in both theoretical (e.g. Drake 1990a; Law and Morton 1993, 1996; Luh and Pimm 1993; Kokkoris et al. 1999) and experimental studies (Robinson and Dickerson 1987; Robinson and Edgemon 1988; Drake 1991; Huxel 1995). Among theoretical demonstrations, the emergence of alternative stable states and invasion resistance are necessarily connected. The strict assumptions under which invasion resistance were found, therefore apply (see Section 2.4.1). Relaxing these assumptions, consequently, precluded their formation (e.g. Huxel 1995; Lockwood 1997; Büssenschütt and Pahl-Wostl 1999). Huxel (1995), however, observed that distinct community trajectories nonetheless emerged from differences in invasion order when invasions either occurred in pairs or at high founder abundance. Among experimental studies, the influence of invasion rate on mediating sequence effects was variable. Consistent with theoretical predictions, Robinson and Edgemon (1988) found that effect

of invasion sequence on community composition was greatest at low rates of invasion. Paradoxically, Robinson and Dickerson's (1987) findings indicated the reverse; the influence of sequence effects on both species richness and community membership was most significant at high rates of invasion. The reason for this contradiction is not clear. Drake (1991) found that the invasion rate had no influence in his systems, but Huxel (1995) found that the same invasion rates did influence competitive hierarchies among a similar set of species. These studies have also shown that differences in habitat quality – such as light availability or nutrient supply – can interact with sequence effects to alter community structure (Dickerson and Robinson 1985; Drake 1991; Huxel 1995). Here conditions of high productivity were found to favor dominant competitors regardless of invasion order. Such findings are consistent with theoretical predictions (e.g. Tilman 1982).

Theoretical predictions on the prevalence of alternative stable states in natural systems in nature vary. Drake (1990a) suggested that alternative states could be a common consequence of differences in invasion history with their likelihood increasing with species pool size. Luh and Pimm (1993) also concluded that number of alternative states is positively correlated with the size of the species pool when assumptions concerning the implausibility of simple community cycles were made. Law and Morton (1993, 1996) predictions, however, were more conservative. They concluded that most species pools probably possess a single invasion resistant state. Situations with two or more alternative states were considered most likely in species-rich systems possessing high connectance. They offer that marine communities may, therefore, be prime candidates for alternative states. Their prediction on the relative scarcity of alternative states must be made with

the caveat that the permanence stability criterion they used does not recognize alternative states of the same species at different relative abundances. Knowlton (1992) offers a theoretical demonstration of how such states could arise in marine ecosystems. If the occurrence of these states can be conclusively demonstrated in natural systems, their estimate would be correspondingly low. Evidence of alternative states in natural systems would seem to support a conservative estimate of their prevalence. Historically the number of case studies reporting such states has been relatively low. Moreover, such cases generally only indicate two possible states. Whether this is a fair representation of nature is uncertain. A significant increase in the number of reported claims over the last decade, however, would suggest not.

2.5.3 Community Cycles

Drake (1990a) and Law and Morton (1993, 1996) found rare occurrences of invasion sequences resulting in persistent community cycles wherein a community continuously oscillated between two or more discrete states. Luh and Pimm (1993) only found evidence of simple ecologically implausible cycles, but conceded that more plausible cycles were possible. A community cycle is formed when each state in the cycle remains vulnerable to invasion by one or more species. Invasion by these species cause the community to move to a different state with its own unique vulnerabilities. A continuous loop is formed when trajectory returns to the initial state and the process repeats. These cycles can be entirely deterministic or stochastic depending on the number of species with which each state is vulnerable (Law and Morton 1993). Evidence of community cycles in nature has been scanty, but has been implicated as causal mechanisms underlying dynamics observed in heathlands (Gimingham 1988) and in lizard populations

of Caribbean islands (Roughgarden 1989).

2.5.4 Humpty-Dumpty Effects

Theoretical studies have found that community endpoints were commonly unreachable without traversing pathways of one or more intermediate states that included transient species not represented in the final community (Drake 1985, 1990a: Luh and Pimm 1993; Law and Morton 1993; 1996). Reassembling the community using only species extant in the final state is not possible. These transient species, therefore, play a critical role in *catalyzing* the outcome (Pimm 1991). Pimm (1991) termed such phenomenon *humpty-dumpty* effects. These findings have potentially serious implications for biological conservation in lieu of the increasing rate of global extinctions. The loss of key *catalyzing* species could spell doom for many communities. Similarly, Lundberg et al. (2000) found that when species were randomly removed from assembled food web, reinvasion by the same species was not always possible. Humpty-dumpty effects also have obvious ramifications for reconstructing damaged ecosystems in that knowledge of these transient community states may be essential for their successful restoration (Luh and Pimm 1993).

2.5.5 Deterministic Versus Indeterministic Assembly Trajectories

Sequence effects are inherently deterministic in theoretical settings. All else being equal, the same order of invasions will always lead to the same community state (Drake 1985,1990a: Luh and Pimm 1993; Law and Morton 1993; 1996). Under experimental conditions, however, Drake (1991) found that such determinism was variable. Some sequences repeatedly led to the same community state with the same composition and invasion vulnerabilities. Other sequences, however, behaved indeterministically in that a

diversity of community states were produced. Such cases may be characterized by the presence of multiple attractors regulating community formation that arise due a heightened sensitivity to subtle variation in environmental or demographic factors. Sequence effects may, therefore, have differential capacities to either dampen or amplify sources of noise. An understanding of such effects arise could contribute to theory of a biological diversity.

2.6 Future Directions in Community Assembly Theory

The various theoretical and experimental studies discussed here have shown that the effect of colonization history can have strong impacts on emergent community dynamics and structure. The scenarios under which such effects have been demonstrated, however, have been relatively limited and many questions still remain unanswered. Most importantly is *when* does history matter? That is, under what conditions are the effects of invasion history most likely to be important? I conclude by offering some possible directions that theoretical and experimental studies could undertake. This list is not meant to be exhaustive. Moreover, these suggestions are motivated by the potential questions they could answer and not by the inherent logistical constraints involved. Some directions, therefore, may be more easily implemented than others.

2.6.1 Modeling Approaches

2.6.1.1 Phenological and Dispersal Constraints

Assembly models have assumed that any species can invade at any time with equal probability and at equal founder abundance. Species, however, clearly demonstrate differences in seasonal patterns of abundance and dispersal abilities (Morin 1999a; Young et al. 2001). Incorporating phenological parameters into species pool

characteristics would delimit the set of potential colonists available at any given invasion step. The addition of dispersal parameters would also allow species to invade at different founder abundance. How would assembly effects play out under such a changing cast of players?

2.6.1.2 Spatial Extension

The effect of the spatial heterogeneity on mediating species coexistence has been shown in a number of studies (Huffaker 1958; Shorrocks and Roswell 1987). The Lotka-Volterra models typically used in theoretical assembly studies, however, assume a mean-field approach. Individuals are assumed to influence one another in proportion to their relative abundance across space (Huston et al. 1988; Judson 1994). Such assumptions provide good approximations of community behavior when organisms are highly mixed or interact over long distances, but break down when such conditions are not met (Law et al. 2000). Current individually-based, spatially explicit modeling techniques (e.g. DeAngelis and Gross 1992; Judson 1994), however, allow the exploration of community dynamics wherein species only interact with a local neighborhood. Incorporating such models into a community assembly approach could, therefore, investigate how invasion sequence effects emerge across time and space. Invasive species once excluded under traditional approaches could find safe harbor when space is considered and thus alter the nature of invasion resistance.

2.6.1.3 Evolution

Community assembly models address the development of community structure over ecological time scales. Fixed species pools are typically generated *a priori* under presumably realistic assumptions concerning trophic interactions. Moreover, species are

considered immutable and do not evolve. Other modeling approaches have addressed the coevolution of species within communities, but don't address the role of assembly effects in structuring assemblages in ecological time (e.g. Hraber and Milne 1997; Caldarelli et al. 1998). By combining these approaches, however, the nature of community assembly processes could be explored in evolutionary time. Sequence effects would delimit which sets of species can coevolve at any given time. Assembly mediated evolutionary changes to the species pool could then alter the spectrum of possible community configurations. Communities once invasion resistant could subsequently become open to new invasions through evolutionary changes brought about within the community itself and/or changes in previously excluded members. How does the prevalence of invasion resistance and alternative community states change over time with an evolving species pool?

2.6.1.4 Critical Dynamics

Theoretical and empirical studies suggest that ecological systems may self-organize to critical states (Solé and Manrubia 1995; Ellner and Turchin 1995; Keitt and Marquet 1996; Keitt and Stanley 1998; Solé et al 1999). To explore such dynamics, McKane et al. (2000) developed a community model of interacting species in which species turnover was driven by species interaction and immigration (see also Solé et al. 2000). They found that at high connectance and low immigration rates, communities reached a critical state characterized by multiple metastable (i.e. long lived transient) states and power-law distributions in species turnover. Theoretical assembly studies have also suggested that alternative states are most probable in systems where connectance is high and immigration rates are low (Law and Morton 1993, 1996). Thus, these same systems may be prime candidates for critical dynamics. The maintenance of critical states, however,

requires a constant immigration of species as a driving force. Stop the flow of immigration and the system will collapse to a subcritical state. As immigration in the Mckane (2000) model was assumed to occur at a fixed rate, the development of invasion resistance was precluded by definition. How a theory of alternative states can be reconciled with critical dynamics is not clear. Two alternatives seem possible: 1) if complete invasion resistance is required for their persistence, then alternative states are necessarily subcritical, or 2) alternative states are metastable, critical systems characterized by a higher proportion of transient species. Further assembly studies could address these possibilities.

2.6.2 Experimental Approaches

Microcosms offer a powerful tool for bridging theory and field studies and have been increasingly used in recent years to address a variety of ecological questions (e.g. Warren 1996; Burkey 1997; Morin 1999b; Fox and Olsen 2000; Petchey 2000). Many of these experiments "assembled" communities in the trivial sense that prey introductions preceded predators. The use of microcosms for formal tests of community assembly, on the other hand, has virtually stopped. This situation is puzzling given the clear influence colonization history was found to have on experimental outcomes. Here, I propose some avenues of potential exploration that further experiments into community assembly could undertake. Some of these are logical extensions of experiments already performed, while others remain untried.

2.6.2.1 Alternative Model Systems

Community assembly in laboratory microcosms has been limited to aquatic systems and to what degree experimental findings are system dependent is unknown.

Demonstration of assembly effects in other system types would, therefore, be valuable. An obvious alternative would be soil system (Verhoef 1996). Such systems are relatively easy to maintain and traditional censusing methods coupled with advancing molecular techniques offer researchers a variety of tools for tracking community changes. The assembly of terrestrial plant communities in either growth chambers or greenhouses might also be possible on a limited scale.

2.6.2.2 Variation in Environmental Factors

Experimental studies have shown that sequence effects can interact with environmental factors to influence resulting community structure (e.g. Dickerson and Robinson 1985; Drake 1991; Huxel 1995) Dickerson and Robinson (1985) and Drake (1991), for example, observed that influence of invasion sequence mediated by scale related arising from microcosm container geometry. These findings are consistent with other microcosm studies in which scaling effects have been also shown to effect experimental results (Wynn and Paradise 2002; see also Peterson et al. 1999). Huxel (1995) also found that competitive hierarchies differed when assembled under alternative nutrient regimes. The evaluation of such factors, however, has been limited to only levels of variation. A more comprehensive understanding of such interactions could be gained by assembling communities on gradients of one or more environmental factors.

2.6.2.3 Disturbance

Disturbance has been demonstrated to have an influential role in altering succession processes and resulting community structure (Huston 1979; Sousa 1984; Pickett and White 1985). Community assembly experiments could explore the effect of disturbance on invasion history by assembling communities either under fluctuating environmental

conditions or through imposed perturbations of varying intensity and frequency. Highly intense or frequent disturbance would likely diminish historical effects. The effect of less frequent or more moderately intense disturbance is unclear. Disturbance of intermediate frequency has been shown to increase species coexistence (Huston 1979) and could therefore enhance sequence effects. Moreover, Fukami (2001) found that when laboratory treehole communities were subjected to a series of repeated disturbances of two types (i.e. drought, mosquito invasion), altering the sequence of disturbance events resulted in changes to community structure. Combining Fukami's approach with that of traditional assembly experiments, would allow the exploration of the interaction of invasion history with disturbance history.

2.6.2.4 Assembly Landscapes

While assembly landscapes of the type constructed by Drake et al. (1993) would seem a natural approach for exploring the influence of invasion history on interconnected community patches, further experiments of this type have not been performed. Similar microcosm landscapes have been recently used to address questions of metapopulation and metacommunity processes on fragmented landscapes (e.g. Warren 1996; Burkey 1997), but such experiments did not consider the effects of colonization historical effects. Landscape assembly approaches could explore the interaction of invasion history and interpatch dispersal on community structure wherein the following factors could be tested: 1) immigration sequence, rate, and source (i.e. multiple species pools), 2) interpatch dispersal rate and distance, 3) patch size and quality, and 4) landscape connectivity.

Chapter 3:
A Test of Invasion Sequence Effects on Community Structure and Invasibility in Soil Microbial Microcosms

This chapter is a revised and expanded version of the following paper:

Zimmermann, C. R., G. S. Sayler, and J. A. Drake. 2001. Community self-organization, alternate community states, and the introduction success of a bioremediative soil microorganism. In: *Understanding Complexity: Proceedings of the 2000 World Congress of the System Sciences* (J. Wilby and g. Ragsdell, eds.), Kluwer Academic Press, London.

My contributions consisted of the following: 1) design of the experimental, 2) microcosm construction and preparation, 3) growth and introduction of microbial strains, 4) sampling and quantification of cell abundance, 5) data analysis and presentation, and 6) writing. My coauthors contribution to this study consisted of financial funding for the project and some technical assistance with the experimental design.

3.1 Introduction

Case studies in natural ecosystems have implicated variation in the history of colonization as a causal mechanism for observed differences in community structure (e.g. Sale 1977; Sutherland and Karlson 1977; Wood and del Moral 1987; Fastie 1995). Controlled laboratory (Gause 1934; Park et al. 1948; Tilman 1977; Gilpin et al. 1986) and field experiments (Paine 1977; Alford and Wilbur 1985) have demonstrated that manipulating the introduction sequence or initial species densities can affect experimental outcomes. Such studies, however, have typically limited the focus of study on two to three species. Research in community assembly processes has evaluated the effect of colonization history by constructing more complex communities through iterative species invasions. A number of theoretical studies, for example, have demonstrated that

permuting the order of species invasions can lead to alternative invasion resistant states (Drake 1990b; Law and Morton 1993, 1996; Luh and Pimm 1993; Kokkoris et al. 1999). Experimental microcosm studies have also shown that invasion sequence effects can alter subsequent community structure and invasion vulnerability (Robinson and Dickerson 1987; Robinson and Edgemon 1988; Drake 1991; Huxel 1995). Laboratory studies taking this latter approach, however, have been limited to aquatic systems. To what degree the influence of sequence effects are system dependent, therefore, is unclear. Here, I test the effect of variation in invasion sequence on community structure and invasibility in a soil microcosm setting within a bioremediation context.

3.1.1 Introduced Species and Bioremediation

A promising, cost effective biotechnological application for the amelioration of environmental contaminants utilizes the capacity of microbial agents to degrade such pollutants to less toxic forms (Bouwer 1992; Forsythe et al. 1995). Where indigenous populations of desirable catabolic genotypes are absent, exogenous organisms can be deployed to implement the bioremediation process (Sayler et al. 1997). In line with this application, prototype remediating organisms are being developed through genetic engineering of natural bacterial strains. A tacit assumption of such an approach is that the remediating organism not only successfully invade the target community, but also persist at a sufficient abundance to insure long term efficacy of cleanup process. While many introduction attempts succeed, however, most have failed (Sayler et al. 1997). As the mechanisms underlying such failures are often complex and system specific, generalizations are difficult. As I have shown, theoretical and experimental studies have demonstrated that differences in history of species colonization can differentially impact

a community invasion vulnerability. To what degree such effects impact soil communities is unknown.

3.1.2 A Community Assembly Experiment in Soil Microcosms

To the best of my knowledge, experiments explicitly testing community assembly theory have not been performed with either bacteria or in soil microbial ecosystems. Experimental results congruent with previous findings would, therefore, add weight to accumulating evidence drawn from different system types that invasion history can play a fundamental role in the altering community structure. Moreover, as successful species colonization is an essential prerequisite for remediative applications where the deployment of exogenous species is involved, the influence of colonization history on invasion vulnerability in soil communities could have important ramifications for effective bioremediation. Here, I perform a community assembly experiment in which microbial communities were assembled by augmenting an existing soil community with sequential introductions of three hydrocarbon degrading strains under alternative sequence permutations. The invasion vulnerability of each assembled community was then tested with the introduction of a bioluminescent reporter bacterium, *Pseudomonas fluorescens* HK44.

P. fluorescens HK44 is the first genetically engineered strain approved for field testing in the United States (Sayler et al. 1995). It carries a transcriptional nahG-luxCDABE fusion (plasmid pUTK21) for naphthalene and salicylate catabolism (King et al. 1990). Exposure to either compound induces the production of visible light. Such a bioluminescent signal could have utility for both the detection of naphthalene deposits and for continuous online monitoring and control of the remediation process. While *P.*

fluorescens HK44 has been shown to successfully colonize a variety of soil types in both microcosm and mesocosm settings (Matrubutham et al. 1997; Zimmermann et al. 1997, 1998; Ripp et al. 1999), what role invasion history could play in a field application remains unexplored.

I test the following hypotheses: 1) Permuting the introduction sequence of competing microbial species can produce alternative community states, and 2) The capacity of a genetically engineered microorganism to successfully colonize a competitive soil environment can be affected by the invasion history associated with the recipient community.

3.2 Methods and Materials

3.2.1 Experimental Design

Three sets of recipient soil communities were assembled by sequential introduction of three competing strains chosen from distinct genera known to mediate transformation of polyaromatic hydrocarbons: *Sphingomonas* (Frederickson et al. 1991), *Mycobacterium* (Boldrin et al. 1993; Heitkamp et al. 1998), and *Rhodococcus* (Walter et al. 1991). The specific strains used were S*phingomonas* sp. A8AN3, *Mycobacterium* sp. Pyr-1, and *Rhodococcus* sp. Sm-1. Previous competition experiments in our lab involving *P. fluorescens* HK44 and these three species found that coexistence of at least three of these species was possible when introductions were made simultaneously (Zimmermann et al. 1997, 1998). Whereas six sequence permutations are possible given a pool of three species, only three were explored for this study. See Table 3.1 for the specific sequences used. These sequences were chosen such that each competitor was represented at a different invasion step in each sequence. *P. fluorescens* HK44 was the last introduction in

each sequence and introductions occurred at 28 day intervals. Following completion of the introduction phase, the microcosms were allowed to equilibrate for 136 days and were periodically monitored for abundance of study strains and indigenous heterotrophic populations. A noninoculated control treatment was included for reference. All four treatments were replicated three times.

3.2.2 Microcosm Design and Preparation

Microcosm containers were constructed from 8.5 OD Schedule-40 PVC pipe cut to a total length of 18". For drainage, an 8" diameter plastic funnel was secured to the inside of the container and sealed with aquarium-grade silicone sealant. Containers were equipped with an airtight lids to minimize contamination. Each lid was fitted with an input port for irrigation and separate input and exhaust ports for aeration. Microcosms were irrigated periodically with a sterile minimal salts media to maintain a 15-18% soil moisture content. A rotating sprinkler head on the interior side of the irrigation port insured a nonintrusive and even distribution of water. Drainage from each microcosm was captured in a airtight bottle to minimize cross contamination. Air inputs were charcoal filtered and metered to a flow rate of 0.25 l min^{-1}. Bacterial air vents (pore size: 1.0 μm) were installed on both aeration ports to prevent microbial contamination and/or escape. See Figure 3.1 for a cross sectional schematic of the container design.

Each container was filled with a stratified bed consisting of a bottom gravel layer, a middle clean sand layer and an upper soil layer. The soil used was a Huntington loam consisting of 42% sand, 40% silt, 18% clay and 1.3% organic matter. A fixed porous partition was installed between the soil and bottom layers to prevent mixing of these layers. All fill materials were autoclaved for one hour prior to installation. My intent in

autoclaving the soil component was not to kill indigenous fauna, but to reduce their level to enhance colonizer success. Plate assays of the soil indigenous populations taken on the following day revealed cell densities of 10^3 cells g^{-1} soil. Following autoclaving, the soil was amended with a mixture of naphthalene and anthracene. While *P. fluorescens* HK44 is only capable of complete degradation of naphthalene, anthracene was added to create a more heterogeneous mixture typical of contaminated soils. These compounds were dissolved in Exxon Univolt 60 transformer oil – also representative of environmental contaminants – and then incorporated into the soil in bulk and mixed overnight in a rotating drum. Assuming uniform distribution of oil in the soil, an approximate loading capacity of 1000 g and 100 mg kg^{-1} of soil for naphthalene and anthracene, respectively, was produced. Fifteen hundred cubic centimeters of contaminated soil were then distributed to each containers. Microcosms were maintained at 27º C in a dark, insulated chamber during the study. Placement of containers within the chamber was randomized.

3.2.3 Species Introduction Protocol

All strains were grown in liquid batch culture at 30º C in a shaking incubator using a yeast extract-peptone-glucose (YEPG) growth media. Prior to the start of the experiment, growth curves relating the optical density of cell cultures with time were developed for each strain. Optical density was then correlated with cell abundance based on serial dilution plate cultures. Based on this information, an appropriate volume of cells was drawn from liquid culture to inoculate each recipient microcosm with an initial cell density of 10^6 cells g^{-1} of soil dry weight. All cells were drawn from log-growth phase to insure high cell vitality. The initial cell density of 10^6 cell g^{-1} was chosen so as to be

sufficient to promote colonization, but not so great as to overwhelm the recipient community. Cells were harvested in a continuous centrifuge and the resulting cell paste resuspended in 300 ml of fresh YEPG media. This suspension was then thoroughly incorporated by hand into the soil layer of the recipient microcosm. Control microcosms received 300 ml of sterile YEPG media.

3.2.4 Soil Sampling and Population Counts

Soil samples for enumeration of cell abundance were taken at 14, 35, 63 and 136 days following the completion of all introductions. Three soil cores, 8 mm in diameter and traversing the entire soil profile, were taken from each microcosm and thoroughly homogenized. From this mixture, a single 1-gram subsample was drawn, suspended in 0.9% saline, and vigorously vortexed for 1 minute to detach the bacterial cells from soil particles. Serial dilutions were then prepared in saline solution and plated in triplicate. A comprehensive regime of selective and nonselective plating was used for isolation and enumeration of the study strains and indigenous populations. Table 3.2 provides a description of plating media used. A general profile of heterotrophic populations was taken using a nonselective YEPG agar plate. *P. fluorescens* HK44 was isolated with a yeast extract/peptone/sodium salicylate (YEPSS) agar plate containing 14 mg l^{-1} tetracycline. As assays of uninoculated soil revealed no indigenous tetracycline-resistant strains, this approach was deemed sufficient for quantification of this strain. A regimen of selective media was developed for the three competitor strains based on plating trials of cells grown in liquid batch culture (Table 3.2). Plates were incubated at 27º C in the dark for 4 days and those containing between 30 and 300 colonies were counted. Heterotrophic populations were quantified based on expressed morphology as a measure

of taxonomic diversity.

3.2.5 Analytical Techniques

Statistical analyses consisted of both standard methods and multivariate analysis. Multivariate analysis was performed on log-transformed data. Hierarchical cluster analysis was performed using the CLUSTR program with Ward's linkage methods and Euclidean distance measure options. Detrended correspondence analysis (DCA) ordination was performed with default options with a modified version of the DECORANA program from the Cornell Ecology Program series (Hill 1979). MRPP group comparisons were performed with recommended options using a program adapted by B. McCune and J. Poston from a subroutine written by P. W. Mielke and K. J. Berry (see Mielke and Berry 1982). All multivariate analyses were performed with the *PC-ORD Version 4.0* software package (McCune and Mefford 1999).

3.3 Results

3.3.1 Heterotrophic Populations

3.3.1.1 Descriptive Community Parameters for Final Sampling Date

The heterotrophic population profile is a nonselective assay of platable resident strains and potentially includes both study strains and populations indigenous to the soil community prior to controlled introductions. Four morphological types were observed among the populations across treatments over the course of sampling. Table 3.3 provides a description of these types. Only Types 1 and 2 were found among control replicates. Type 3 showed temporal variability among replicates of all sequence treatments, but was present in all replicates at the conclusion of sampling. Notably, Type 4 was only found among all replicates of Sequence A systems from Day 35 to Day 136. Using

heterotrophic census data, the following community descriptive parameters were calculated for each community replicate by sampling date: 1) total cell abundance, 2) species richness, 3) Shannon diversity, and 4) species equitability. Morphological types were used in place of true species. Figures 3.2-3.5 show time series of each parameter by treatment. Table 3.4 gives for a tabular summary of all parameters by treatment and replicate for the final sampling date. An examination of the time series figures reveals apparent differences between treatments for all four parameters emerging over time. Differences in species richness between treatments for the final sampling date were distinct and without variation between replicates. Variation in the remaining three parameters, however, was exhibited between treatment replicates. Analysis of variance tests were therefore performed to assess the treatment effects on these three parameters for the final sampling date (Table 3.5a-3.7a). Analyses indicated that for an α=0.05, treatment effects were significant for all parameters. Tukey HSD[4] post hoc tests were subsequently conducted to test for pairwise differences between treatments (Table 3.5b-3.7b). Table 3.8 provides a summary of pairwise treatment comparisons for all four community parameters. While differences between treatments were variable depending on the particular parameter, all treatments exhibited differences in at least two parameters. Communities assembled under Sequence A were consistently the most distinct in comparison with the remaining three treatments.

3.3.1.2 Cluster Analysis of Census Data for Final Sampling Date

Hierarchical cluster analysis was performed on heterotrophic census data for the final sampling date (Figure 3.6). Analysis revealed a high degree correspondence of replicates

[4] Honestly Significant Difference

within treatment with complete linkage of all treatments occurring with >99% of information still remaining. Communities assembled under Sequence B and Sequence C showed the strongest similarity between treatments with linkage between these groups occurring with approximately 97% information remaining. Sequence A exhibited the greatest dissimilarity to the remaining treatments. Complete linkage between this and the remaining three treatments did not occur until all information was exhausted.

3.3.1.3 Community Ordination of Census Data for Final Sampling Date

Detrended correspondence analysis (DCA) of community composition was performed for the final sampling date. Figure 3.7 shows the arrangement of communities for the first two ordination axes. The percent variance explained by the first two axes was 0.922 and 0.076. Three morphological types showed a strong positive correlation with the first axis: Type 2 (r=0.899), Type 3 (r=0.871) and Type 4 (r=0.837). Note the strong similarity between communities assembled under Sequences B and C.

3.3.1.4 MRPP Analysis of Community Composition by Sampling Date

To assess the difference in community composition between treatments over the entire sampling period, MRPP group comparisons were performed on census data grouped by treatment for each sampling date. Figure 3.8 shows a time series of results. Plotted values include the probability that the observed differences in treatments can be attributed to chance alone (p) and the within-treatment agreement (A). Within-treatment agreement (A) is a measure of homogeneity within treatments and ranges between values of 0-1. A value of 1 would indicate identical members within treatments. Results indicate a highly significant difference between treatments for the entire period with p-values ranging between 0.0003 (Day 136) to 0.0056 (Day 35). Agreement within treatments declined

over Day 14 to Day 63 indicating of a divergence in composition within treatments during this period. This was followed, however, by a sharp convergence within treatments over the last 73 days. Agreement within treatments reached a peak value on the final sampling date with a value a 0.875.

3.3.2 Population Dynamics of *P. fluorescens* HK44

Figure 3.9 shows a time series of HK44 population dynamics over the 136 day equilibration period. HK44 successfully colonized all replicates of communities assembled under Sequences A and C. It was, however, completely absent in all replicates of Sequence B for the full sampling period. Contrasting dynamics in cell abundance, however, were expressed between the two sequence treatments in which HK44 was successful. Mean population abundance for Day 14 was nearly three orders of magnitude greater among replicates assembled under Sequence A than those assembled under Sequence C. Abundance values among Sequence A replicates subsequently displayed a rapid decline over the post-introduction period. Abundance among Sequence B replicates, on the other hand, exhibited an approximately linear increase over the same time period. A two-factor analysis of variance test was performed on the effect of treatment and sampling date on HK44 abundance (Table 3.9a). For an α=0.05, treatment and interaction effects were significant. A Tukey HSD post hoc test was then conducted to determine the significance of pairwise comparisons between treatments by sampling date (Table 3.9b). Significant differences were found for Day 14 and Day 35, but were insignificant over the last two sampling dates.

3.3.3 Population Dynamics of *Sphingomonas* A8AN3, *Mycobacterium* Pyr-1, and *Rhodococcus* Sm-1

The selective plating regime used for isolation of the three competing strains failed to find any significant growth of these species. Failure to detect these species, however, does not preclude their occurrence. The choice of selective media was based on plate culture trials using strains grown in liquid batch culture. Isolation of bacterial from soil colloids, however, is difficult and the added stress involved with the selective media could have arrested growth. In addition, cell morphology consistent with *Sphingomonas* A8AN3 and *Mycobacterium* Pyr-1 were found among heterotrophic population counts. Previous competition experiments involving these same strains found conclusive evidence of coexistence of at least three of these four species where introductions were made simultaneously (Zimmermann et al. 1997; Zimmermann et al. 1998). A different plating schedule, however, was used in this these studies.

3.4 Discussion

A series of analyses on heterotrophic sampling data were undertaken to elucidate the effect of experimental treatments on community composition. All results indicated that manipulations of invasion sequence had a strong influence on microbial community structure. Differences between treatments were found in four descriptive parameters for the final community state: 1) species richness, 2) total cell abundance, 3) Shannon diversity, and 4) species equitability. While the specific differences between treatments were variable, all treatments were found to differ in two or more parameters. Sequence A communities were the most distinct having the highest mean values for species richness, total cell abundance, and species diversity. Notably, of the four morphological types

observed across treatments, a single type was only found among Sequence A communities. As this type did not match the morphology of any of the study strains, it represents a strain indigenous to the soil community prior to introductions.

Cluster analysis and community ordination of final compositional values indicated a high degree of similarity within treatments as well as distinct differences between treatments. A strong similarity between Sequence B and Sequence C communities was indicated in both analyses. Moreover, MRPP analysis found: 1) a significant difference in composition existed between treatments for the full equilibration period, and 2) a changing degree of similarity within treatments characterized by a divergence in composition over the first 63 days followed by a sharp convergence over the last two sampling dates.

Analysis of HK44 census data indicated distinct differences in invasion vulnerability arising from sequence effects. Complete invasion resistance was exhibited in Sequence B. No cells were detected in any of the treatment replicates for the full course of sampling. While communities assembled under Sequences A and C were both invasible by HK44, distinct differences in HK44 dynamics were demonstrated between treatments. Sequence A communities were highly receptive to invasion. An approximately 20-fold *increase* over inoculation density levels was exhibited over the first 14 days following introduction. Communities assembled under Sequence C, on the other hand, were considerably less vulnerable. Here, HK44 exhibited a 35-fold *decrease* in inoculation density during the same time period. In total, a difference in abundance of nearly three orders of magnitude was displayed between these treatments on Day 14. Abundance levels among Sequence A communities declined exponentially over the following 122

days. In contrast, abundance among Sequence C communities exhibited a approximately linear increase over the same period. By the last day of sampling, these trajectories had converged to nearly the same value.. Notably, although Sequence B and Sequence C communities exhibited the highest degree of between-treatment similarity, distinct differences were exhibited in their respective vulnerability to invasion by HK44.

A mechanistic explanation for the observed patterns was beyond the scope of this current study. Moreover, the lack of supporting population data for the competing strains prevents a simple correlative analysis of relative abundance among study strains. A possible explanation, however, may lie the timing of invasion for *Sphingomonas*. Final heterotrophic population abundance as well as post-introduction HK44 levels appear positively correlated with the length of elapsed time between the introduction of *Sphingomonas* and the end of the introduction phase. Among communities where *Sphingomonas* was the initial introduction (Sequence A), final heterotrophic abundance was highest and HK44 most successful. Among communities where *Sphingomonas* was the final competitor introduction (Sequence B), on the other hand, final heterotrophic abundance was lowest and all HK44 invasions failed. It might be proposed that *Sphingomonas* introduction have an initial inhibitory influence on growth that diminishes with time. Among communities were Sphingomonas was introduced first, however, HK44 reached abundance levels as much as three orders of magnitude greater than those obtained in previous microcosm experiments where HK44 was the sole introduction (Sayler et al. 1997; Zimmermann et al. 1997; Ripp et al. 2000). This suggests a more complex solution is required.

I have demonstrated that sequence effects can influence both the structure and

invasibility of microbial communities. Beyond the theoretical value of such finding, these results could have obvious ramifications for bioremediative applications where introduced species are employed. Some knowledge of community history might, therefore, prove valuable for effective implementation. Perhaps more significantly, the effects arising from sequence permutations could find potential application in field deployment scenarios. Achieving sufficient cell biomass of bioremediating organisms is crucial for effective bioremeidation. My findings show that sequential introductions can not only impact HK44 biomass levels, but in some cases appeared to actively promote cell abundance above levels obtained in previous experiments. This result suggests a possible facilitative or synergistic effect operating between species (see Simberloff and Von Holle 1999; Ricciardi 2002). Such effects could be harnessed through the sequential deployment of a consortium of remediating organisms.

Chapter 4:
The Interaction of Invasion Sequence Effects with a Productivity Gradient in Assembled Protist Communities

4.1 Introduction

Investigation into the processes of *community assembly* seeks the general principles or rules by which species combine to form communities (see Drake 1990a; Samuels and Drake 1999; Belyea and Lancaster 1999). One line of research in this area has sought how variation in colonization history can affect community structure and invasion vulnerability. Controlled experiments involving the manipulation of the introduction sequence and/or initial species densities have a long history in ecological research (e.g. Gause 1934; Park et al. 1941; Paine 1977; Alford and Wilbur 1985). More recent experiments involving the construction of more trophically complex communities have shown that changing the order of species arrival can alter the resulting community state (Robinson and Dickerson 1987; Robinson and Edgemon 1988; Drake 1991; Zimmermann 2001). The interaction of sequence based effects within a variable environment, however, has received limited attention. Dickerson and Robinson (1985) assembled protistan communities with different invasion rates and container sizes in order to test various predictions made by MacArthur and Wilson's (1963; 1967) island biogeography theory. Contrary to expectations, they found that the smaller microcosms sustained a greater species richness. Attributing this finding to habitat differences generated by container geometry, a followup experiment (Dickerson and Robinson 1986)

involving modifications to correct for these confounding effects produced results more consistent with theoretical predictions. Drake (1991) tested the effects of invasion history on phytoplankton communities constructed in laboratory microcosms of two sizes: a small 250 mL system and a large 40 L system. While he found that sequence effects had limited impact on competitive relationships among phytoplankton in the small systems, such effects played a significant role in the large systems, altering both community composition and invasibility. Drake concluded that variation in light regimes between the systems may have may influenced the outcome. Huxel (1995) explored the effect of invasion parameters – sequence, timing, and founder abundance – on the competitive relationships of four phytoplankton species under batch and continuous flow conditions. He found that media replenishment readily altered the competitive hierarchies obtained under batch conditions. Finally, Flum (1997) assembled lotic periphyton communities in artificial streams to examine the role of invasion history on species composition, biomass accumulation, and water chemistry. Communities assembled under alternative sequences initially diverged in all properties, but ultimately converged at the species composition level. Surprisingly, however, biomass and water chemistry properties remained divergent. Once an apparent equilibrium was attained, nutrient loading regimes were either increased or decreased by an order of magnitude to assess the impact on community composition. No change was found.

Using laboratory microcosms, I explore the interaction of a variable invasion sequence against a gradient of primary productivity. Detritus based protist communities consisting of three trophic levels were assembled through ordered species introductions under two alternative introduction schedules. This assembly protocol occurred along a full

productivity gradient comprised of five discrete levels of nutrient concentration. Censusing of community composition was carried out periodically over 64 days. In addition, a suite of environmental variables was quantified for each microcosm at the conclusion of the experiment to assess the impact of alternative invasion sequence on chemical properties. The following questions are asked: 1) Does change in the order of species invasion order produce alternative community states? 2) Does a given invasion sequence produce alternative communities with changing productivity level? Community states are evaluated for changes in species composition and/or species rank abundance. 3) What is the interaction of sequence and productivity effects? and 4) Does changing the order of invasion have differential effects on abiotic parameters?

4.2 Methods and Materials

4.2.1 Experimental Design

A two-factor experimental design consisting of two introduction sequences and five productivity levels was used. Each combination consisted of five replicates. Communities consisted of three trophic levels with detritus as the nutrient source. The three trophic levels consisted of: 1) bacteria, 2) three bacterivores (*Colpidium striatum*, *Tetrahymena pyriformis*, and *Cinetochilum* sp.), and 3) two omnivores that consume both bacteria and bacterivores (*Euplotes* cf. *patella* and *Blepharisma* cf. *americanum*). While *Euplotes* does consume bacteria, persistence of a bacteria-alone diet was not attained in preliminary growth trials. *Blepharisma*, on the other hand, was found to persist on a diet of only bacteria. Preliminary predator-prey feeding trials indicated that both *Euplotes* and *Blepharisma* fed on all bacterivores.

Each invasion sequence consisted of a variation of the basic following sequence:

Bacterivore → Omnivore → Bacterivore → Omnivore → Bacterivore. The two invasion sequences were (see also Table 4.1):

Sequence I: *Colpidium, Euplotes, Cinetochilum, Blepharisma, Tetrahymena*

Sequence II: *Colpidium, Blepharisma, Tetrahymena, Euplotes, Cinetochilum*

A period of 5 days between introductions followed bacterivore introductions, while a period of 10 days followed omnivore introductions to compensate for their slower growth rate. *Colpidium* comprised the first introduction in both sequences as it demonstrated consistently good growth in all preliminary growth trials. While an exploration of more than two sequences would have been desirable, labor constraints limited this number.

The productivity gradient consisted of five discrete nutrient concentrations: 1.00, 0.75, 0.50, 0.10, and 0.05 grams of protist pellet per liter of water (see Section 4.2.3). As the prescribed strength for this media calls for a single pellet (≈0.72 g) per liter of water, this range represents a full gradient ranging from low to high productivity. Preliminary growth trials indicated that all three bacterivores as well as *Blepharisma* could persist in pure culture at this full range of concentrations.

4.2.2 Culturing of Species

Colpidium, Tetrahymena, Euplotes and *Blepharisma* were cultured from commercial starter cultures from Carolina Biological Supply. *Cinetochilum* was isolated from pond samples collected in Knox County, Tennessee. Culture methods follow those prescribed by Lawler and Morin (1993a). Stock cultures of each species were maintained in approximately 100 mL protist media in 240 mL glass jars with loose lids. Media consisted of a single protist pellet (≈0.72 g) per liter of spring water with added wheat seed (see Section 4.2.3). Bacterivores and *Blepharisma* were grown on a strict diet of

bacteria. *Euplotes* was grown on a diet of bacteria and *Paramecium.* A rotating inventory of fresh stock cultures was maintained throughout the introduction period to insure high vigor among founding populations.

4.2.3 Microcosm Preparation

Microcosm containers consisted of covered 240 mL glass jars with 100 mL of media. Media was prepared as follows. A 3.0 liter concentrate of full strength media (i.e. 1.0 g L^{-1}) was created by mixing 3.0 grams of Carolina® protist pellet (Carolina Biological Supply #WW-13-2360) with 3.0 liters of sterilized Carolina® spring water (Carolina Biological Supply #WW-13-2450) in a single large flask. This mixture was then inoculated with bacteria. To insure a source of prey for all bacterivores, this inoculation was made with a 10 ml bacteria slurry consisting of equal portions of media drawn from stock cultures of each bacterivore and filtered to remove the protist component. This culture was gently stirred at room temperature for 24 hours to allow for bacterial growth. A sufficient volume for each nutrient concentration was then created in batch by diluting this concentrate with an appropriate volume of sterile dH_2O in an 1800 mL open mouth flask, i.e. media for the 0.5 g L^{-1} treatment was created by mixing equal portions of concentrate and water. Here, dH_2O was used instead of spring water to ensure proportional distribution of spring water constituents. During this process, the concentrate source flask was continuously mixed to insure an equal distribution of bacteria and sediments among all transfers. The appropriate media strength was then distributed to each microcosm. The source container was again continuously stirred to insure an even distribution of all constituents. Wheat seeds were added to each microcosm to improve nutrient availability. Seeds were sterilized by boiling for five

minutes and distributed by weight. Replicates of the 1.0 g L^{-1} treatment each received 0.16 grams of seeds, i.e. the wet weight of four average sized wheat seeds. The remaining treatments received amendments normalized to their relative nutrient concentrations, e.g. each 0.5 g L^{-1} treatment replicate received 0.08 grams. During the experiment, microcosms were maintained on a darkened lab bench at room temperature

A set of five sterile control standards were established for each nutrient concentration as a reference against which biologically mediated chemical changes occurring within experiment treatments could be made. Standards were produced as described above without the bacterial inoculation step and stored under refrigeration to deter microbial growth.

4.2.4 Introduction of Species

4.2.4.1 Bacterivores

Stock cultures of the each bacterivore were censused just prior to their introduction to obtain an estimate of population density (see Section 4.2.5). Based on this estimate, a variable volume of media and suspended organisms needed to target the desired founder abundance was drawn and transferred to each recipient microcosm. *Colpidium* was introduced at ≈500 individuals per microcosm. *Tetrahymena* and *Cinetochilum* were introduced at ≈1000 individuals per microcosm to compensate for differences in cell biomass. Note: Stock cultures of bacterivores were grown in protist media of concentration 0.72 g L^{-1} protist pellet. The volume of media transferred with each introduction would, consequently, have either a slightly enriching or diluting effect on the concentration of the recipient community. The relative effect of this contribution was greatest for the 0.05 g L^{-1} treatment. As the largest volume involved in any single

introduction was around 0.2 ml, this would produce at most a ≈3% increase in nutrient concentration for this nutrient concentration with each bacterivore introduction.

4.2.4.2 Omnivores

Euplotes and *Blepharisma* were introduced at 20 individuals per microcosm. Omnivores were triple rinsed in spring water prior to introduction to reduce the risk of contamination.

4.2.5 Sampling of Species Abundance

Protist populations were censused just prior to each new introduction and at 10, 20, and 34 days following the completion of the introduction phase. Bacterial abundance was not considered. Table 4.1 shows a schedule of sampling dates for the full experiment. Sampling protocols were adopted from Lawler (1993). Each microcosm was thoroughly stirred and a ≈0.5 ml volume drawn with a sterile Pasteur pipette. This volume was distributed in uniform droplets onto a laboratory balance with tared Petri dish until a total weight of ≈0.3 g was attained. Sample weight was recorded to the nearest 0.001 g. Extant species were then censused under magnification for the full sample. As protist media was assumed to have a density that approximated water, population abundance (# mL^{-1}) was calculated by dividing population counts by the sample weight. Abundance for a given species occasionally exceeded the capacity for reliable estimation. These samples were diluted with a known volume of water and a weighed subsample drawn for enumeration. When individuals of an introduced species were not found, the entire microcosm was scanned under magnification for presence of the species. Species indicated as being present were given an arbitrary abundance of 1 individual/ml. Upon completion of the experiment, census counts were checked for

internal consistency. Cases in which a species was not found on sampling dates following its introduction were changed to a "present" status if such species were found on subsequent sampling dates.

4.2.6 Media Replenishment

Media was replenished at 10 day intervals with the last replenishment occurring on Day 60 (see Table 4.1). Each microcosm was thoroughly mixed and a 10 ml volume drawn and replaced with 10 ml of sterile media of the appropriate concentration. To maintain the total volume at 100 ml, an additional variable volume of ≈1.0 ml was added to each microcosm to compensate for sampling and evaporative losses. When media replenishment occurred on the same day as species introductions (i.e. Day 20 and Day 30), replenishments were performed prior to introductions. Wheat seeds were neither removed nor replaced during the experiment.

4.2.7 Chemical Analysis of Protist Media for the Final Sampling Date

Upon completion of the experiment, media for both treatment microcosms and control standards were analyzed for chemical properties. Microcosms were first measured for pH and dissolved oxygen (DO) using standard electronic meters. The entire aqueous contents of each microcosm and each control standard was then filtered (0.2 µm Millipore® nylon filter) to remove debris and living components and refrigerated in sterile EPA vials until further analysis. Media was later analyzed for total dissolved carbon, total inorganic nitrogen, and an anion/cation profile consisting of the following elements: Ca, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Zn, and Zr. Total organic carbon (TOC) was determined with a DC-80 Series modular TOC analysis system. Inorganic nitrogen (NH_4^- and NO_3^-) was determined colormetrically by microplate method (see Sims et al. 1995).

Anion/cation concentrations were determined using a Model 61 Thermo-Jarrell Ash (Franklin, MA) inductively coupled argon plasma-optical emission spectrometer.

4.2.8 Analytical Techniques

Statistical analyses consisted of both standard methods (analysis of variance, t-tests) and MRPP group comparisons. The Multiple Response Permutation Procedure (MRPP) is a nonparametric multivariate technique for testing a hypothesized difference between two or more predefined groups. The program was adapted by B. McCune and J. Poston from a subroutine written by P. W. Mielke and K. J. Berry (see Mielke and Berry 1982). Recommended options were used. MRPP was performed with the *PC-ORD Version 4.0* software package (McCune and Mefford 1999).

4.3 Results

4.3.1 Species Composition

4.3.1.1 Population Dynamics

Time series of mean abundance by species were produced for each treatment combination (Figures 4.1-4.5). A discussion of population dynamics follows for each species.

4.3.1.1.1 Bacterivores

Colpidium successfully colonized all replicates for all treatment combinations. All *Colpidium* populations in the 0.05 g L^{-1} and 0.10 g L^{-1} media treatments, however, went extinct for both sequences. This species demonstrated a stable abundance at these levels in pure culture. Declines in abundance appeared correlated with the timing of introduction for *Blepharisma*. The shortest time to extinction occurred in the 0.05 g L^{-1} treatment where *Blepharisma* was the first omnivore introduced. While small declines in

Colpidium abundance followed omnivore introductions at higher nutrient concentrations, populations appeared stable by the end of the experiment.

Cinetochilum was the most successful colonist overall. This species successfully invaded all systems among Sequence II replicates. While abundance level exhibited a slight decrease over the last two sampling dates at the 0.05 g L^{-1} nutrient concentration, abundance was otherwise increasing among the remaining media treatments during this period. With the exception of a single replicate at the 1.00 g L^{-1} nutrient concentration, *Cinetochilum* also successfully invaded all Sequence I systems. While an extinction did occur in a single replicate of the 0.05 g L^{-1} level, abundance levels were increasing over the last two sampling dates for media treatments 0.05 g L^{-1} through 0.75 g L^{-1}. Extinction of this species, however, occurred in all replicates of the 1.00 g L^{-1} treatment. No explanation for these extinctions is apparent (however, see Section 4.3.5). Note: While *Cinetochilum* was found to occur in the water column, most cells were typically found grazing in the sediment layer. As *Cinetochilum* was the smallest of the species used, sediment constituents likely offered this species some refuge from predation. As sediments loads among nutrient concentrations were proportional to the overall concentration, an unintended "hidden treatment" (sensu Huston 1997) in the form of a habitat complexity gradient was created. As treatments without media sediment were not considered, such a refuge effect cannot be assessed.

Tetrahymena failed to invade any system. No cells were observed post-introduction in any system in either sequence treatment.

4.3.1.1.2 <u>Omnivores</u>

Blepharisma, with the exception of a single replicate (Sequence I; Media 0.05 g L^{-1})

successfully invaded all systems and was present in all these systems at the conclusion of the experiment. Population abundance was stable or increasing among higher nutrient concentrations for both sequences. Population levels at the two lowest nutrient concentrations for Sequence II, however, were declining concurrent with declines in *Colpidium* abundance. While longer time series may have determined their fate, the continued persistence of these species at these levels is questionable. While abundance levels among Sequence I systems of the same concentrations appeared more stable, this is likely the result of the 15-day lag in the timing of their introduction. As *Colpidium* also went extinct in these systems, persistence of these populations is therefore questionable.

Euplotes was, next to *Tetrahymena*, the least successful colonist and presence among treatments was highly variable. *Euplotes* was the second omnivore introduction in Sequence II treatments and it performed most poorly among these systems. No cells were found post-introduction in any replicate of the 0.50, 0.75, or 1.0 g L^{-1} media treatments. *Euplotes* was found in three and four replicates, respectively, for the two lowest nutrient concentrations. However, both the occurrence and abundance declined over the course of sampling and their eventual extinction in all replicates is likely. When *Euplotes* was the first omnivore introduction, colonization by this species was more successful. *Euplotes* was found in one and two replicates, respectively, of the 1.00 and 0.75 g L^{-1} nutrient concentrations. All these populations, however, went extinct by last sampling date. In contrast, *Euplotes* successfully invaded all replicates of the 0.05, 0.10, and 0.50 g L^{-1} treatments for this sequence. By sampling end, however, occurrence and/or abundance was decreasing among all replicates of 0.05 and 0.10 g L^{-1} media treatments. Declines in abundance among these treatments coincided with *Blepharisma*

introductions indicative of interspecific competition for prey. Strong evidence for the persistence of *Euplotes* was only found for the 0.50 g L^{-1} treatment. A stable coexistence of both omnivore species was only found for this nutrient concentration.

4.3.1.2 Species Abundance for the Final Sampling Date

Figure 4.6 shows a graph of final species abundance by sequence and nutrient concentration. While the final abundance of *Colpidium* and *Blepharisma* across nutrient concentrations was generally similar between sequences, clear differences were evident for *Euplotes* and *Cinetochilum*. To assess the influence of experimental treatments on final abundance, a two-factor analysis of variance test of the effects of sequence permutation and nutrient concentration on abundance values was performed for all species except *Tetrahymena* (Table 4.2-4.5). Nutrient concentration effects were highly significant for all four species. Sequence effects, however, were only significant for *Euplotes* and *Cinetochilum*. A Tukey HSD post hoc pairwise comparison of mean abundance between sequences was conducted by nutrient concentration for these two species (Table 4.3-4.4). These tests indicated the following: 1) mean abundance for *Euplotes* differed for the 0.50 g L^{-1} nutrient concentration, and 2) mean abundance for *Cinetochilum* differed at both the 0.50 and 1.00 g L^{-1} levels.

4.3.1.3 Rank Abundance for the Final Sampling Date - Sequence II

Examination of final population abundance values for Sequence II systems suggest a change in rank abundance of constituent species over the 0.50 g L^{-1} through 1.00 g L^{-1} nutrient concentrations. To evaluate this apparent change, populations in each of these respective systems were assigned a rank depending on relative abundance. A two-factor analysis of variance was conducted for the effect of species and nutrient concentration on

mean rank values (Table 4.6a). Results indicated that species effects and the interaction term were significant. Tukey HSD post hoc pairwise comparisons of mean rank by species were conducted for each nutrient concentration (Table 4.6b). These comparisons indicated the following change in rank abundance with nutrient concentration: 1) for the 0.75 g L^{-1} and 1.00 g L^{-1} nutrient concentrations, species ranking were *Colpidium* > *Cinetochilum* > *Blepharisma*, and 2) for the 0.50 g L^{-1} level, *Colpidium* and *Blepharisma* were codominant and *Cinetochilum* was ranked last.

4.3.2 Community Properties for the Final Sampling Date

The following community parameters were calculated for each set of replicates based on census data for the final sampling date: 1) species richness, 2) Shannon diversity, and species equitability. Table 4.7 provides a tabular summary of parameter values by treatment and replicate. Figures 4.7-4.9 provide graphs of parameter values by sequence and nutrient concentration. Among Sequence I systems, all three parameters showed well defined hump-shaped relationship with nutrient concentration – except for a slight increase at the lowest nutrient concentration for species equitability – with peak values for all parameters occurring at the 0.50 g L^{-1} level. These relationships were more variable among Sequence II systems. Species richness was essentially flat across higher nutrient concentrations with slight declines over the 0.10 and 0.05 g L^{-1} nutrient concentrations. Species diversity and species equitability in Sequence II systems also showed hump-shaped relationships with nutrient concentration – though flatter than Sequence I systems – with exceptions in both parameters at the lowest nutrient concentration. Here, peak values for both parameters occurred at the 0.75 g L^{-1} level. To evaluate the influence of treatments effects on these parameters, two-factor analysis of

variance tests for the effect of sequence permutation and nutrient concentration on each parameter was performed (Tables 4.8a-4.10a). Media treatment effects were significant for all three parameters. While sequence effects were only significant for species equitability, interaction terms were significant for both species richness and Shannon diversity. Tukey HSD post hoc pairwise comparison of means were conducted for the following: 1) differences in mean parameter values between sequences by nutrient concentration, and 2) differences in mean parameter values between nutrient concentrations within sequences (Tables 4.8b-4.10b). Between sequence comparisons found: 1) differences in species richness at the both lowest and highest nutrient concentrations, and 2) differences in species diversity and equitability at the highest nutrient concentration only. Within Sequence I comparisons found 1) mean species at the 0.50 g L^{-1} level was greater than all remaining concentrations, 2) peak diversity at the 0.50 g L^{-1} level was greater than both the lowest and highest nutrient concentrations, and 3) peak equitability at the 0.50 g L^{-1} level was only greater than the highest nutrient concentrations. Within Sequence II comparisons found no difference in either species richness or diversity between nutrient concentrations. Differences in species equitability were only found between the two lowest nutrient concentrations.

4.3.3 Community Trajectories Within and Between Sequences

4.3.3.1 Convergence/Divergence Within Sequences

To evaluate convergent trends within sequences, pairwise Sorensen dissimilarity was calculated between replicates for each treatment combination by sampling date. Mean pairwise dissimilarity was then calculated for each set of replicates and plotted by time (Figure 4.10). To more readily distinguish differences in dissimilarity between

sequences, mean dissimilarity values were also plotted for both sequences by nutrient concentration (Figures 4.11-4.15). An inspection of these figures reveals the following: 1) a relatively high degree of similarity among all treatment replicates throughout the sampling period, 2) an increase in divergence within replicates for both sequences over the post-introduction period with apparent differences in the degree of divergence between nutrient concentrations and between sequences permutations, and 3) a convergence in similarity within all treatments over the last two sampling periods. To assess treatment effects on these changes in community similarity, I considered two quantities: 1) mean dissimilarity by sequence and nutrient concentration for the final sampling date, and 2) mean maximal dissimilarity by sequence and nutrient concentration This latter value is the highest dissimilarity value expressed between replicates over the post-introduction period. Figure 4.16 and 4.17 show graphs of these quantities by sequence and nutrient concentration. No apparent trend in final mean dissimilarity is evident either between sequences or between nutrient concentrations. Mean maximal dissimilarity, on the other hand, displayed a stronger relationship with nutrient concentration. Among Sequence I systems, a broad U-shaped pattern across nutrient concentrations is exhibited with the nadir occurring at the 0.50 g L^{-1} level. A similar, though flatter, relationship is shown among Sequence II systems, although this pattern is violated at the lowest nutrient concentration. Notably, mean maximal dissimilarity was greater among Sequence I communities for all nutrient concentrations. Two-factor analysis of variance tests of the effect of sequence and nutrient concentration on mean final dissimilarity and on mean maximal dissimilarity were performed (Tables 4.11 and 4.12a). Test results indicated that all effects were insignificant for final dissimilarity

values. All treatment effects, on the other hand, were significant for maximal dissimilarity. Followup Tukey HSD post hoc tests were therefore conducted for the following: 1) differences in mean maximal dissimilarity between sequences by nutrient concentration, and 2) differences in mean maximal dissimilarity between nutrient concentrations within sequences (Table 4.12b). Between sequence comparisons indicated that maximal dissimilarity was significantly greater among Sequence I communities for both the lowest and the highest nutrient concentrations. Between nutrient concentrations comparisons within Sequence I communities found that maximal dissimilarity at the 0.50 g L^{-1} was significantly less than either the 0.05 or the 1.00 g L^{-1} levels. No difference in maximal dissimilarity was found between for nutrient concentrations within Sequence II.

4.3.3.2 Convergence/Divergence Between Sequences

MRPP comparisons of compositional data between invasion sequences were performed to assess the effect of alternative invasion sequences on community trajectories. Similar to t-tests, the MRPP test is a nonparametric multivariate procedure for testing the hypothesis of no difference between two or more predefined groups. Reported p-values indicate the probability that the differences observed among groups are attributable to chance alone. Separate comparisons were made by sampling date and nutrient concentration. Significance values were plotted by time to expose trends in convergence/divergence between sequence treatments (Figure 4.18). The sharp divergence (i.e. decrease in p-value) between sequences through Day 30 is an artefact of the differences in introduction schedules. Following the final introduction on Day 30, however, productivity-dependent compositional differences were found to arise between

sequence permutations. For an α=0.05, sequence effects were highly significant throughout the equilibration period for both the 0.10 g L^{-1} and 0.50 g L^{-1} treatments. Trajectories between sequences for the 0.75 g L^{-1} and 1.00 g L^{-1} nutrient concentrations displayed an increasing convergence over Day 20-Day 50, but both diverged sharply over the last two sampling periods. Compositional differences between sequences, however, were only significant for 1.00 g L^{-1} nutrient concentration for the last sampling date. Trajectories between sequences at the lowest nutrient concentration (i.e. 0.05 g L^{-1}) converged over the last two sampling dates and differences were insignificant by the conclusion of sampling.

4.3.4 Summary of Biotic Comparisons Between Sequences

Table 4.13 shows a summary of the biotic comparisons made between sequences. The strongest evidence for sequence effects on community states occurs at the 0.50 and 1.00 g L^{-1} nutrient concentrations. Sequence effects for these levels were indicated for the following analyses: 1) analysis of variance of final abundance levels by species, 2) analysis of variance of community parameters for the final sampling date, and 3) MRPP analysis of final community composition. MRPP analysis also indicated a difference in final species composition arising between sequences for the 0.10 g L^{-1}. Analysis of variance tests of final abundance values by species, however, indicated no significant differences between sequences. In addition, an inspection of abundance values for the final date (see Figure 4.6) indicates no difference in rank abundance among constituent members.

4.3.5 Chemical Properties for the Final Sampling Date

Tables 4.14-4.16 provide a tabular summary of pH/DO and elemental composition

values by treatment and replicate. Figures 4.19-4.22 show graphs of H^+, DO, dissolved carbon, and inorganic nitrogen concentrations by nutrient concentration for both the assembled communities and the control standards[5]. Inspection of these graphs reveal: 1) individual sequences had a disproportional effect in modifying H^+ and DO concentrations dependent on nutrient concentration, and 2) apparent differences between sequences in four parameters at the highest nutrient concentration. A two-factor analysis of variance test was therefore performed to assess the effects of sequence permutation and nutrient concentration on each parameter (Tables 4.17a-4.20a). Sequence effects were significant for all parameters except H+ concentration. A Tukey HSD post hoc comparison of means between sequences was performed for the three remaining parameters by nutrient concentration (Tables 4.18b-4.20b). Results indicated that all three parameters were significantly different between sequences at the highest nutrient concentration. MRPP comparisons of anion/cation concentrations between sequence treatments were performed by nutrient concentration. See Table 4.21 for reported p-values by nutrient concentration. For an α=0.05, significant differences in concentrations were found for the 1.0 g L^{-1}, 0.50 g L^{-1}, and 0.05 g L^{-1} media treatments. Table 4.22 shows a summary of comparisons of abiotic properties between sequences.

4.4 Discussion

4.4.1 Effect of Primary Productivity on Community States

Of the two sequence permutations tested, communities assembled under Sequence II exhibited the least variety in community states with changes in nutrient concentration. For the three highest concentrations, all communities were composed of *Colpidium*,

[5] pH values were converted to H+ concentration prior to analysis.

Cinetochilum, and *Blepharisma*. Analysis of mean rank abundance, however, did indicate that a change in rank abundance among species at these nutrient concentrations. At the two lowest nutrient concentrations, steady declines in the presence and abundance of *Euplotes* suggest extinction by this species is likely. Declines in *Blepharisma* populations indicate that extinction of this species is also probable. Given the extinction of both species, communities composed of strictly *Cinetochilum* would arise at these concentrations. No significant differences in either species richness or diversity with changing nutrient concentration were found. Species equitability only differed between the two lowest nutrient concentrations.

Communities assembled under Sequence I, on the other hand, displayed a greater degree of variation in community states with nutrient concentration. As population dynamics of systems at the two lowest nutrient concentrations appeared similar to those of Sequence II, a collapse to a *Cinetochilum*-alone community state appears likely. In contrast to Sequence II, however, communities assembled at higher concentrations exhibited distinct variation in species composition at all three nutrient concentrations. Communities at the intermediate concentration had the highest species richness, species diversity, and species equitability. Only at this nutrient level was evidence for a stable coexistence of four of the five study species found. Species composition at successively higher nutrient concentrations resulted in a shrinking subset of species. Composition at the 0.75 g L^{-1} nutrient concentration consisted of *Colpidium*, *Cinetochilum*, and *Blepharisma*. At the highest concentration, *Cinetochilum* went extinct in all systems leaving a final composition of *Colpidium* and *Blepharisma*. As *Cinetochilum* was found to maintain a stable population with both *Colpidium* and *Blepharisma* at the 1.0 g L^{-1} in

Sequence II systems, the cause for its extinction among Sequence I communities is unclear. As sequence permutation effects had distinct effects on chemical properties at this nutrient concentration, an abiotic cause may be responsible for this extinction.

4.4.2 Sequence Effects and Alternative Community States

Evidence for invasion sequence effects on community structure was sought with between-sequence comparisons of: 1) total community composition with time, 2) final sampling date species abundance, and 3) final sampling date values for species richness, Shannon diversity, and species equitability. Time series of multivariate comparisons of compositional data indicated a significant difference between sequences for the 0.10 g L^{-1} and 0.50 g L^{-1} nutrient concentrations emerged throughout the post-introduction period. Similar analysis of systems at the 1.00 g L^{-1} level indicated a convergence in composition over Day 40-50, followed by a sharp divergence over the last two sampling periods concomitant with the rapid extinction of *Cinetochilum* among Sequence I systems at this concentration. In total, multivariate analysis indicated significant differences in final community composition between sequences for the 0.10 g L^{-1}, 0.50 g L^{-1}, and 1.00 g L^{-1} nutrient concentrations. ANOVA results also indicated differences between sequences in the final abundance by species for the 0.50 g L^{-1} and 1.00 g L^{-1} concentrations. Communities at these concentrations were distinguished by: 1) the presence of *Euplotes* and a greater mean abundance of *Cinetochilum* among Sequence I systems at the 0.50 g L^{-1} nutrient concentration, and 2) the presence of Cinetochilum among Sequence II systems at the 1.00 g L^{-1} nutrient concentration. ANOVA tests also indicated differences in the final values of descriptive community parameters at these same concentrations. Significant differences in species richness were found at the 0.50 g L^{-1} and 1.00 g L^{-1}

nutrient concentrations. Differences in species diversity and equitability were also found at the 1.00 g L^{-1} level.

In total, the best evidence for sequence effects on community composition was found at the median and highest nutrient concentrations. While MRPP analysis also indicated a difference between sequences at 0.10 g L^{-1} nutrient concentration, the reason for this distinction is uncertain. No difference in the final abundance of any constituent species was found. In addition, inspection of final abundance data for these systems indicated no difference in relative abundance.

4.4.3 The Interaction of Sequence Effects with Primary Productivity

Within Sequence I systems, four community states of distinct species composition were found to emerge with changes in primary productivity – states of the same composition arising at the 0.05 and 0.10 g L^{-1} nutrient concentrations and states of unique composition at each of the remaining three levels. Within Sequence II systems, two community states of distinct composition were found – a single state at the 0.05-0.10 g L^{-1} nutrient concentration and single state at the 0.50-1.00 g L^{-1} levels. Among the communities at the three highest nutrient concentrations, however, changes in rank abundance of *Cinetochilum* and *Blepharisma* were found between the communities at the median level and those at the two highest levels. Between sequence comparisons found alternate states arising from sequence effects at the median and highest nutrient concentrations (Section 4.4.2). Five unique community states emerge from the interaction of sequence and primary productivity effects when the overlap in states between sequences is eliminated (Table 4.23). The emergence of these states appeared to be primarily the result of the following dynamics:

- Under low productivity conditions, *Colpidium* was unable to sustain a population under predation pressure by *Blepharisma*. As *Blepharisma* can persist on a diet of strict bacteria, this species reached populations levels that ultimately drove *Colpidium* extinct. *Blepharisma* abundance levels declined sharply following *Colpidium* extinctions and its persistence in questionable. While *Blepharisma* was shown to persist at this nutrient concentration in preliminary growth trials, competition by *Cinetochilum* populations could be impacting this species. Preliminary feeding trials did indicate that *Blepharisma* preyed on *Cinetochilum*. The refuge provided *Cinetochilum* by media sediments combined with a low degree of vigor observed among *Blepharisma* cells at these concentrations may have precluded effective predation among these treatments.
- *Euplotes* failed to successfully colonize higher productivity levels. Bakciunas and Lawler (1995) explored the predator-prey relationship between *Euplotes* and *Colpidium* under low and high nutrient conditions. They found *Euplotes* had a significantly lower capture rate under high nutrient conditions due to the increased mean cell length of *Colpidium* and eventually went extinct. While cell length was not explicitly measured in this study, cell size of *Colpidium* was observed to be positively correlated with nutrient concentration.
- Priority effects were exhibited by both omnivores. *Blepharisma* exhibited strong priority effects. Among systems where *Blesphrisma* was the first omnivore introduced, *Euplotes* failed to successfully colonize any system. *Euplotes*, on the other hand, displayed a weak priority effect. Where *Euplotes* was the first omnivore introduction, a stable coexistence between omnivores was achieved at the median concentration. No strong reverse effect on *Blepharisma*, however, was apparent.

- *Cinetochilum* successfully invaded all systems, but went abruptly extinct among Sequence I systems at the highest productivity level. While a reason for this extinction is not clear, it may have arisen from chemical changes arising within this treatment (Section 4.4.4)
- *Tetrahymena* failed to successfully invade any system. Whether this was due to the effects of competition or predation cannot be determined.

4.4.4 Treatment Effects on Chemical Properties

In addition to effects on species composition, experimental treatments were found to have differential effects in modifying media chemical properties. Media parameters quantified for the final sampling date consisted of pH, dissolved oxygen, total dissolved carbon, total inorganic nitrogen, and a profile of anion/cation concentrations. A comparison against control standard values indicated that disproportional changes in H^+ and DO concentrations arose between nutrient concentrations within each sequence. Strong evidence of distinct changes to media properties as a result of sequence effects occurred at the 1.00 g L^{-1} nutrient concentration. Here, differences in dissolved oxygen, total dissolved carbon, total inorganic nitrogen, and anion/cation concentration were found between sequences. Sequence effects on anion/cation concentrations were also indicated for the 0.05 g L^{-1} and 0.50 g L^{-1} nutrient concentration. Note, however, that no evidence of sequence effects on species composition was found at the 0.05 g L^{-1} nutrient concentration. This suggests that colonization history can have impacts on ecosystem properties that are not directly translated into changes at the species composition level. These finding are consistent with those of Flum (1997).

4.4.5 Determinism and Indeterminism in Community Trajectories

Drake (1991) makes a distinction between deterministic and indeterministic trajectories. Deterministic trajectories arise when a given invasion sequence repeatedly leads to the same community state. Indeterministic trajectories, on the other hand, occur when the same sequence results in a diversity of community endpoints. Drake (1991) argues that such diversity of states may arise from a sensitivity to subtle variation in factors other than sequence effects alone (i.e. founder vagility). If variation in the degree of similarity within replicates is used as a measure of the relative determinism within trajectories, differences in the degree of determinism within trajectories can be evaluated. Ultimately, all community trajectories converged over the last two sampling dates and differences in mean final dissimilarity between treatments were statistically insignificant. We could therefore conclude that the degree of determinism as well as the time to convergence was equal among treatments. A general decrease in similarity between replicates, however, was observed in all community trajectories during the first 20 days of the post-introduction period. The extent of this divergence was dependent on both sequence permutation and nutrient concentration. Maximal dissimilarity values among Sequence I communities were greater than those of Sequence II for all nutrient concentrations. For Sequence I systems, maximal dissimilarity exhibited a strong U-shaped correlation with nutrient concentration with the nadir occurring at the median concentration. The greatest maximal dissimilarity occurred at the highest nutrient concentration. While the relationship of maximal dissimilarity with nutrient concentration was less defined for Sequence II systems, the greatest maximal dissimilarity also occurred at the highest concentration. In addition, dissimilarity peaked

on Day 40 for both sequences at this concentration. With a single exception, dissimilarity for the remaining treatments peaked on Day 50. One hypothesis for these observations is that small differences among replicates were amplified by fast growth rates associated with higher productivity. A cause for the higher maximal values among Sequence I communities at the lower nutrient concentrations is unclear. Sampling effects arising from lower abundance observed at these concentrations could be responsible. Small differences in the measure absolute abundance would be reflected as larger differences in relative abundance. That dissimilarity values followed a similar trajectory with time, however, makes this explanation less than satisfactory.

We can conclude from these results that, even among assembly trajectories that are ultimately equally deterministic in terms of the variation in the final community state, sequence effects can generate different degrees of variability in community states en route to this state. The extent of this variation can be critically dependent on the environmental conditions and history of invasion. This raises the possibility that some putative indeterministic assembly trajectories are more deterministic at longer time scales. Rather, these trajectories may produce a greater degree of variation during the equilibration phase (e.g. Sequence I systems) and/or possess longer equilibration periods. Either case presents scenarios in which the imposition of external factors – e.g. invasion, disturbance, environmental fluctuation – could have differential impacts on community trajectories dependent on the timing of the event. This suggests a simple followup experiment incorporating an additional invasion manipulated to occur during periods of peak dissimilarity and during periods of convergence. Would an invasion occurring during a period of peak dissimilarity result in the production of greater variability in

community states? Or do species have differential ability to either amplify or dampen intrinsic variation?

Chapter 5:
Do Similar Communities Develop on Similar Sites? - A Test of Community Convergence in a Long Term Field Microcosm Study

5.1 Introduction

McCune and Allen (1985) examined the species composition of adjacent canyon forest communities of western Montana. They asked: "Do similar communities develop on similar sites?" The authors found that little of the variation in composition could be attributed to site conditions and concluded that a variety of alternative community states emerged from differences in a host of historical factors. At the heart of the McCune and Allen (1985) question are the relative roles that determinism and indeterminism play in the production of community structure. Supporters of community convergence have held that, – due to such factors as environmental tolerances, species interactions, life history traits – species are replaced in a relatively deterministic fashion until a stable species configuration is attained (Cowles 1901; Clements 1916; Margulef 1963; Odum 1969). Moreover, such a process is held to be repeatable. That is, following a disturbance, developing communities will traverse trajectories of similar ecological structures culminating in the same climax state. Evidence of convergence in both natural and artificial systems has been found (Cowles 1901; Myster and Pickett 1990; Nilsson and Wilson 1991; Sommer 1991; Wilson and Whittaker 1995). That convergence is the only outcome of community development, however, has been increasingly challenged (e.g. Gleason 1926; Elton 1930; Engler 1954; Chesson and Case 1986; Wu and Loucks

1995). Many ecologists currently hold that historical factors including physical (i.e. the timing of disturbance and/or environmental fluctuations) and biological events (i.e. colonization sequence, priority effects) can have a strong influence on the development of community structure (Lewontin 1969; Chesson and Case 1986; Drake 1991; Tanner and Hughes 1996; Berlow 1997).

In this study, I revisit the question posed by McCune and Allen (1985) using a field microcosm approach wherein the effect of differences in history, more so than gross environmental similarities, on community composition could be evaluated. Fifteen artificial ponds arranged in five clusters of three replicates were established under the same initial conditions and at a wooded location north of Knoxville, Tennessee, and allowed to develop undisturbed for eight years. Canopy closure was complete at the time of establishment leading to similar light conditions among all microcosms. Overstory vegetation, however, was variable across the study area offering a potential difference in both biotic and abiotic inputs between clusters. Here, manipulations of community history were not explicitly performed. Rather, historical factors are implicit in the spatial arrangement of microcosms in that cluster replicates are assumed to have environments (i.e. light conditions, leaf litter and rainfall inputs, etc) and propagule inputs that are more similar than more spatially distant systems. Given these similar conditions, do we find that similar communities emerge? Based on census data collected over the course of the eighth growing season, I examine patterns of changing species composition for evidence of convergence or divergence among communities for the following scenarios: 1) the overall study site, 2) within experimental clusters, and 3) among microcosms independent of spatial proximity. In addition, convergence was evaluated using five alternative

classification schemes. Phytoplankton communities are generally believed to reach a stable equilibrium in 30-60 days under stable conditions and successional dynamics are similar in many respects to those of terrestrial plant communities (Reynolds 1984; Sommer et al. 1986, Sommer 1991b, Reynolds 1993). However, as the generation time of algae is roughly a thousand times shorter than terrestrial vegetation (1-14 days), a single season of plankton dynamics can be comparable to several centuries of plant succession (Sommer et al. 1986). Results obtained from eight years of undisturbed development should, therefore, offer a suitable basis in which to evaluate community convergence.

5.2 Methods and Materials

5.2.1 Study Site Description and Experimental Setup

The study site was established in a mixed hardwood woodlot located on a private farm in northern Knox County, Tennessee. The study area was bordered on the northwestern edge by an active horse pasture and on the southern edge by a shallow gully. Overstory species consisted of American sycamore, elm, red maple, ash and yellow-poplar. Middle and understory species consisted of red maple, elm and miscellaneous woody shrubs. Canopy closure was complete during the study period and light availability at the forest floor consisted only of diffuse light and occasional sunflecks.

In the spring of 1987, fifteen 20 liter polypropylene buckets were distributed in five clusters of three replicates along a transect approximately 8 meters in length. Distance between neighboring clusters was variable and ranged between 2.0-3.5 meters. See Figure 5.1 and Figure 5.2 for a scale map and photographs of the study site, respectively. At the time of establishment, each microcosm was filled with 20 liters of sterile Woods

Hole MBL media (see Stein 1975). This is a nutrient-rich medium to which no subsequent amendments were made. The site was subsequently undisturbed for 8 years following establishment.

5.2.2 Data Collection

Microcosm biota were censused a total of six times at two week intervals between May 11 and July 20, 1995 (Julian dates: 130-200). Each microcosm was gently mixed and a single 500 ml aliquot collected from the middle-deep region using standard plankton sampling methods. Collected samples were promptly preserved with Lugol's iodine for later identification. Additional living samples were also retained to aid in the classification process. Precautions were taken during sampling process to avoid cross contamination between microcosms.

Environmental data was not collected from the study site during the course of the study. However, daily rainfall records for the a period extending from one week prior to the beginning of sampling through the end of sampling were obtained from the archives of the National Climatic Data Center. Data from two nearby collection points, the Knoxville Experimental Station in Knox County and the Norris Experimental Station in Anderson County, were obtained and averaged by day. Total weekly rainfall values were then calculated by summing mean daily values for 7-day periods with the last day of every other week coinciding with microcosm sampling dates.

5.2.3 Species Identification and Community Classification

Collected samples of microcosm biota were identified under magnification with a light inverted microscope. Where possible, organisms were classified to species. Classification to the nearest possible taxa was performed when identification to the

species level was problematic. Species were also classified to both higher taxonomic and functional categories for purposes of alternative community classification. Functional classification of organisms was based on characteristics of morphology, type of locomotion, and trophic class. Finally, the greatest axial linear dimension (GALD; Lewis 1976) was measured on 10+ individuals from each population as an index of body size

Using the above classification and body size data, five separate classification schemes of community composition were developed for analysis of convergent trends. The five schemes consisted of 1) species level – abundance (#/ml), 2) species level – presence/absence, 3) higher taxonomic level – abundance (#/ml), 4) functional level – abundance (#/ml), and community features. The community features consisted of the following parameters for each community: a) total species abundance (#/ml), b) species richness, c) Shannon diversity, d) autotroph:heterotroph abundance ratio, e) edible:nonedible abundance ratio, and f) community size defined by the weighted average of species GALD data. Given the largest predators were shorter than 200 μm GALD, 20 μm GALD was considered the upper limit of edible food size (Downing and Rigler 1984).

5.2.4 Analytical Methods

Data analyses consisted of both standard statistical methods (analysis of variance, Student t-test) and multivariate analysis. Multivariate analysis consisted of MRPP group comparisons and cluster analysis. Multiple Response Permutation Procedure (MRPP) is a nonparametric multivariate technique for testing a hypothesised difference between two or more predefined groups. Comparisons were performed using a program adapted by B. McCune and J. Poston. (see Mielke and Berry 1982). Cluster analysis was performed

using the CLUSTR program. Both multivariate programs were part of the *PC-ORD Version 4.0* software package (McCune and Mefford 1999). For purposes of multivariate analysis, species abundance values were log-transformed prior to analysis (Gauch 1983). Also, species found in less than four samples over the entire sampling record were removed per recommendations of Sneath and Sokal (1973).

5.3 Results

5.3.1 Community Description Parameters

The following descriptive community parameters are discussed: 1) community composition, 2) community history, 3) species richness, diversity, and equitability, 4) species abundance, and 5) trophically related variables.

5.3.1.1 Community Composition

In total, 41 species in 12 higher taxonomic and 13 functional groups were found among the 15 microcosms over the 70 days of sampling (Table 5.1). Of these 33 species were successfully identified to the genus level or higher. Species composition among systems was notably variable. Only *Astasia* sp. (a colorless form of *Euglena*) and an unidentified ciliate were found to occur in all 15 microcosms and no single species occupied more than 11 microcosms simultaneously. Eleven species were found to occur in 10+ microcosms. Frequency distributions are provided that indicate the number of microcosms in which occurrence was found on at least one sampling date by individual species (Figure 5.3) and by number of species (Figure 5.4). Of the 12 higher taxonomic groups found, representatives from four groups were found to occur in all 15 systems: *Chlorophyceae, Euglenophyta, Bacillariophyta,* and *Ciliophora*. These groups cumulatively accounted for approximately half of the total species count. Eight groups

were found in 10 or more of the microcosms accounting for 85% of all species. Of the 11 functional categories, members from three groups were found in all microcosms: flagellates (>20 μm), flagellated heterotrophs (<20 μm), and ciliates (>20 μm). These groups accounted for approximately 22% of all species. Eight of the 11 groups were found in two-thirds or more of the microcosms accounting for 66% of all species. Frequency distributions are supplied that show the number of microcosms wherein at least a single occurrence was recorded for higher taxonomic (Figure 5.5), and 2) functional classification (Figure 5.6).

5.3.1.2 Colonization History

Table 5.2 provides a schedule of colonization by species for the full sampling period. The following values are reported: 1) the number of microcosms occupied by species and sampling date, 2) the number of microcosms involving an initial occurrence by species and sampling period, and 3) the total number of microcosms in which at least a single occurrence was observed by species. While an overall trend is evident in timing and turnover of species, the colonization history among individual microcosms was diverse. The timing of *initial* occurrence between microcosms was notably variable among species. Of the 11 more common species (i.e. those that occurred in 10 or more microcosms), 4 species were founds which had their initial occurrence on the same sampling date in ≥75% of systems in which they were found overall: *Achnanthes* sp., *Chlorella* sp., *Collodictyon triciliatum,* and *Gomphonema olivaceum.* Of the 28 more rare species (i.e. those found in 2-9 microcosms), 10 species exhibited a simultaneous initial occurrence in ≥75% of the communities in which they found overall. Notably, 7 of these last 10 species were only found on a single sampling date.

5.3.1.3 Richness, Diversity, and Equitability

Figure 5.7 provides a time series of species richness for the full sampling period. Plotted variables include: 1) species richness by individual microcosm, 2) mean species richness per microcosm, 3) mean species richness per individual cluster, and 4) total species richness. Total species richness – i.e. the total number of species found sitewide on a given date – was 20 species at the start of sampling (Day 130). This value peaked at 24 species on Day 172 and declined to 21 species at the conclusion of sampling on Day 200. Richness values among individual microcosms were highly variable, but in all cases consisted of only a small subset of the total overall number of species present. Mean richness per microcosm was only 6.7 species at the start of sampling and, despite the slight increase in total species richness over the first 42 days of sampling, declined steadily to the value of 4.4 species by the end of sampling.

Figure 5.8 and Figure 5.9 show time series of Shannon diversity and species equitability, respectively Presented variables include: 1) diversity/equitability by individual microcosm, 2) mean diversity/mean equitability per microcosm, and 3) mean diversity/mean equitability per individual cluster. Species diversity and equitability remained relatively constant throughout the collection period, but both declined to their lowest values over the last two sampling dates. Initial diversity was 1.07 and declined to value of 0.63 on the last sampling date. Equitability at the start of sampling was 0.58 and declined to 0.50 over the same time period. The observed decline in species diversity, therefore, resulted from both a decline in species richness and equitability.

5.3.1.4 Species Abundance

Figure 5.10 provides a time series of total species abundance (i.e. cumulative

abundance of all present species). The following variables are presented: 1) total abundance by individual microcosm (#/L), 2) mean total abundance per microcosm (#/L), and 3) mean total abundance per individual cluster (#/L). Like species richness, total abundance was highly variable over time with as much as a two orders of magnitude difference in abundance values found between sampling dates. Note the synchronous oscillation in abundance exhibited in most of the 15 systems with peaks in abundance peaks occurring on Day 130 and Day 158. This pattern was repeated in mean total abundance by cluster for a majority of the five experimental clusters.

5.3.1.5 Trophic Variables

Figures 5.11-5.13 show times series for the following trophic variables: 1) mean community size per microcosm (defined by the weighted average of species GALD data), 2) mean edible:nonedible ratio per microcosm, and 3) mean heterotroph:autotroph ratio per microcosm. Mean community size showed a general increase over Day 130-186 resulting in a decline in the mean edible:nonedible ratio over this same period. These trends are consistent with successional trophic changes observed in freshwater plankton communities (see Sommer et al. 1986).

5.3.1.6 Statistical Analysis of Community Parameters

Two-factor analysis of variance tests were performed to assess the effects of 1) microcosm and sampling date, and 2) cluster and sampling date on values of species richness, Shannon diversity, species equitability, total species abundance, community size, edible:nonedible ratio, and heterotroph:autotroph ratio. Results are collated in Table 5.3 (microcosm and sampling date) and Table 5.4 (cluster and sampling date). Both microcosm and cluster effects were insignificant for all seven parameters. The effect of

sampling date was significant only for species richness and community size. Tukey HSD post hoc pairwise comparison of means found that the observed decline in mean species richness was significant between Days 130 and 200 (diff=-2.27, Q=-4.58, Q crit=4.16) and the increase in mean community size was significant between Day 130 and Day 186 (diff=16.55, Q=4.19, Q crit=4.16).

5.3.2 Rainfall Data and Correlation with Observed Patterns

Figure 5.14 provides a time series of weekly rainfall totals for the study period. Note the apparent correlation of periods of heavy rainfall with similar peaks in total species abundance values. To evaluate this correlation, rainfall/abundance data pairs were plotted for each of the six sampling dates (Figure 5.15). A fitted logarithmic trendline accounts for 77% of the observed variation. The asymptotic nature of the relationship suggests an upper bound on the effect of rainfall inputs on promoting abundance levels. In addition, observed decreases in mean community size (Figure 5.11) and concomitant increases in the mean edible:nonedible ratio (Figure 5.12) were synchronous with large rainfall events. Such changes would be consistent with an associated increase in smaller autotrophs (i.e. <20 μm) during these periods either through growth or immigration. Heavy rainfall presumably could play a role in both cases, either through enhancing microcosm nutrient levels or as a vector of propagule transport.

5.3.3 Tests of Community Convergence

Using census data was organized into five alternative classification schemes (see Section 5.2.3), evidence for convergent trends among communities was sought for the following scenarios: 1) for the overall study site, 2) among the five experiment clusters, and 3) among microcosms independent of spatial proximity. Each of these will be

discussed in turn.

5.3.3.1 Community Convergence: Overall Site

Community convergence for the overall site was evaluated using Sorensen's dissimilarity index. As this parameter can be used with either abundance or presence/absence data, data from all community classification schemes were considered except *Community Features*. Dissimilarity values were first determined for each community pair by sampling date and classification data type. Mean dissimilarity per microcosm was then quantified by sampling date by averaging all 105 pairwise values and the resulting means plotted by time to expose temporal changes in community similarity (Figure 5.16). Note a similar pattern exhibited among classification data types characterized by a sharp increase in dissimilarity over Day 144-158, concurrent with a rainfall peak during this period, followed by a general levelling trend over the last 42 days. While some variation in mean dissimilarity was expressed during this latter period, an inspection of associated confidence intervals indicated no significant differences in mean values.

5.3.3.2 Community Convergence: Cluster Effects

Convergence among communities within clusters was evaluated with three methods: 1) MRPP analysis, 2) Sorensen's dissimilarity index, and 3) cluster analysis. A discussion of these analyses follows.

5.3.3.2.1 <u>MRPP Analysis: Cluster Effects on Community Composition</u>

Comparisons of compositional data grouped by cluster were performed using the Multiple Response Permutations Procedure (MRPP). Similar to t-tests, MRPP is a nonparametric multivariate procedure for testing the hypothesis of no difference between

two or more predefined groups. The p-statistic reported by this test indicates the probability that the differences observed among groups can be attributed to chance alone. MRPP comparisons were performed by classification data type and sampling date and reported p-values plotted by time to expose trends in compositional differences between clusters (Figure 5.17). This time series reveals an oscillating pattern among data types with periods of decreasing similarity *between* clusters during Days 130-144 (except *Taxonomic* data) and Days 158-186 (all data types). These periods correspond with periods of decreased rainfall. Periods of increasing similarity, in contrast, coincided with rainfall peaks. For an α=0.05, three comparisons were significant: 1) Day 144 for *Community Features* data (p=0.010), 2) Day 186 for *Taxonomic* data (p=0.022), and 3) Day 186 for *Functional* data (p=0.014). Despite the general lack of statistically significant results, the consistent pattern displayed among data types suggest an underlying dynamic of alternating convergence/divergence within clusters driven by changes in rainfall inputs.

5.3.3.2.2 Sorensen Community Dissimilarity: Convergent Trends Within Clusters

Here, evidence for convergence arising within cluster communities was sought using Sorensen's dissimilarity index. In order to assess the overall influence of spatial-clustering on changes in community composition, I considered both the effect of each individual cluster as well as the net effect of all clusters combined. The effect of individual clusters is addressed first. Sorensen dissimilarity values were determined for each community pair by sampling date. Mean dissimilarity was then calculated by sampling date for the following: 1) within each cluster (3 pairwise values each), and 2) between clusters (90 pairwise values). This process was repeated for all classification

data types except *Community Features*. Figures 5.18-5.21 show time series of these mean values for *Species (abundance)*, S*pecies (presence/absence)*, T*axonomic* and *Functional* data types, respectively. To summarize this time series data, a frequency histogram was produced that shows the number of clusters that exhibited an increase in mean similarity between adjacent sampling dates by data type (Figure 5.22). While some variation is expressed between data types, an overall oscillating pattern of convergence/divergence is exhibited that is consistent with that observed in the MRPP analysis. Here, a majority of the five clusters increased in similarity for all data types during Days 130-144 (except *Taxonomic* data), Days 158-172, and Days 172-186. These periods again correspond with periods of decreased rainfall. To assess the effect of spatial-clustering on community trajectories during these periods, changes in similarity *within clusters* were compared against changes in similarity *between clusters*. If site-specific differences arising at cluster scales impact community trajectories, the convergence within clusters should exceed that emerging between clusters. Indeed, an examination of Figures 5.18-5.21 reveals that of the clusters that increased in similarity over the three periods in question (i.e. Figure 5.22), all cases also had a net increase in similarity greater than that occurring between clusters. To assess the significance of these differences, the change in community dissimilarity over adjacent sampling dates was determined for each community pair for each of the periods in question. The mean change was then determined by period for each cluster (3 pairwise values each) and between clusters (90 pairwise values). Within cluster means were then compared with the between cluster mean using a single–factor analysis of variance. Separate analyses were conducted for each period and classification data type. Also, only those clusters

that had a mean decrease in dissimilarity greater than that between clusters were considered to strengthen the analysis. For an α=0.05, however, all comparisons were insignificant (Table 5.5). Therefore, the null hypothesis that cluster effects had no influence on convergence in composition during these periods cannot be rejected.

The small number of replicates associated with each cluster reduced the statistical power of the above analyses. To increase the power, a second comparison was performed that considered the net effect of all clusters combined on changes in dissimilarity values. Sorensen dissimilarity values were again determined for each community pair by sampling date. Mean dissimilarity was then calculated by sampling date for the following: 1) within all clusters (15 pairwise values), and 2) between clusters (90 pairwise values). Figures 5.23-5.26 shows time series of mean dissimilarity values for within all clusters and between clusters for *Species (abundance)*, *Species (presence/absence)*, *Taxonomic* and *Functional* data types, respectively. An examination of these figures reveal that the decrease in the mean dissimilarity within clusters was greater than that between clusters for the following cases: 1) Days 130-144 (*Species-level)*, 2) Days 158-172 (all data types), and 3) Days 172-186 (*Functional-* and *Taxonomic-level*). To assess the significance of these observed differences, a t-test comparison of the mean change in dissimilarity within clusters with the mean change between clusters was performed for each case (Table 5.6). For an α=0.05, all comparisons were again insignificant.

5.3.3.2.3 <u>Cluster Analysis and Information Loss</u>

The final test of the influence of spatial clustering on community compositional was performed with cluster analysis. First, cluster analysis (Euclidean distance, nearest

neighbor linkage) was performed by sampling date for each of the five classification data types. For each analysis, the distance on the objective function (Wishart 1969) was then determined for the point at which each individual cluster was completely linked. All distances were normalized to represent a "percent information lost" value. Information loss by individual cluster was plotted by time to reveal changes in the relative effect of clustering over the course of the sampling. Figures 5.27-5.31 show time series of percent information loss values for each of the five clusters for *Species (abundance)*, *Species (presence)*, *Taxonomic*, *Functional*, and *Community Features* data types, respectively. In order to summarize this time series data, a frequency histogram was constructed to show the number of clusters that exhibited a decline in information loss was exhibited by sampling period and data type (Figure 5.32). A decline in information loss would indicate an increase in the meaningful relationship of microcosms based on a grouping by cluster. While the proportion of clusters indicating such a decline was variable among data types, two sampling periods – Days 130-144 and Days 158-172 – showed a decline in information loss for a majority of clusters for the *Species (abundance)*, *Species (presence)*, and *Community Features* data types. These periods again coincided with periods of decreased rainfall.

To assess the overall mean effect of spatial-clustering on compositional changes, mean information loss per cluster values were calculated by sampling date. Figure 5.33 shows a time series of these mean values by data type. Here, an oscillating pattern – consistent with that observed in previous analyses – is exhibited with troughs and peaks in information loss well correlated with changes in rainfall patterns. To determine whether the observed changes in mean values with time were significant, a single-factor

analysis of variance was conducted by data type (Table 5.7). For an α=0.05, all comparisons were insignificant. An examination of Figures 5.27-5.31, however, reveals that Cluster 3 showed an opposing trend in changes in information loss for many sampling dates. To assess the effect of this cluster on analysis of variance results, tests were repeated with this cluster removed prior to analysis. With this amended data set, the analysis of variance test was significant for *Species-level (abundance)* data (Table 5.7). A Tukey HSD post hoc pairwise comparison found that mean information loss for Day 144 was significantly less than Day 172 (mean diff=-0.425, Q=4.727, Q crit=4.490). An inspection of the cluster dendrogram for *Species-level (abundance)* data for Day 144 (Figure 5.34) reveals a good correspondence between cluster members and the created linkages. A strong linkage is also noted between members of Clusters 1 and 2 and between members of Clusters 3, 4, and 5. As clusters were numbered based on spatial contiguity, the linkage of these clusters suggests that larger scaled factors may have been affecting community development during this period.

5.3.3.3 Community Convergence: Spatially Independent Trends

The previous analysis indicates that a weak degree of convergence may have been generated from cluster-scaled effects. Here, I sought evidence for stronger patterns of convergence arising between communities that were independent of spatial proximity.

5.3.3.3.1 <u>Trends in Species Co-occurrence: Astasia and Chlorella</u>

An examination of species data for the final sampling date revealed a strong trend in the relative co-occurrence of two phytoplankton species – *Astasia* and *Chlorella*. Whereas these species combined occupied 14 of the 15 microcosms, coexistence of these species was only found in a single microcosm. Of the remaining 13 microcosms,

Chlorella was found alone in six systems and *Astasia* was found alone in seven systems. One microcosm lacked both species on this sampling date. To evaluate the association of this pattern with overall community composition, cluster analysis (Ward's method, Euclidean distance) was performed on *Species-level (abundance)* data for Day 200 and correspondence between the occurrence of these species and the formed linkages evaluated. See Figure 5.35 for a cluster dendrogram. With the exception of a single microcosm (4-B), correspondence with the two largest linked groups was excellent.

Given these results, an examination of trends in the co-occurrence of these species was carried out for the full data set. *Astasia* and *Chlorella* were among the more cosmopolitan community members, being found in 15 and 13 microcosms, respectively. In characterizing the occurrence of these species, the frequency of the following scenarios were quantified by sampling date: 1) *Astasia* occurs alone, 2) *Astasia* occurs alone on two consecutive dates, 3) *Chlorella* occurs alone, 4) *Chlorella* occurs alone on two consecutive dates, 5) *Astasia* and *Chlorella* occur together, and 6) *Astasia* and *Chlorella* occur together on two consecutive dates. The frequency of these six scenarios was then plotted by time to expose temporal trends (Figure 5.36). An inspection of this figure reveals that cases of each species occurring alone as well as cases of each species occurring alone on consecutive sampling dates both increased with time. Cases involving the co-occurrence of both species, on the other hand, were highest over the first two sampling dates and generally decreased over the remaining sampling period. These collective trends suggest an underlying pattern of community divergence. The 15 microcosms were therefore reassigned to two groups for further analysis based on the pattern of *Astasia*/*Chlorella* occurrence for the final sampling date:

Group 1 (*Chlorella* present on Day 200): Microcosms 1-A, 2-B, 3-A, 3-C, 5-A, 5-B, and 5-C.

Group 2 (*Astasia* present on Day 200): Microcosms 1-B, 1-C, 2-A, 2-C, 3-B, 4-A, 4-B, and 4-C.

Microcosms 3-B and 3-C were exceptions to the occurrence pattern of *Astasia/Chlorella*. These systems, which either lacked or possessed both species on the last day of sampling, were assigned to groups based linkages generated by cluster analysis of *Species-level (abundance)* data for Day 200 (Figure 5.35). Note that only two of the five original clusters remained intact with this reassignment.

5.3.3.3.2 MRPP Analysis: Group Effects on Community Composition

An MRPP comparison of compositional data between these two groups was performed by sampling date and reported p-values plotted by time to expose temporal changes in significance values (Figure 5.37). This process was repeated for all five classification schemes. Prior to Day 158, varying patterns in the convergence/divergence in similarity between groups were exhibited among classification data types. Comparisons based on *Species-level* data decreased steadily in significance over the first three sampling dates indicating an increasing degree of similarity between groups during this period. Following Day 158, however, the differences between groups rapidly increased in significance for all day types with p-values falling below the 0.05 threshold for four of the five data types. While comparisons based on *Community Features* data also exhibited a trend of increasing significance over this same period, significance levels failed to drop below 0.05 before the end of sampling. These trends indicate a period of divergence in community composition between Group 1 and Group 2 microcosms

stemming from Day 158. As records indicate a rainfall peak in the week preceding Day 158, high rainfall inputs may have played a role in initiating this divergence.

5.3.3.3.3 Sorensen Community Dissimilarity: Convergent Trends Within Groups

Here, the convergence within groups was evaluated using Sorensen's dissimilarity index. All classification schemes, except *Community Features,* were considered. To evaluate convergence among groups, dissimilarity *within groups* was again compared against dissimilarity between groups. Dissimilarity values were first determined for each microcosm pair by sampling date. Mean dissimilarity by date was then calculated for the following: 1) within Group 1 microcosms (21 pairwise values), 2) within Group 2 microcosms (28 pairwise values), and 3) between Group 1 and Group 2 microcosms (56 pairwise values). Figures 5.38-5.41 shows time series of these mean values for *Species (abundance)*, *Species (presence/absence)*, *Taxonomic* and *Functional* data types, respectively. An inspection of these figures reveals a consistent pattern among means for all classification data types. First, a sharp decrease in similarity both within and between Group 1 and Group 2 systems occurred during Days 144-158 concomitant with the rainfall peak of this period. Following Day 158, the following trends emerge: 1) Group 1 microcosms diverged until Day 172, but subsequently converged to the final sampling date, 2) Group 2 microcosms converged until Day 186, but diverged over the last two sampling dates, 3) similarity between Group 1 and Group 2 microcosms decreased steadily until the end of sampling. Indeed, an examination of pairwise dissimilarity values *between* groups for the final sampling date revealed a large proportion of values equal to 1 indicating complete dissimilarity. These proportions by data type were: 1) *Species (abundance)* – 0.339, 2) *Species (presence)* – 0.339, 3) *Taxonomic* – 0.143, and

4) *Functional* – 0.321.

The trends of increasing similarity within groups coupled with sharp decrease in similarity between groups suggest the emergence of two distinct community structures. Single-factor analysis of variance tests were performed to assess whether the mean dissimilarity within each group was less than that between groups. Separate tests were Day 130 and Day 200 to assess the degree of similarity between groups for the initial and final sampling dates (Table 5.8). For Day 130, only comparisons based on *Functional-level* data were found to be significant. For Day 200, however, significant differences were indicated for all data types. Tukey-Kramer post hoc tests were carried out for all significant analysis of variance results (Table 5.9). These test indicated that: 1) mean functional similarity within Group 2 communities was significantly greater than mean similarity within Group 1 communities for Day 130, and 2) mean similarity within groups was greater than that between groups for all data types for Day 200. We can, therefore, conclude while significant differences in initial makeup between groups were not indicated for most measures, a pattern of increasing divergence between groups was exhibited that culminated in compositional differences on Day 200 that were significant for all data types tested.

5.3.3.3.4 Temporal Trends in Functional Groups

The above analyses indicate a divergence of community trajectories arising between Group 1 and Group 2 systems. To illuminate a possible explanation for this divergence, time series of presence/absence data for the 11 functional groups for these groups were constructed. See Figure 5.42 for all coccals, Figure 5.43 for small filaments and all flagellated autotrophs, Figure 5.44 for all flagellated heterotrophs, and Figure 5.45 for

ciliates, rhizopods, and rotifera. An examination of these times series reveal qualitative differences in the occurrence of four functional groups following Day 158: coccals (6-20 μm), flagellated autotrophs (>20 μm), flagellated heterotrophs (<20 μm), and ciliates (>20 μm). An inspection of these figures reveals the following differences between groups. Group 1 systems exhibited an increase in the occurrence of edible coccals over Days 186-200 coupled with deceases in flagellated autotrophs, flagellated heterotrophs, and ciliates over Days 158-200. Group 2 systems, on the other hand, exhibited contrasting trends for all four functional categories. Note that Chlorella and Astasia are members of the coccals (6-20 μm) and flagellated autotrophs (>20 μm) groups, respectively.

A possible explanation for these patterns of turnover may lie in the relationship between the ciliates and edible coccals (i.e. 6-20 μm) functional groups. Ciliates can play a powerful role in altering community structure through selective feeding of phytoplankton (Harris 1986; Sommer 1989). Chlorella, the single largest component of the edible coccals category, comprised approximately 80% of the abundance for all recorded occurrences of this functional group. While ciliates are known to consume *Chlorella*, many ciliate species – including *Euglena* – internally retain *Chlorella* for purposes of endosymbiotic production of photosynthate (Bold and Wynne 1985). Ciliates can also obtain oxygen from the retained cells for use in oxygen stressed environments. To more closely examine the dynamics of these two groups, additional time series were constructed that present both abundance and presence data. See Figures 5.46 and 5.47 for coccals (6-20 μm) and ciliates, respectively. Following the rainfall

peak of Day 158, edible coccals increases in both mean abundance and occurrence for both groups. While occurrence was approximately equal between groups between Day 158-186, mean abundance was greater among Group 2 systems during this period with peaking mean abundance occurring on Day 172. The difference in mean abundance for this date, however, was not significant (t-test: t=1.36, df=13, p[one-tail]=0.098). When only occupied systems were compared, however, the difference in mean abundance was significant (t-test: t=1.82, df=10, p[one-tail]=0.049). Ciliate occurrence within Group 1 decreased steadily from Day 130-172 with a slight increase over Days 172-200. Ciliates in Group 2 systems, on the other hand, increased in occurrence from Day 130 until Day 186 when all microcosms in this group were occupied. While both groups exhibited an increase in mean ciliate abundance over Days 144-158, a greater increase occurred among Group 2 systems. A t-test comparison of mean abundance between groups, however, was insignificant for Day 158 (t-test: t=1.36, df=13, p[one-tail]=0.098.

5.4 Discussion

The aim of this study was to create an experimental basis in which community convergence could be tested. By establishing multiple artificial communities at the same site and under the same initial conditions, the same physical environment could be approximated among systems. Given similar settings, do we find a convergence in compositional factors? From data gathered after eight years of undisturbed development, evidence for convergent trajectories was sought among three sets of microcosms: 1) the overall site, 2) within experimental clusters, and 3) among communities independent of spatial location. As this study comprises a natural experiment, it is essentially descriptive.

Diversity was notably high among microcosms. In total, 41 species were found over the full course of sampling. Total species count per sampling date ranged between 20-24 species. Mean species richness per microcosm, however, was significantly lower ranging from 6.7 species on the initial day of sampling to low of 4.4 species at sampling end. Only two species – *Astasia* and an unidentified ciliate – had at least a single occurrence in all 15 systems. Approximately two-thirds of all species occurred in less than half of the microcosms and no single species was found to occupy more than 11 microcosms simultaneously. Occurrence, however, was greater at higher levels of classification. Of the 12 higher taxa groups observed, representatives from four were found in all systems: *Chlorophyceae* (green algae), *Euglenophyta* (euglenoids), *Bacillariophyta* (diatoms), and *Ciliophora* (ciliates). These fours groups accounted for ≈50% of all species. Of the 11 functional groups, representative from three groups were found in systems: flagellates (>20 μm), flagellated heterotrophs (<20 μm), and ciliates (>20 μm). These groups accounted for ≈ 22% of all species.

Colonization history among systems was highly variable with the initial occurrence of species between microcosms spread over multiple sampling dates for most species. Total abundance per microcosm was also variable with large oscillations in abundance well correlated with changes in rainfall. While differences between microcosms or clusters were not found in terms of basic descriptive community parameters (i.e. species richness, diversity, trophic variables), significant temporal trends were found that indicated both a significant decline in mean species richness as well as a significant increase in community size. The trend toward increasing mean body size and concomitant changes in the edible portion of the extant autotrophic population are consistent with established

models of plankton succession (see Sommer et al. 1986). Thus, an overall convergence among systems was indicated based on these crude measures of community structure.

Evidence of convergence for the overall study site was not found. Rather, similarity between communities displayed a sharp decrease over the Day 144-158 apparently driven by the disturbance generated by the heavy rainfall during this period. Thereafter, similarity remained relatively constant for the remainder of the study period. The asymptotic nature of this relationship suggests a possible upper bound on allowable species combinations. Cumulative evidence drawn from MRPP, Sorensen dissimilarity, and cluster analyses, however, suggest that a weak convergence at the cluster scale occurred during periods of reduced rainfall. The weak statistical power arising from the small number of replicates within clusters prevents a clear assertion of this effect. Whether this convergence was driven by difference in physical environment or propagule inputs in uncertain. Differences between clusters in terms of their relative proximity to potential sources of colonists – either from canopy washout or otherwise – is possible. Among environmental factors, differences in light availability seem unlikely. Canopy closure was complete at the time of establishment and only diffuse light and occasional sunflecks were observed at the microcosm level during the course of sampling. One potential candidate is a difference in leaf litter inputs. Leaf litter quantity and quality have been shown to affect the colonization and distribution of aquatic biota (Stout et al. 1985; Yanoviak 1999). Overstory composition was variable across the study site and differential inputs of leaf litter are likely.

Stronger evidence was found for a divergence in community trajectories emerging between communities independent of spatial proximity. Differences in the occurrence of

four functional categories over Days 158-200 were found between two groups of microcosms. The first group exhibited an increase in edible coccals and decreases in flagellated autotrophs (>20 μm), flagellated heterotrophs (<20 μm) and ciliates. The second group showed contrasting trends for all four categories. Statistical analysis of compositional changes between groups indicated a consistent pattern of divergence arising between these groups that were initiated on Day 158. While elucidating cause and effect in such a case can be difficult, a possible explanation for the observed patterns may lie in the relationship between *Chlorella* and ciliates. The rainfall event preceding Day 158 appeared to have had a disproportional effect on increasing Coccals (6-20 μm) abundance among Group 2 microcosms. This led to a disproportionate increase in the occurrence of ciliates in these systems. Preferential grazing of coccals by ciliates could have then shifted dominance to flagellated autotrophs and flagellated heterotrophs in these systems. Clearly, it would be presumptuous to assert that the difference observed among these communities constitute alternative stable states. While alternate stable states have been demonstrated in a number of phytoplankton systems (Drake 1991; Drake et al. 1993; Huxel 1995; Robinson and Edgemon 1988), the length of my sampling record precludes such an assertion in this case. Telling, however, is the fact that MRPP comparisons for the final sampling date indicate that composition difference between these groups remained significant despite the heavy rainfall preceding this date. In addition, rainfall records indicate that 7-day totals did not exceed 0.75 inches for nine weeks following the conclusion of sampling. Whether these communities would remain stable given decline in environmental fluctuation, however, would be speculative.

One explanation for the high diversity observed in my systems may lie in the intrinsic

nature of plankton communities. Scheffer (1991) has argued that accumulating evidence of the past two decades suggest that planktonic systems possess a strong potential for nonequilibrium dynamics. Plankton communities often possess high diversity and erratic population fluctuations. Oscillating dynamics have been observed in field data (e.g. Reynolds 1980, 1990; Sommer 1986; McCauley and Murdoch 1987), laboratory experiments (e.g. Goulden and Hornig 1980), and in simulation models (Kooijman 1986). Long-term laboratory experiments in plankton dynamics, in particular, have shown that multispecies systems can display highly irregular behaviour that persists for many years (Ringelberg 1977; Kersting 1985). Field experiments similar to this study have also shown high level of species diversity among systems possessing identical physical conditions (Jenkins and Buikema 1998). Indeed, Scheffer (1991) suggests that planktonic systems may be prime candidates for chaotic dynamics. While convincing evidence for chaos from natural systems has been generally lacking, one notable exception is an analysis performed by Sugihara and May (1990) on a large data set of marine plankton counts taken over a 20 year period at Scripps Pier, San Diego. Their analysis concluded that dynamics expressed by the diatom community were at least partly governed by a strange attractor.

Nonequilibrium dynamics in phytoplankton systems open the door for a powerful influence of historical events such as environmental fluctuations to override the tendency for internal order and stability (Harris 1986). Disturbance in the form of periodic heavy bouts of rainfall had a strong impact on influencing community development in my systems. Heavy rainfall appeared to have a multiple effect in stimulating growth and dispersion as well as disrupting community structure. Atmospheric inputs have been

shown to have a dramatic effect altering water chemistry and community properties in similar systems (Carpenter 1982, Walker et al. 1991). Large levels of accumulated leaf litter were observed in all microcosms and the high concentration of carbon resulting from slow decomposition would effectively tie limited nutrients to the bacterial component. The effect of rainfall inputs – including wet deposition and washout of dry deposition from the forest canopy – could therefore have a profound, but likely short lived, effect in enhancing nutrient availability in these systems. As deposition of atmospheric nitrogen from manmade emissions is known to be particularly high in this region, nitrogen inputs would likely be disproportionately high. While a concomitant effect of nitrogen inputs on decreasing pH is likely, such an effect cannot be quantified without water chemistry data. As phytoplankton are known to have different pH tolerances that can affect both growth and competitive relationships (Kroes 1971; Goldman et al. 1982a, 1982b), differential changes to pH among microcosms could have played a role in initiating the divergence of communities exhibited after the rainfall event preceding Day 158.

5.5 Prospectus

This study has a number of shortcomings. First and foremost, a segregation of physical and biotic factors is impossible without supporting environmental data. The assumption that conditions within clusters are more similar than between clusters can therefore not be tested. Second, the sample record is too brief. While available data offers a glimpse of underlying community dynamics, only with a comprehensive data record – i.e. full seasonal data preferably collected over several seasons – can any hope of generality be ascribed to the observed patterns of convergence/divergence. Sampling on

a shorter time interval may also be warranted given the rapid changes in species composition observed in relation to rainfall inputs. A third shortcoming is the relatively small experimental design. A larger number of replicates would increase sampling power and allow the detection, if any, of subtle processes operating at small scales. Establishment of microcosms on multiple sites would also allow a cross comparison of communities established under alternative conditions. Clearly, however, the logistics of such an "ideal" experiment could be prohibitive.

Chapter 6: Conclusions

6.1 Introduction

A continuing debate over the nature of ecological communities has persisted in which two opposing viewpoints have struggled for supremacy. The first viewpoint holds that, under stable environments, communities are structured largely through forces within the community (Cowles 1901; Clements 1916; Margulef 1963; Odum 1969). Successional trends in community development are viewed as self-organizing processes wherein community structure emerges from the interactions among community members. The second viewpoint holds that community structure arises largely through the imposition of extrinsic processes (Gleason 1926). Here, the vagaries of fluctuating environments, disturbance, and random species recruitment mediate the formation of species assemblages.

Clearly, these respective positions represent highly polarized views on the nature of ecological communities and case evidence for each has been offered by supporters of both schools. Evidence increasingly suggests, however, that the roles of equilibrium and nonequilibrium processes and that of determinism and chance can play variable roles in the development of community structure (Chesson and Case 1986; Cody 1989; Wu and Lockes 1995). As no single theory of the succession appears to capture the rich nuances of this process, some ecologists have called for a more pluralistic approach (e. g. Cody 1989; Berlow 1997). In this regard, Berlow (1997) describes three categories of successional processes that potentially arise in natural systems: 1) externally driven, 2)

canalized, and 3) contingent. *Externally driven succession* – consistent with the Gleasonian viewpoint – stresses the disproportionate role that extrinsic forces play in maintaining community structure. *Canalized succession* occur when early colonizers have strong and consistent effects on following species and/or when phenological or dispersal constraints generate consistent patterns of recruitment (Berlow 1997). These effects result in a relatively deterministic and predictable turnover of species wherein communities starting under different initial conditions converge toward a common endpoint. Following a disturbance, these communities return via similar pathways to the same predisturbance state. Canalized successional trends are, therefore, consistent with Clement's (1916) single climax community model. *Contingent succession* occurs when the sign and/or magnitude of species interactions strongly depend upon the context in which they occur (Berlow 1997). Here, effects arising from the timing and/or sequence of invading species can produce multiple successional pathways that ultimately diverge to alternative stable states. An understanding of the variation produced among community states requires a detailed knowledge of species interactions and how they are influenced by historical events. If such a pluralistic approach is viable, then a critical challenge to a basic ecological theory must be to determine under what conditions such situations can be expected to arise.

6.2 The Where, When, and How of Colonization History Effects

Evidence from theoretical and experimental community assembly studies has shown that variation in colonization history *can* have lasting impacts on patterns of species composition (Law and Morton 1996; Drake et al. 1999; Morin 1999a; Sait el al. 2000). Empirical support from natural systems comes from case studies of alternative

community states as well as long term investigations of successional trends that have implicated variation in either initial species composition and/or species arrival order as mechanisms underlying the divergence of community states (e.g. Engler 1954; Sale 1977; Sutherland and Karlson 1977; Wood and del Moral 1987; Fastie 1995). How prevalent such effects are, however, remains unclear and many questions remain unanswered. Here, I discuss what I perceive to be three key questions: the where, when, and how of invasion history effects.

6.2.1 Where Are Effects of Invasion History Mostly Likely to Emerge?

Are some ecosystem types more prone to historical effects than others? Alternative community states arising from variation in invasion sequence have been predicted to be more prevalent in species-rich systems with high food web connectance and/or interaction strength (Gilpin and Case 1974; Luh and Pimm 1993; Law and Morton 1996). Marine and aquatic ecosystems may, therefore, be prime candidates for such phenomena (Law and Morton 1996; but see Moyle and Light 1996). Indeed, Knowlton (1992) indicates that a disproportionate number of cases of alternative states have been reported from marine environments. Numerous examples of priority effects have also been demonstrated in various marine and aquatic systems (Turner 1983; Alford and Wilbur 1985; Hart 1992; Lawler and Morin 1993a; Blaustein and Margalit 1996; Berlow 1997; Fincke 1999; Benedetti 2000). Moreover, the emergence of alternative states found in experimental assembly studies have all occurred in aquatic microcosms (e.g. Robinson and Dickerson 1987; Robinson and Edgemon 1988; Drake 1991; Huxel 1995). As experimental assembly studies have been *limited* to aquatic systems, however, the generality of their results to other system types is unclear. In Chapter 3, I therefore

addressed the role of community assembly effects in an alternative system type by assessing the influence of invasion history on community structure in assembled soil microbial communities. As evidence of comparable studies was not found in the literature, this experiment may be the first exploration of sequence effects in such systems. I found that altering the sequence of invasion of microbial competitor resulted in significant impacts on both community composition and invasibility. Thus, invasion history may also play an influential role in soil ecosystems.

The findings of my study as well as previous experimental studies in community assembly (e.g. Robinson and Dickerson 1987; Robinson and Edgemon 1988; Drake 1991; Huxel 1995), however, may be contingent on the life history traits of constituent species (Young et al. 2001). Community assembly models treat the establishment and growth of invading species as a population level phenomenon (e.g. Post and Pimm 1983; Drake 1988, 1990a; Law and Morton 1993, 1996). Rare propagules arrive, and if successful, establish a growing population. Priority effects emerge when population abundance reach levels that exert an effect on subsequently arriving species. Young et al. (2001), therefore, suggests that the mechanisms of establishment among invertebrates, algae, and microbes – e.g. determinate growth, high intrinsic growth rates, and generation times typically much shorter than species residence times – may make aquatic and soil systems more prone to priority effects and, therefore, more susceptible to the influence of invasion history.

The influence of invasion history in terrestrial systems is more difficult to assess. Models of community succession in plant communities treat the growth and establishment of species as an individual-based phenomenon (Young et al. 2001). Many

plant species populations are in decline by the time that reproductive maturity is reached. Young et al. (2001), therefore, argues that the establishment characteristics of vascular plants – indeterminate growth, low growth rates, and residence times not much longer than individual life spans – likely reduce the potential for priority effects in plant communities except for cases of limited dispersal. Consequently, the impact of difference in species arrival may be more limited in these systems. Evidence of priority effects, nonetheless, have been found. For example, the early colonization of species that are highly suppressive of later successional species have been observed to delay or even halt successional processes in some systems (Tappeiner et al. 1991; Mallik 1995). Controlled field experiments have also shown the effect of timing of species arrival can influence competitive outcomes (e.g. Harper 1961; D'Antonio et al. 2001) and seed germination rates (e.g. Eriksson and Eriksson 1998) among plant species. Facelli and Facelli (1993) found that, even after death, the leaf litter of early dominant species could inhibit the growth of later species. Moreover, field studies in terrestrial systems have also attributed the formation of alternative climax communities to variation in initial floristic composition (Engler 1954) or to differences in species arrival times (McCune and Allen 1985; Fastie 1995; Honnay at al. 2001). To date, the intrinsic time constraints involved in long-term field studies, however, have precluded a comprehensive test of assembly effects in plant communities. In that regard, ecological restoration of such systems may offer the potential of expanding assembly theory in terrestrial systems when permutation of species introductions are considered.

Theoretical work by McKane et al (2000) suggest a potential diagnostic for identifying systems susceptible to invasion history effects (see also Solé et al. 2000). The

authors explored the nature of species turnover in model communities wherein extinctions were driven by both species interactions and random immigration events. They found that communities evolved to a dynamic species equilibrium with the distribution in species lifetimes contingent on the relative influence of food web connectance and immigration rate. At very low connectance and high immigration, turnover was driven exclusively by random immigration events and lifetimes were Gaussian. At higher connectance and high immigration, species lifetimes became lognormal. Finally, at high connectance and low immigration rates, communities came to a critical state characterized by multiple long-lived transient states with power law distributions in turnover. As theoretical studies indicate that assembly driven alternative community states are likely more prevalent under these same conditions (Law and Morton 1993, 1996), power-law distributions in turnover may indicate the types of systems where invasion history is likely to be most influential.

6.2.2 When Are the Effects of Invasion History Most Likely to Emerge?

What conditions or circumstances are likely to promote historical effects? I address three conditions: 1) site productivity, 2) invasion characteristics, and 3) environmental stability.

6.2.2.1 Site Productivity

Theoretical studies predict that the prevalence of alternative stable states increases with the size of the regional species pool (Drake 1990b; Law and Morton 1993, 1996; Luh and Pimm 1993). It follows that environmental conditions that promote species diversity on a local scale might also enhance the potential for invasion history effects. Conditions that highly limit community membership, on the other hand, should constrain

the effect of historical effects. Species diversity, for example, has been shown to peak at intermediate productivity (Tilman 1982; Rosenzweig 1995; Waide et al. 1999). Alternative stable states may also, therefore, be most likely at these productivity levels (Van der Koppel et al. 1996; Chase 1999). While an exploration of environmental factors among experimental assembly studies has been limited, findings do indicate that the effects of invasion history are contingent on abiotic conditions (e.g. Dickerson and Robinson 1985; Drake 1991; Huxel 1995). Drake (1991), for example, observed that sequence effects led to alternative community states when communities were assembled in large-scale systems. When assembled in small-scale systems, on the other hand, increased light availability resulted in a single species dominating all communities regardless of invasion order. Similarly, Huxel (1995) found that initial invaders exhibited priority effects when communities were cultured under batch nutrient conditions. When nutrients were replenished, however, a single competitor again dominated all sequence treatments. Tests of such factors in these studies, however, has been limited to two experimental levels. In Chapter 4, I sought a more comprehensive test of the interaction of invasion sequence with productivity by assembling detritus based protist communities on a gradient of five productivity levels. Two results emerged. First, a diversity of community states emerged with changes in productivity within sequences. Second, and more significantly, the influence of invasion history on community structure was dependent on productivity level. Sequence effects were not found at the two lowest nutrient concentrations. At higher concentrations, however, sequence effects led to alternative community states. Two mechanisms may be operating in this case. First, as already indicated, higher productivity levels allow the participation of a greater number

of species (Rosenzweig 1995). The more species, the greater the spectrum of potential priority effects. Lower productivity sites, therefore, may be less influenced by colonization history (Chase and Leibold 2002). Second, more productive sites enhance growth rates and, thereby, amplify priority effects. Very high productivity levels, however, may favor superior competitors that can ultimately dominate a system regardless of invasion order (e.g. Drake 1991; Huxel 1995). Alternative states were found at the highest level tested in this study. Whether such states would be maintained at still higher productivity levels or at higher rates of invasion could be explored. Another key finding of this study was that mean species diversity peaked at intermediate productivity levels in both sequences consistent with some theoretical predictions (Tilman 1982). That this peak occurred at different levels for each sequence emphasizes the potential role of invasion history in mediating local biodiversity.

6.2.2.2 Invasion Characteristics

The emergence of alternative stable states in community assembly models appear contingent on two key assumptions regarding the nature of invasion events. The first assumption regards communities as relatively closed to invasion. Invasions are treated as single species events that occur at frequencies low relative to equilibrium times and at low founder abundance (e.g. Post and Pimm 1983; Drake 1988, 1990a; Law and Morton 1993, 1996). Theoretical studies that have tested this assumption have shown that opening the community to a greater influence of invasion – either by increasing species number, invasion frequency, or founder abundance – can subvert the formation of alternative states (Huxel 1995; Lockwood et al. 1997; Büssenschütt and Pahl-Wostl 1999). Experimental assembly studies have observed similar findings. Robinson and

Edgemon (1988), for example, found that high invasion rates greatly diminished the influence of sequence effects. Huxel (1995) also observed that dominance hierarchies arising from manipulations of invasion order were contingent on the time interval between invasion and founder population abundance. All species exhibited priority effects when introduced at low initial densities and low invasion rates. At higher invasion rates, however, priority effects were only maintained by increasing founder abundance. Thus, the influence of invasion history is likely to be greatest in relatively closed systems or where population growth rates are rapid relative to the arrival of new species. As growth rates are contingent on productivity levels, lower productivity sites should necessitate lower invasion rates than more productive sites for priority effects to emerge.

The second assumption is that all species have equal access to communities (Young et al. 2001). That is, any species can potentially invade at any time step with equal probability. When invasions are random, the number of possible sequence permutations increases factorially with species pool size. Thus, even a small pool of just 10 species has 3,628,800 possible sequence permutations where only a single invasion attempt per species is allowed. As species frequently demonstrate differences in phenological patterns of activity and dispersal abilities (Morin 1999a; Young et al. 2001), random colonization is likely unrealistic for many natural systems. Scenarios involving species with well defined differences in phenological attributes would constrain the number the potential colonizers at any given time step and, thereby, delimit the number of possible sequence permutations. The effects of invasion history should, therefore, be diminished in such cases. Moreover, many species display tradeoffs in life history attributes that

either favor colonization or competition abilities. Huston (1979) found that when such traits were inversely correlated, successional patterns exhibited a predictable sequence of turnover (i.e. canalized) that culminated in a competition-structured community reminiscent of Clements' (1916) climax successional model. To date, theoretical assembly studies have not considered the role of life history traits in mediating invasion history effects. Incorporating life history attributes in community assembly models would contribute to tying community assembly and community succession approaches into a more comprehensive theory of community development.

6.2.2.3 Environmental Stability

The canonical assembly models (e.g. Post and Pimm 1983; Drake 1990b; Law and Morton 1993, 1996) took an equilibrium approach to community dynamics. Experimental assembly studies have also all assumed a stable environment. When the conditions of equilibrium have been tested by increasing the rate of species arrival, the influence of invasion history in generating alternative community states was either diminished or negated (e.g. Huxel 1995; Lockwood et al. 1997; Büssenschütt and Pahl-Wostl 1999). While the impact of disturbance on community dynamics has been well documented (Huston 1979; Sousa 1984; Pickett and White 1985), a similar investigation of the interaction of invasion sequence effects with disturbance factors has yet to be addressed.

The function of disturbance in reinitiating the successional process is a common element of succession models and is, therefore an implicit component of community assembly theory. Disturbance has been also implicated as a generative event in many case studies of alternative stable states in natural systems (e.g. Barkai and Branch 1988.

Dublin et al. 1990). The effect of various disturbance or environmental fluctuations on community trajectories incurred *during* the assembly process, however, is unclear. Presumably, some degree of environmental stability is required for assembly effects to fully emerge. Very frequent and intense disturbance, therefore, should override historical effects. In Chapter 5, for example, I tested for convergent trends in species composition among a suite of field microcosm communities based on spatial proximity. While evidence suggested a weak degree of convergence among neighboring systems, disturbance in the form of periodic bouts of heavy rainfall had a significant randomizing effect on community structure. Moreover, consistent with some cases of alternative stable states, a particular rainfall event appeared to play a key role in generating a pattern of divergent composition between two sets of communities that were independent of spatial location.

How disturbance regimes of less intensity or frequency interact with sequence effects requires investigation. A number of possible interactions seem possible. Huston (1979) demonstrated theoretically that, where disturbance was assumed to have a proportionate effect on reducing all resident species, the effect of disturbance on mediating competitive interactions was dependent on the rate of disturbance frequency relative to species growth rates. Competitive exclusion was found to be slowed or even halted when invasion frequency is rapid relative to growth rates. Here, simultaneous establishment was assumed for all species. Presumably, priority effects could still arise under some regimes of intermediate disturbance when colonization rates are slow enough for early arrivals to overcome induced setbacks in population abundance. Where disproportionate effects on community members arise, disturbance could potentially enhance invasion history effects

by: 1) amplifying priority effects where later species arrivals are more negatively impacted, or 2) creating species configurations with unique invasibilities that when successfully colonized divert communities down alternative trajectories. Where species display well-defined differences in phenological attributes, the timing of particular disturbance events could also act as humpty-dumpty processes that catalyze unique community states. The dependence of recruitment on the timing of disturbance, for example, has been shown to affect the trajectory of community development (Sousa 1980; Pickett and White 1985; McCune and Allen 1985)

6.2.3 How Do Effects Of Invasion History Emerge?

What are the mechanisms underlying the effects of invasion history? How do differences in the order of invasion result in alternative stable states? While a number of theoretical and experimental studies in community assembly have demonstrated that differences in invasion order can alter community structure (Robinson and Dickerson 1987; Robinson and Edgemon 1988; Drake 1990b; Drake 1991; Law and Morton 1993, 1996; Luh and Pimm 1993; Huxel 1995; Kokkoris et al. 1999), an investigation of the mechanisms involved have remained largely unexplored. Here, I discuss: 1) priority effects, 2) deterministic versus indeterministic assembly trajectories, and 3) keystep species.

6.2.3.1 Priority Effects

The influence of differences in invasion order on community properties are broadly attributed to priority effects wherein species that colonize a community earlier have differential impacts on subsequently arriving species (Morin 1999a). Such effects can arise through direct (e.g. Hart 1992; Lawler and Morin 1993b) or indirect species

interactions (e.g. Kim 1997) or through biologically mediated changes to abiotic conditions (Hart 1992; Facelli and Facelli 1993; Chapter 4). The emergence of priority effects have been shown to be a complex phenomenon that can be variably dependent on: 1) the timing of species arrivals (Alford and Wilbur 1985; Lawler and Morin 1993b; Shorrocks and Bingley 1994; Huxel 1995; Hodge et al. 1996), 2) abiotic conditions such as pH (Warner et al. 1991), nutrient concentration (Huxel 1995; Chapter 3), and system scale (Drake 1991; Finke 1999), or 3) the presence of other species (Kim 1997).

Most experimental explorations of priority effects have addressed the inhibitory effect of early arrivals on subsequent species (Alford and Wilbur 1985; Facelli and Facelli 1993; Lawler and Morin 1993b; Shorrock and Bingley 1994; Ehmann and MacMahon 1996). Inhibitory effects have been shown to be asymmetrical, i.e. *Species A* effects *Species B* when *Species A* arrives first, but *Species B* has no effect on *Species A* when *Species B* arrives first (Lawler and Morin 1993b). In Chapter 4, for example, I assembled replicate protistan communities under two sequence permutations and at five nutrient concentrations. I observed that when *Blepharisma* was the first omnivore introduction, *Euplotes* was unsuccessful at all productivity levels. *Euplotes* was only found to persist at a single intermediate productivity level when it was the first omnivore introduction. A strong reciprocal effect on *Blepharisma*, however, not was found. Priority effects can also have facilitative effects wherein in the survival and/or growth of later species is enhanced by early species (e.g. Small et al. 1971; Werner and Harbeck 1982; Kim 1997). In Chapter 2, I explored the effect of permuted invasions of three competing microbial strains on community composition and vulnerability to invasion by a target strain, i.e. *P. fluorescens* HK44. I found that the post-introduction abundance HK44 as well as the

abundance of other indigenous heterotrophic populations appeared correlated with the timing of introduction of one of the three competing strains, i.e. *Sphingomonas*. When *Sphingomonas* was introduced first, HK44 and heterotrophic abundance was high. Conversely, when *Sphingomonas* was introduced last, HK44 failed to invade and remaining heterotrophic abundance was low. The reason for this correlation is unclear. One hypothesis is that *Sphingomonas* had an inhibitory priority effect that diminished with time. The abundance of HK44 in sequences where *Sphingomonas* was introduced first, however, was 1-3 orders of magnitude greater than those obtained when HK44 was introduced alone in similar soil environments (e.g. Sayler et al. 1997; Zimmermann et al. 1997; Ripp et al. 2000). An alternative hypothesis, therefore, is that a facilitative effect among invaders (sensu Simberloff and Von Holle 1999; Ricciardi 2002) emerged from this sequence that promoted HK44 biomass levels.

To date, most explorations of priority effects have been limited in scope. Typically, only taxonomically or trophically related (i.e. guilds) species have been considered. Time scales are also usually short in duration. Successional models that incorporate mechanisms of facilitative or inhibition (e.g. Clements 1916; Engler 1954; Connell and Slatyer 1977), on the other hand, generally address the broad cumulative effect of various stages of development on subsequent stages. Moreover, such models (e.g. Connell and Slatyer 1977) assume the repeated operation of a single mechanism – i.e. facilitation *or* inhibition – that results in a particular pathway (Pickett et al. 1987). The multiplicity of pathways that could arise from mixed mechanisms or from variation in species colonization history are not addressed. Studies of priority effects have also generally only focused on the operation of a single mechanism between species. Species that have

differential effects on subsequent invaders, however, could act as "switches" that shunt successional trajectories down particular pathways. Future investigations could address such effects by expanding the focus of study to the interactions – both direct and indirect – of a larger group of species and at longer time scales.

6.2.3.2 Deterministic and Indeterministic Trajectories

Among communities assembled under experimental conditions, Drake (1991) found that varying degrees of determinism in the trajectories emerged under different invasion sequences. Some assembly trajectories behaved deterministically in that a given sequence of invasion consistently led to the same community state. Other trajectories, in contrast, behaved indeterministically wherein the same sequence produced a variety of community endpoints. Drake (1991) suggests that this indeterminism may result from an inherent sensitivity to subtle variation in environmental or demographic (i.e. founder vagility) variables that emerges as a property of the sequence in question. Given this dichotomy of community behavior, the potential for alternative community states can be seen to emerge from sequence related effects in two ways: 1) from variation in the order in which species arrive wherein the trajectories of individual permutations behave deterministically, and/or 2) from the amplification of variation in environmental factors due to a sensitivity to such variation inherent within some sequences. For the latter case, what factors govern this sensitivity requires investigation. Is it synergetic effect of the entire sequence? Or are there key species, or perhaps key combinations of species, whose timing of invasion play a fundamental role in generating such sensitivity (see Section 6.1.3.3)? How context dependent are such effects? In Chapter 4, for example, I assembled replicate protistan communities under two sequence permutations and at five

nutrient concentrations. While the degree of community similarity between replicates was statistically insignificant between treatment combinations by the conclusion of the experiment, communities assembled under the sequence where *Euplotes* was the first omnivore introduced were found to exhibit a greater degree of variation in community composition during the equilibration period at all productivity levels tested. When *Blepharisma* was the first omnivore introduction, strong priority effects generated by this species also had the effect of reducing variation between replicates. Thus, while both sequences produced community trajectories that were ultimately equally deterministic (sensu Drake 1991) in that replicates were found to converge to a single community state, the differential degree of variation generated between sequences en route to such convergence could provide the necessary conditions onto which further variation could be created. What effect, for example, would an additional invasion – timed to occur during the period of peak divergence – have on subsequent community structure? Moreover, the maximum degree of dissimilarity produced within sequences was correlated with nutrient concentration with the greatest peak dissimilarity occurring at highest nutrient concentration for both sequences.

6.2.3.3 Keystone and Keystep Species

A number of authors have recognized a need for a distinction between the processes or mechanisms that generate the divergence between alternative community states and those that maintain them (Sutherland 1974; Drake 1990a; Petraitis and Latham 1999). The keystone species of Paine (1966) or the foundational species of Dayton (1971) represent species that play a critical role in *maintaining* community structure. Field experiments have demonstrated that artificial exclusion of such species can produce a cascade of

extinctions that alters the community structure (e.g. Paine 1966, 1977). Keystone species have been identified in a number of natural systems including the starfish in rocky intertidal communities (Paine 1966), the elephant in the African savannah (Laws 1970), and the crayfish in lotic communities (Hart 1992).

Similarly, it might be possible to identify one or more species whose timing of colonization plays a fundamental role in *generating* alternative community states. I term these *keystep species*. In general, two types of such species could be recognized: 1) *canalizing species* that act to converge community trajectories toward a single community state, and 2) *bifurcation-producing species* that act to promote divergent trajectories leading to alternative stable states. As any given invasion sequence could potentially contain multiple representatives of each of these types of species, the ultimate community state attained could depend on their respective timing of invasion. While keystep species could potentially be found for both deterministic and indeterministic community trajectories (see Section 6.1.3.2), the nature of the mechanisms underlying each would appear to be fundamentally different.

Among indeterministic trajectories, alternative states have been observed to emerge as a result of increased sensitivity to environmental or demographic (i.e. species vagility) variables that arises as a function of some sequences. Here, keystep species operate by either amplifying (i.e. bifurcation-producing species) or dampening (canalizing species) environmental variation. The production of alternative states among such trajectories would appear to require one or more noise amplifying species that generate a degree of requisite variation from which divergent pathways could emerge. Alternative community states could thus potentially emerge in two ways: 1) positive-feedback mechanisms push

divergent trajectories into the basins of alternative community attractors (Wilson and Agnew 1992), or 2) subsequent invasions by canalizing species draw subsets of these diverse trajectories together toward alternative endpoints. Figure 6.1 offers for a graphical representation of this latter concept. Here, the invasion of Species A acts as a noise amplifying process that generates a diversity of divergent trajectories. For trajectories leading to State I, the invasion of Species B acts to dampen noise and canalize a subset of trajectories toward this state. Similarly, the invasion of Species C draws the remaining trajectories toward State II.

Among deterministic trajectories, in contrast, operate by defining the topography of possible community states based on the variation in the order of all subsequent invasions. Among species pools that contain but a single community state (see e.g. Law and Morton 1993, 1996), differences in invasion order have no effect on outcomes and keystep species do not exist. Among species pools containing multiple states, canalizing species act to constrain all possible subsequent sequences toward the same community state. That is, differences in the order of invasion following this species have no effect on altering community structure. In contrast, bifurcation-producing species act as "switching" nodes around which pathways to alternative states are generated. Here, the ultimate state remains undetermined until one or more subsequent invasion events are played out. These concepts are graphically demonstrated for a small pool of six species (Figure 6.2). While such a pool contains a total of 6! (e.g. 720) sequence permutations where species are allowed a single invasion attempt, 10 sequences are illustrated for conceptual purposes. Here, Species D acts a canalizing species in Sequences 1-2 and Sequences 5-10. When Species D is the third invasion following Species A and B (e.g.

Sequences 5-10), Community State IV is the result regardless of the order of invasion of following species. Species B, D, and E, on the other hand, act as bifurcation and subsequent invasion are necessary before the final state can be predicted. Community State III, for example, is not determined until all invasions have occurred. In this example, the potential role of individual species as keystep invasions can be dependent on the timing of invasion. Species D has a similar canalizing effect on subsequent invasions when it is either the third or fourth invasion. The community state produced in each case, however, is different. In Sequences 3 and 4, this species occurs too late in the sequence to produce any effect. The bifurcation producing effect of Species C and E, on the other hand, depends critically on timing. When these species follow Species D (Sequence 1 and Sequences 5-10), for example, bifurcation effects do not emerge. Note that I distinguish keystep species based on their relative effect *across* all possible trajectories in defining the overall assembly space. This does not diminish, however, the importance of the timing of invasion of other species in producing a given state. In my example, Community States II can only be arrived at by a single invasion sequence. Here, the timing of invasion of all constituent species is, therefore, essential for the emergence of this state.

If the above conceptualization of the assembly process is an accurate reflection of the assembly processes, a number of questions need to be addressed: 1) Are their some individual species that consistently behave as keystep species regardless of where they are found? Or such properties a intrinsic function of the regional species pool and/or environmental context? 2) Are their species characteristics – i.e. trophic level, interaction strength, connectivity to other species – that distinguish canalizing or bifurcation species?

Law and Morton (1993, 1996), for example, found that the prevalence of alternative community states increase with species pool connectance. Species that increase overall connectivity (i.e. omnivores), therefore, may be likely candidates for bifurcation species. Strong competitors, on the other hand, may act as canalizing species (e.g. Tilman 1982). 3) How dependent are such effects on the relative timing of invasion? Are there periods during the assembly process, for example, when keystep species are most likely to play an influence? Clearly, the answers to such questions would necessitate an exploration of a fairly large number of alternative and studies of this nature would have to be performed in a theoretical setting. Findings could then be used to generate hypotheses that could be tested in natural systems.

6.3 Applied Community Assembly

In addition to the theoretical contributions community assembly can offer community ecology, assembly theory may also offer avenues of practical application to the field of ecological engineering. The results of such application could, in turn, contribute to the theoretical realm. Ecological engineering is a broad area of applied ecological theory that encompasses such diverse fields as ecological restoration, environmental bioremediation, waste treatment, food and fuel production, and the design of sustainable habitats (Mitsch 1993; Todd and Josephson 1996). Here, I finish with a discussion of possible avenues in which knowledge gained from community assembly research could have application.

6.3.1 Ecological Restoration

Restoration ecology has largely progressed as a haphazard collection of individual, site specific cases with little development of general theory or repeatable principles that would allow the transfer of methodologies between situations (Hobbs and Norton 1996;

Keddy 1999). As a consequence, a majority of restoration attempts fall short of predetermined goals (Lockwood and Pimm 1999). While restoration as a technique for mediating damaged ecosystems has increased worldwide (Cairns 1988; Jordon et al. 1988), restoration attempts are likely to continue to meet with limited success without the development of a sound theoretical principles. Recent attempts towards building a such conceptual framework, however, are being made (see Pickett and Parker 1997; Hobbs and Norton 1996; Allen et al. 1997; Keddy 1999; Egan and Howell 2001). In particular, restoration ecologists are beginning to recognized the influence that issues of complexity and nonequilibrium dynamics can have on restoration efforts. The impact of historical factors and initial conditions on current community states, for example, has been recognized (Hobbs and Norton 1996; Egan and Howell 2001). In addition, as the prevailing approach to restoration involves directing ecosystem development through forced transitions along some desired pathway (Luken 1990), the potential influence of threshold effects in producing undesirable community endpoints is also being addressed (Hobbs and Norton 1996). As the reversal of such effects can be difficult or impossible, knowledge of such threshold points could be crucial to achieving desired goals.

Ecological restoration often assumes a limited form of community assembly wherein species are added to a system with the aim of reconfiguring the system to some desired goal. Given the poor success rate of most restoration projects, however, a more explicit application of the tenets of community assembly theory could prove worthwhile (see Keddy 1999; Young et al. 2001). While the role that controlled sequential introductions could play in restoration efforts has been acknowledged, the application of such effects has received little attention. (Hobbs and Norton 1996). Clearly, many questions would

require addressing were such an approach be undertaken. These would include but are not limited to: 1) What is the appropriate species pool? 2) What are the appropriate introduction parameters, i.e., timing and rate of introductions, founder abundance, etc.? 3) How much knowledge of historical factors is required? Is the entire tortuous history of a community required for its replication or are there shortcuts? and 4) Are humpty-dumpty effects (sensu Drake 1985; Pimm 1991) operating such that intermediate community states involving transitory species may be required to achieve the target community? As a knowledge of community history is likely to be limited in many restoration projects, some trial-and-error would be required. The knowledge gained from such an intentionally experiment approach could enhance assembly theory as well as generate an assembly rule base to guide future restoration attempts.

6.3.2 Environmental Remediation

A current application of biotechnology involves employing microbial agents to ameliorate environmental contaminants (Bouwer 1992; Forsythe et al. 1995). Deployment of exogenous organisms have been utilized where indigenous populations of desirable catabolic genotypes are lacking (Sayler et al. 1997). Applications of this type have typically involved the introduction of a single remediating organism. An alternative approach could involve a consortium of remediating strains in which sequential introduction could play a role in both enhancing colonization success and bioremediation efficacy. In Chapter 2, for example, I found that permuting the introduction sequence of a suite of hydrocarbon catabolizing strains significantly impacted the biomass levels of a target organism, i.e. HK44. Moreover, the biomass levels achieved under one assembly sequence permutation exceeded those obtained in previous experiments where HK44 was

introduced alone. These differences suggest that a facilitative or synergistic effect may have been operating between species (sensu Simberloff and Von Holle 1999; Ricciardi 2002). Such an effect, if clearly demonstrated, could be harnessed in field application by manipulating the order of introduction of a host of remediating microorganisms

6.3.3 Other Applications

- The approach of modern industrialized agriculture to grain production has largely been based on strict monocultures of annual crops whose productivity is artificially maintained through application of chemical fertilizers and herbicides. Research colleagues of the Land Institute in Salina, Kansas are developing viable alternatives to the prevailing agricultural paradigm by creating perennial grain polycultures through mimicry of prairie community dynamics. Part of their current research program includes controlled field experiments wherein polyculture communities are being constructed through manipulated species introductions (Piper 1996). Results are currently pending.
- John Todd of Ocean Arks International has engineered a water treatment system consisting of a series of allied aquatic ecosystems (Todd. and Josephson 1996). Waste products are fed through the system and are gradually degraded in a stepwise fashion resulting in purified potable water. Controlled assembly of such systems could potentially enhance operational efficacy by: 1) assembling alternative communities drawn from pools of species selected for functional characteristics, and 2) selecting the optimal community for the task at hand.

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Appendices

Appendix A: Figures

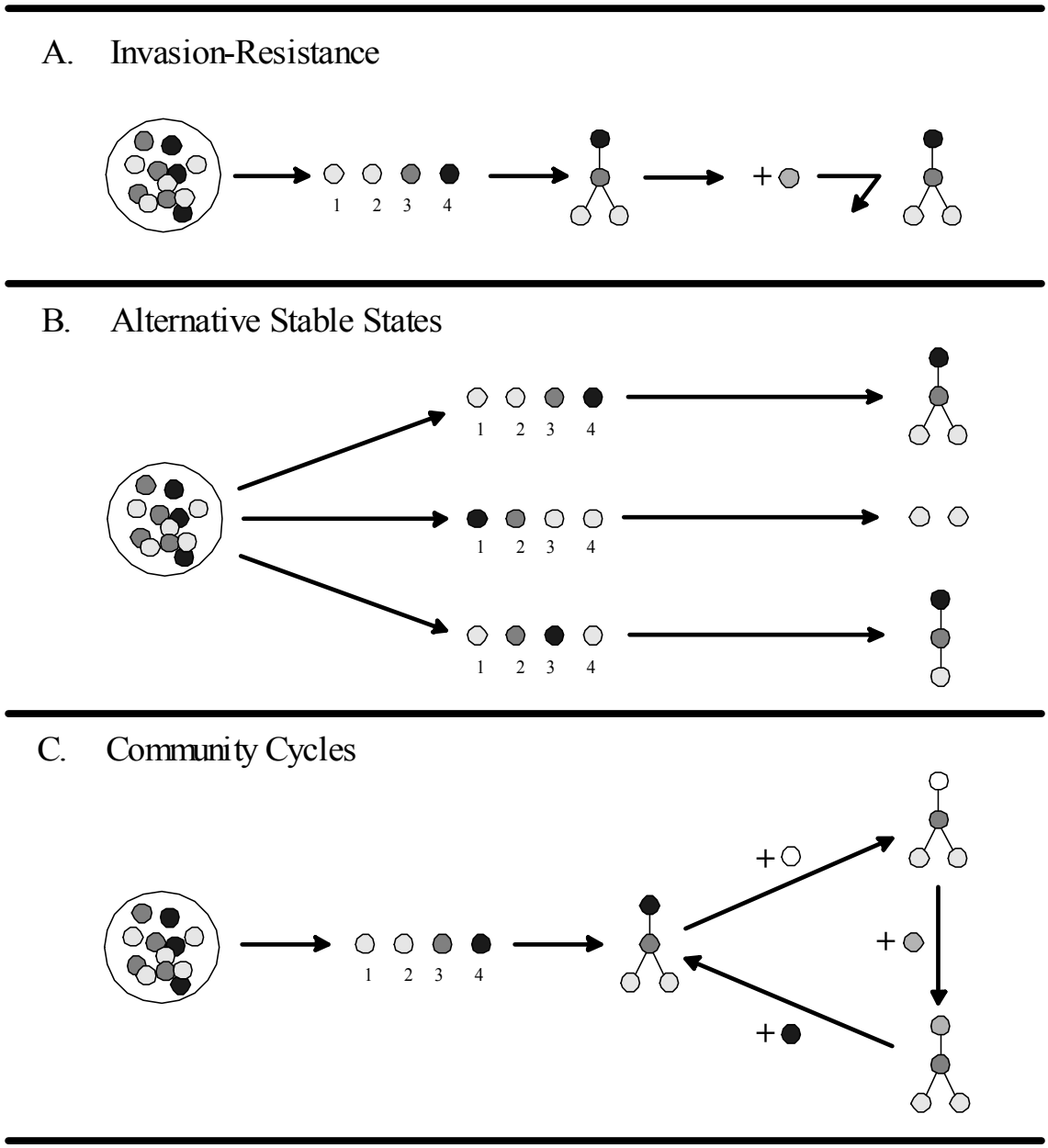


Figure 2.1. Graphical representation of assembly related community phenomena: A) Invasion-resistance – community assembly can result in invasion-resistant states that limit community membership; B) Alternative stable states – Changing the sequence of invasion can lead to alternate stable states; C) Community cycles – Rare invasion sequences can result in persistent community cycles wherein a community continuously oscillates between two or more states. (continued on next page)

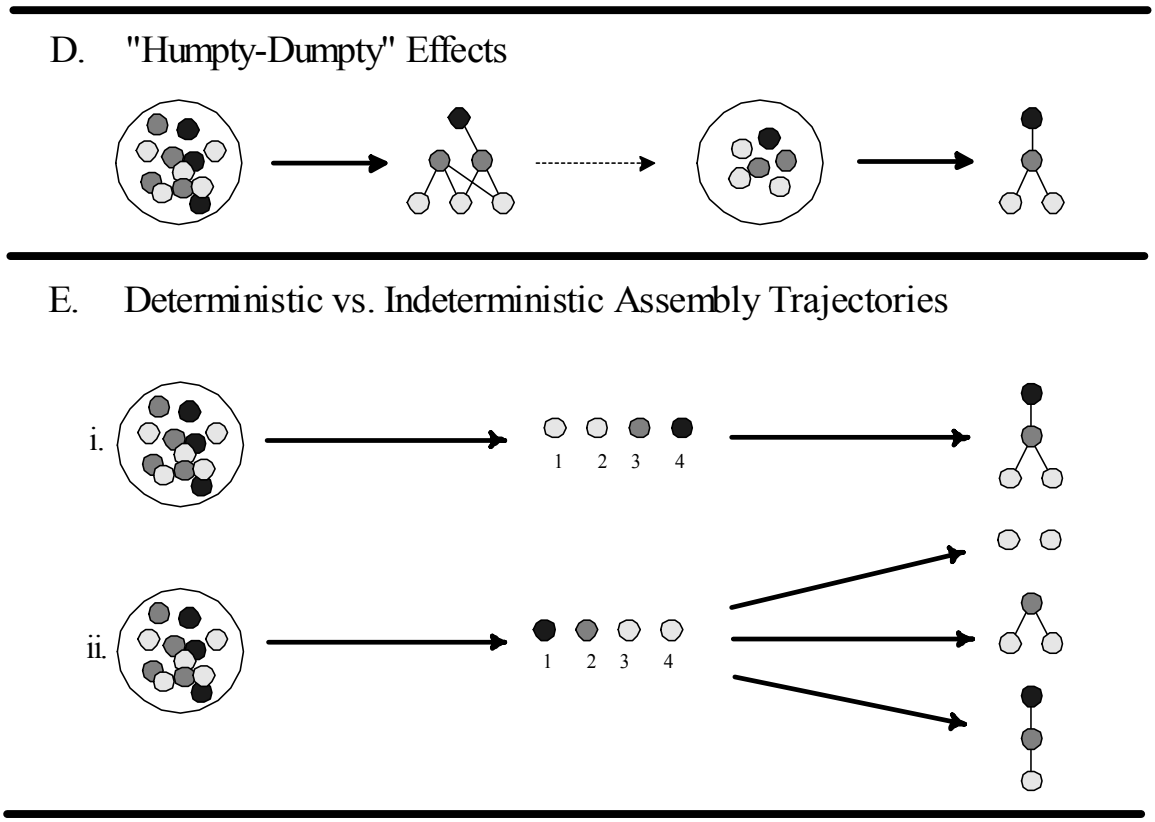


Figure 2.1 continued. Graphical representation of assembly-related community phenomena: D) Humpty-dumpty effects – Some communities cannot be reconstructed using only species extant in the final state; E) Deterministic vs. indeterministic trajectories – Some sequences consistently produce the same community state, while others can lead to multiple states.

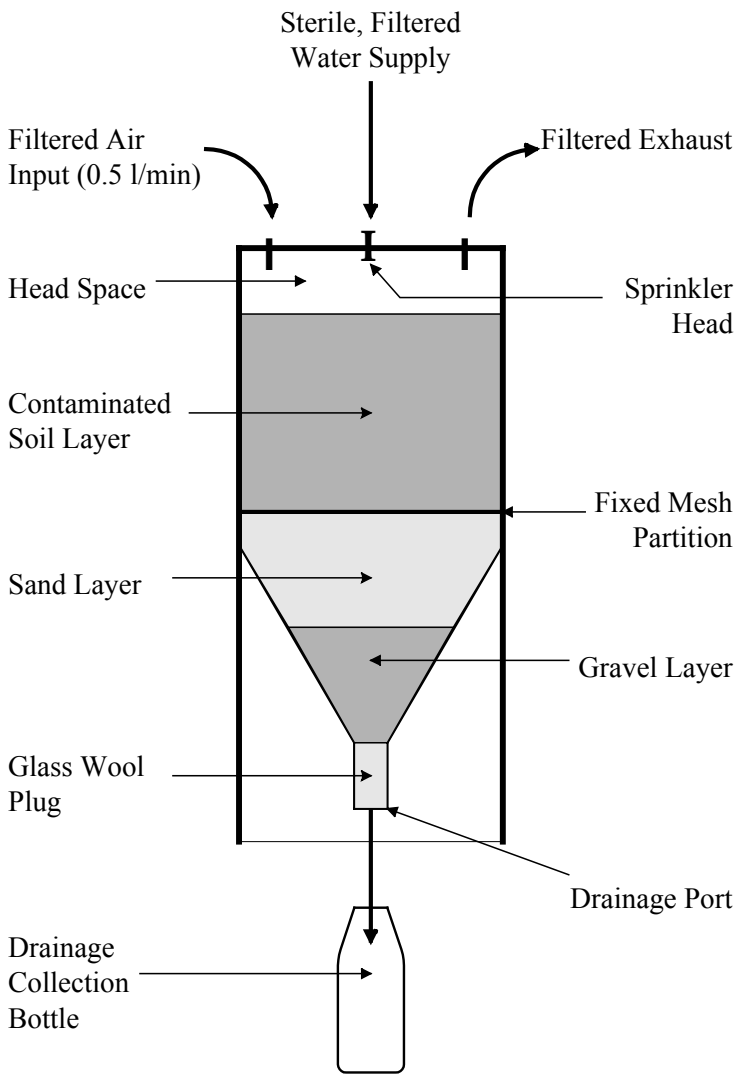


Figure 3.1. A cross-sectional schematic of the soil microcosm chamber.

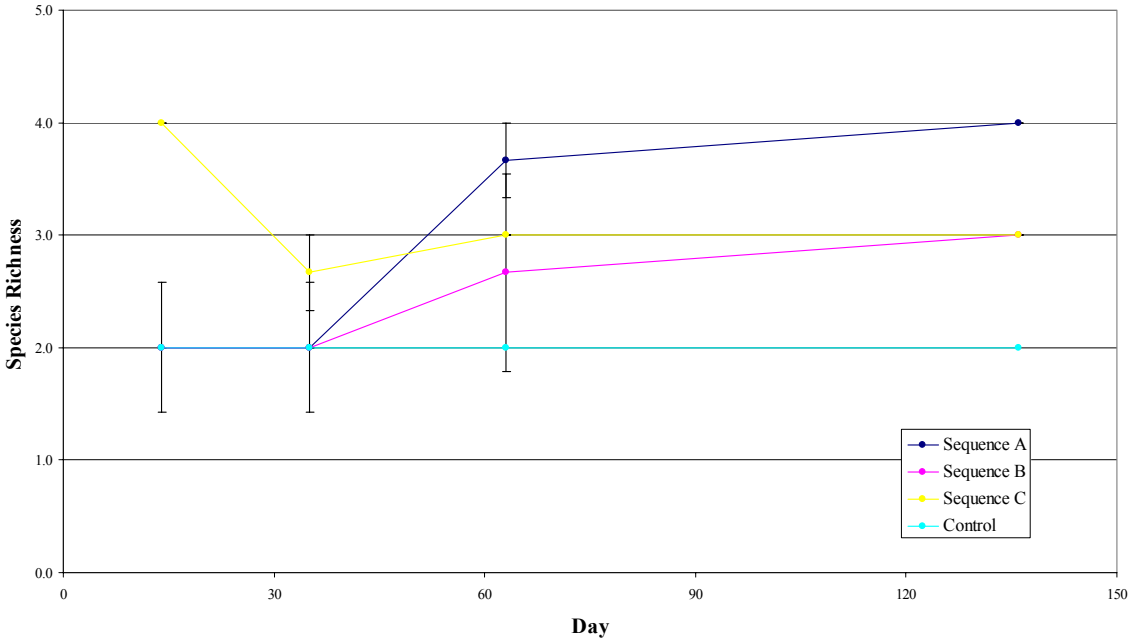


Figure 3.2. Time series of mean species richness by treatment. Error bars represent stand error of the mean.

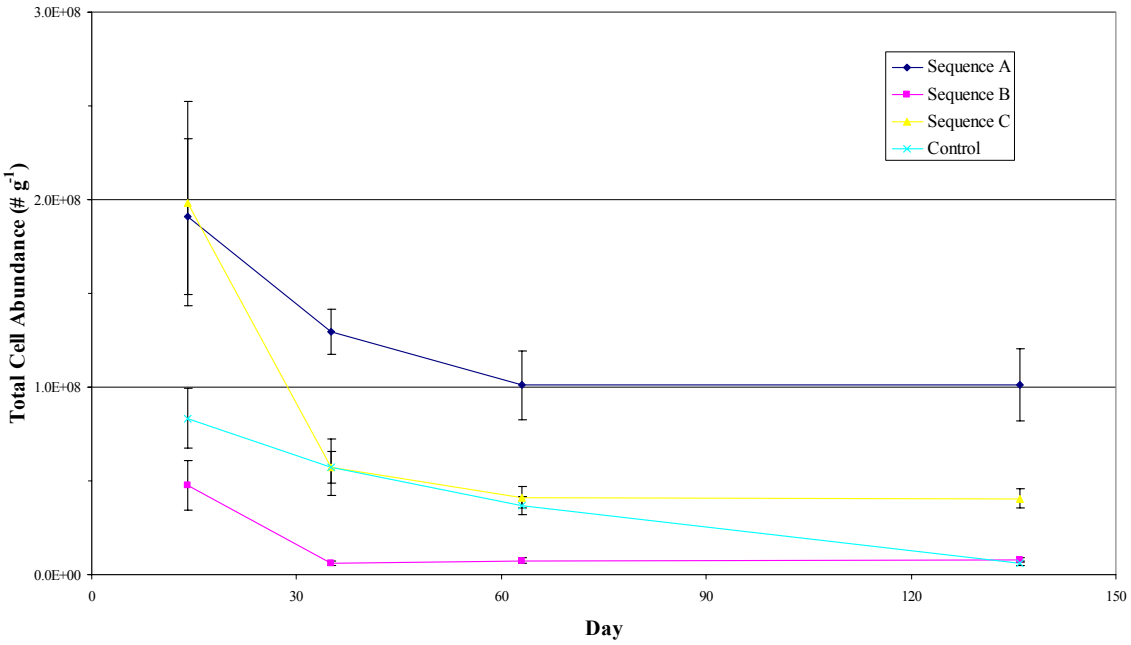


Figure 3.3. Time series of mean total cell abundance by treatment. Error bars represent stand error of the mean.

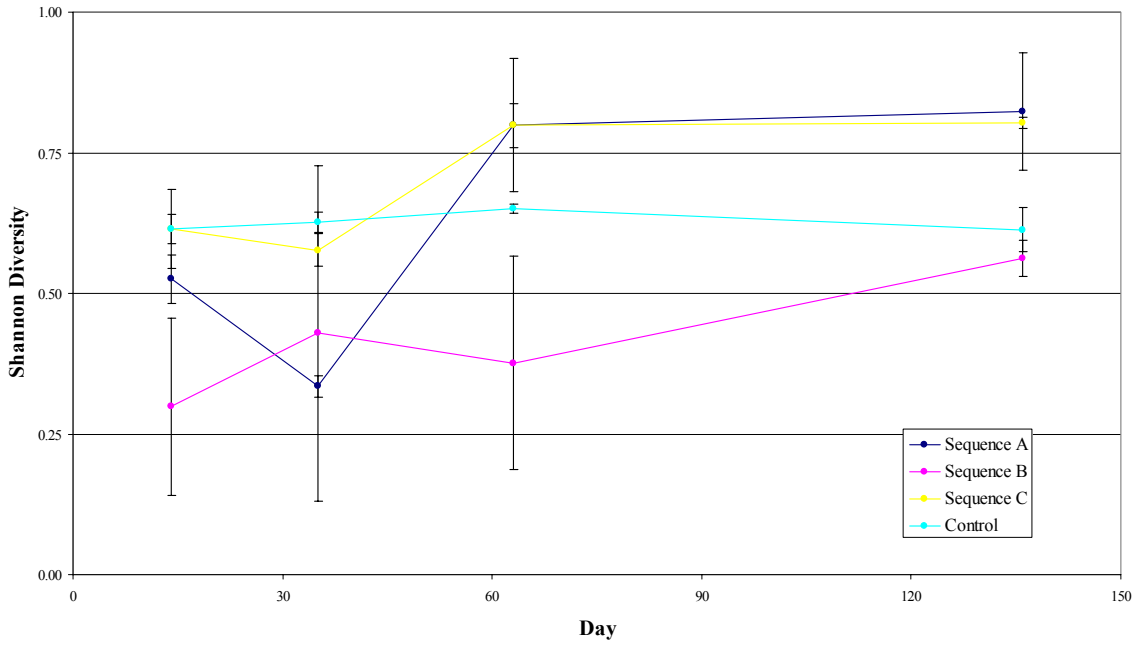


Figure 3.4. Time series of mean Shannon diversity by treatment. Error bars represent stand error of the mean.

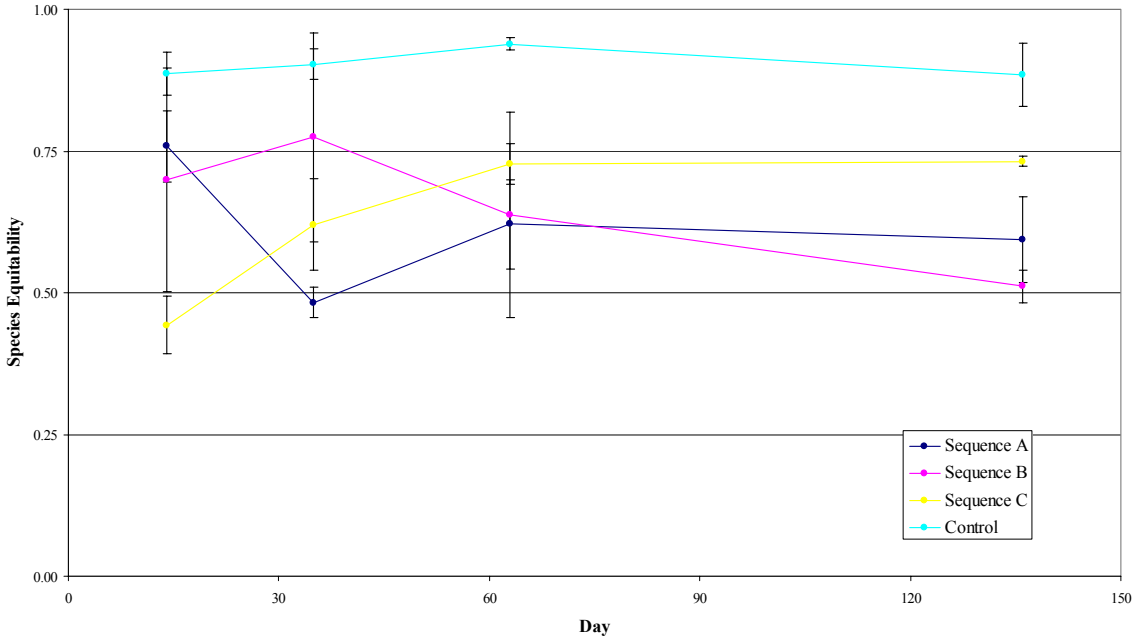


Figure 3.5. Time series of mean species equitability by treatment. Error bars represent stand error of the mean.

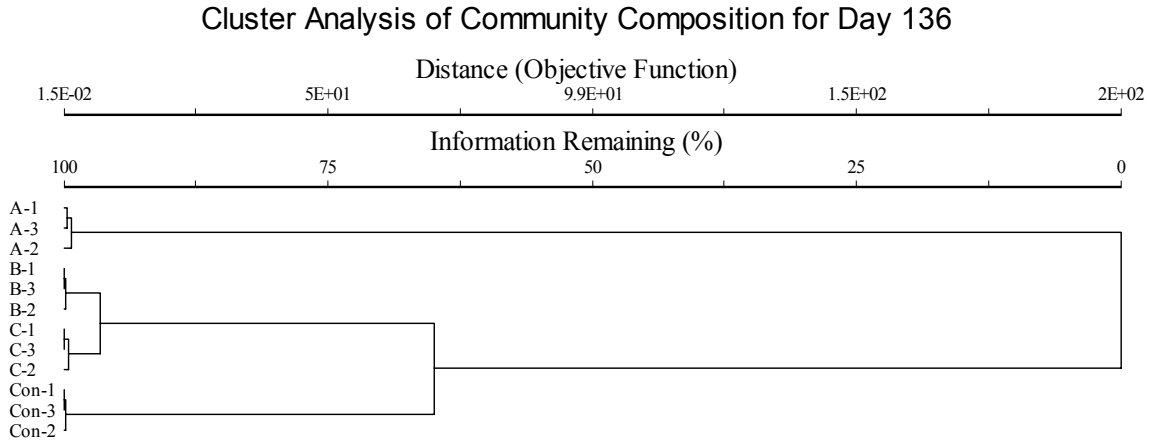


Figure 3.6. Cluster analysis dendrogram of community membership data taken at the conclusion of the experiment. Communities are labeled by sequence and replicate number. With >99% information remaining, all twelve microcosms were cleanly grouped by experimental treatment. Note the strong similarity between microcosms of Sequence B and Sequence C.

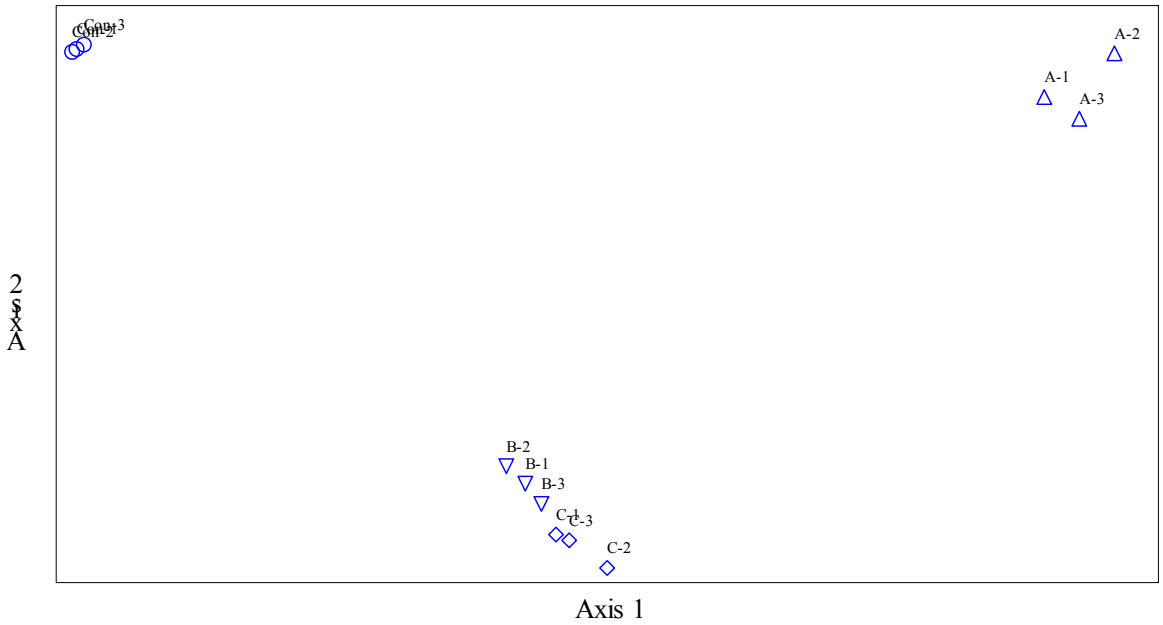


Figure 3.7. Detrended correspondence analysis ordination diagram showing the position of the twelve final community states relative to the first two ordination axes. Community points are labeled by sequence and replicate number. Note the strong similarity between microcosms of Sequence B and Sequence C.

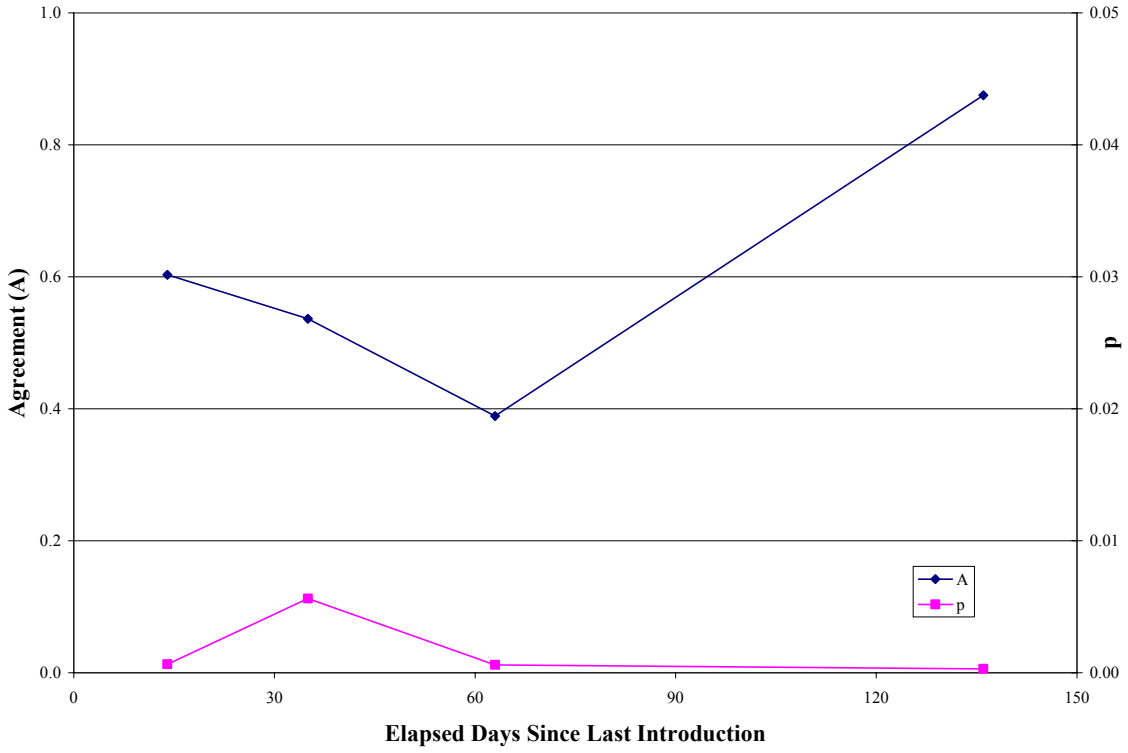


Figure 3.8. Time series analysis of MRPP comparisons of species compositional data grouped by treatment. Plotted values include the probability that the observed differences in treatments can be attributed to chance alone (p) and the chance-corrected within-treatment agreement (A). Note the sharp increase in agreement (A) within treatments over the last two sampling dates.

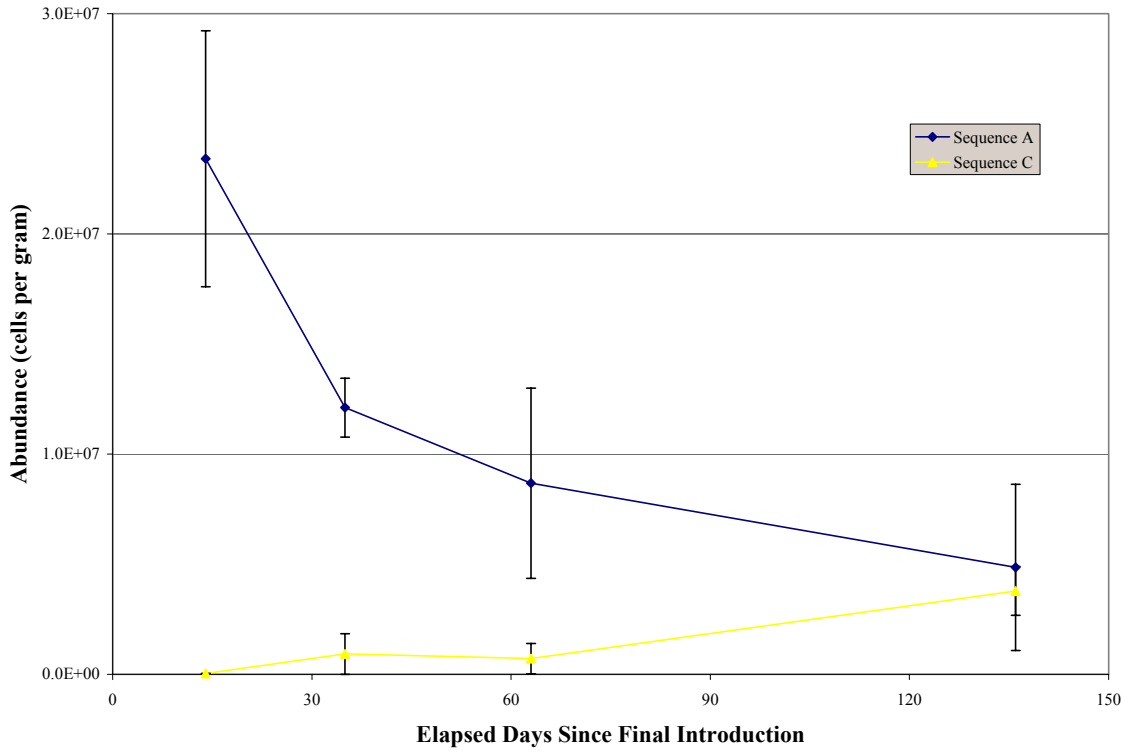


Figure 3.9. Time series of HK44 population dynamics. Sequence B was not invasible by HK44. Error bars represent standard error of the mean.

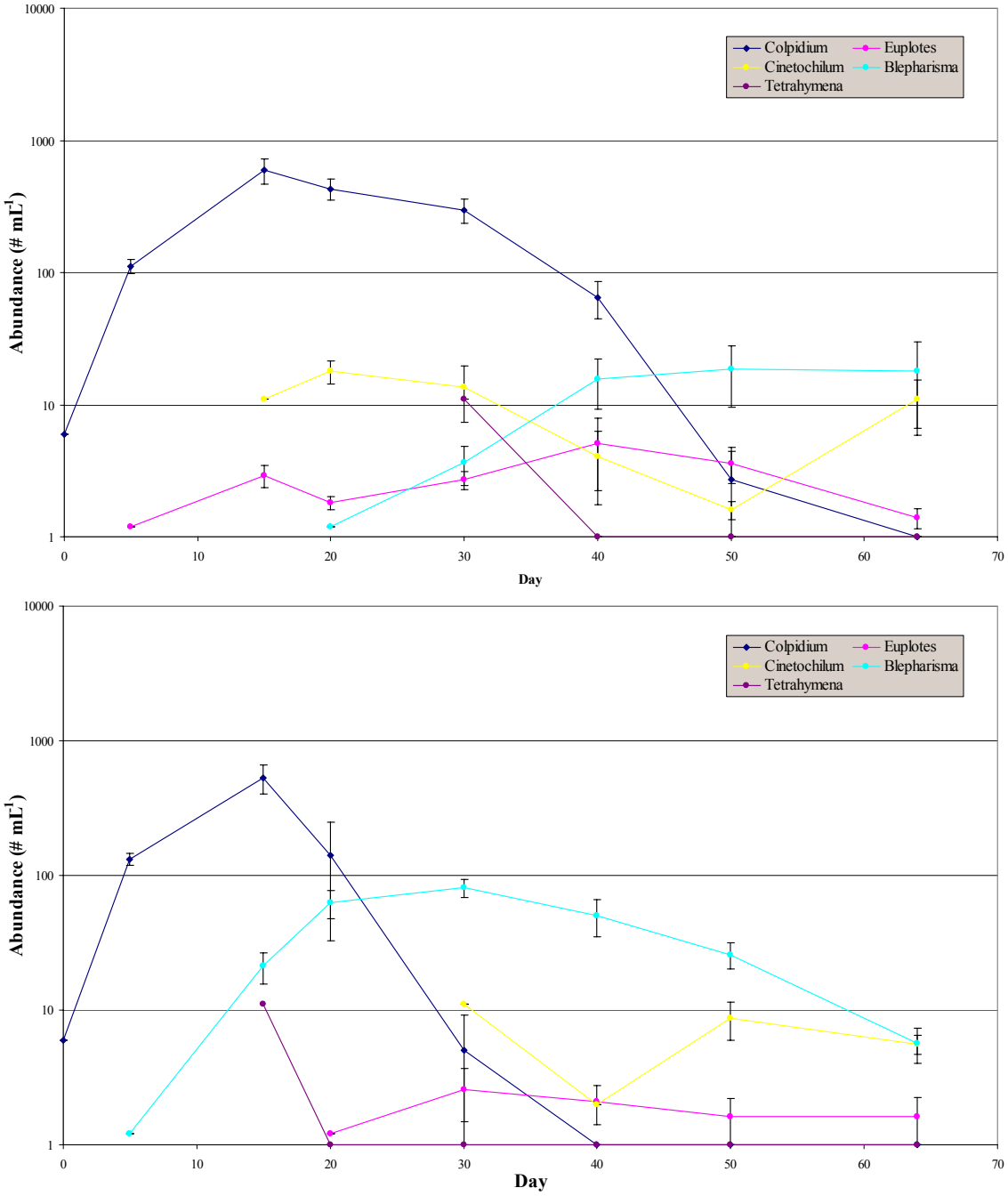


Figure 4.1. Time series of abundance values for five study species by sequence for 0.05 g L^{-1} nutrient concentration. Top and bottom figures show Sequence I and Sequence II values, respectively. Abundance for the point of each introduction represent inoculation density values. Error bars represent standard error of the mean.

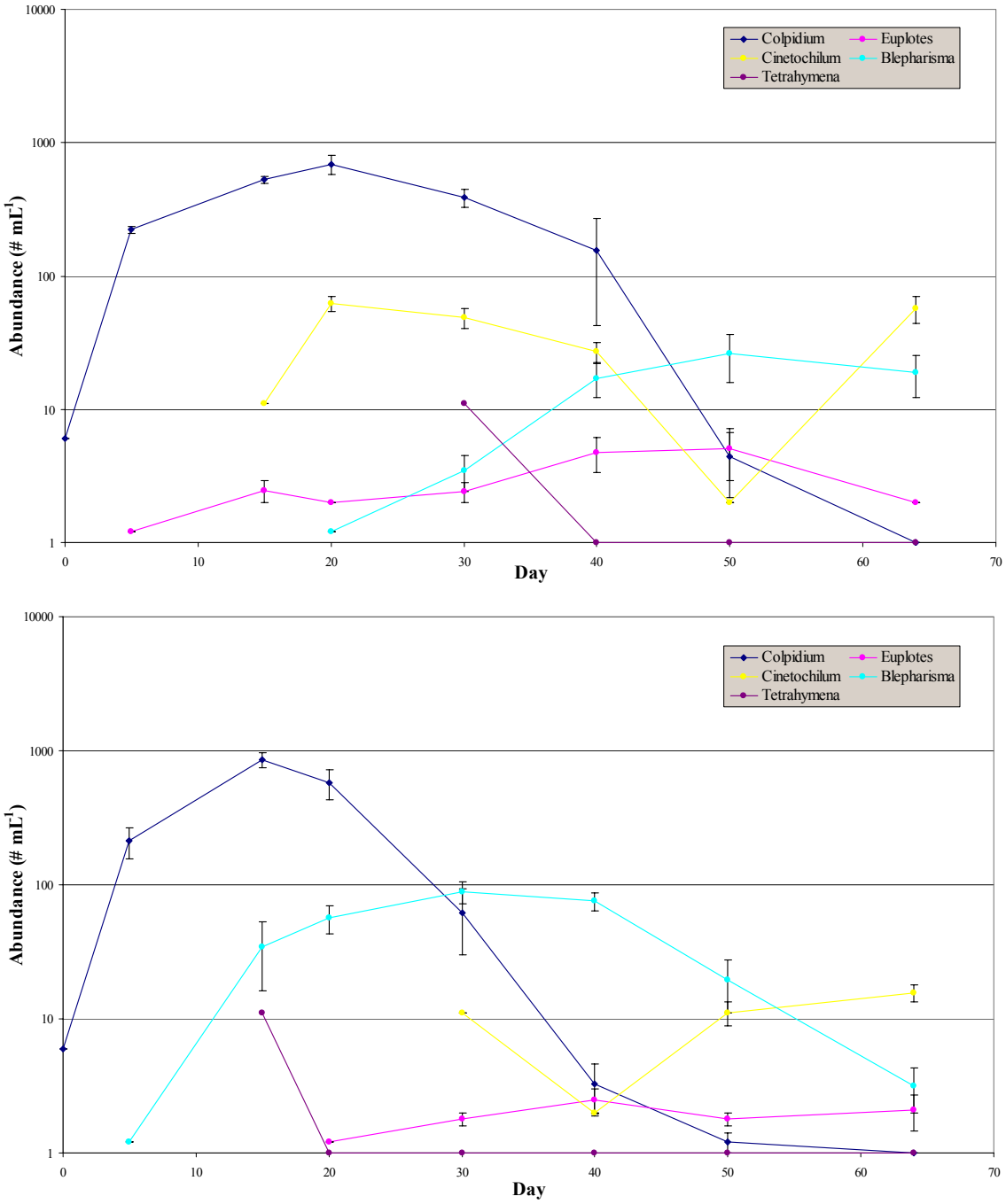


Figure 4.2. Time series of abundance values for five study species by sequence for 0.10 g L^{-1} nutrient concentration. Top and bottom figures show Sequence I and Sequence II values, respectively. Abundance for the point of each introduction represent inoculation density values. Error bars represent standard error of the mean.

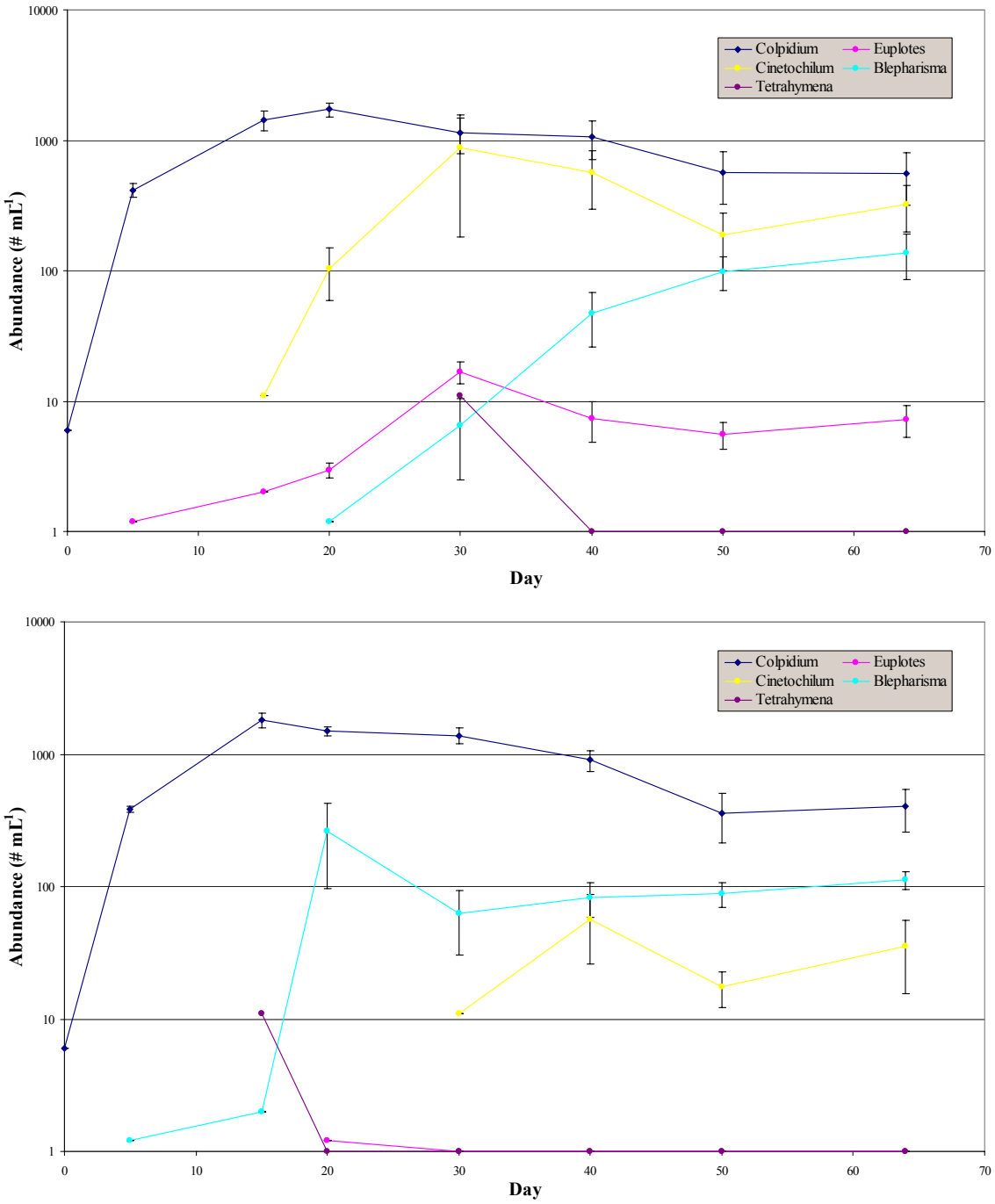


Figure 4.3. Time series of abundance values for five study species by sequence for 0.50 g L^{-1} nutrient concentration. Top and bottom figures show Sequence I and Sequence II values, respectively. Abundance for the point of each introduction represent inoculation density values. Error bars represent standard error of the mean.

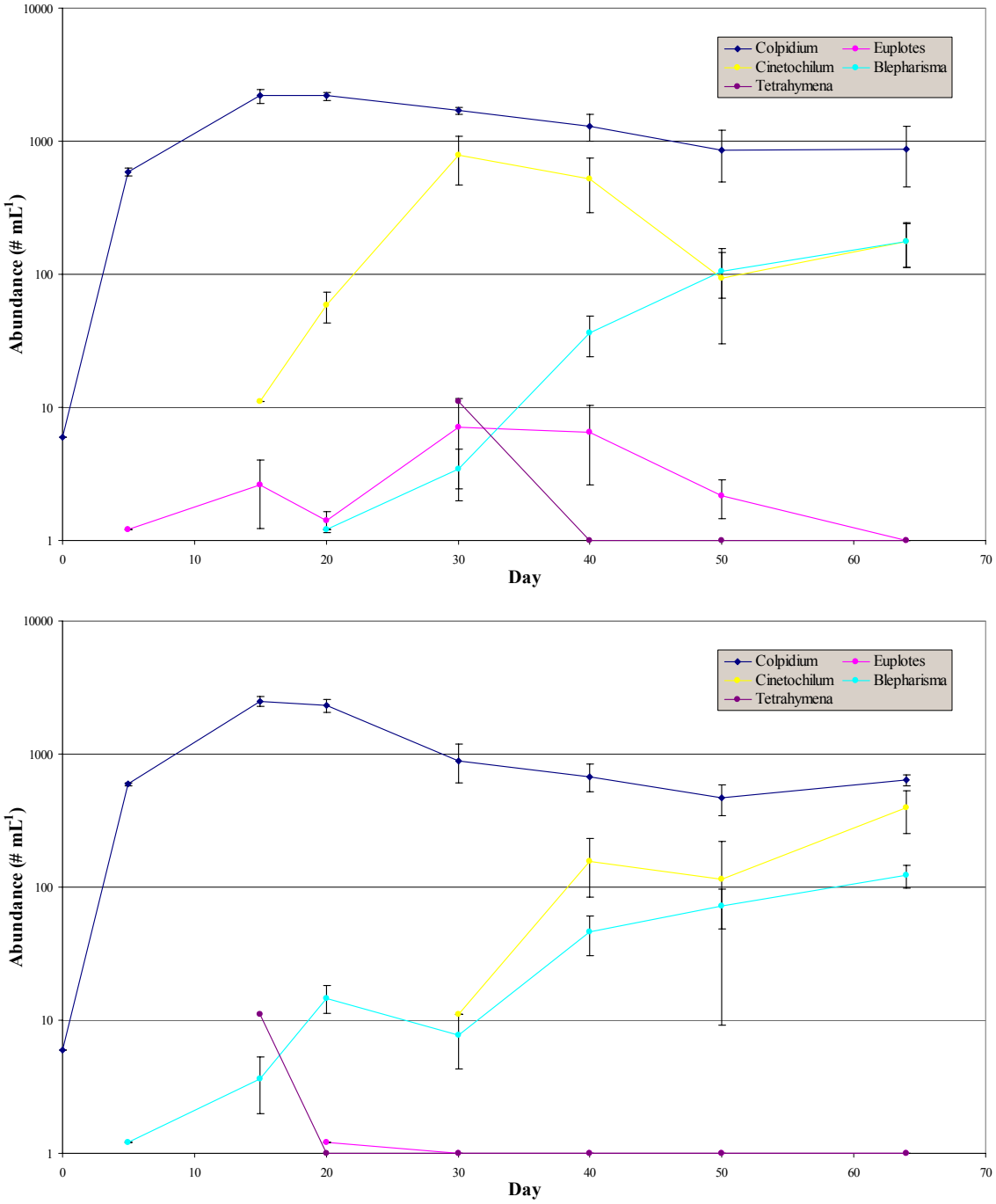


Figure 4.4. Time series of abundance values for five study species by sequence for 0.75 g L^{-1} nutrient concentration. Top and bottom figures show Sequence I and Sequence II values, respectively. Abundance for the point of each introduction represent inoculation density values. Error bars represent standard error of the mean.

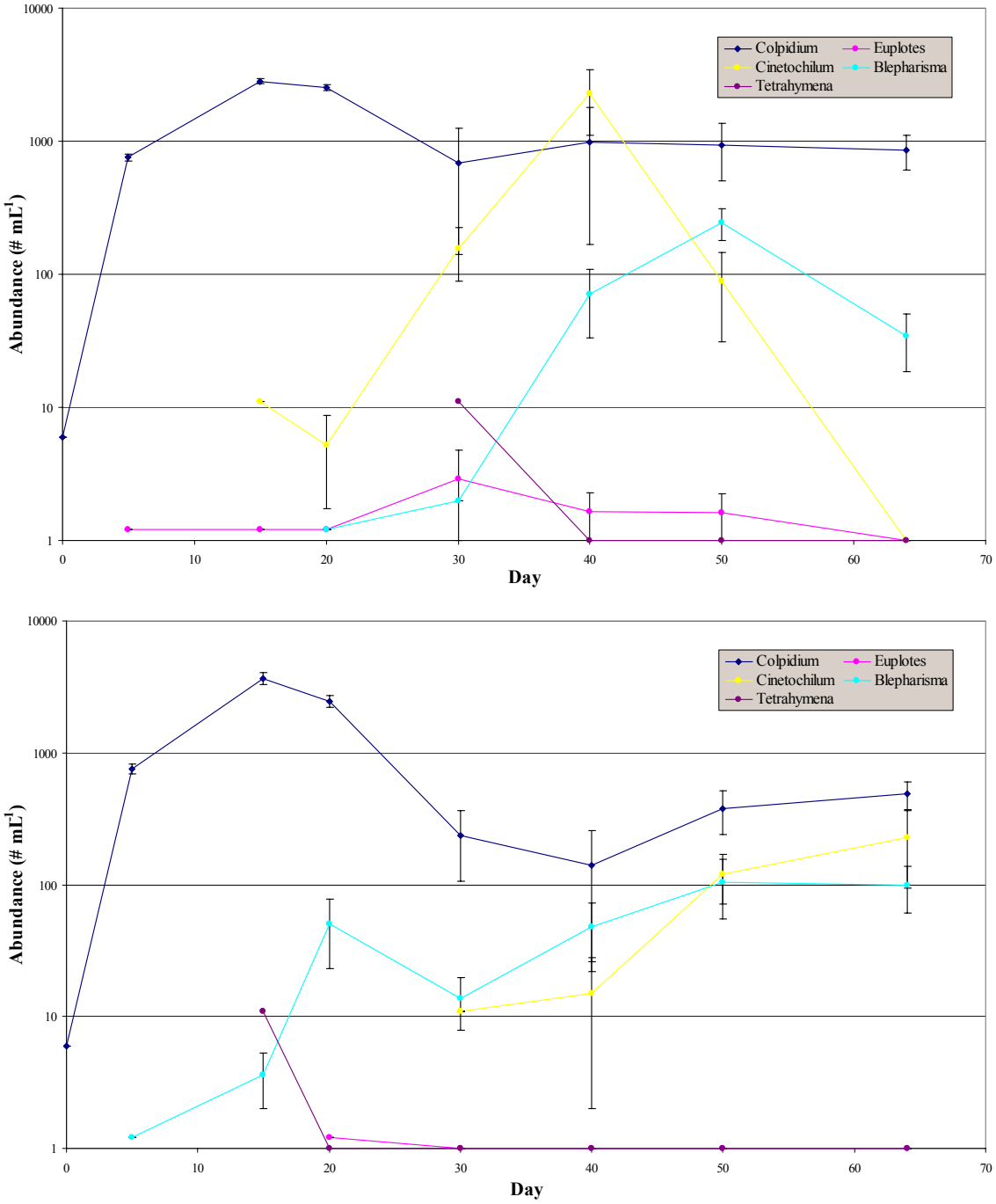


Figure 4.5. Time series of abundance values for five study species by sequence for 1.00 g L^{-1} nutrient concentration. Top and bottom figures show Sequence I and Sequence II values, respectively. Abundance for the point of each introduction represent inoculation density values. Error bars represent standard error of the mean.

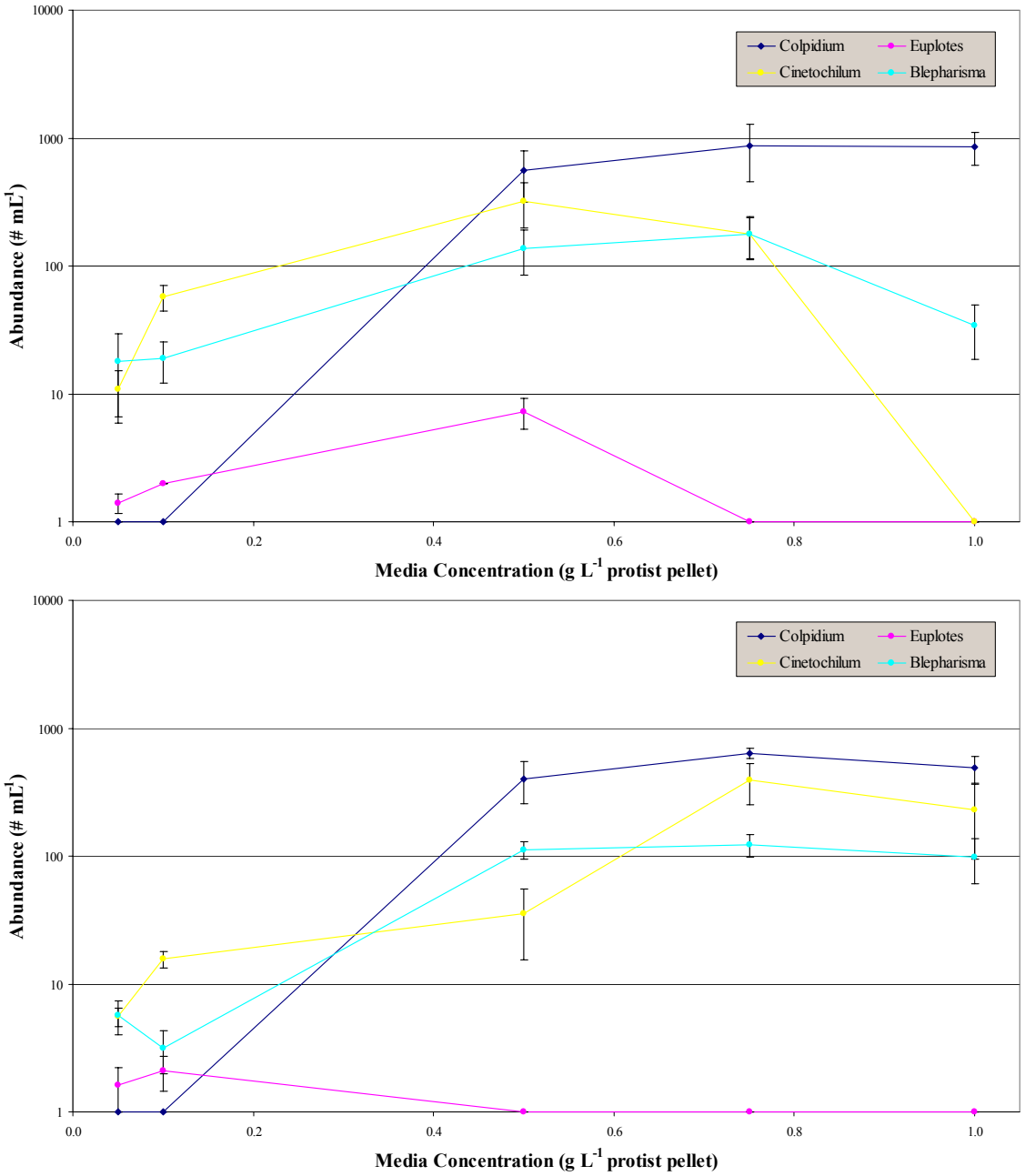


Figure 4.6. Abundance values by species and nutrient concentration for the final sampling date. The top and bottom figures show abundance values for Sequence I and Sequence II, respectively. *Tetrahymena* failed to invade any treatment. Error bars represent standard error of the mean.

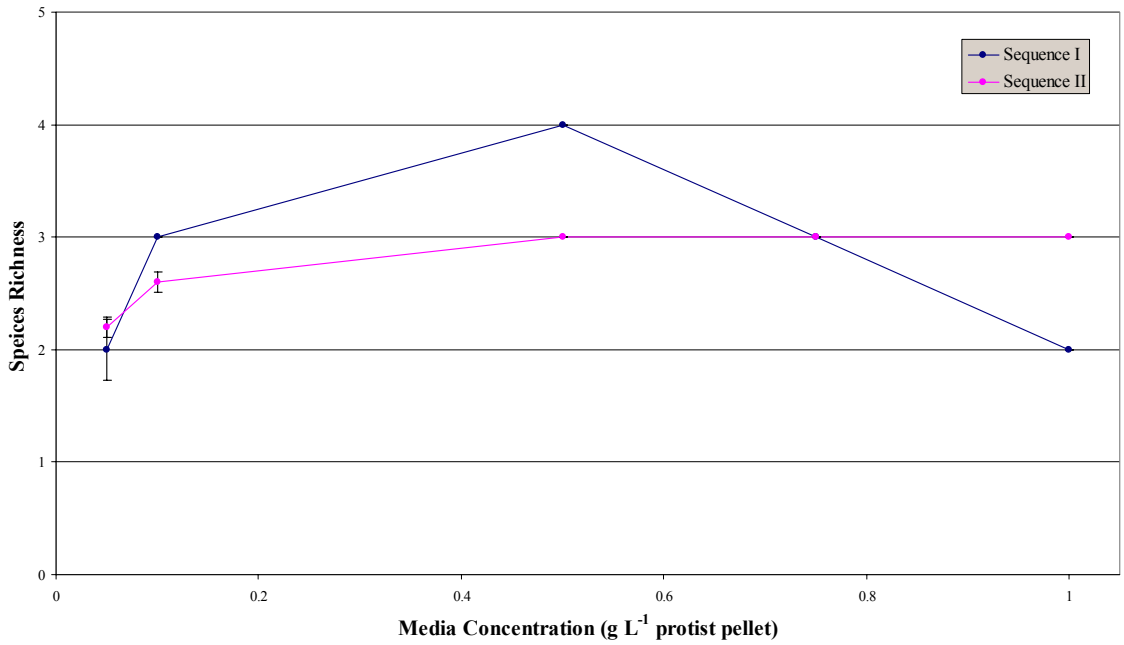


Figure 4.7. Species richness for the final sampling date by sequence and nutrient concentration. Error bars represent standard error of the mean.

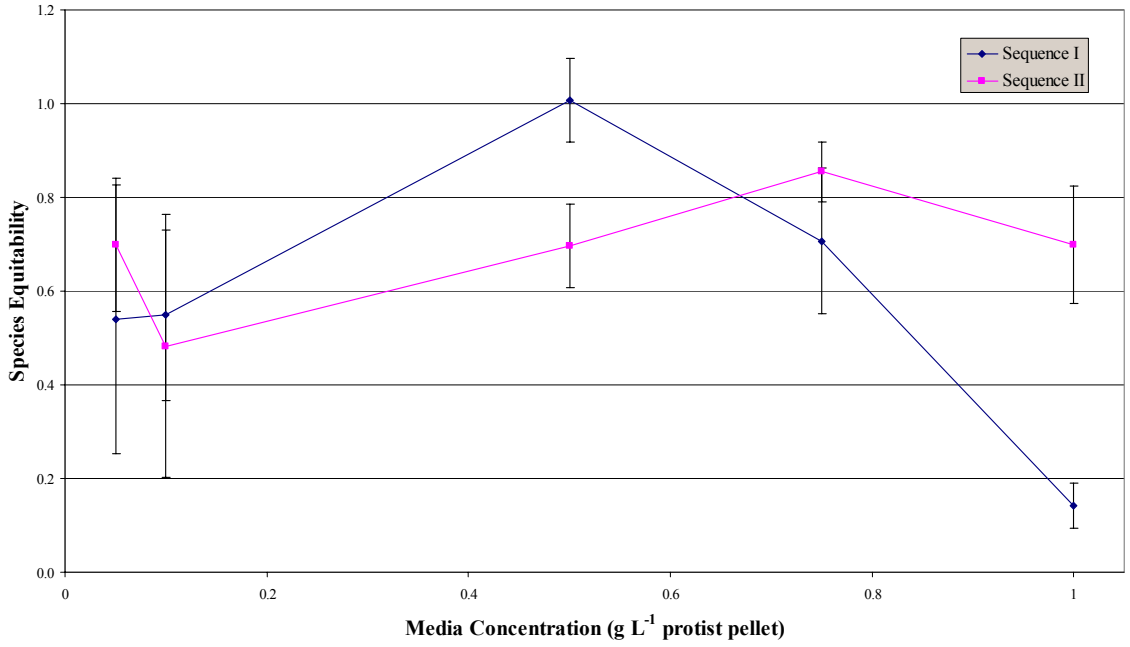


Figure 4.8. Shannon diversity for the final sampling date by sequence and nutrient concentration. Error bars represent standard error of the mean.

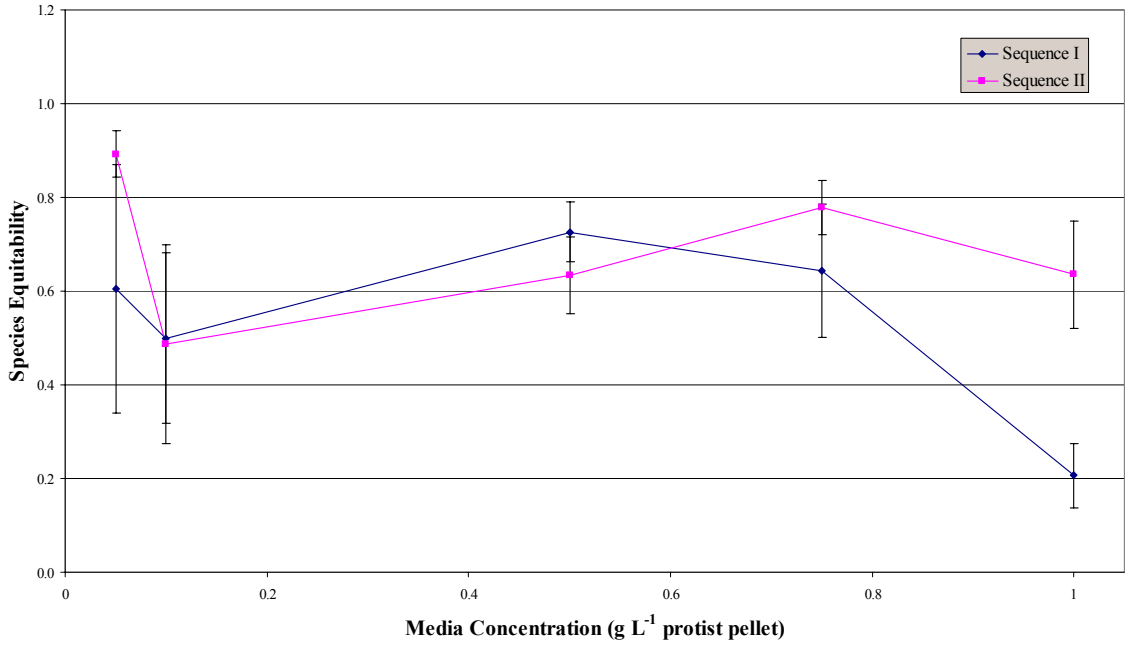


Figure 4.9. Species equitability for the final sampling date by sequence and nutrient concentration. Error bars represent standard error of the mean.

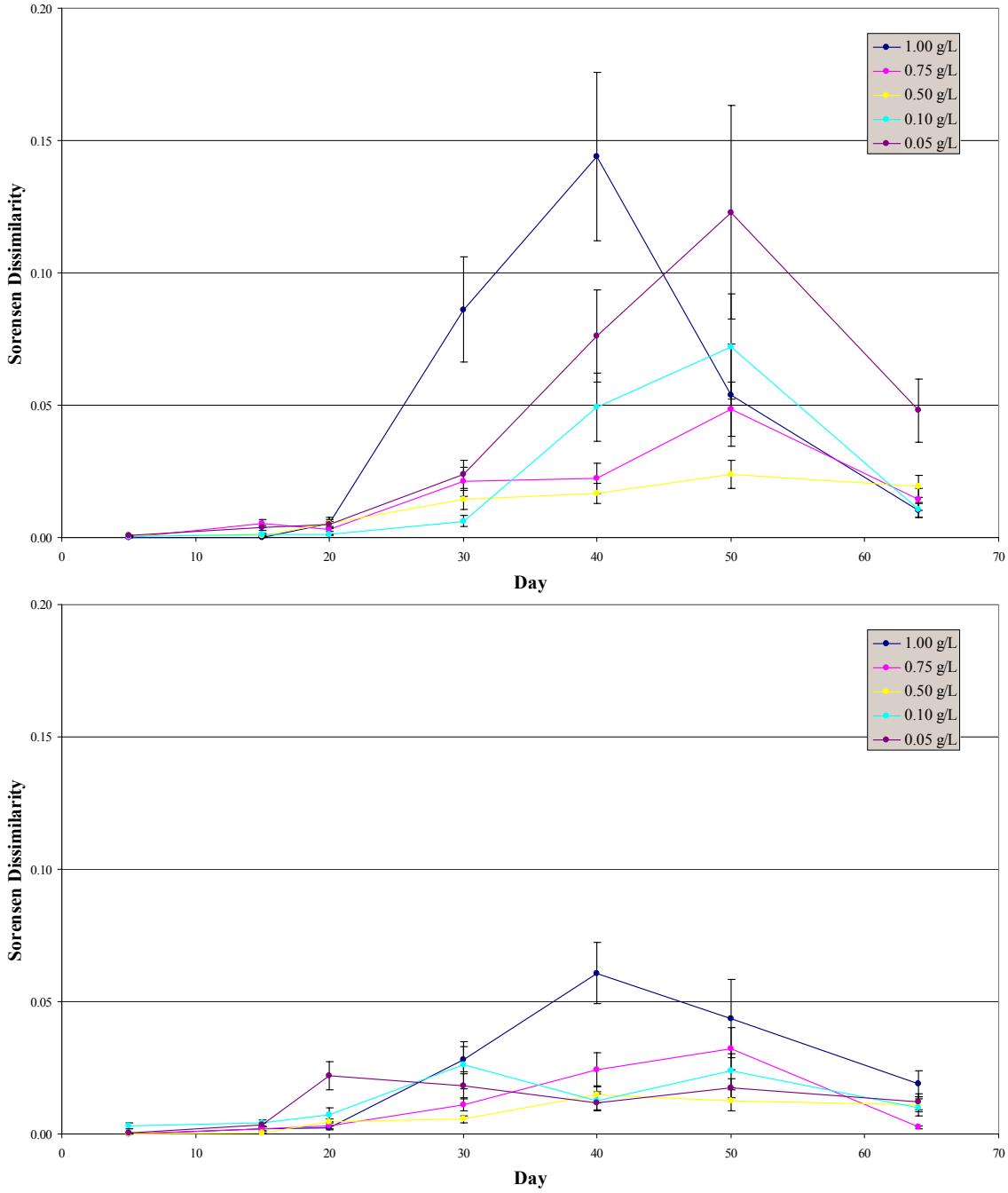


Figure 4.10. Time series of mean Sorensen dissimilarity by sequence and nutrient concentration. The top and bottom figures show Sequence I and Sequence II values, respectively. Error bars represent standard error of the mean.

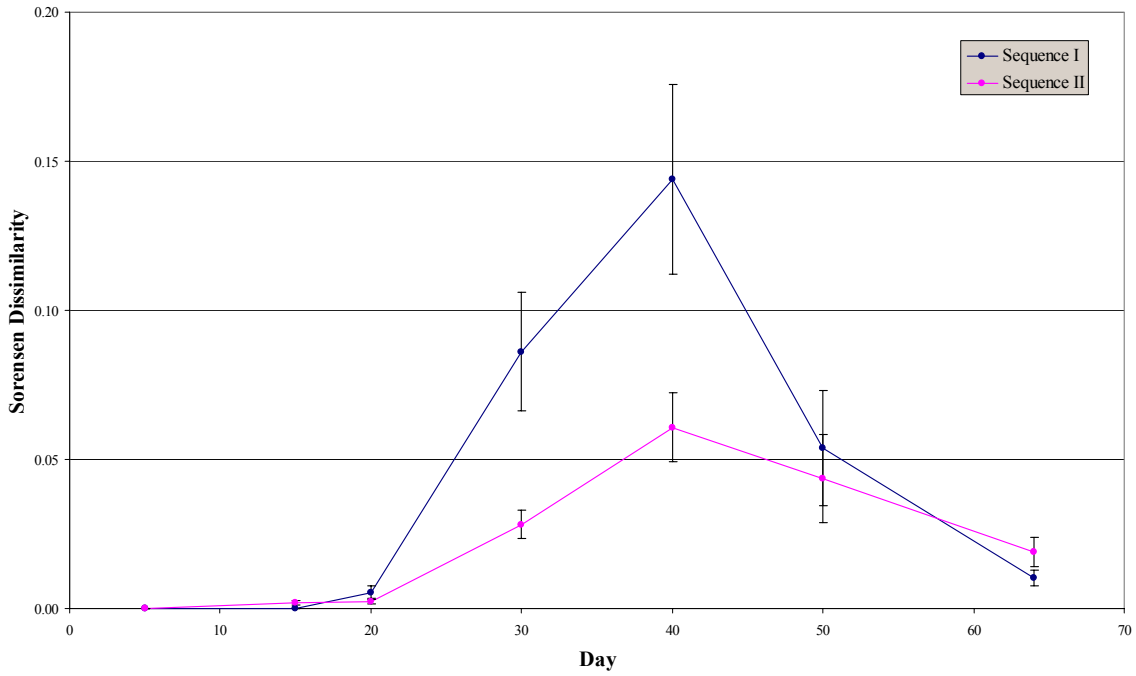


Figure 4.11. Time series of mean Sorensen dissimilarity by sequence for 1.00 g L^{-1} nutrient concentration. Error bars represent standard error of the mean.

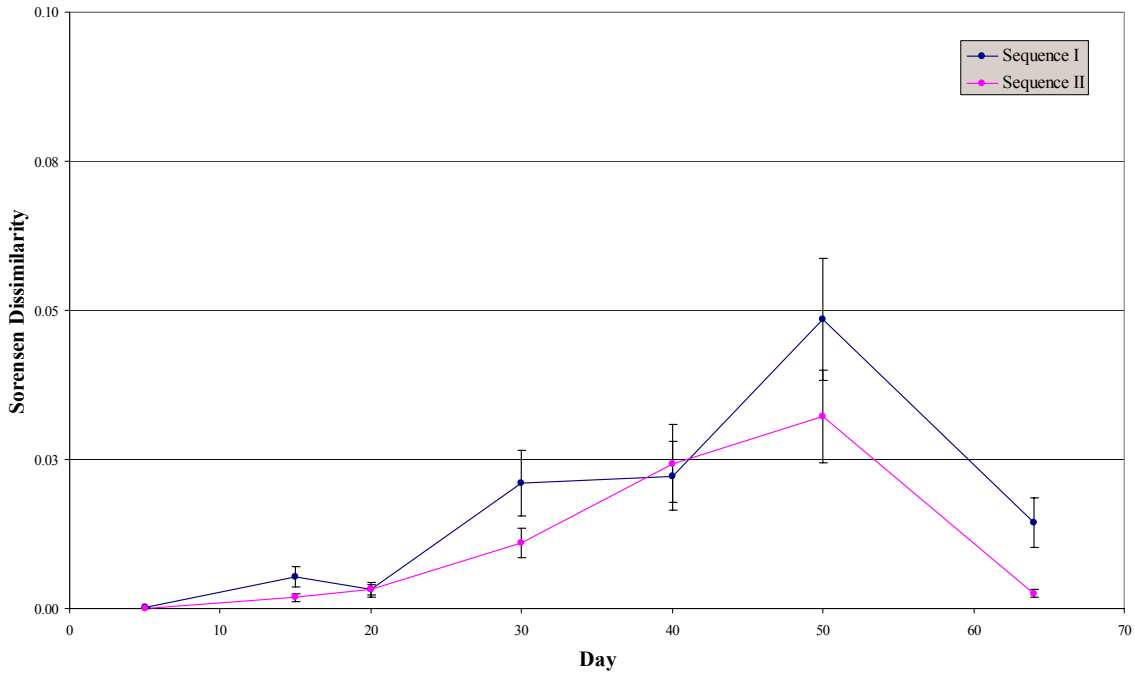


Figure 4.12. Time series of mean Sorensen dissimilarity by sequence for 0.75 g L^{-1} nutrient concentration. Error bars represent standard error of the mean.

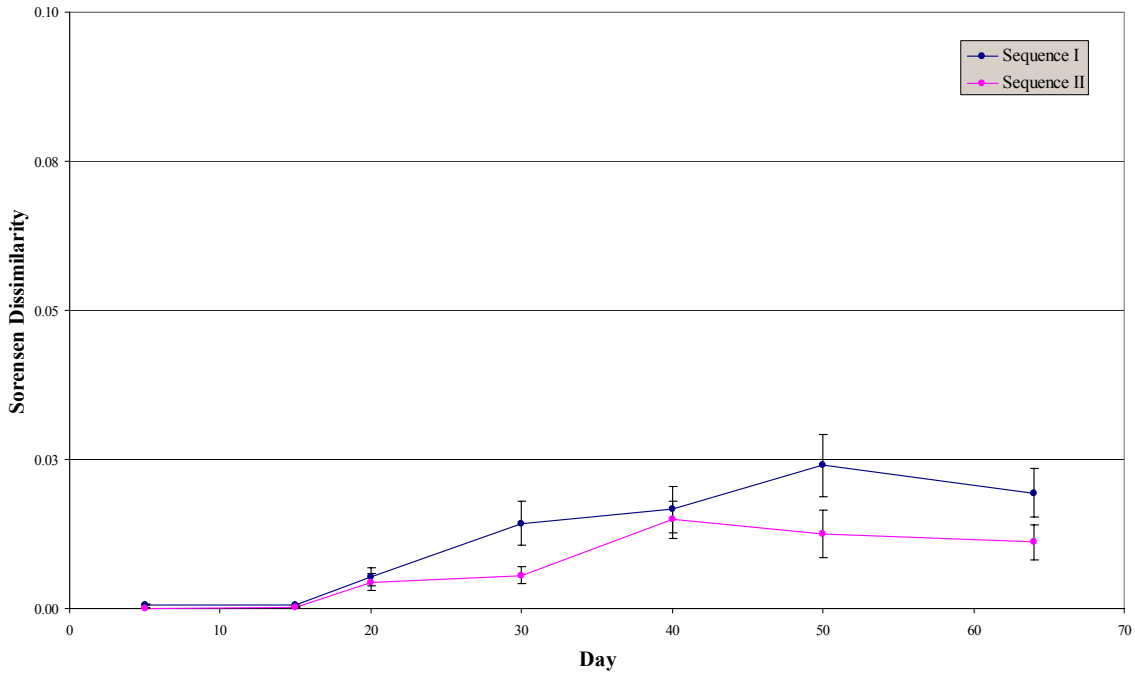


Figure 4.13. Time series of mean Sorensen dissimilarity by sequence for 0.50 g L^{-1} nutrient concentration. Error bars represent standard error of the mean.

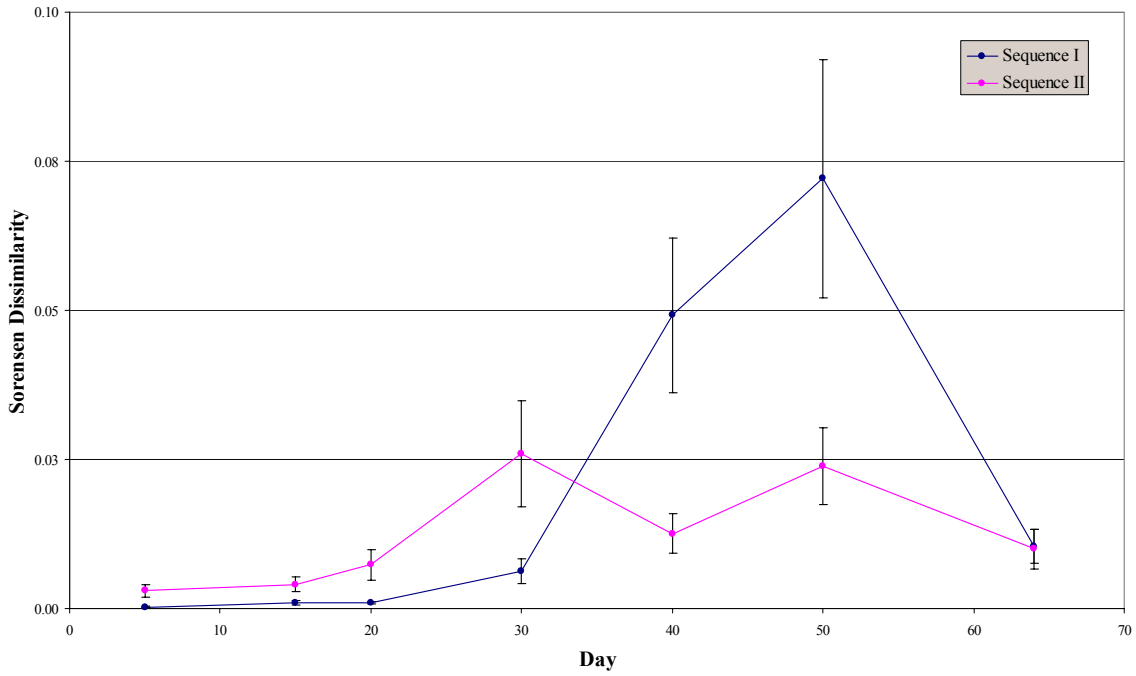


Figure 4.14. Time series of mean Sorensen dissimilarity by sequence for 0.10 g L^{-1} nutrient concentration. Error bars represent standard error of the mean.

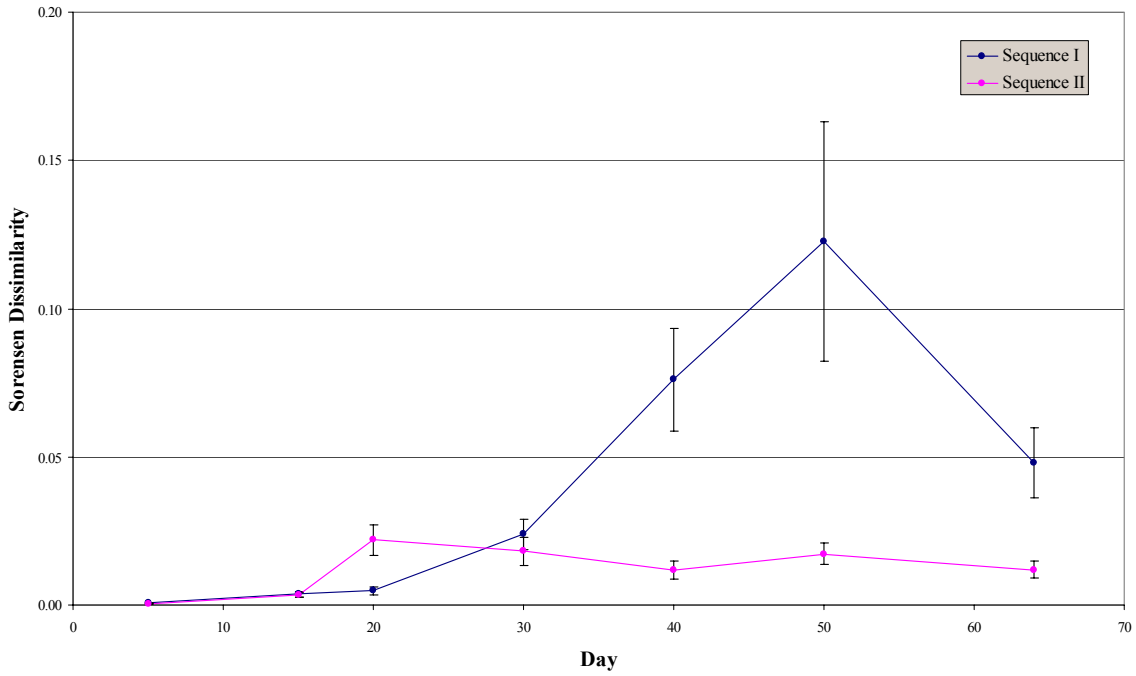


Figure 4.15. Time series of mean Sorensen dissimilarity by sequence for 0.05 g L^{-1} nutrient concentration. Error bars represent standard error of the mean.

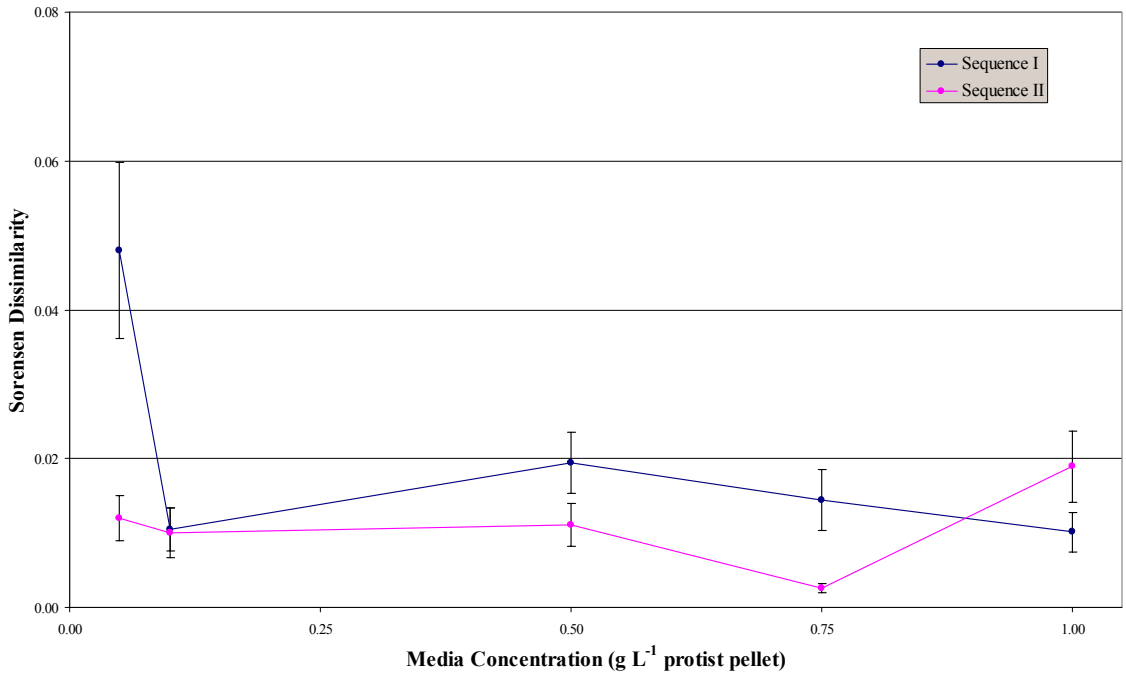


Figure 4.16. Mean dissimilarity for the final sampling date by sequence and nutrient concentration. Error bars represent error of the mean.

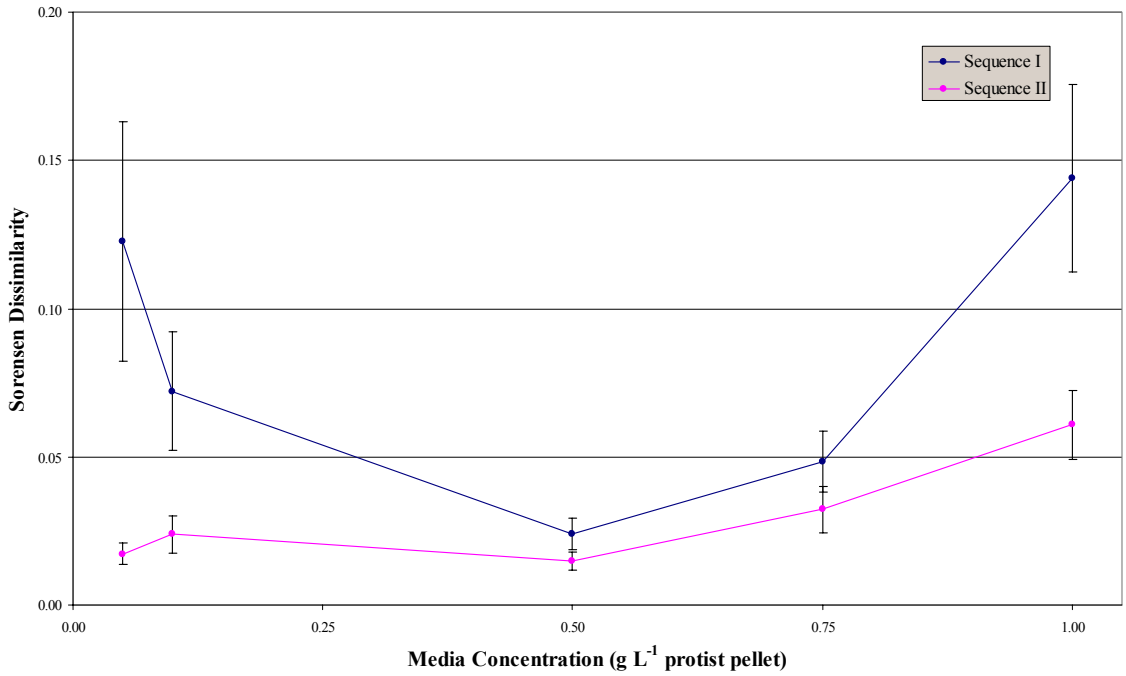


Figure 4.17. Mean maximal dissimilarity by sequence and nutrient concentration. Error bars represent error of the mean.

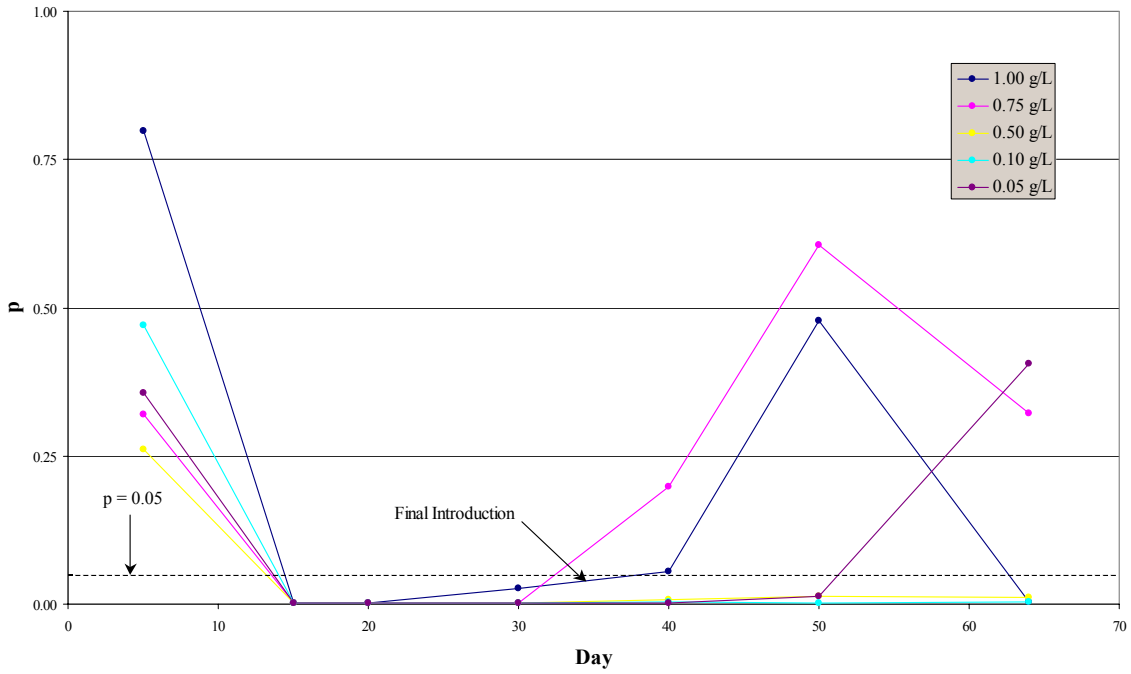


Figure 4.18. Time series of reported p-values by nutrient concentration and sampling date of an MRPP comparison of community composition between invasion sequences.

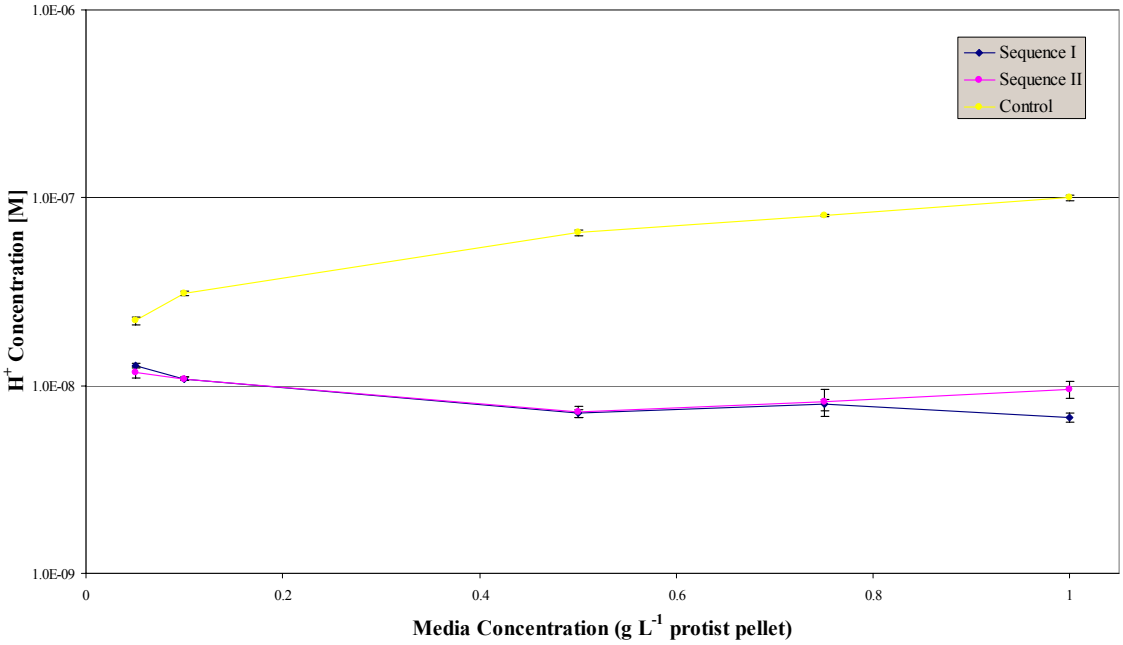


Figure 4.19. H^+ concentration by nutrient concentration for Sequence I, Sequence II and Control standards. Error bars represent the standard error of the mean.

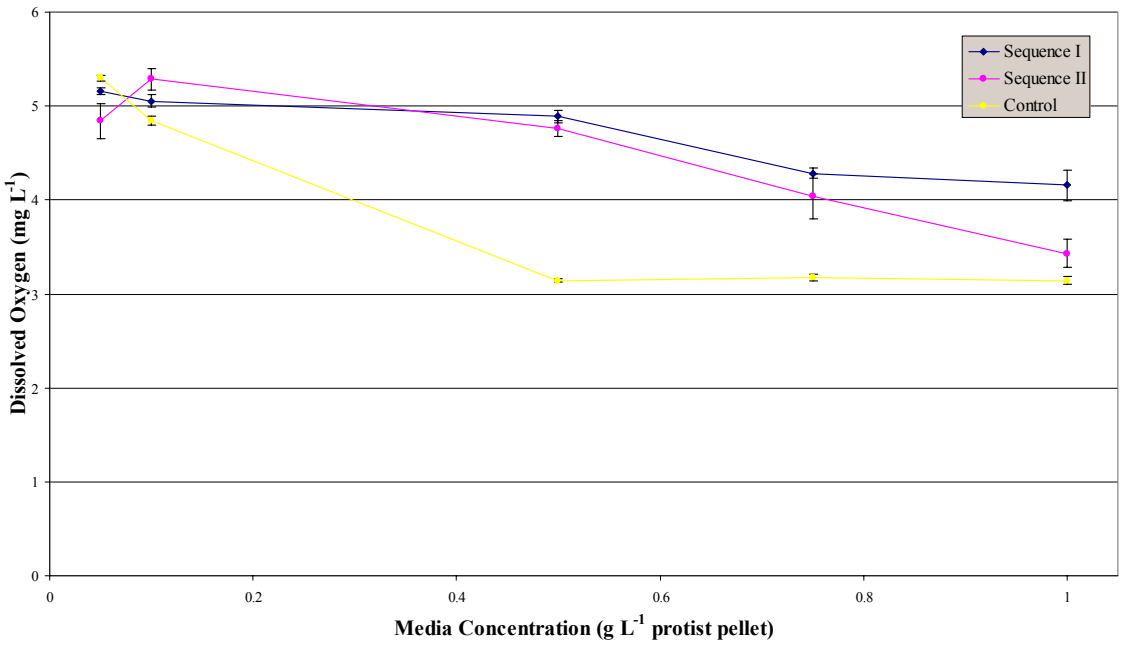


Figure 4.20. Dissolved oxygen concentration by nutrient concentration for Sequence I, Sequence II and Control standards. Error bars represent the standard error of the mean.

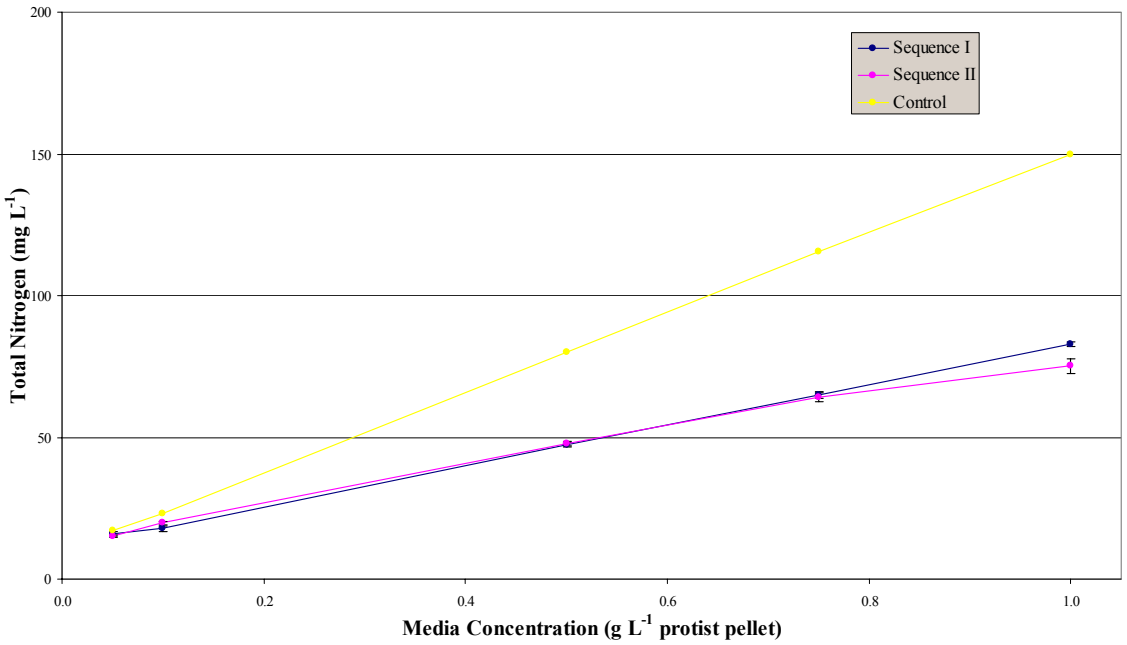


Figure 4.21. Total dissolved carbon concentration for final sampling date by sequence and nutrient concentration. Error bars represent the standard error of the mean.

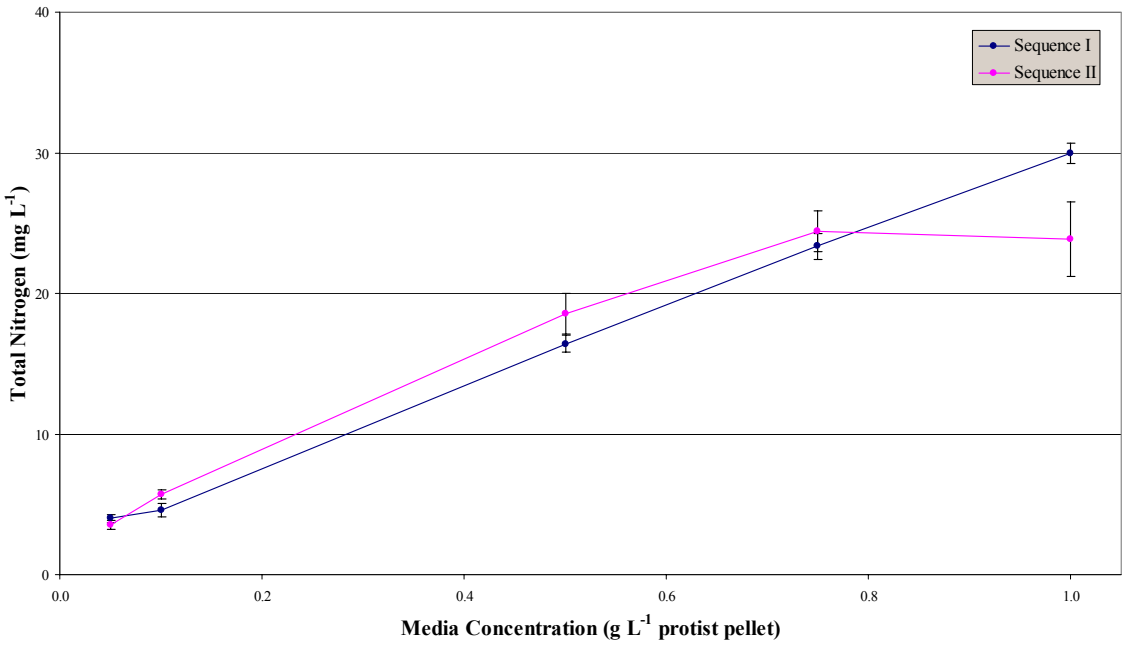


Figure 4.22. Total inorganic nitrogen concentration for final sampling date by sequence and nutrient concentration. Inorganic nitrogen was not found in media control standards. Error bars represent the standard error of the mean.

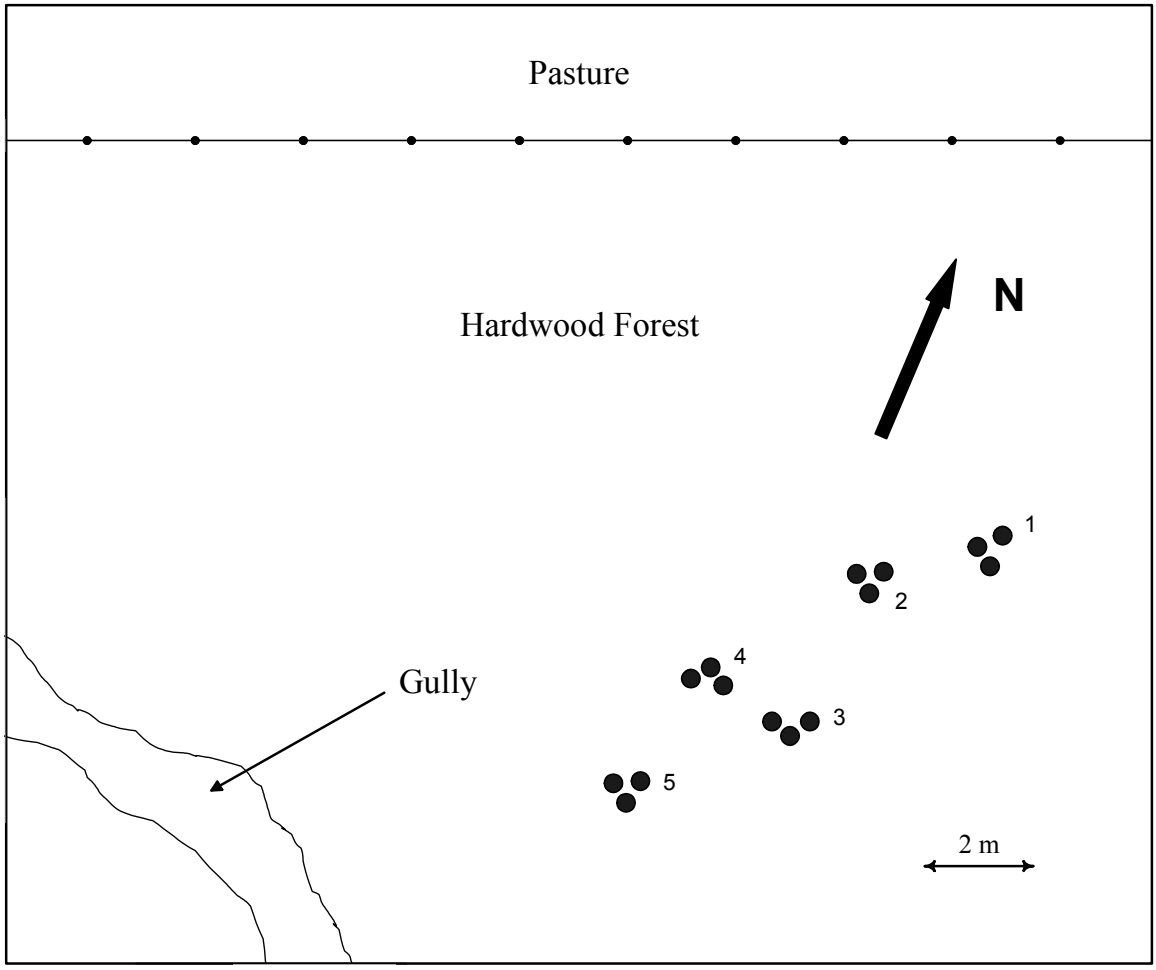


Figure 5.1. Scale map of study site showing positioning of the 15 experimental microcosms.

Figure 5.2. Photographs of study site. Long shot (top) and medium shot (bottom) of study site from adjacent pasture. Microcosms were located approximately 10 meters in from the pasture fence. (Continued on next page)

Figure 5.2 continued. Photographs of study site. Close-up shots of study area showing two neighboring clusters (top) and a single cluster (bottom).

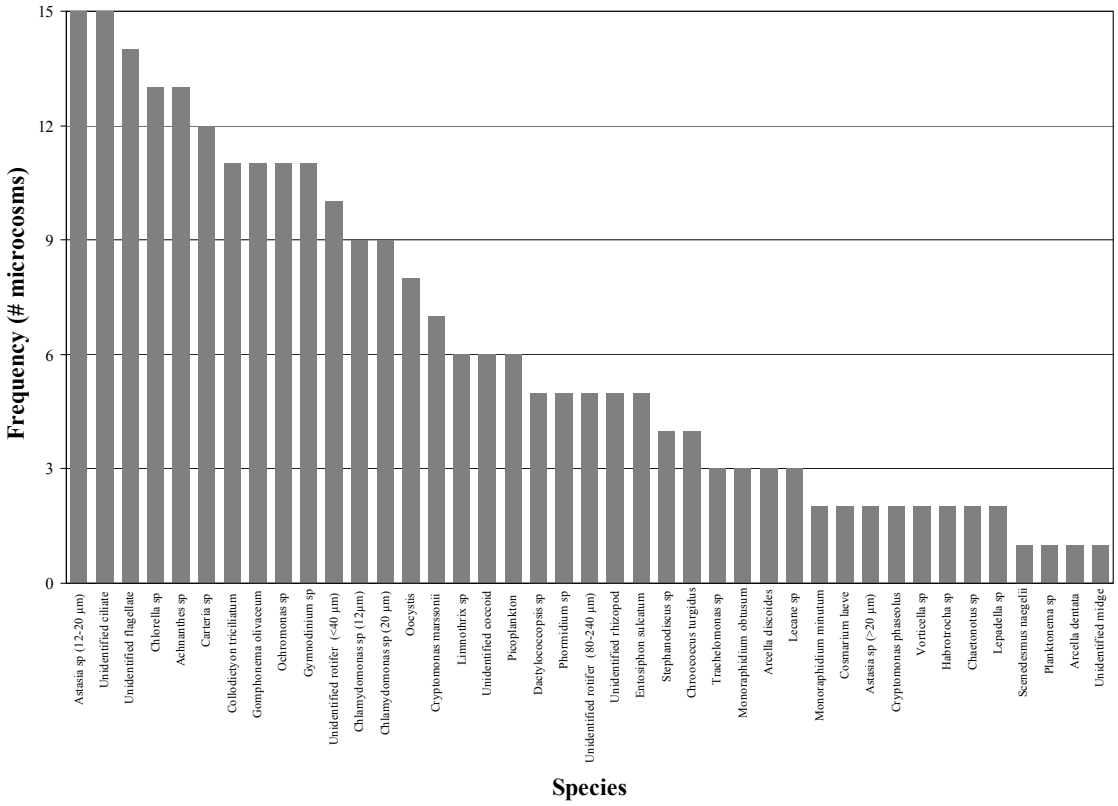


Figure 5.3. The number of microcosms in which at least a single occurrence was found by species.

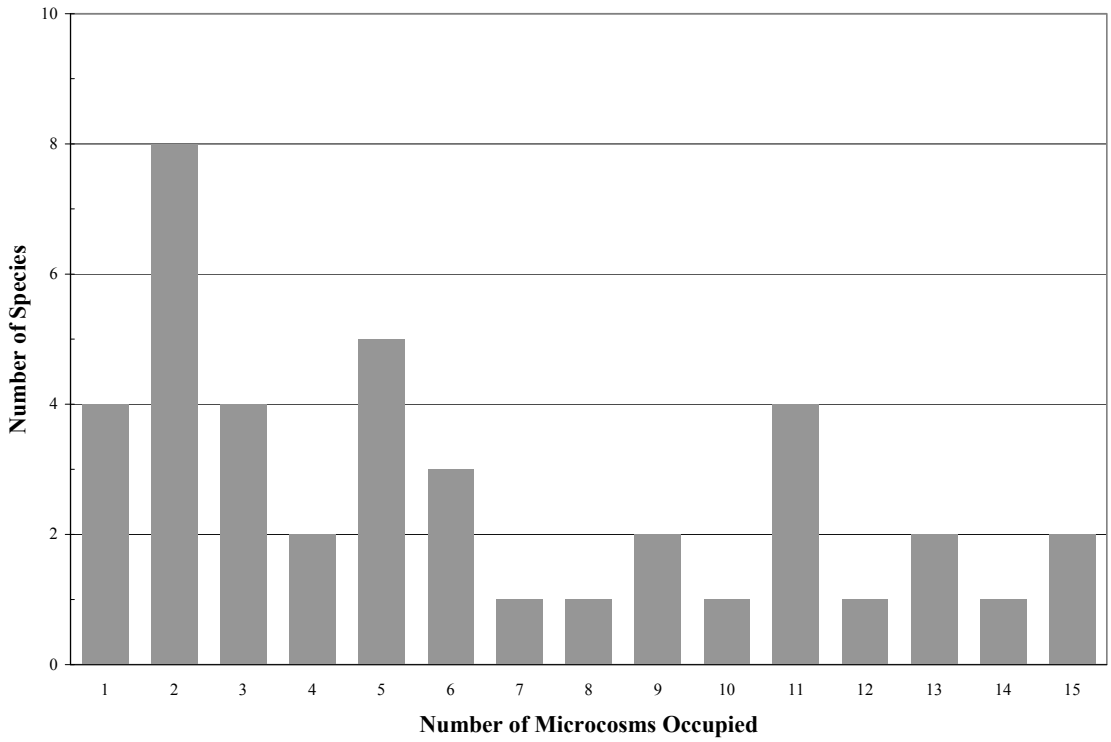


Figure 5.4. Frequency distribution showing the number of species by the number of microcosms in which at least a single occurrence was reported. For example, three of the 41 species were found in six of the 15 microcosms.

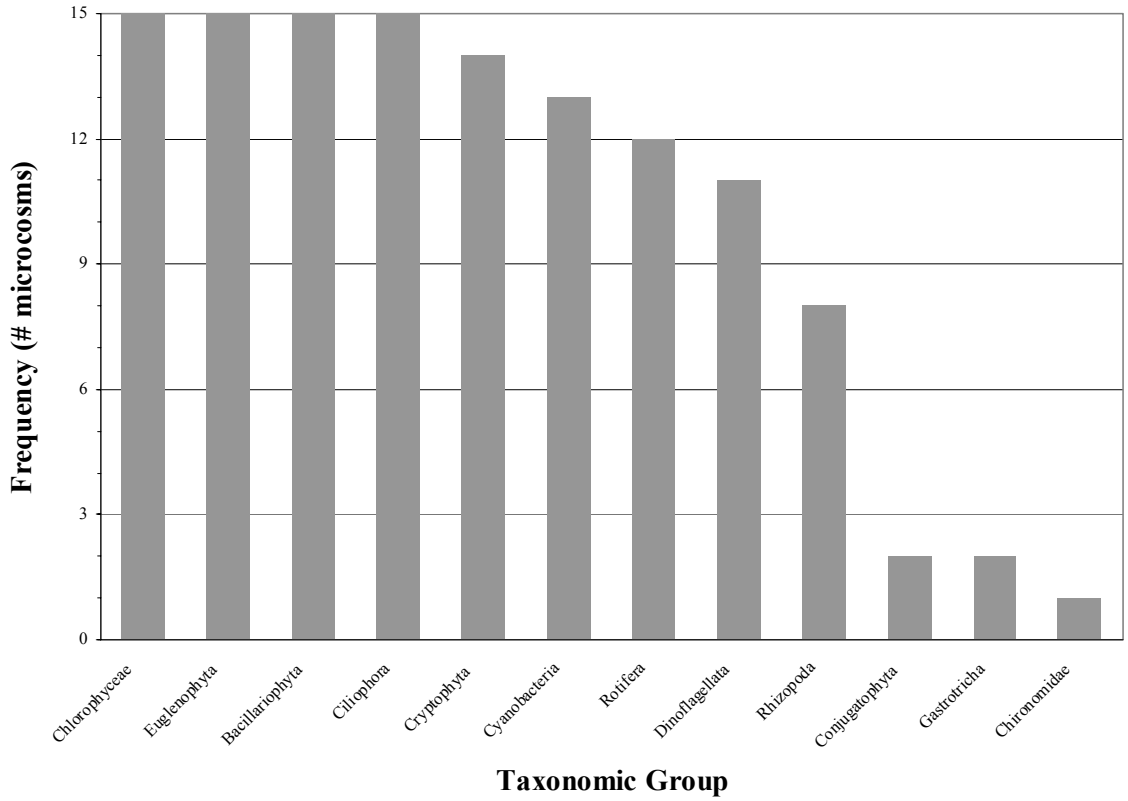


Figure 5.5. Frequency distribution indicating the number of microcosms in which at least a single occurrence was found by higher taxonomic groups.

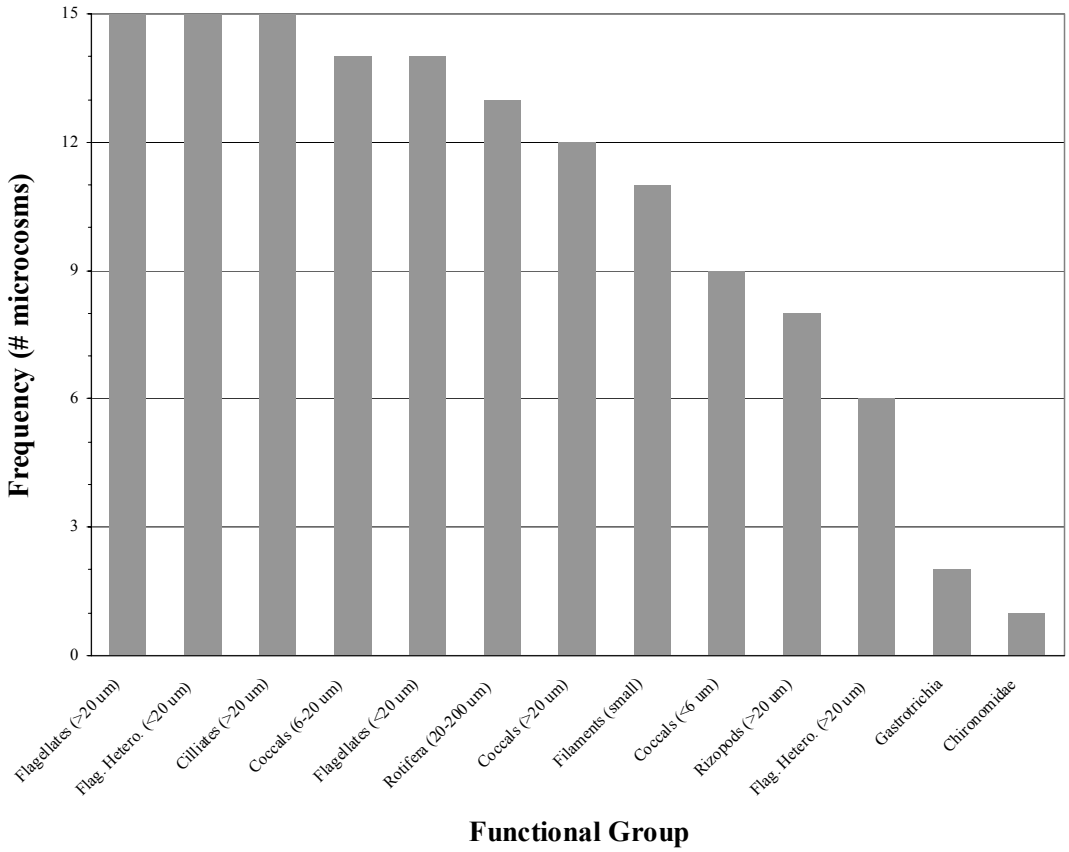


Figure 5.6. Frequency distribution indicating the number of microcosms in which at least a single occurrence was found by functional groups.

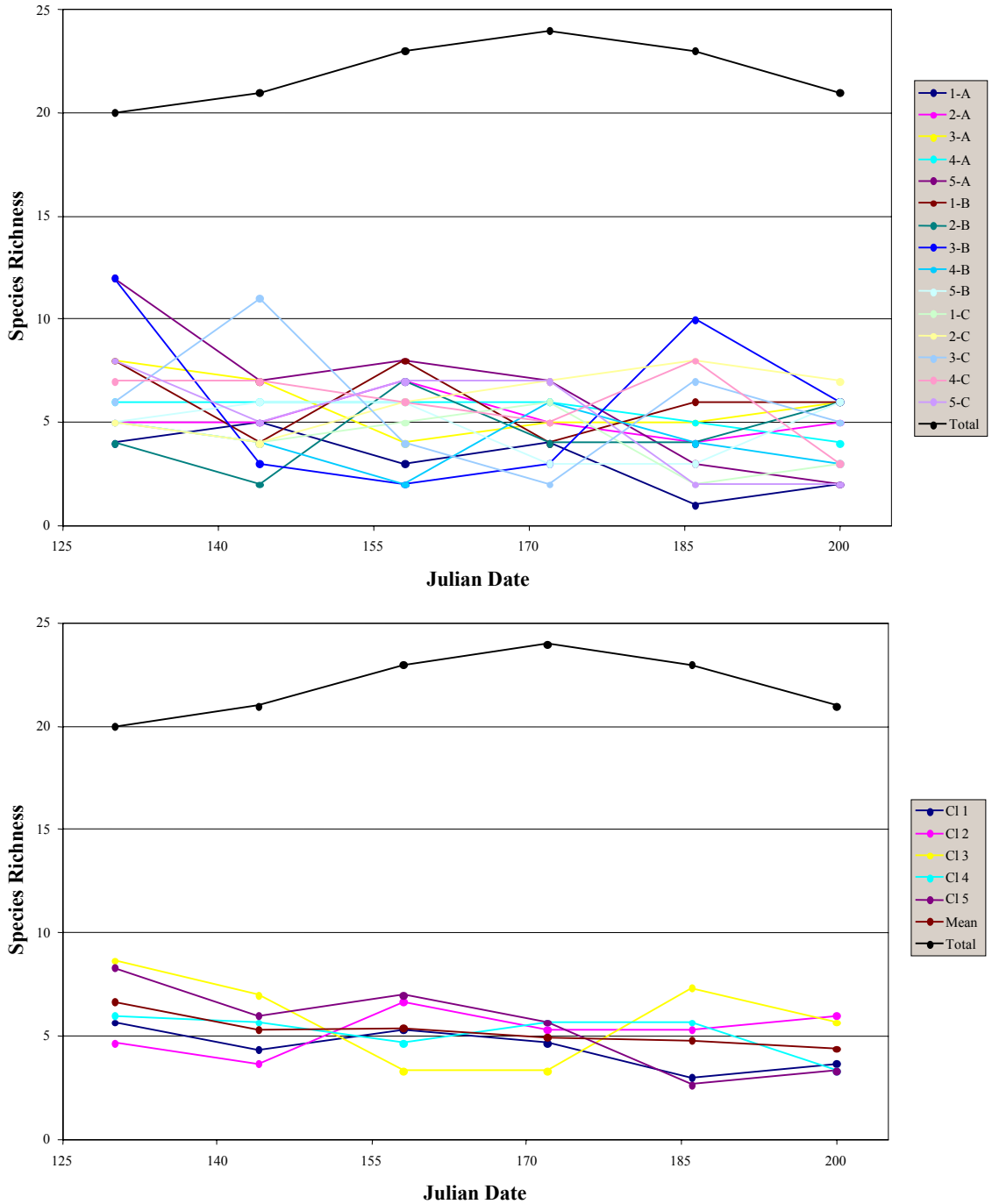


Figure 5.7. Time series of species richness by microcosm and by cluster. The top graph shows species richness by individual microcosm. The bottom graph shows mean species richness by individual cluster. Total species richness for the overall site is shown in black in both graphs.

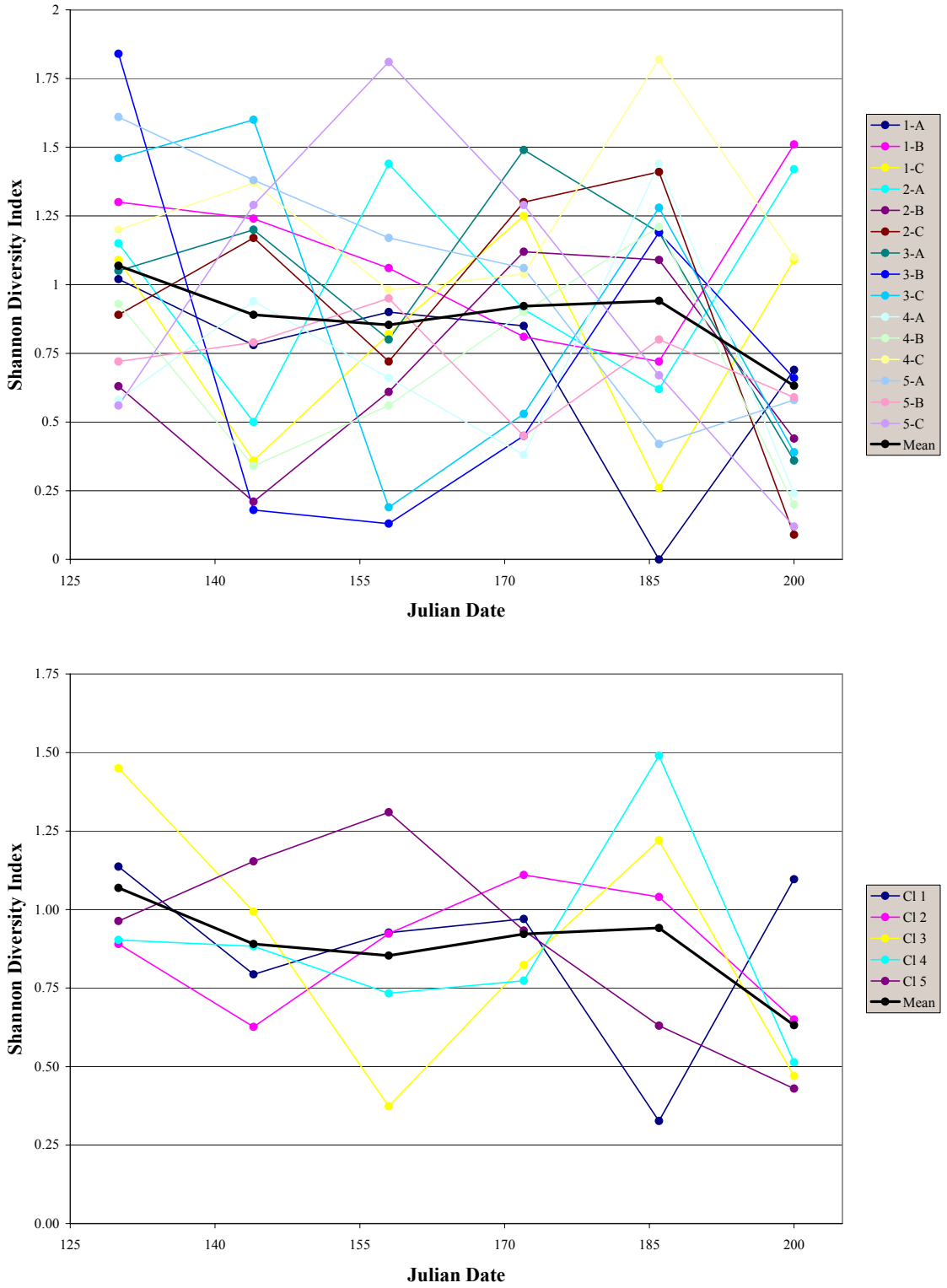


Figure 5.8. Time series of Shannon diversity by microcosm and by cluster. Top graph shows diversity by individual microcosm and the bottom graph shows mean diversity by individual cluster. Mean diversity per microcosm for the overall site is shown in black in both graphs.

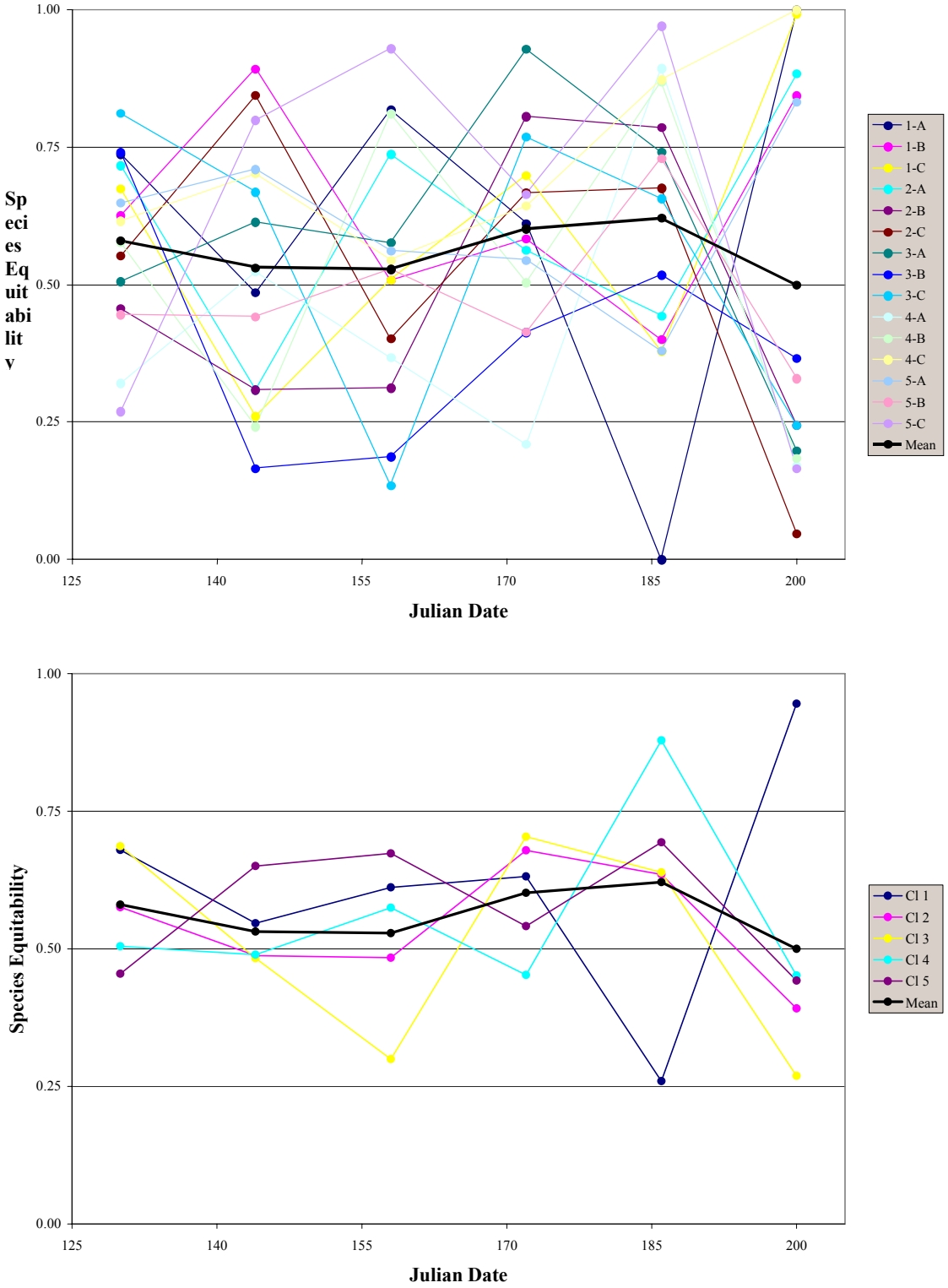
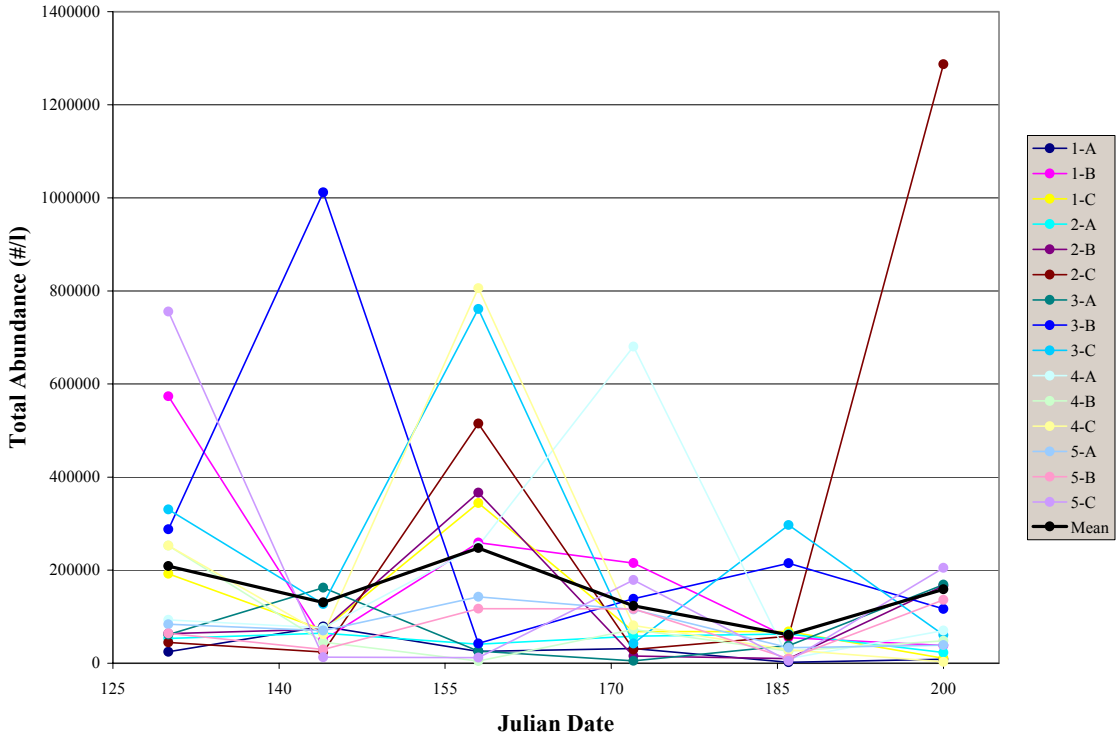


Figure 5.9. Time series of species equitability by microcosm and by cluster. Top graph shows equitability by individual microcosm and the bottom graph shows mean equitability by individual cluster. Mean equitability per microcosm for the overall site is shown in black in both graphs.



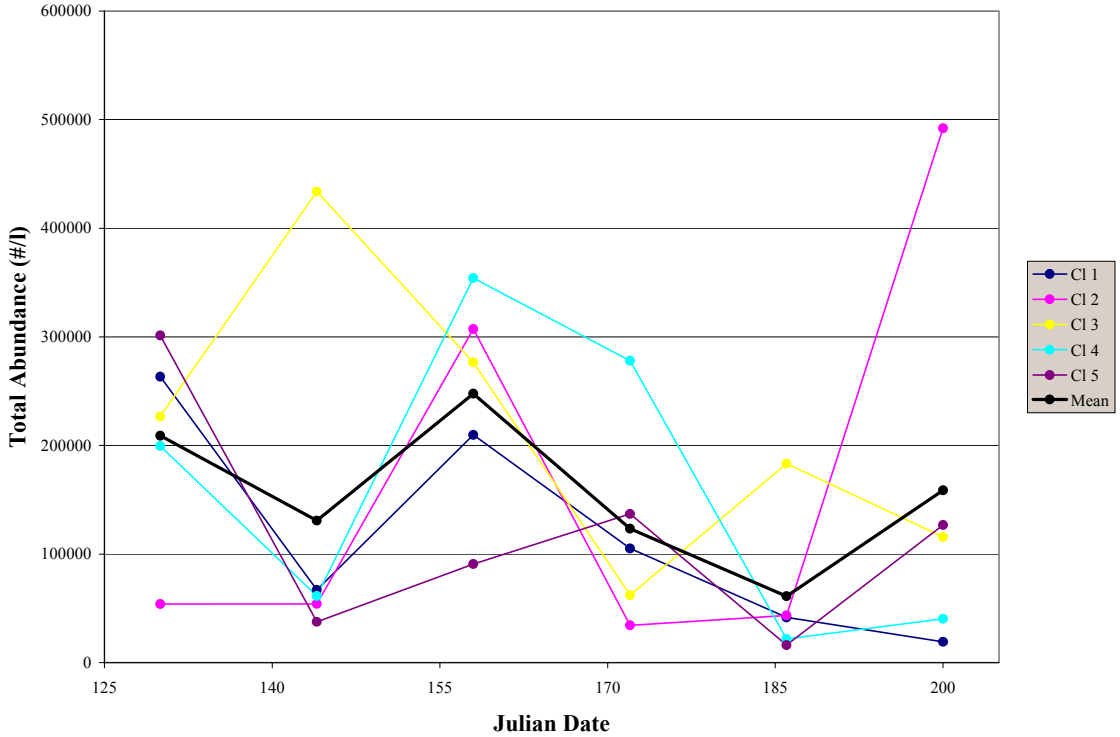


Figure 5.10. Time series of total abundance (#/L) by microcosm and by cluster. Top graph shows total abundance by individual microcosm and the bottom graph shows mean total abundance by individual cluster. Mean total abundance per microcosm for the overall site is shown in black in both graphs.

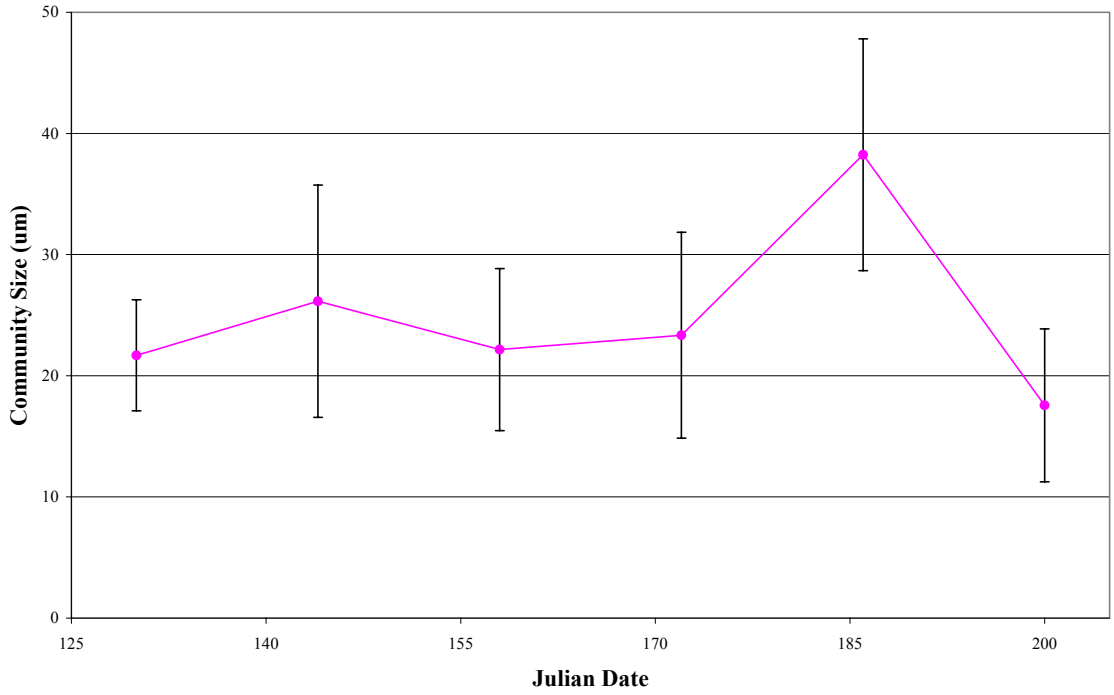


Figure 5.11. Time series of mean community size per microcosm. Community size is defined by the weighted average of species GALD data. Error bars represent 95% confidence intervals.

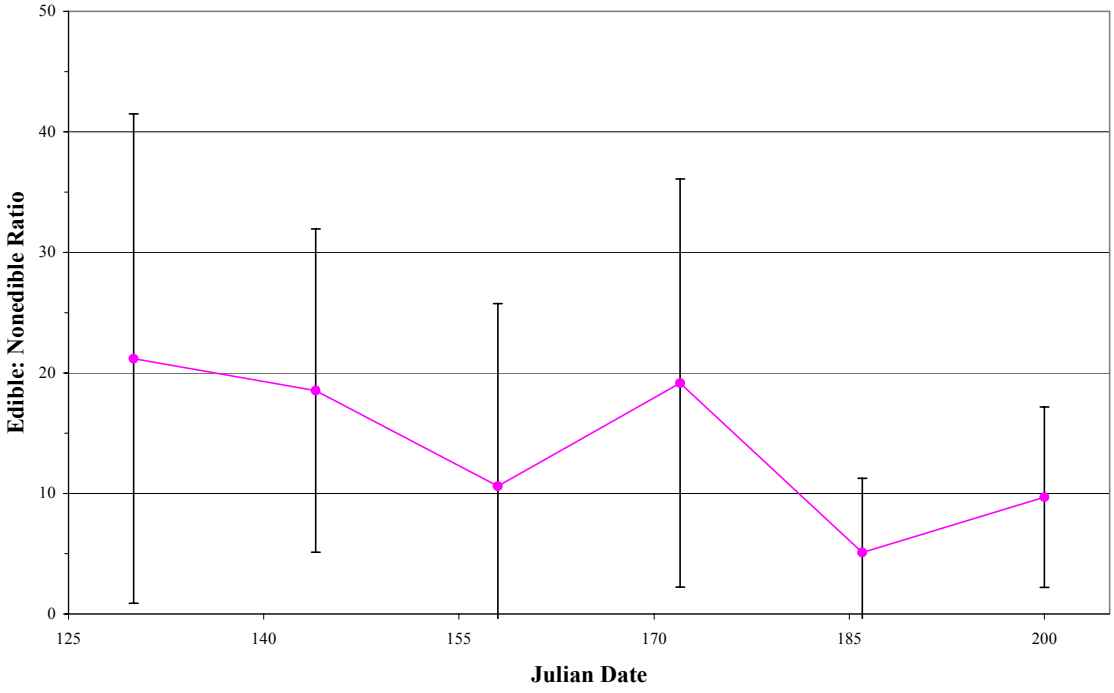


Figure 5.12. Time series of mean edible:nonedible ratio per microcosm. Error bars represent 95% confidence intervals.

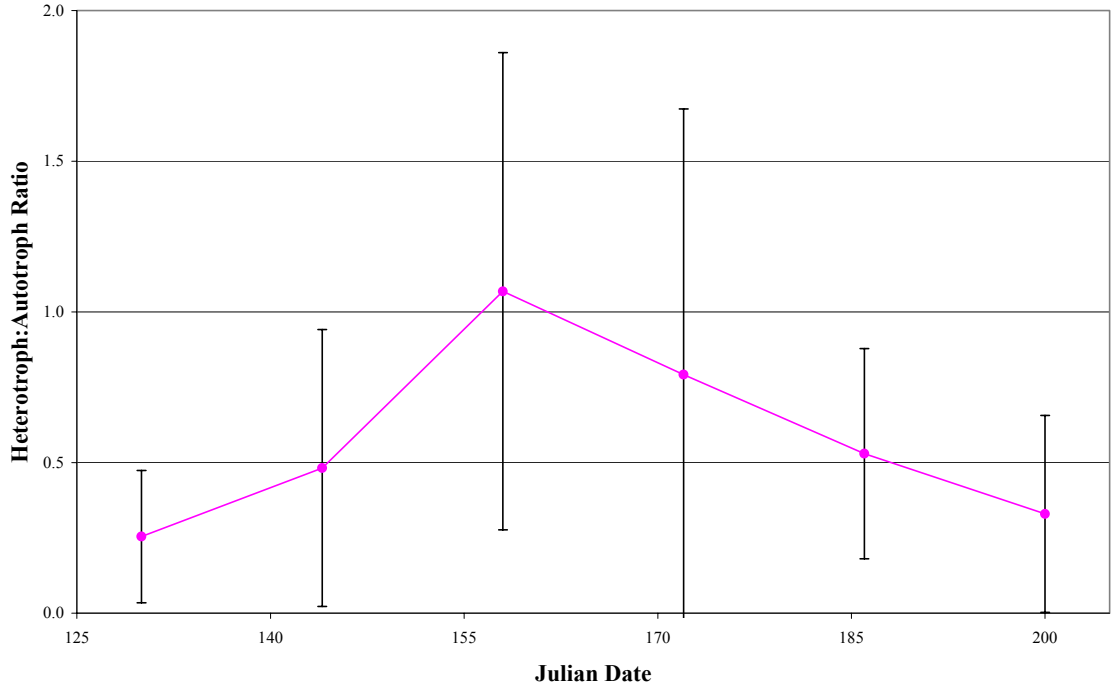


Figure 5.13. Time series of mean heterotroph:autotroph ratio per microcosm. Error bars represent 95% confidence intervals.

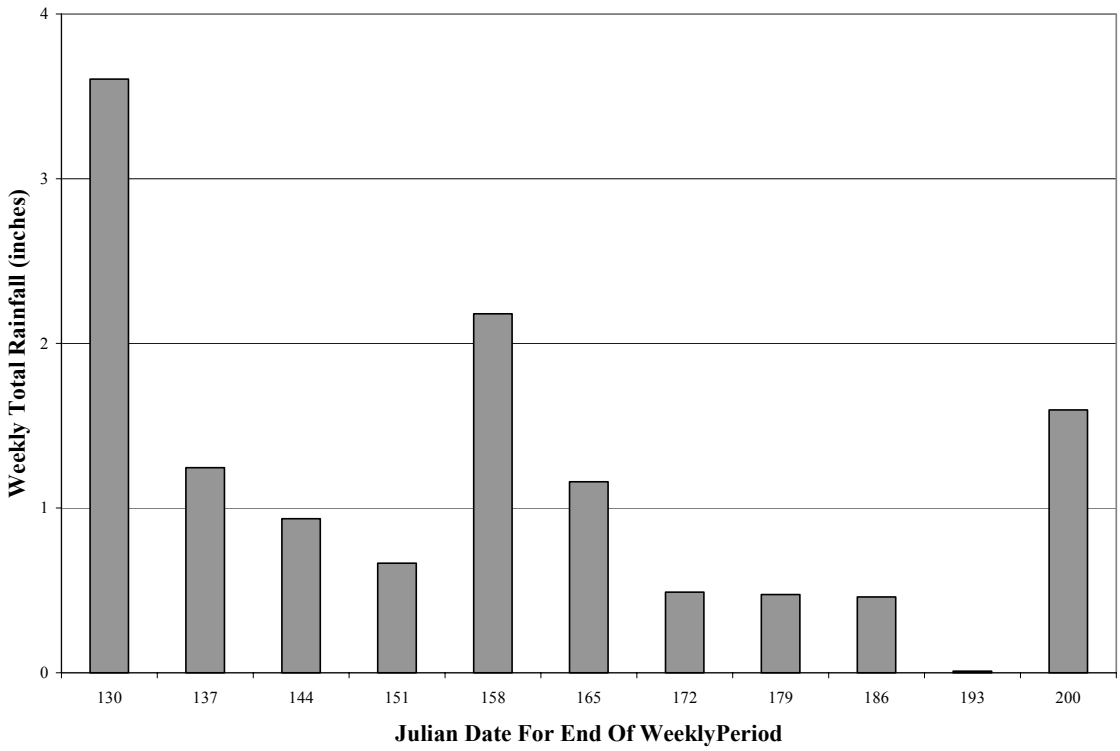


Figure 5.14. Weekly rainfall totals for the study area. Rainfall for the study site was obtained by averaging daily rainfall records for two nearboring collection points – the Knoville and Norris Experimental Stations. Daily values were then summed for 7-day periods with the end of the every other weekly record corresponding with microcosm sampling dates.

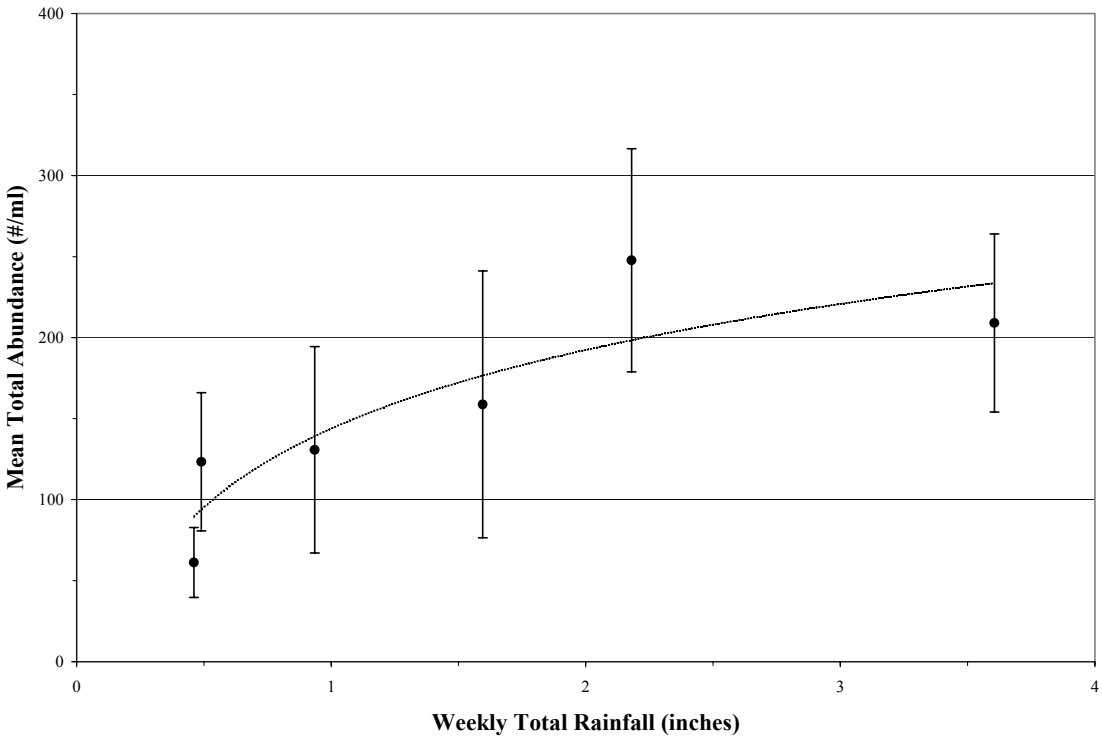


Figure 5.15. Correlation of mean total abundance with weekly total rainfall. A logarithmic trendline had an $r^2 = 0.77$. Error bars for abundance values represent standard error of the mean.

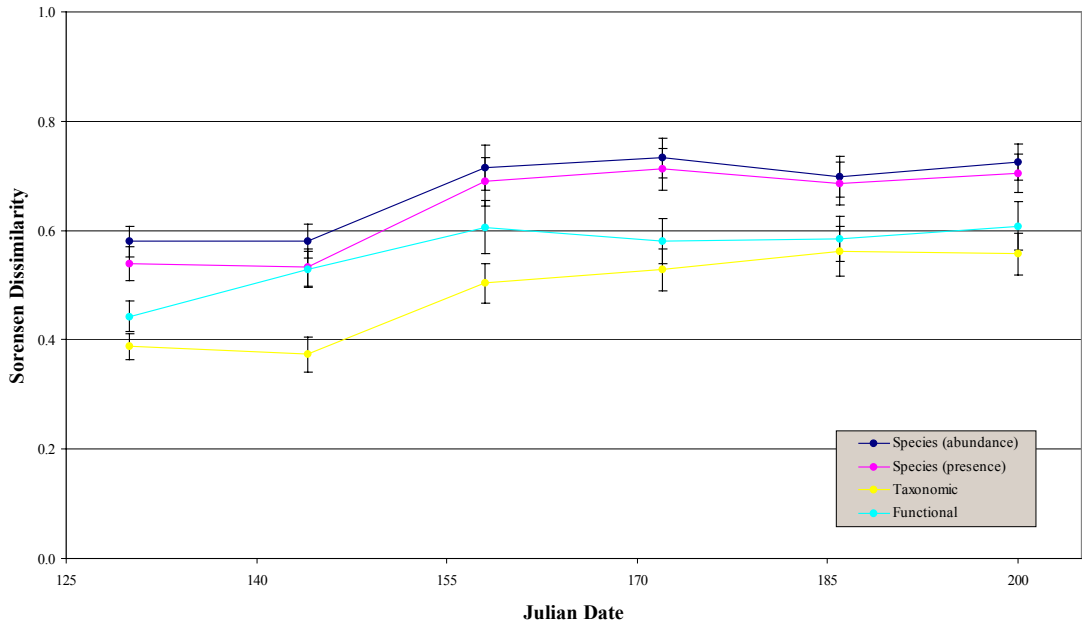


Figure 5.16. Time series of mean dissimilarity for the overall site for four of the five classification data types. *Community Features* data was not considered. Error bars represent 95% confidence intervals.

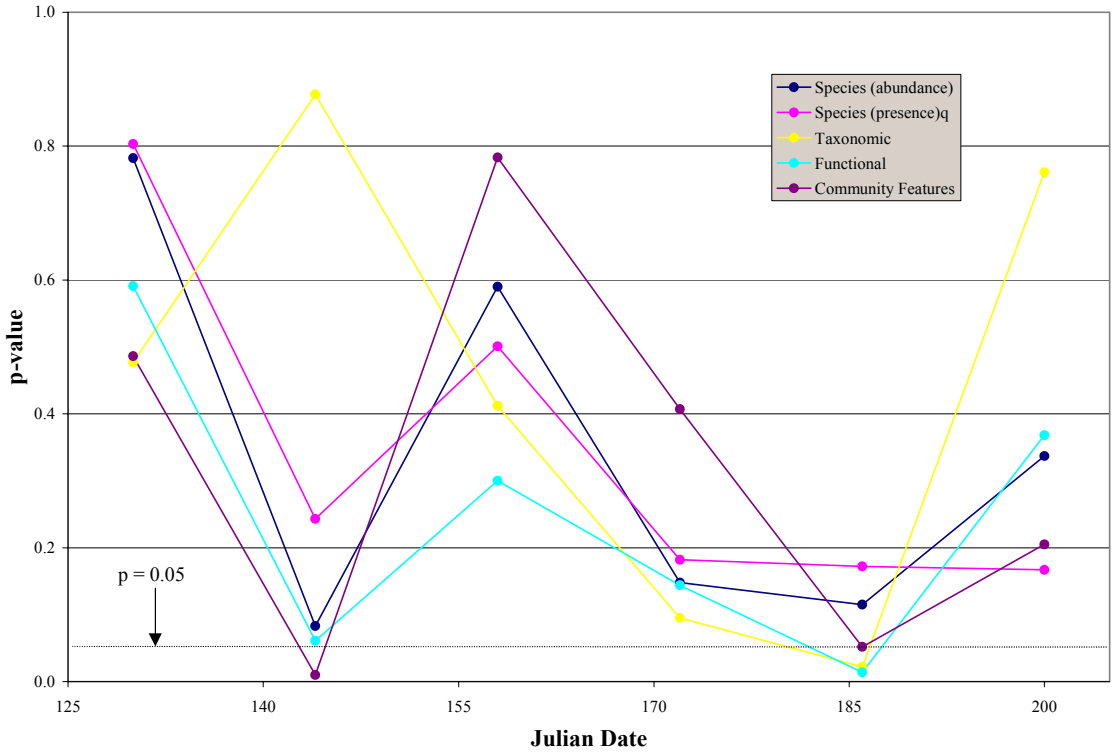


Figure 5.17. Time series of reported p-values of MRPP comparisons by sampling date of compositional data grouped by cluster. All five classification schemes are shown.

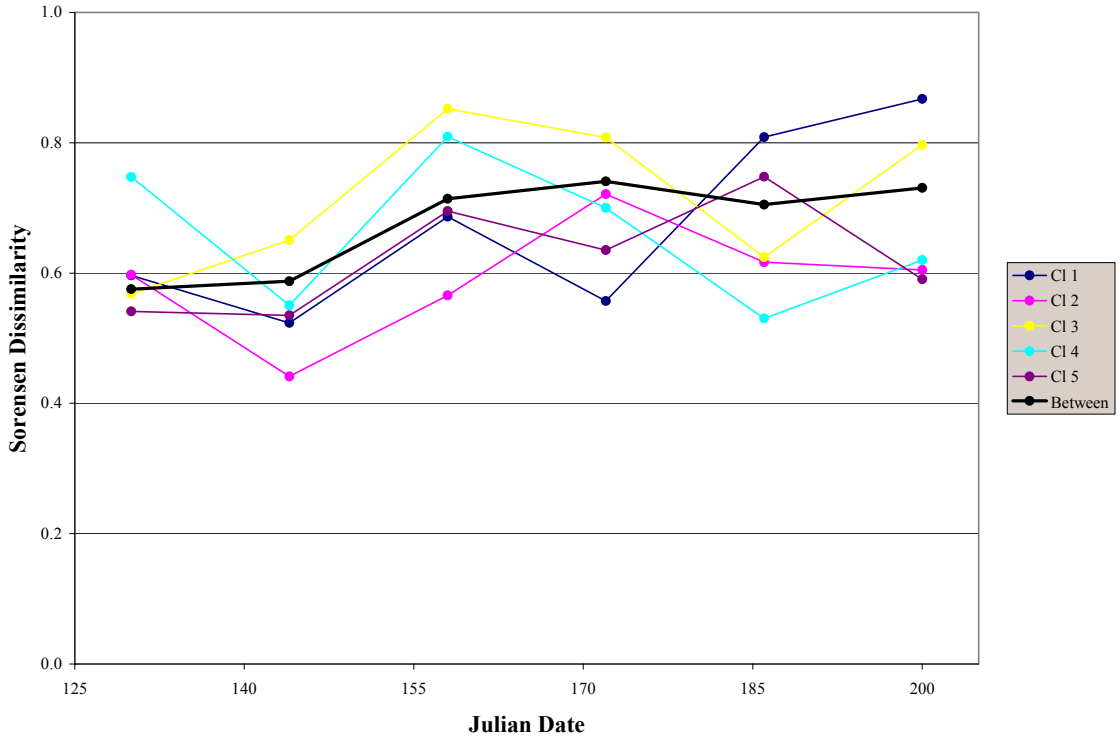


Figure 5.18. Time series of mean dissimilarity for *Species-level (abundance)* classification data. Mean dissimlarity between clusters is shown in black. Cluster labels are abbreviated, i.e "Cl1" corresponds to Cluster 1. Error bars are omitted for clarity.

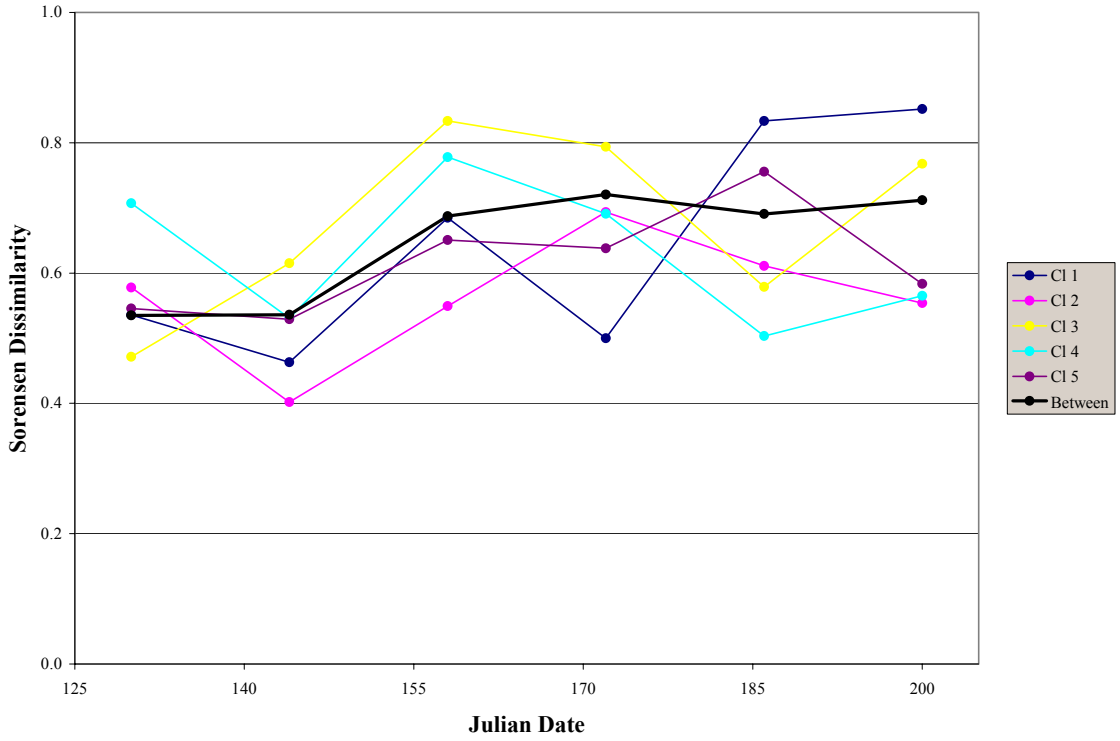


Figure 5.19. Time series of mean dissimilarity for *Species-level (presence/absence)* classification data. Mean dissimlarity between clusters is shown in black. Cluster labels are abbreviated, i.e "Cl1" corresponds to Cluster 1. Error bars are omitted for clarity.

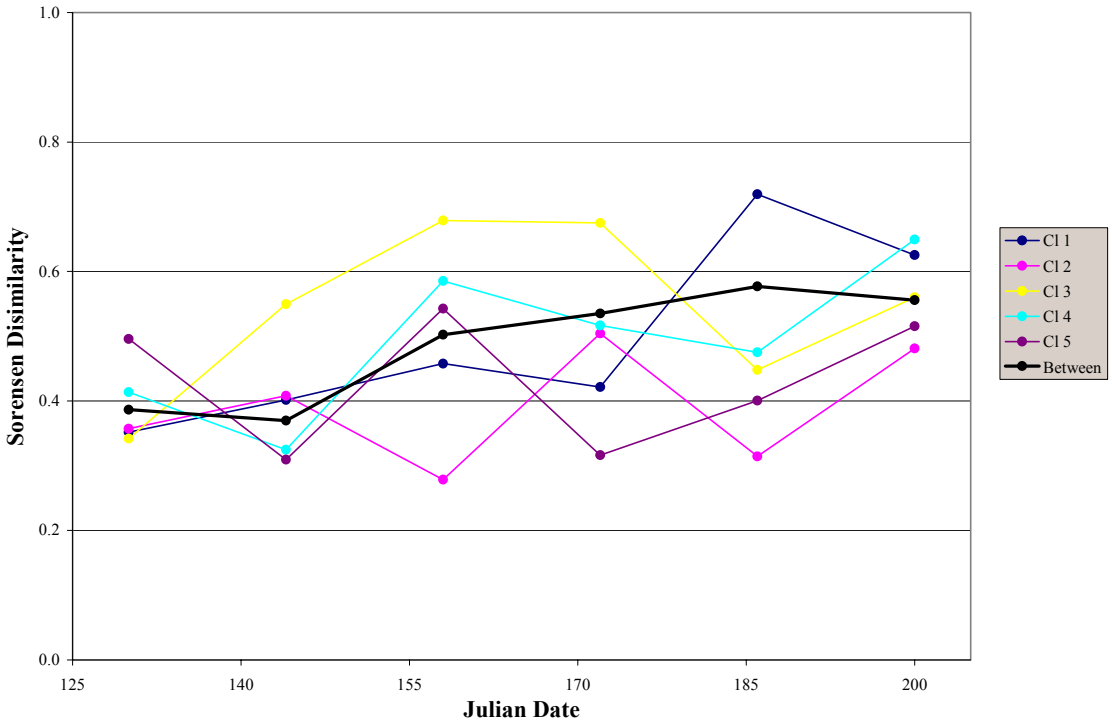


Figure 5.20. Time series of mean dissimilarity for *Taxonomic-level* classification data. Mean dissimlarity for the overall site is shown in black. Cluster labels are abbreviated, i.e "Cl1" corresponds to Cluster 1. Error bars are omitted for clarity.

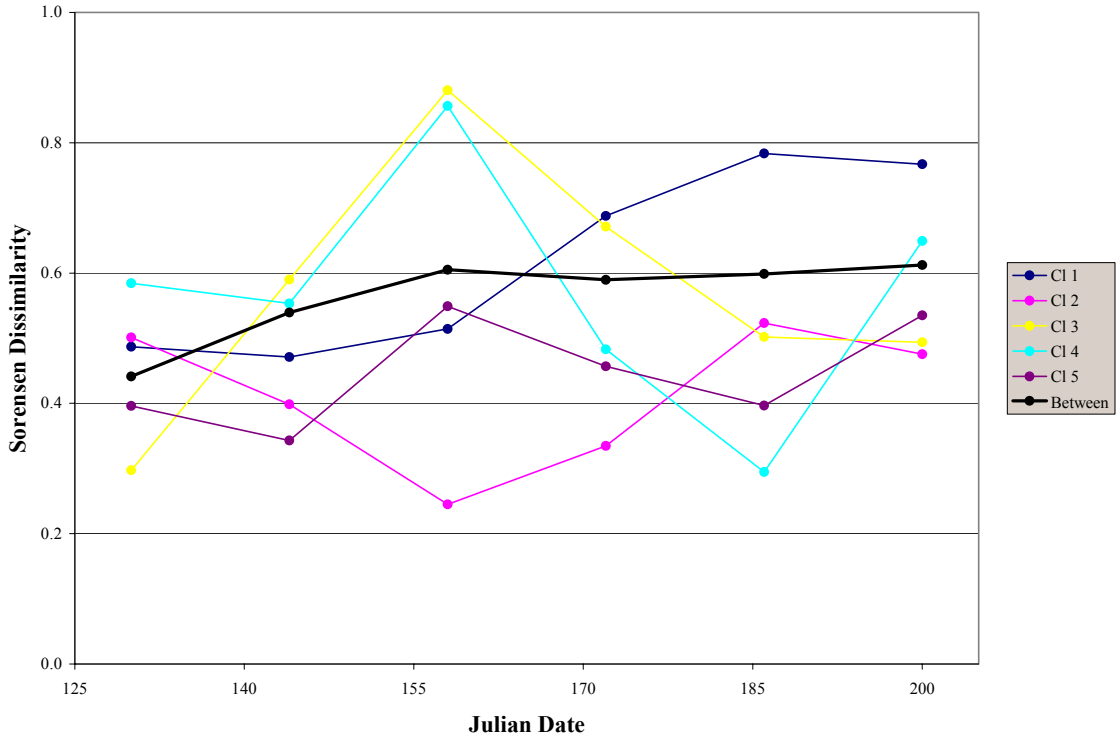


Figure 5.21. Time series of mean dissimilarity for *Functional-level* classification data. Mean dissimlarity between clusters is shown in black. Cluster labels are abbreviated, i.e "Cl1" corresponds to Cluster 1. Error bars are omitted for clarity.

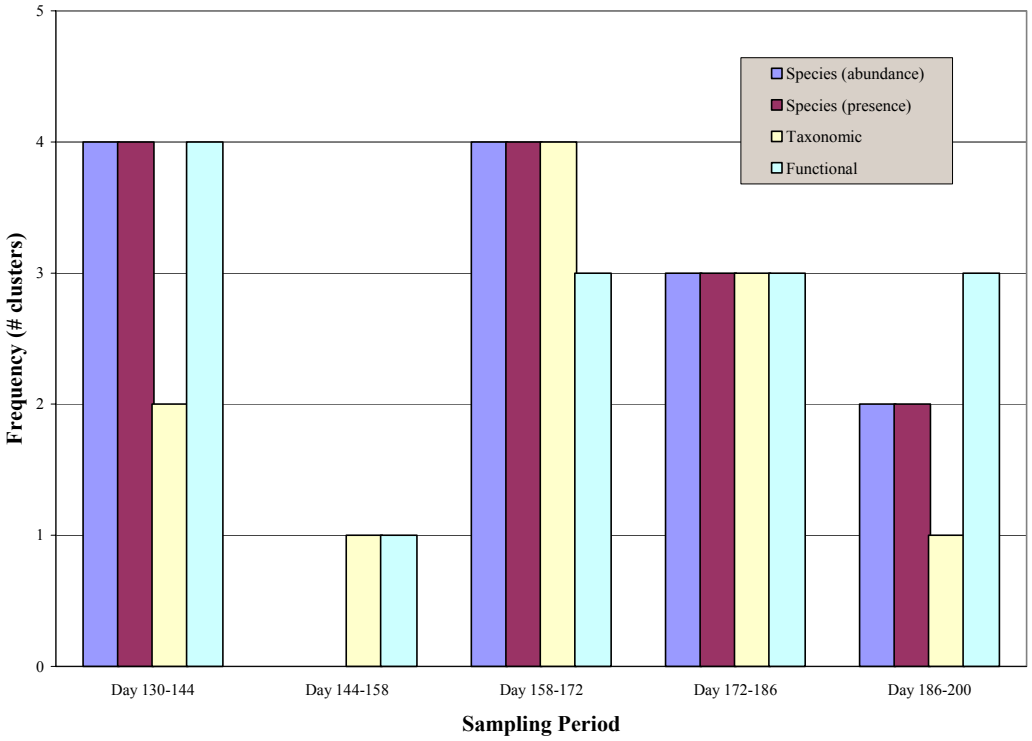


Figure 5.22. Frequency histogram indicating the number of clusters by sampling period and data type that exhibited a decrease in mean pairwise dissimilarity.

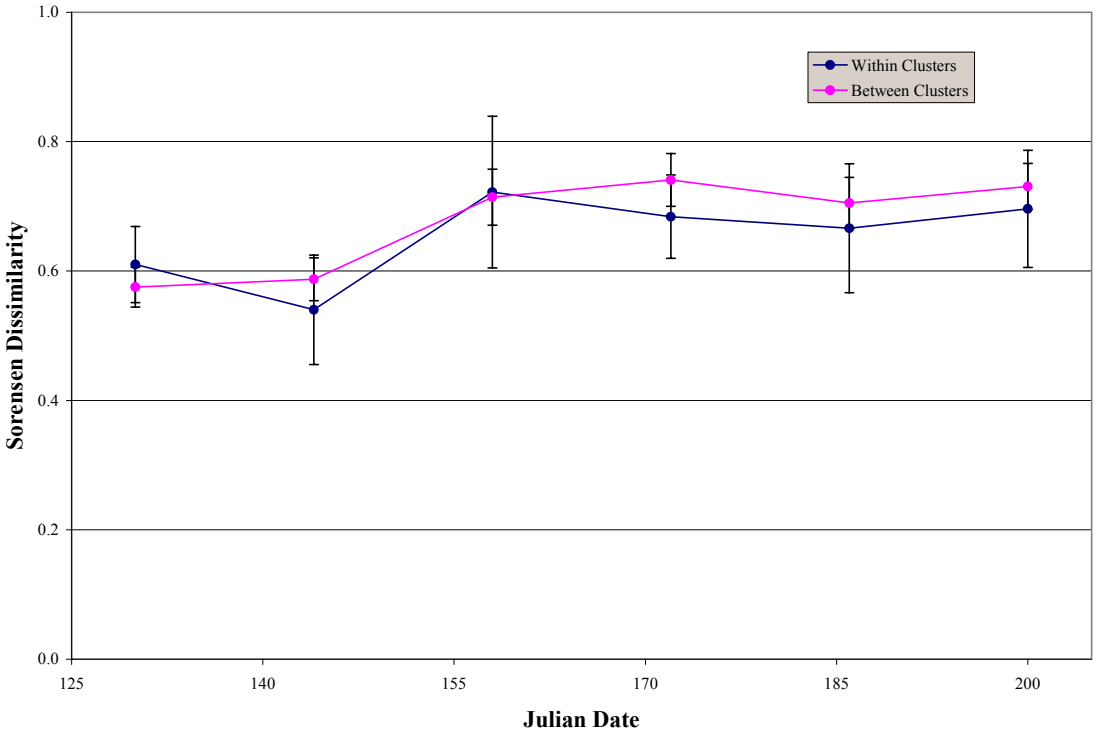


Figure 5.23. Time series of mean dissimilarity *within clusters* versus mean dissimilarity *between clusters* for *Species-level (abundance)* classification data. Error bars represent 95% confidence intervals.

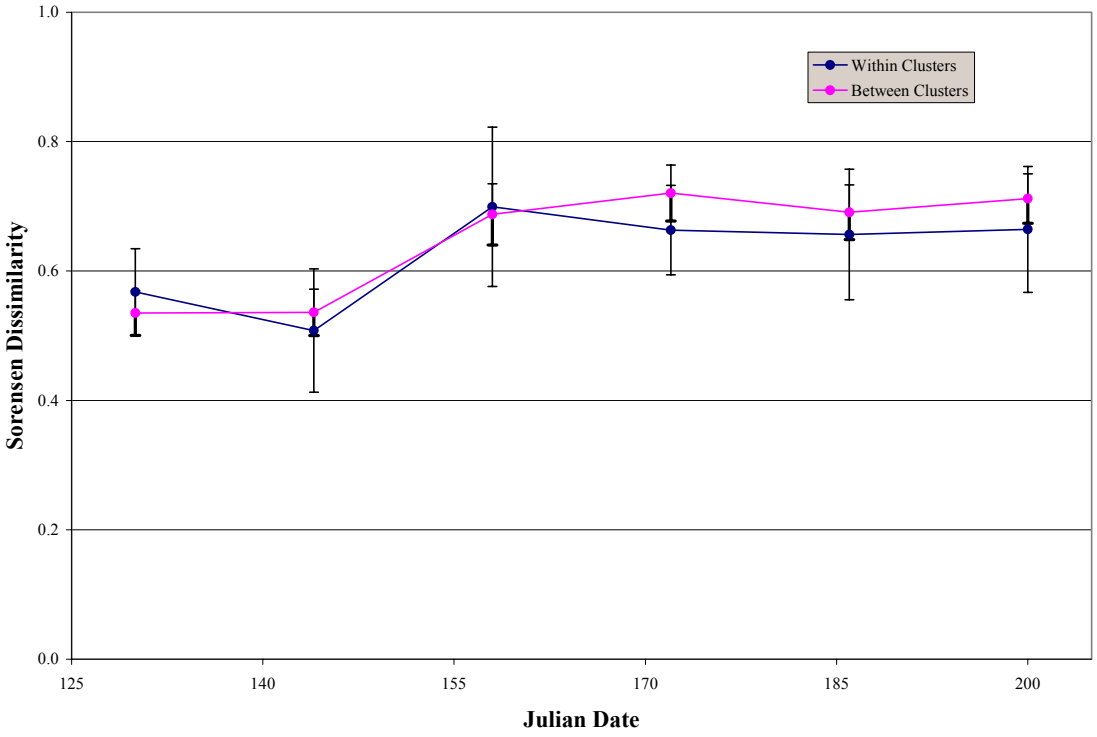


Figure 5.24. Time series of mean dissimilarity *within clusters* versus mean dissimilarity *between clusters* for *Species-level (presence/absence)* classification data. Error bars represent 95% confidence intervals.

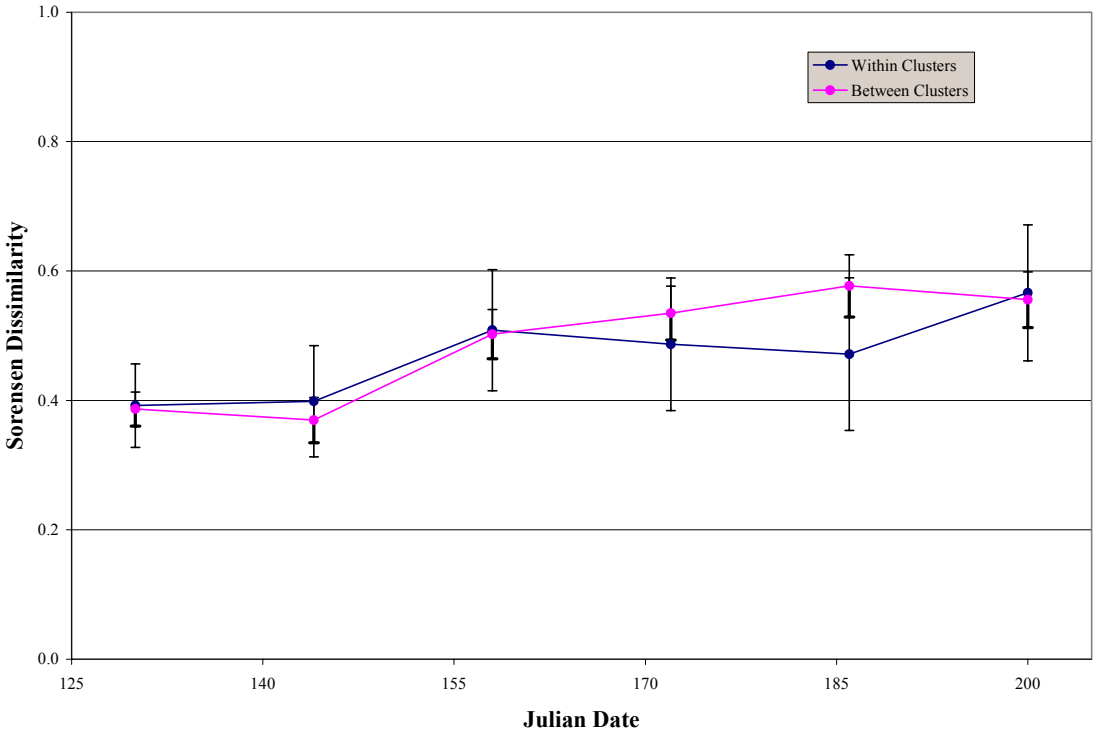


Figure 5.25. Time series of mean dissimilarity *within clusters* versus mean dissimilarity *between clusters* for *Taxonomic-level* classification data. Error bars represent 95% confidence intervals.

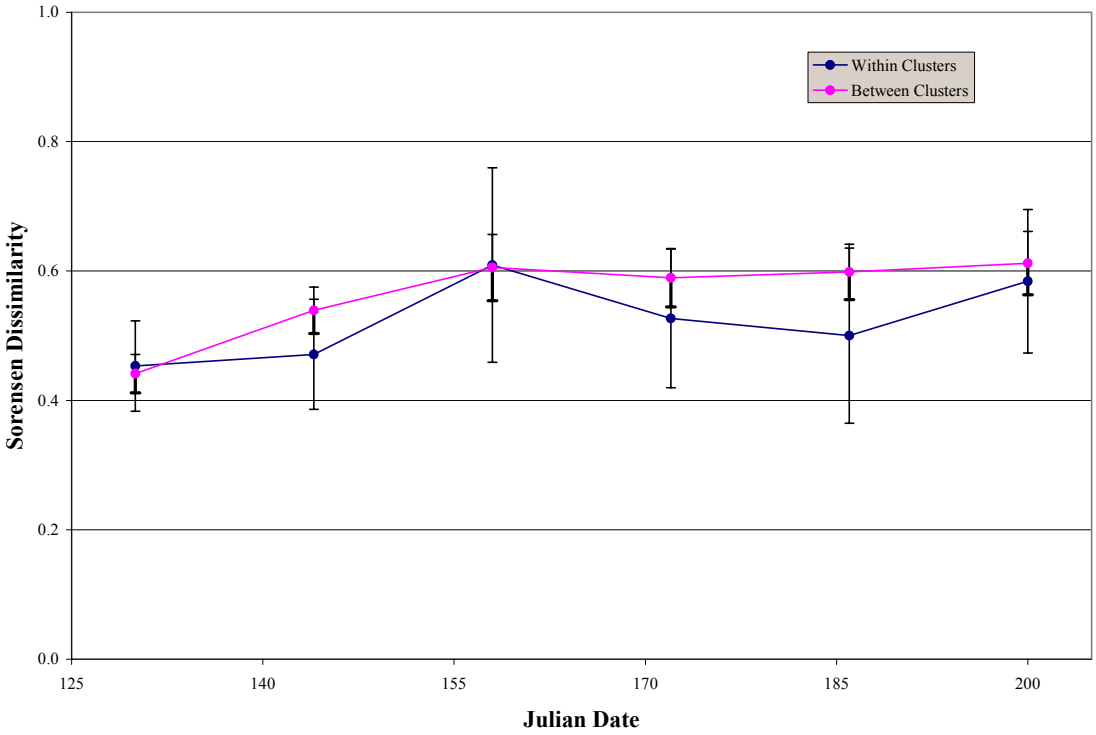


Figure 5.26. Time series of mean dissimilarity *within clusters* versus mean dissimilarity *between clusters* for *Functional-level* classification data. Error bars represent 95% confidence intervals.

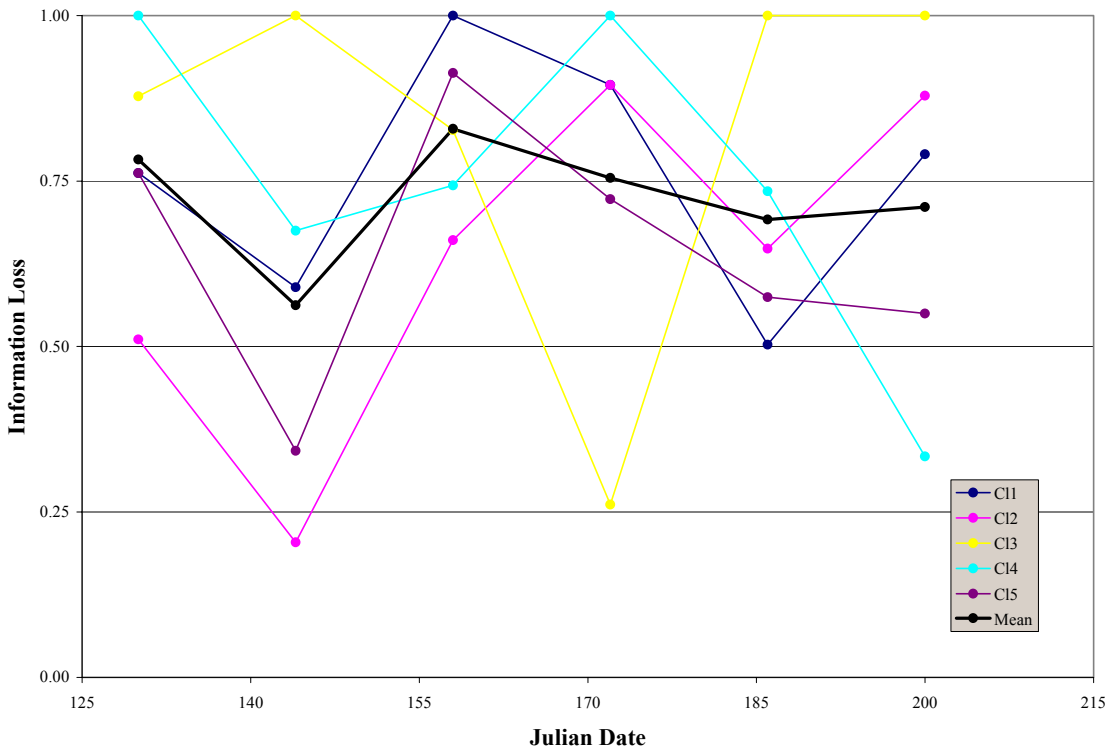


Figure 5.27. Time series of information loss for complete cluster linkage by cluster for *Species-level (abundance)* classification data. Overall mean information loss per cluster is shown in black.

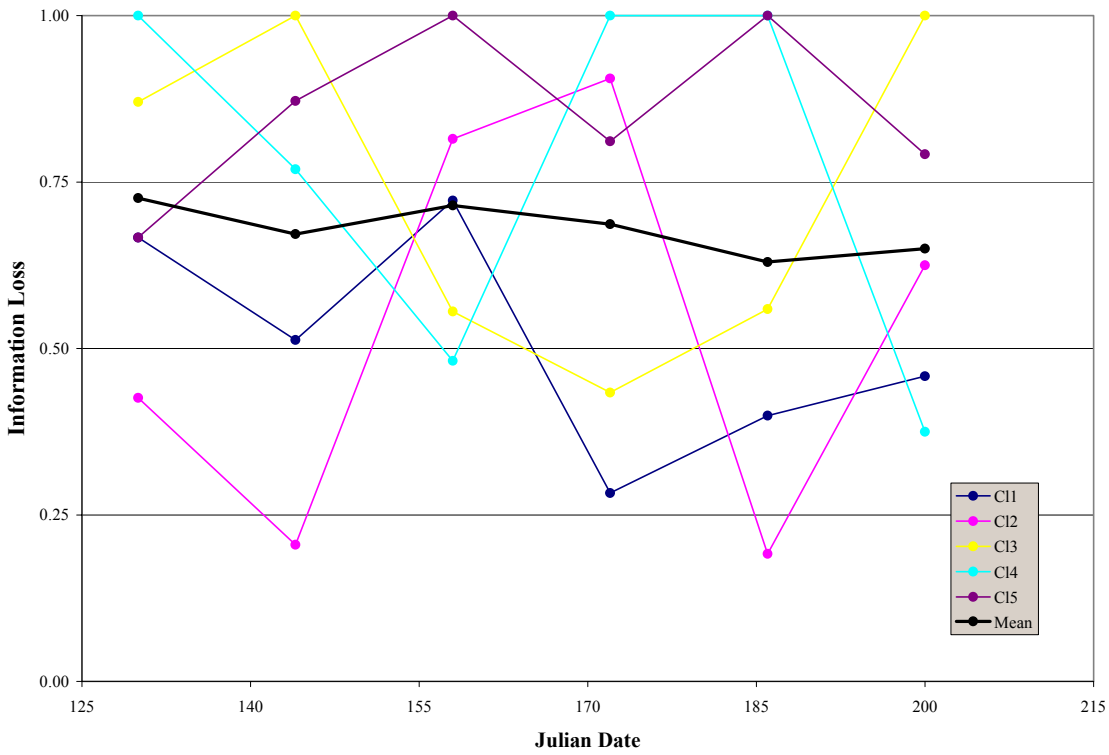


Figure 5.28. Time series of information loss for complete cluster linkage by cluster for *Species-level (presence)* classification data. Overall mean information loss per cluster is shown in black.

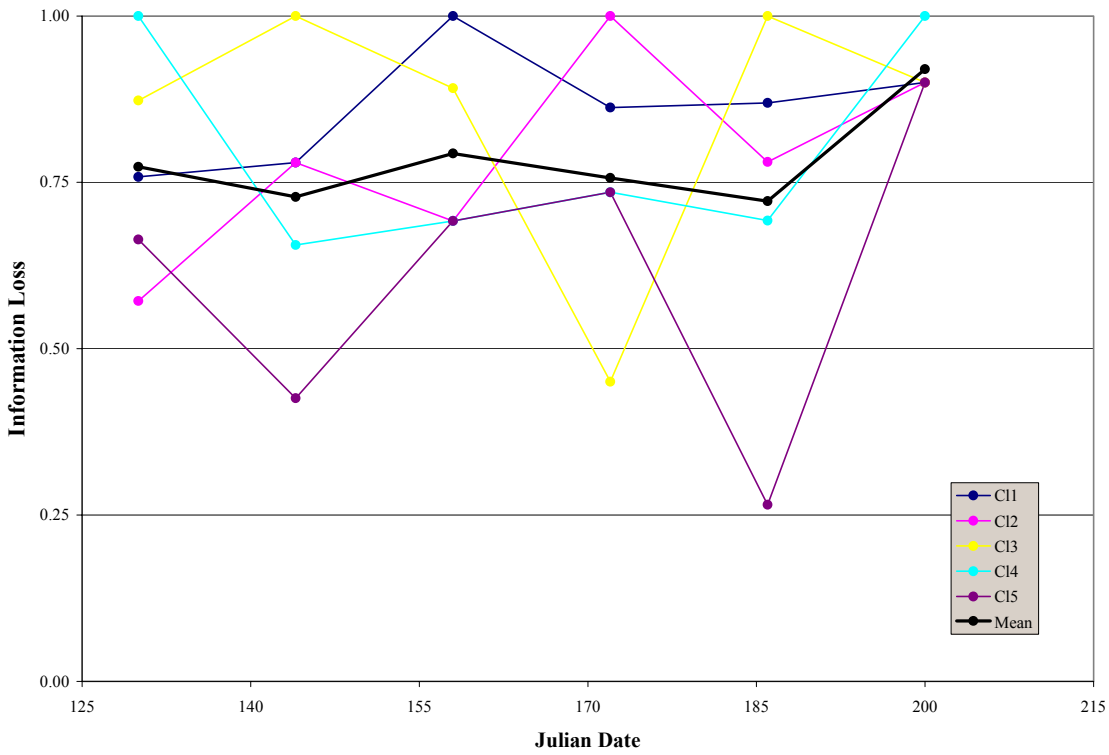


Figure 5.29. Time series of information loss for complete cluster linkage by cluster for *Taxonomic-level* classification data. Overall mean information loss per cluster is shown in black.

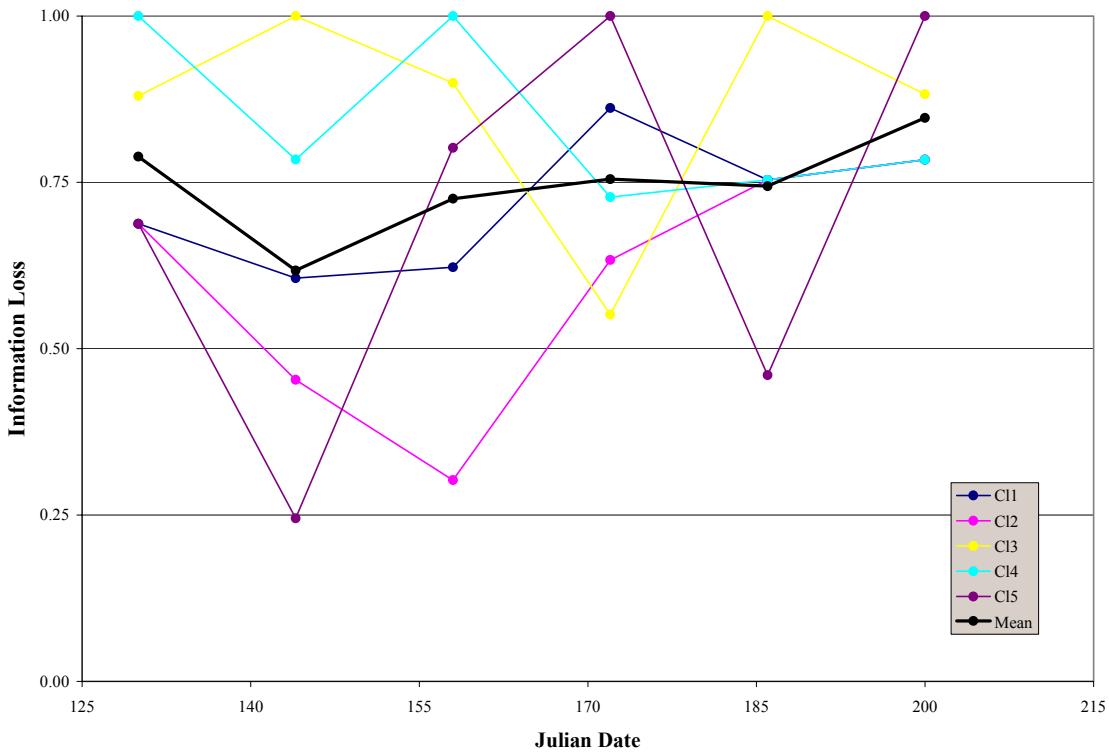


Figure 5.30. Time series of information loss for complete cluster linkage by cluster for *Functional-level* classification data. Overall mean information loss per cluster is shown in black.

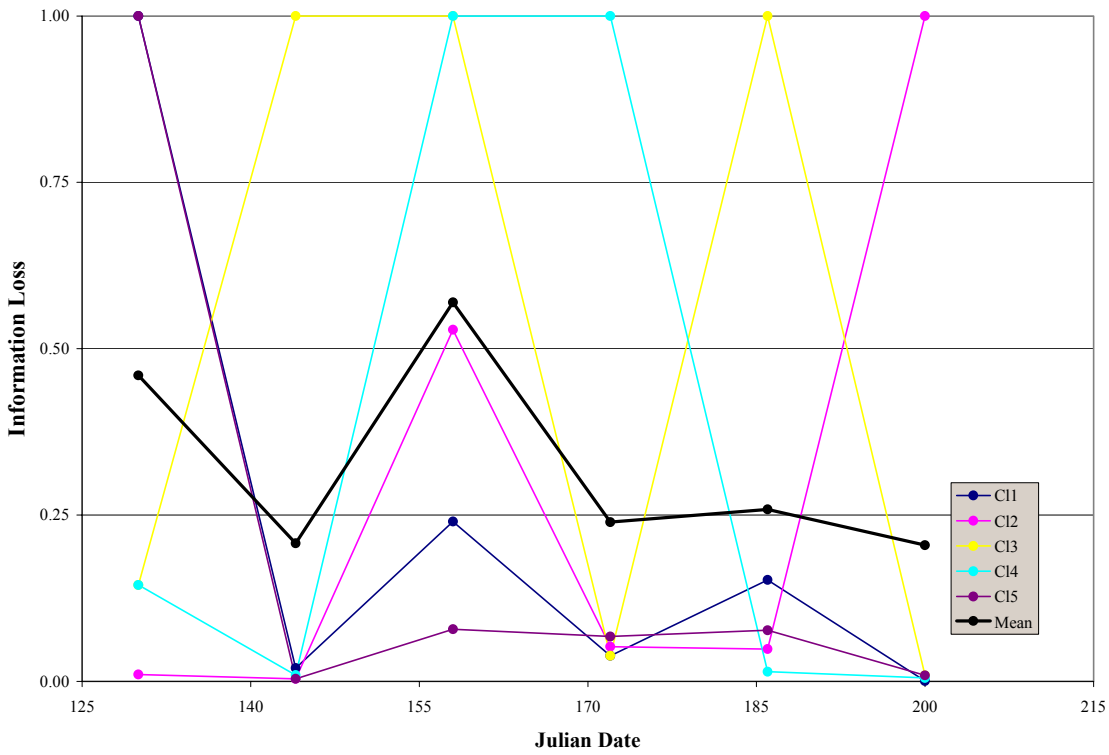


Figure 5.31. Time series of information loss for complete cluster linkage by cluster for *Community Features* classifcation data. Overall mean information loss per cluster is shown in black.

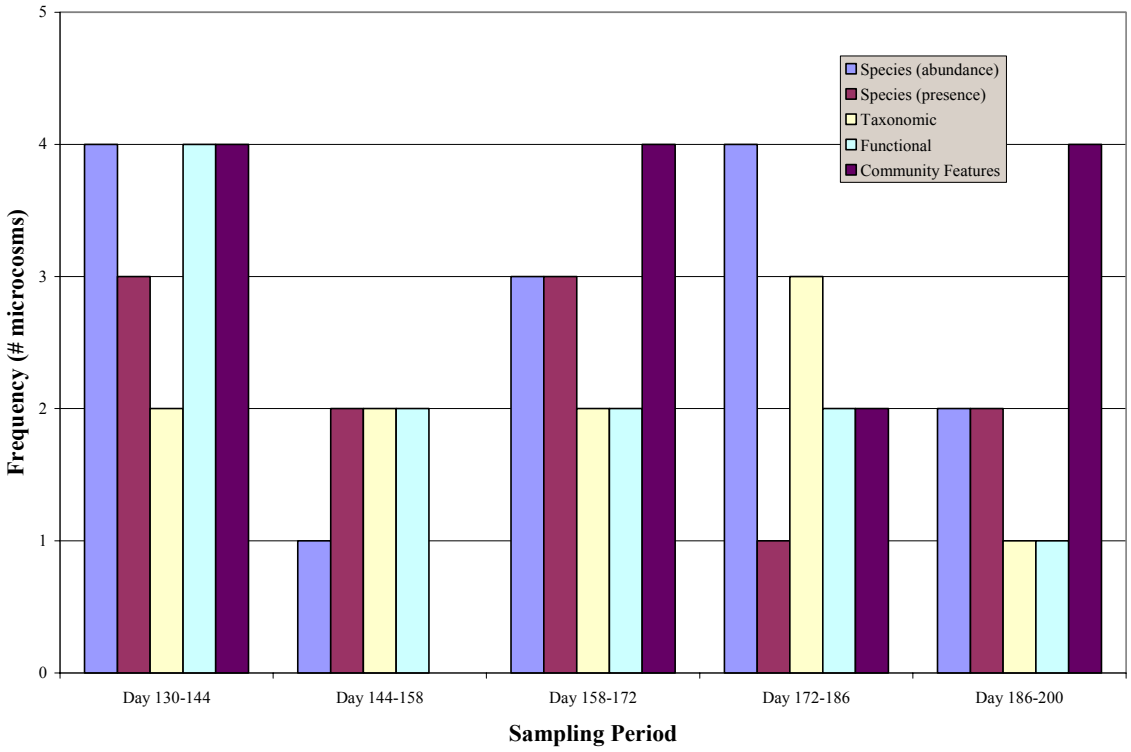


Figure 5.32. Frequency histogram indicating the number of clusters by sampling period and classification data type that exhibited a decrease in percent information loss for complete linkage.

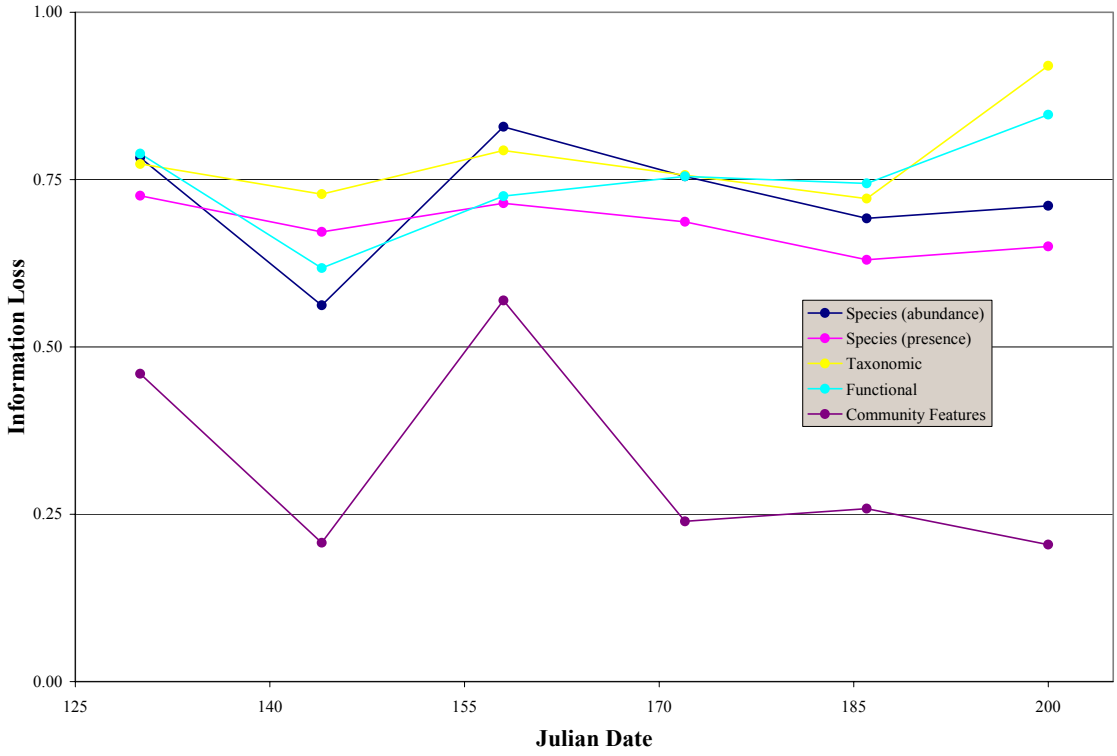


Figure 5.33. Time series of mean information loss for complete by cluster for all five classification data types.

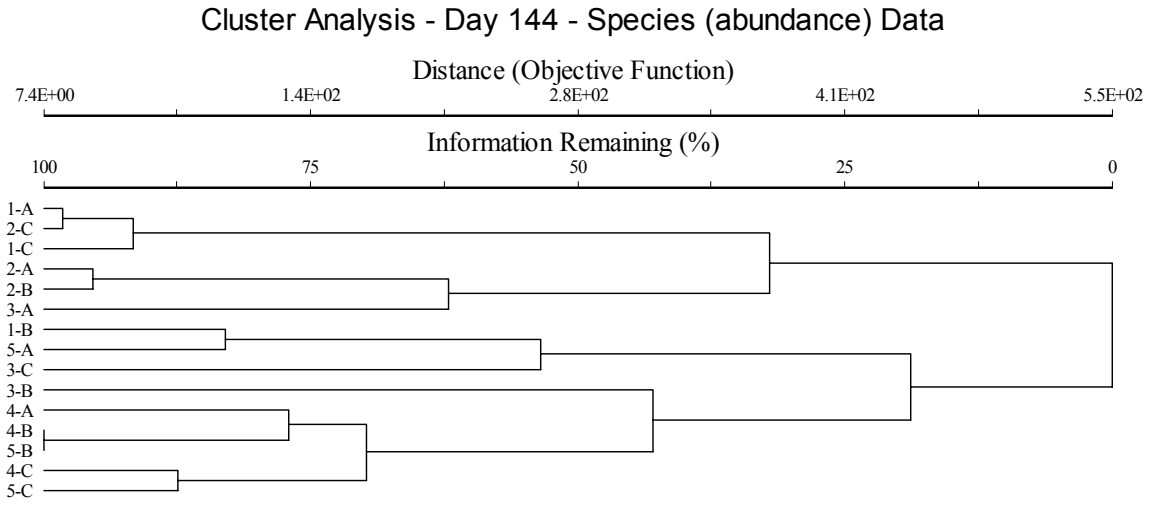


Figure 5.34. Cluster analysis of *Species-level (abundance)* classification data for Day 144.

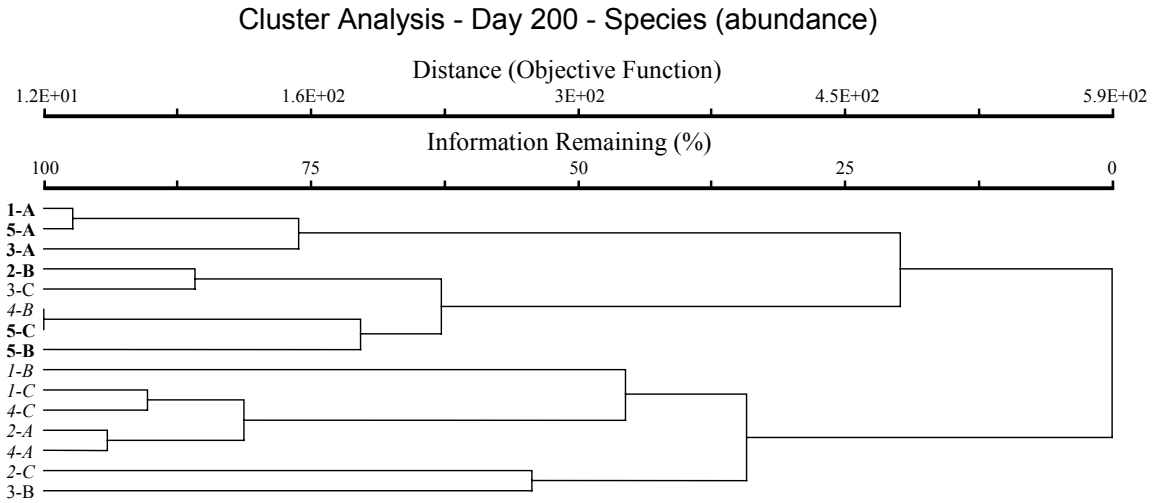


Figure 5.35. Cluster analysis (Ward's method, Euclidean distance) of *Species-level (abundance)* classification data for Day 200. Microcosms denoted in **bold** are characterized by the presence of *Chlorella* sp. and the absence of *Astasia* sp. Microcosms denoted in *italics* are characterized by the presence of *Astasia* sp. and the absence of *Chlorella* sp. Microcosm 3-C lacked both species and microcosm 3-B possessed both. Note the strong correlation of the presence/absence of these species with the two main linked groups.

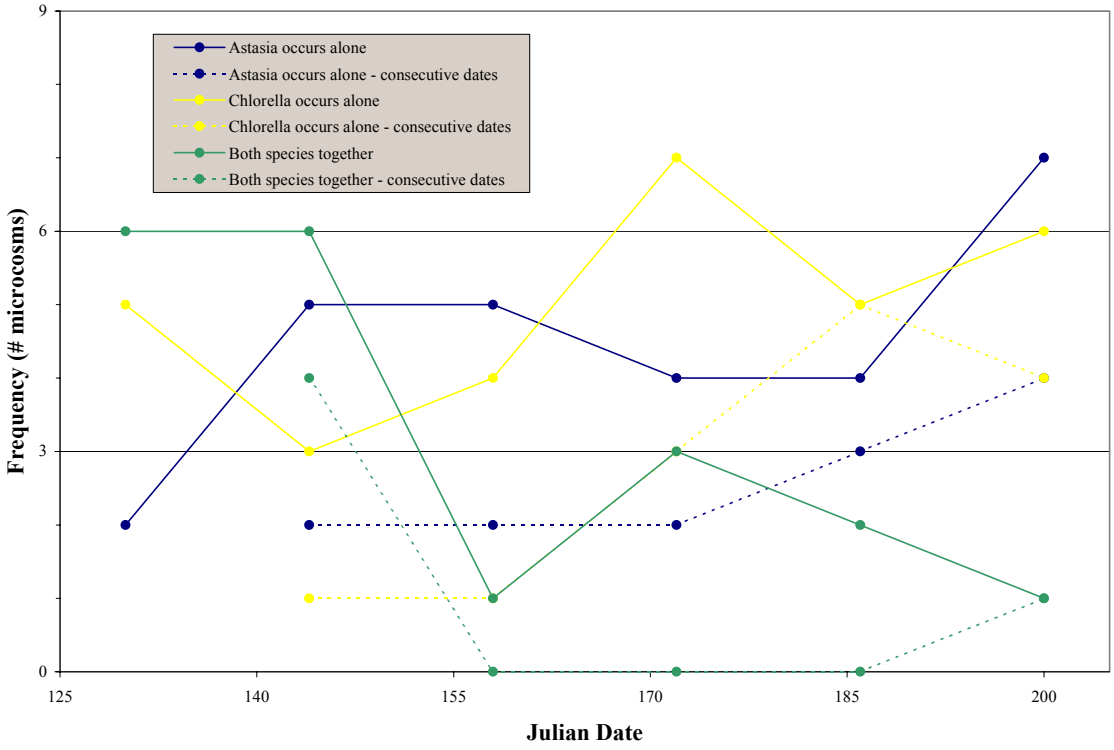


Figure 5.36. Time series of frequency of occurrence of *Astasia* and *Chlorella*. The following scenarios were quantified by sampling date: 1) *Astasia* occurs alone, 2) *Astasia* occurs alone on two consecutive dates, 3) *Chlorella* occurs alone, 4) *Chlorella* occurs alone on two consecutive dates, 5) *Astasia* and *Chlorella* occur together, and 6) *Astasia* and *Chlorella* occur together on two consecutive dates.

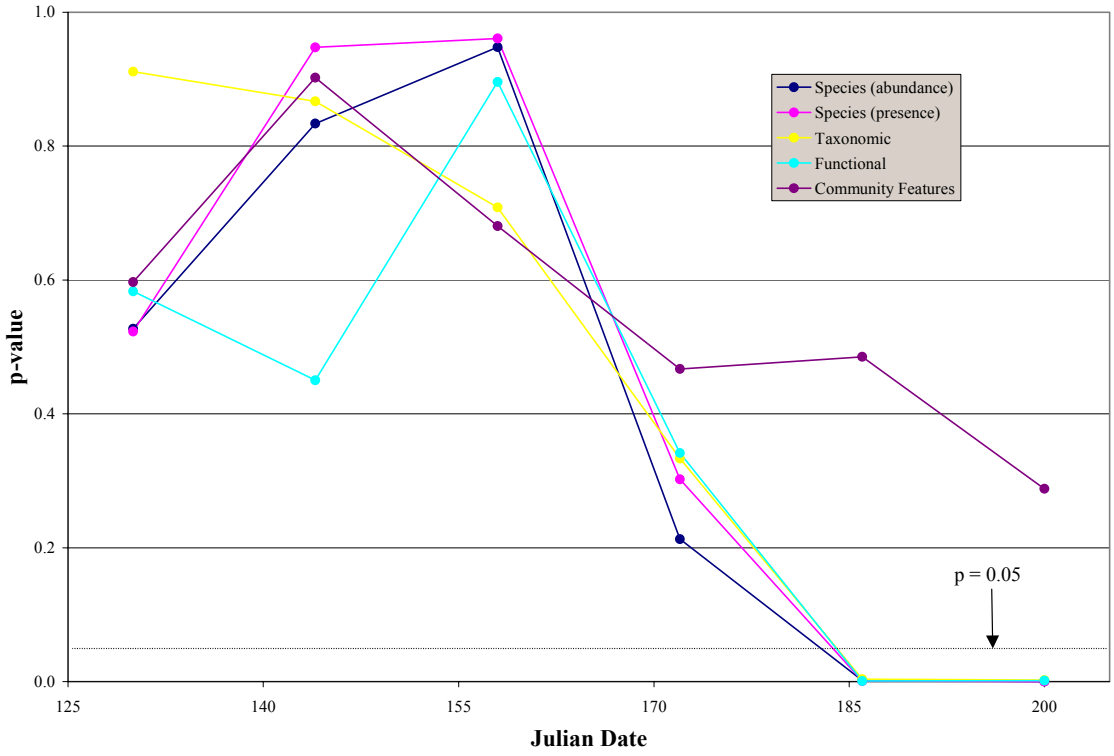


Figure 5.37. Time series of reported p-values of MRPP comparisons of Group 1 versus Group 2 microcosms by classification data type.

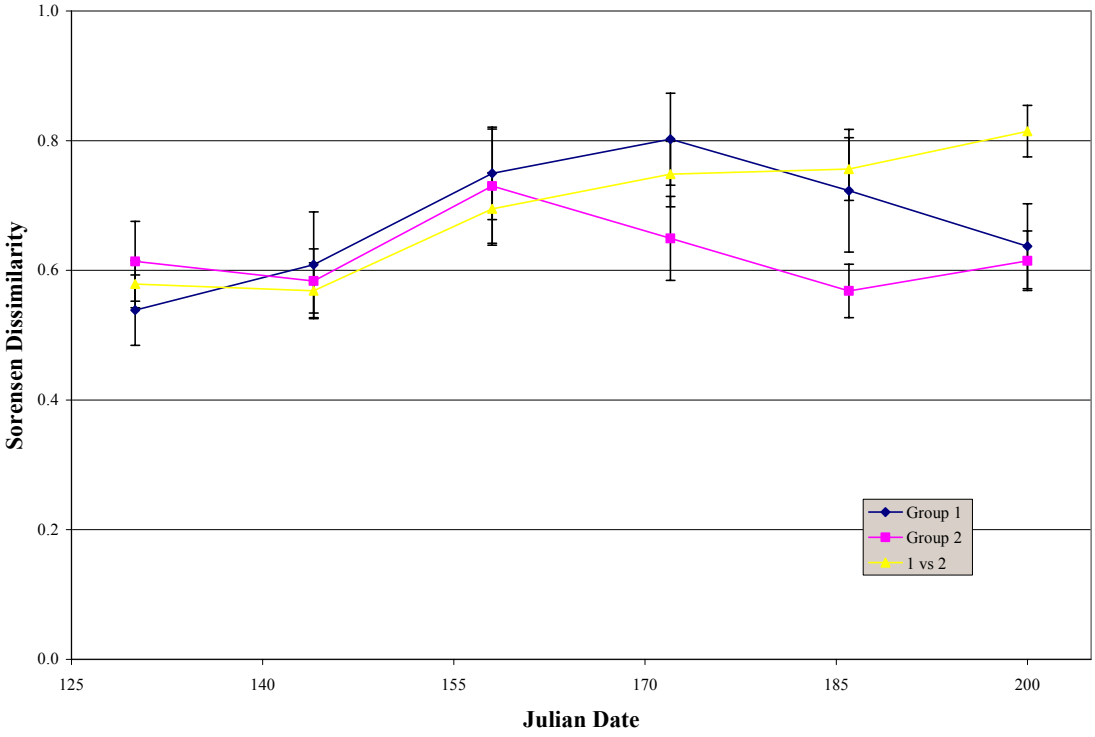


Figure 5.38. Time series of mean dissimilarity for *Species-level (abundance)* classification data for the following groups: 1) Within Group 1 microcosms, 2) Within Group 2 microcosms, and 3) Between Group 1 and Group 2 microcosms. Error bars are 95% confidence intervals.

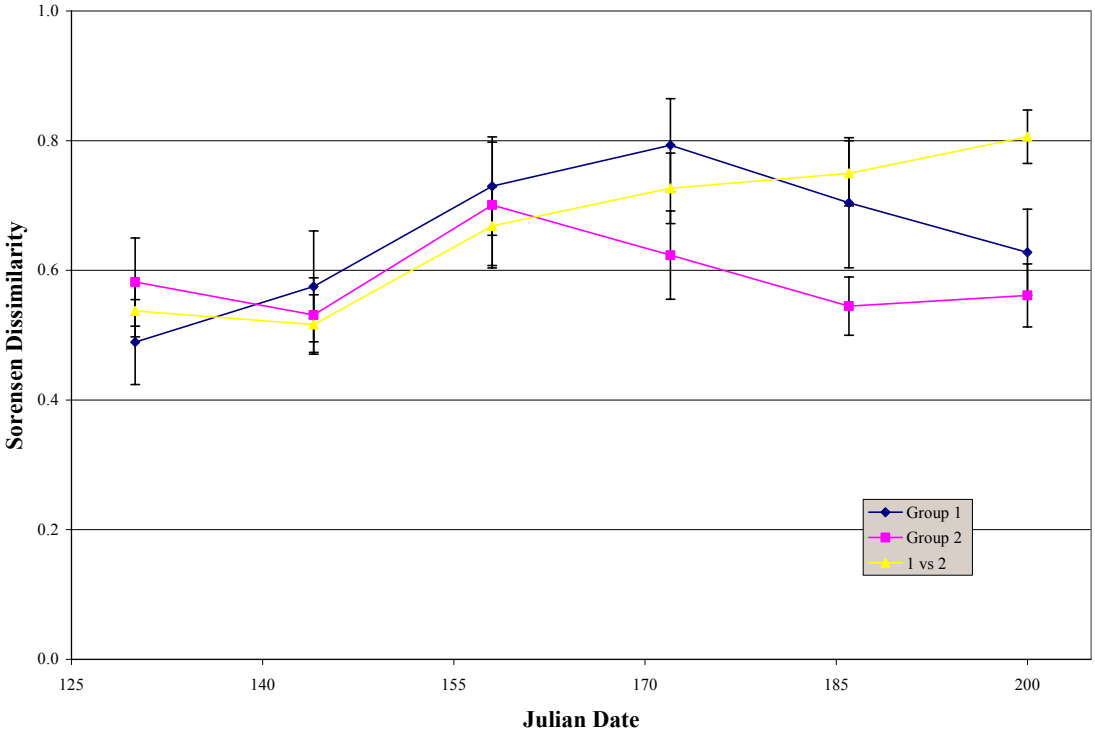


Figure 5.39. Time series of mean dissimilarity for *Species-level (presence/absence)* classification data for the following groups: 1) Within Group 1 microcosms, 2) Within Group 2 microcosms, and 3) Between Group 1 and Group 2 microcosms. Error bars are 95% confidence intervals.

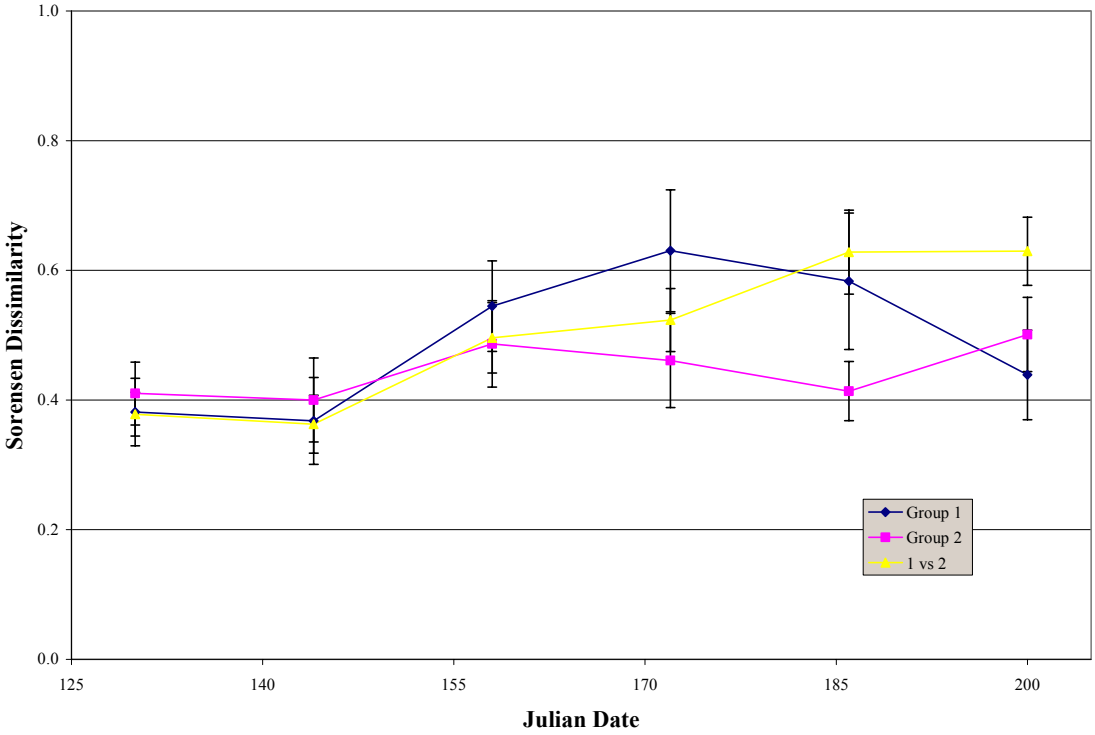


Figure 5.40. Time series of mean dissimilarity for *Taxonomic-level* classification data for the following groups: 1) Within Group 1 microcosms, 2) Within Group 2 microcosms, and 3) Between Group 1 and Group 2 microcosms. Error bars are 95% confidence intervals.

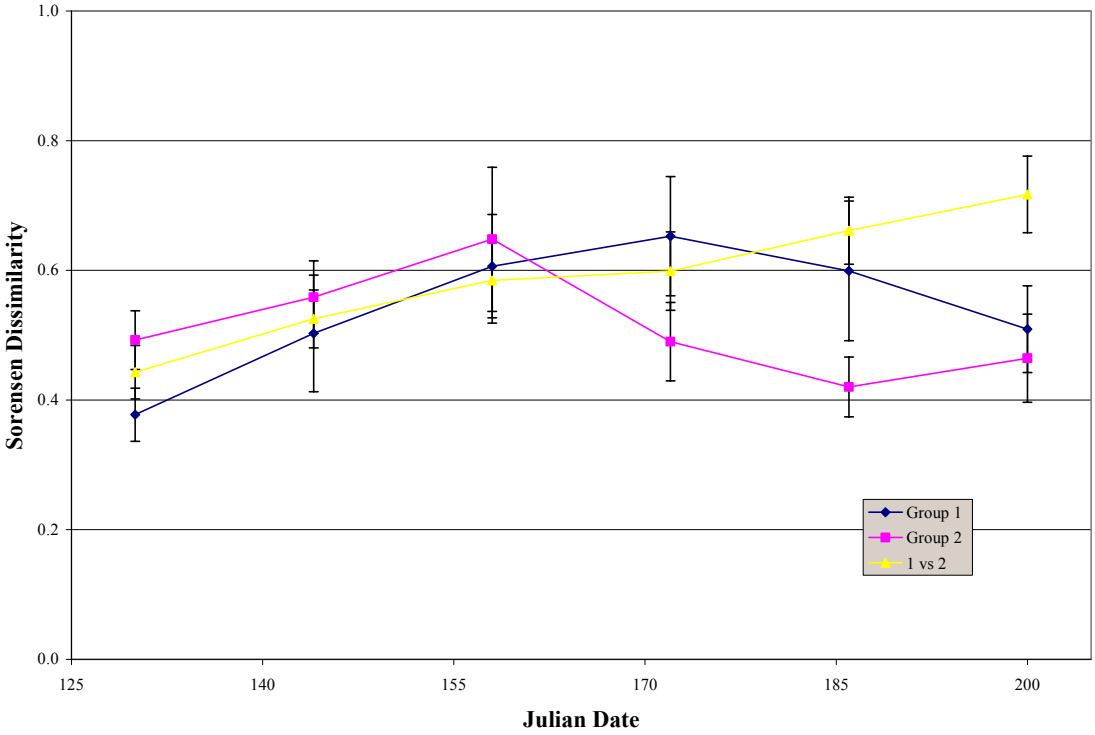


Figure 5.41. Time series of mean dissimilarity for *Functional-level* classification data for the following groups: 1) Within Group 1 microcosms, 2) Within Group 2 microcosms, and 3) Between Group 1 and Group 2 microcosms. Error bars are 95% confidence intervals.

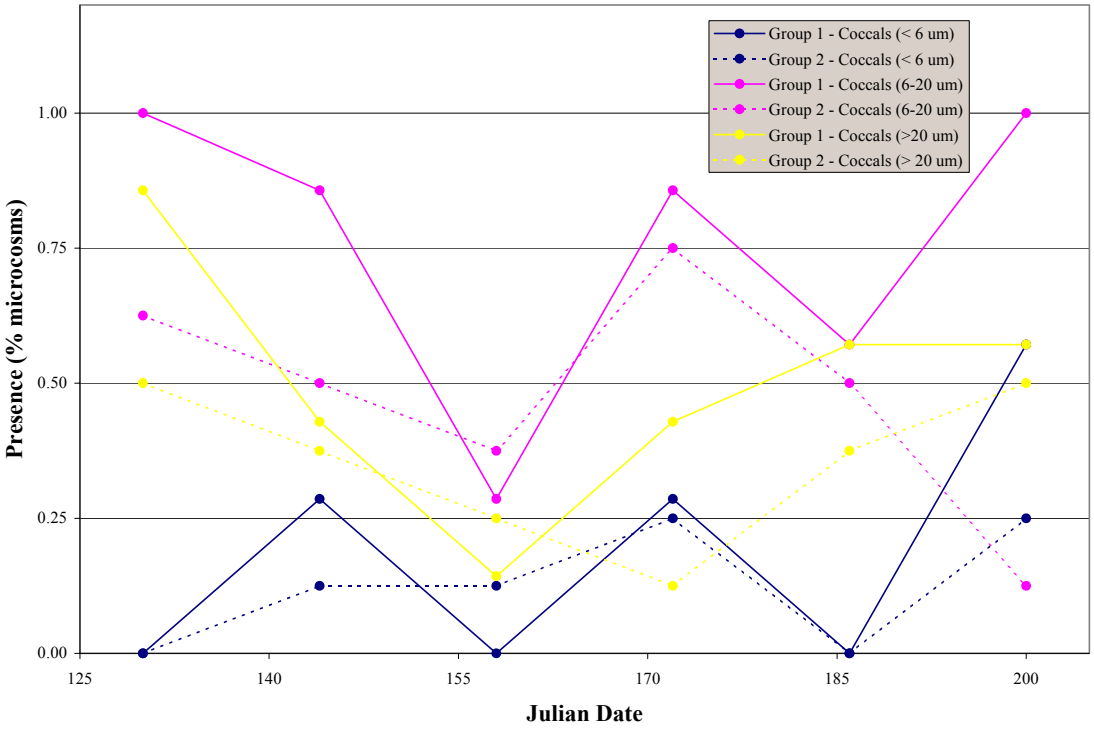


Figure 5.42. Time series of presence data for Group 1 and Group 2 microcosms for coccals (<6 µm), coccals (6-20 µm), and coccals (20 µm) functional groups.

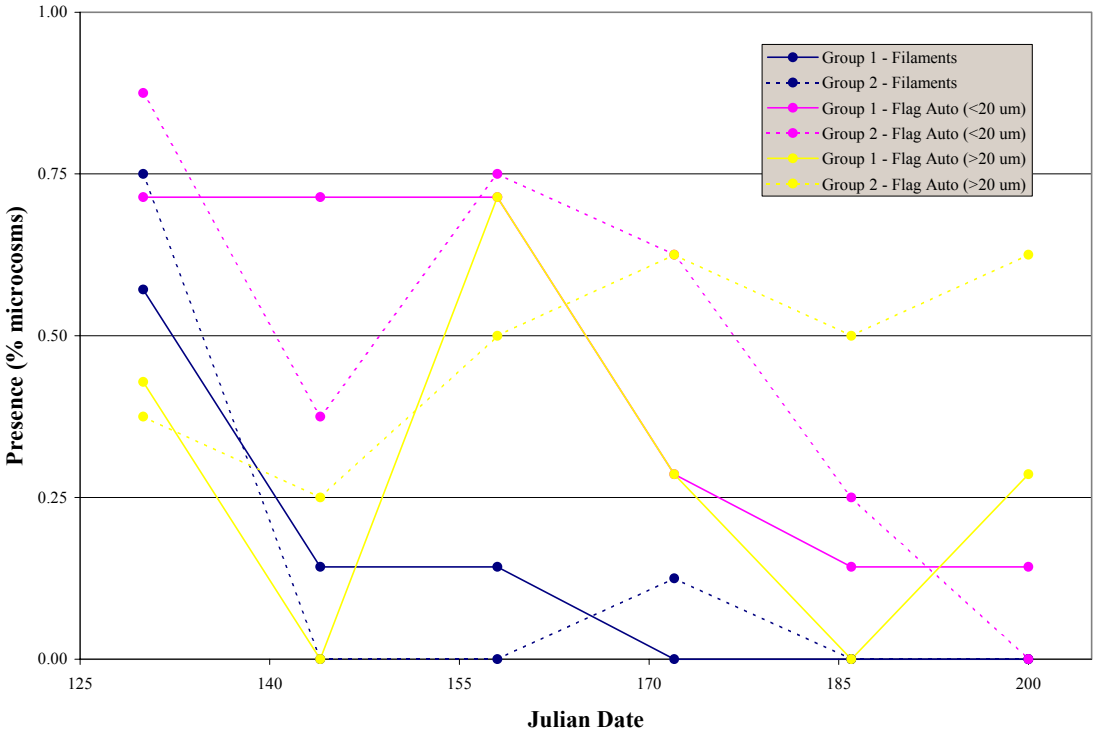


Figure 5.43. Time series of presence data for Group 1 and Group 2 microcosms for small filaments, flagellated autotrophs (<20 µm), and flagellated autotrophs (>20 µm) functional groups.

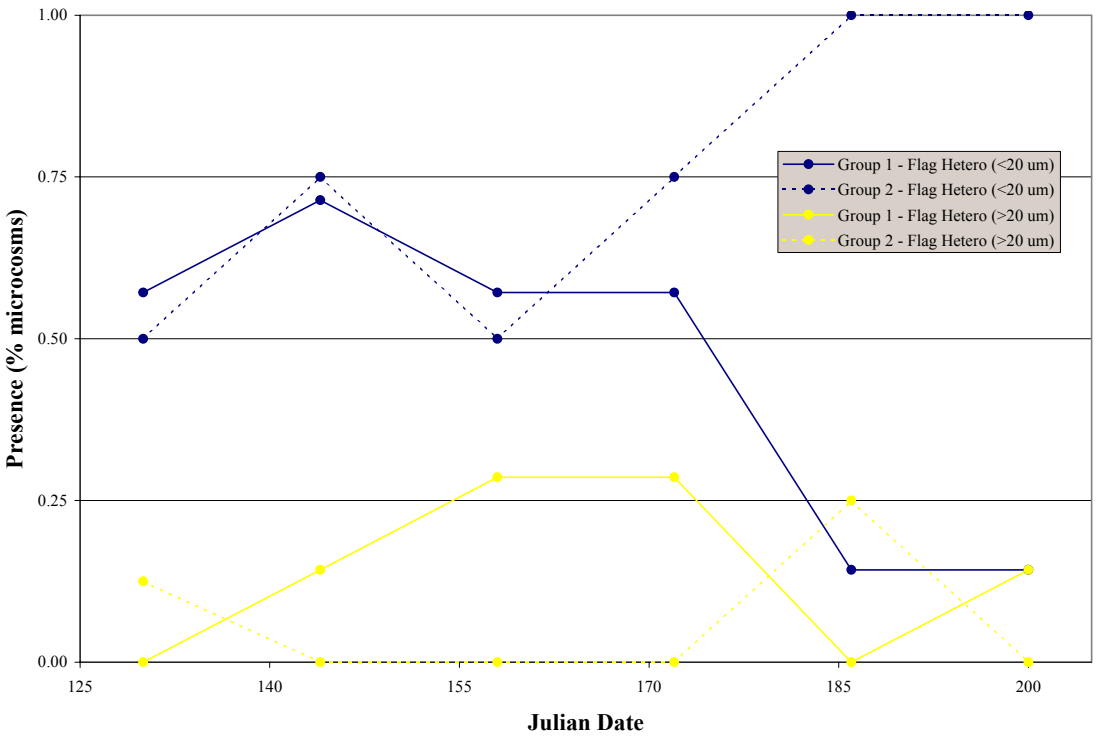


Figure 5.44. Time series of presence data for Group 1 and Group 2 microcosms for flagellated heterotrophs (<20 µm) and flagellated heterotrophs (>20 µm) functional groups.

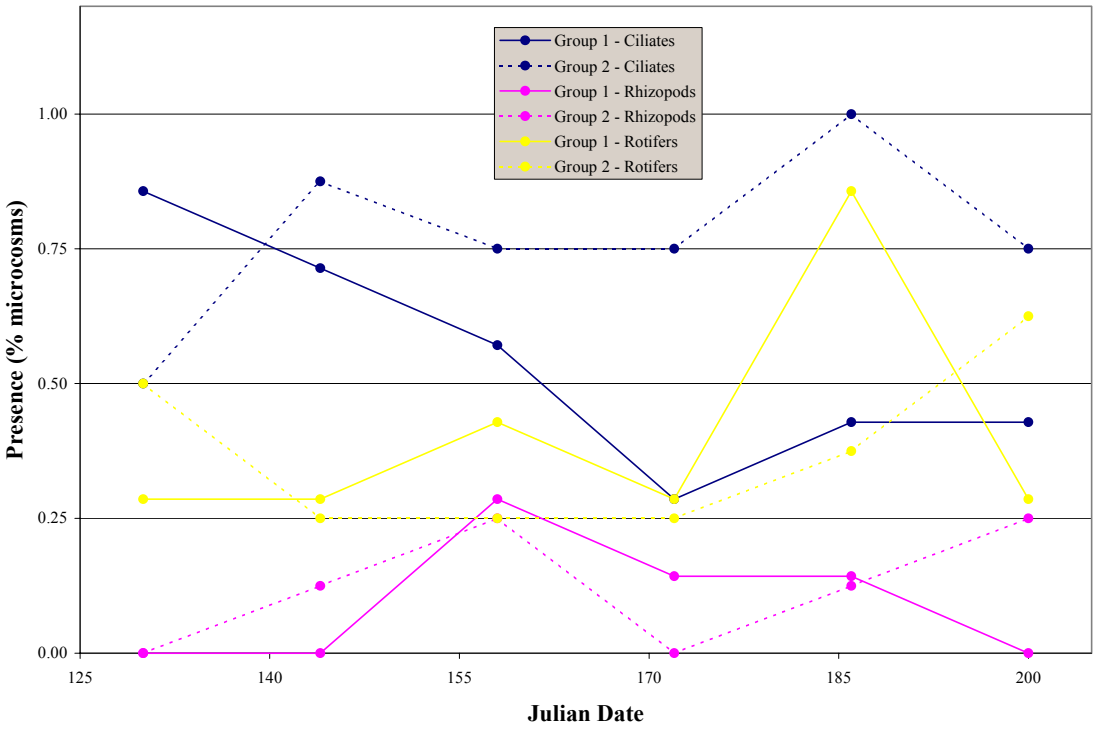


Figure 5.45. Time series of presence data for Group 1 and Group 2 microcosms for ciliates (>20 µm), rhizopods (>20 µm), and rotifera (20-200 µm) functional groups.

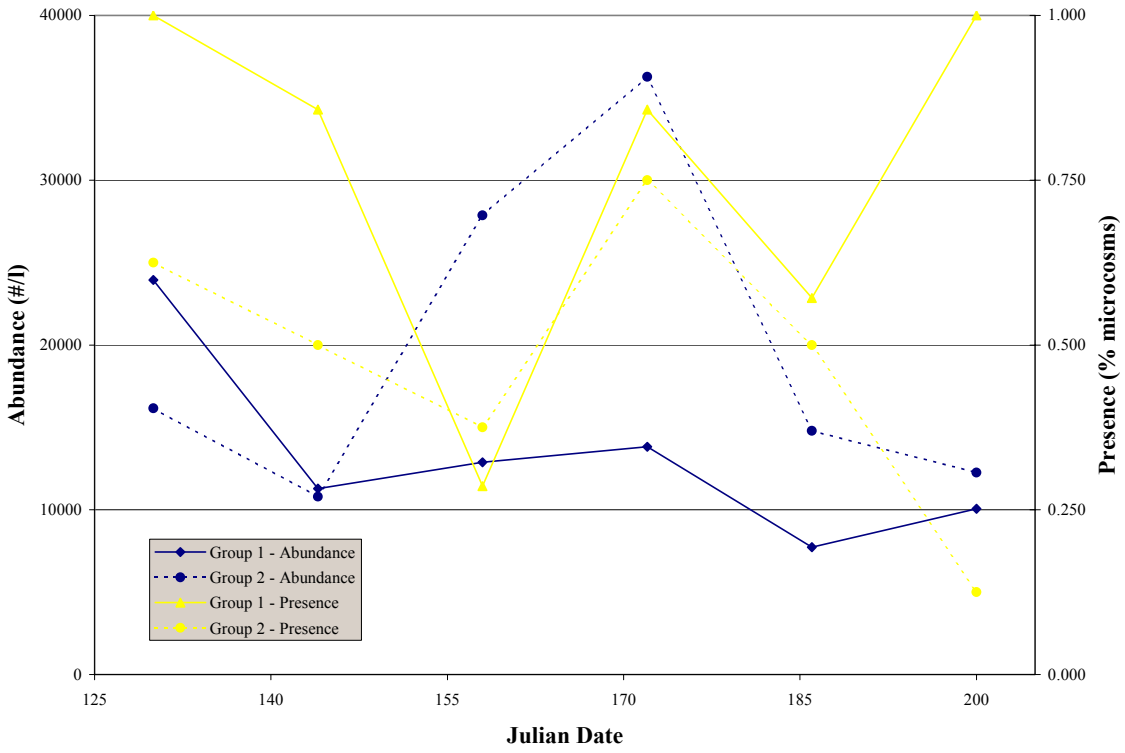


Figure 5.46. Time series of coccals (6-20 µm) abundance and occurrence by Group 1 and Group 2.

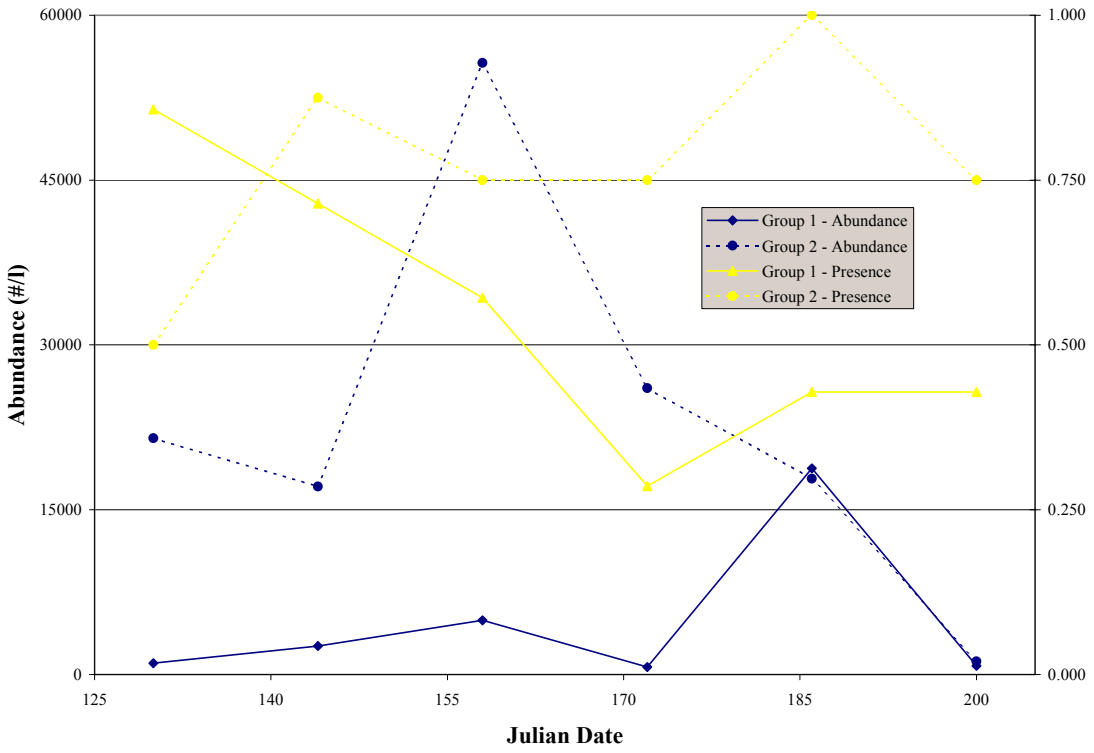


Figure 5.47. Time series of ciliate abundance and occurrence by Group 1 and Group 2 for ciliates.

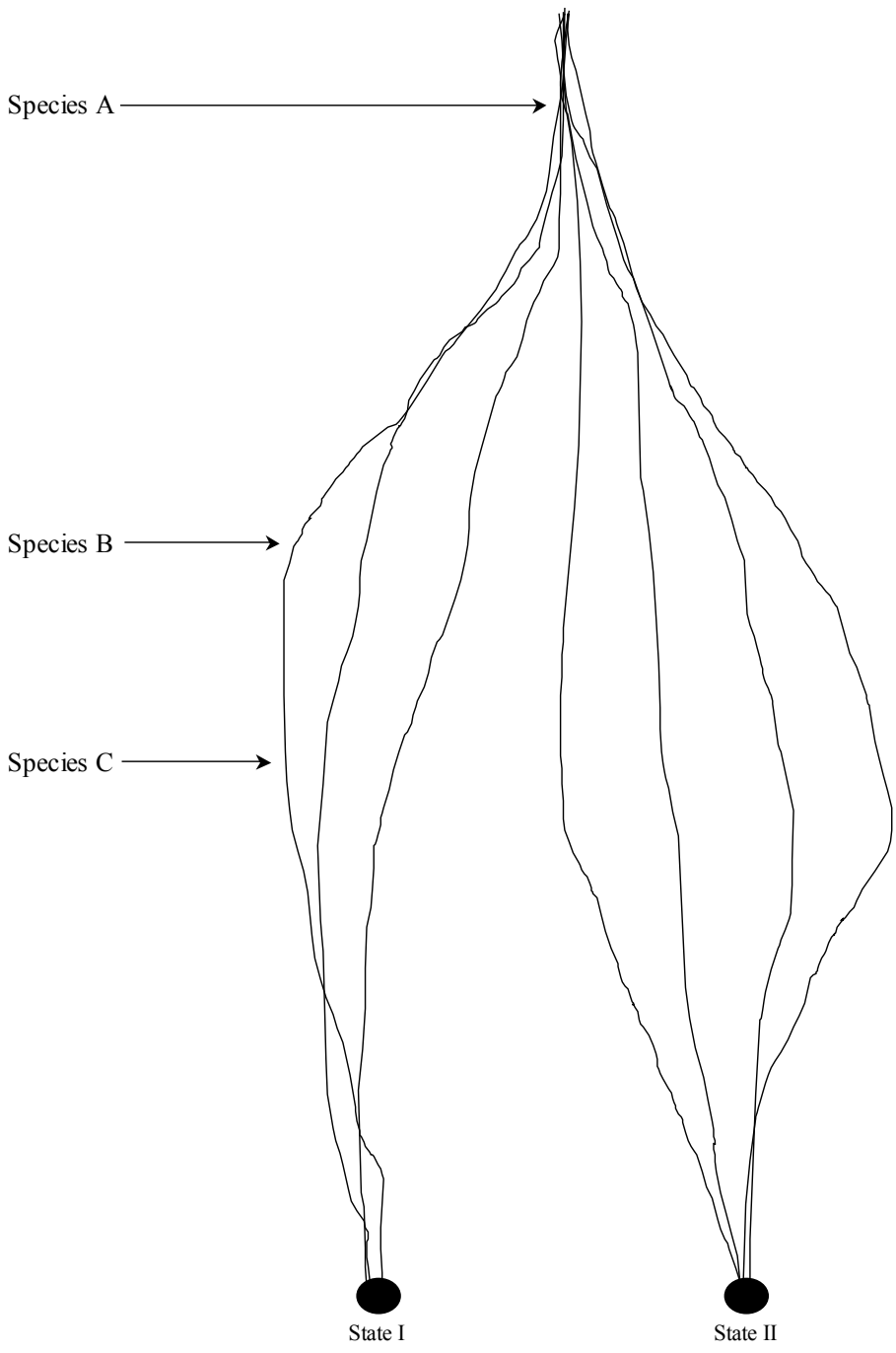
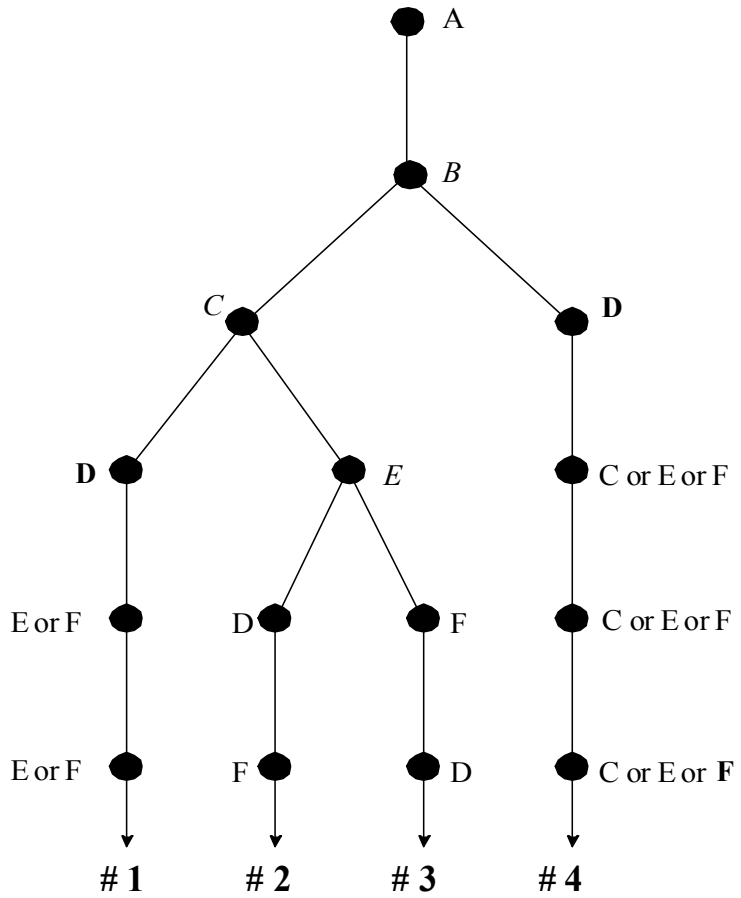


Figure 6.1. Demonstration of alternative states arising from an indeterministic assembly sequence. The invasion of Species A acts as a noise-amplifying process that generates a diversity of divergent trajectories. For trajectories leading to State I, the invasion of Species B acts to dampen noise and canalize a subset of trajectories toward this state. Similarly, the invasion of Species C draws the remaining trajectories toward State II.



Community State	Sequence
I	Sequence 1: A → *B* → *C* → **D** → E → F Sequence 2: A → *B* → *C* → **D** → F → E
II	Sequence 3: A → *B* → *C* → *E* → D → F
III	Sequence 4: A → *B* → *C* → *E* → F → D
IV	Sequence 5: A → *B* → **D** → C → E → F Sequence 6: A → *B* → **D** → C → F → E Sequence 7: A → *B* → **D** → E → C → F Sequence 8: A → *B* → **D** → E → F → C Sequence 9: A → *B* → **D** → F → C → E Sequence 10: A → *B* → **D** → F → E → C

Figure 6.2. A graphical representation illustrating the effect of keystep species in deterministic assembly trajectories. Canalizing and bifurcation species are shown in italics and bold, respectively. The table beneath the figure shows four possible community states and their respective assembly sequences.

Appendix B: Tables

Table 2.1. Case studies of alternative stable states in natural systems.

Authors	Community Type	Hypothesized Mechanism
Sutherland and Karlson (1977)	Fouling communities	Order of species arrival
Hatcher (1984)	Benthic community	Size-dependent response by grazers
McCune and Allen (1985)	Canyon forests	Historical factors during early succession
Barkai and Branch (1988)	Sublittoral communities	Presence/absence of top predator
Dublin et al. (1990)	Woodland savanna	Fire and elephant browsing.
Blindow et al. (1993) & Romo et al. (1996)	Freshwater lakes	Effect of submerged vegetation on water quality
Fastie (1995)	Coniferous forests	Order of species arrival
Stromayer and Warren (1997)	Deciduous forests	Deer overgrazing
Augustine et al. (1998)	Deciduous forests	Nonmonotonic functional response by herbivore
Petraitis and Dudgeon (1999)	Intertidal communities	Threshold effect in the predation rates
Van de Koppel et al. (2001)	Tidal flats	Positive feedback between silt erosion and diatom growth

Table 3.1. The three introduction sequences used in soil microcosm community assembly experiment.

	Sequence		
Introduction	**A**	**B**	**C**
1	*Sphingomonas* A8AN3	*Mycobacterium* Pyr-1	*Rhodococcus* Sm-1
2	*Mycobacterium* Pyr-1	*Rhodococcus* Sm-1	*Sphingomonas* A8AN3
3	*Rhodococcus* Sm-1	*Sphingomonas* A8AN3	*Mycobacterium* Pyr-1
4	*P. fluorescens* HK44	*P. fluorescens* HK44	*P. fluorescens* HK44

Table 3.2. Plating medium for isolation of study strains assembly experiment.

Plating Medium	Species Selectivity
YEPG	Non-specific heterotrophic populations
YEPG + 5% NaCl	Isolates *Rhodococcus* and *Mycobacteria* from HK44 and *Sphingomonas*
YEPG + 5% NaCl + Ampicillin	Isolates *Mycobacterium*
YEPG + Nalidixic acid	Isolates *Sphingomonas*
YEPSS + Tetracycline	Isolates HK44

Table 3.3. Four cell morphological types found among heterotrophic populations.

Type	**Morphological Characteristics**		
	Color	*Shape*	*Transmittance*
1	white	round	opaque
2	gray	round	translucent
3	yellow	irregular	opaque
4	iridescent	irregular	translucent

Table 3.4. Final sampling date community parameters for twelve microcosms by treatment. Communities are labeled by sequence and replicate number.

	Community Parameter			
Microcosm	*Total Cell Abundance (cell gram^{-1})*	*Species Richness (S)*	*Shannon's Diversity Index (H')*	*Species Evenness (E)*
		Sequence A		
Replicate 1	1.28E+08	4	0.656	0.473
Replicate 2	1.12E+08	4	0.800	0.577
Replicate 3	6.37E+07	4	1.014	0.731
Mean	1.01E+08	4	0.823	0.594
C.V.	0.333	0	0.219	0.219
		Sequence B		
Replicate 1	9.23E+06	3	0.540	0.491
Replicate 2	8.27E+06	3	0.522	0.476
Replicate 3	6.33E+06	3	0.624	0.568
Mean	7.94E+06	3	0.562	0.512
C.V.	0.186	0	0.097	0.097
		Sequence C		
Replicate 1	4.37E+07	3	0.807	0.735
Replicate 2	4.73E+07	3	0.819	0.746
Replicate 3	3.07E+07	3	0.785	0.715
Mean	4.06E+07	3	0.804	0.732
C.V.	0.216	0	0.021	0.021
		Control		
Replicate 1	5.07E+06	2	0.627	0.571
Replicate 2	7.45E+06	2	0.673	0.613
Replicate 3	4.77E+06	2	0.539	0.491
Mean	5.76E+06	2	0.613	0.558
C.V.	0.26	0	0.111	0.111

Table 3.5. Test for the effect of sequence permutation on total cell abundance for the final sampling date.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Treatments	1.78E+16	3	5.92E+15	19.83	<0.000	4.07
Within Treatments	2.39E+15	8	2.99E+14			
Total	2.02E+16	11				

b. Tukey HSD post-hoc test of differences in pairwise means of total cell abundance for the final sampling date. For an α=0.05, Q critical (k=4, df=8) = 4.53. Significance differences are underlined.

	Sequence B	Sequence C	Control
Sequence A	9.33	6.06	9.55
Sequence B		3.27	0.22
Sequence C			3.49

Table 3.6. Test for the effect of sequence permutation on Shannon diversity for the final sampling date.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Treatments	0.158	3	0.053	5.21	0.028	4.07
Within Treatments	0.081	8	0.010			
Total	0.238	11				

b. Tukey HSD post-hoc test of differences in pairwise means of Shannon diversity for the final sampling date. For an α=0.05, Q critical (k=4, df=8) = 4.53. Significance differences are underlined.

	Sequence B	Sequence C	Control
Sequence A	10.93	0.81	8.80
Sequence B		10.12	2.13
Sequence C			7.99

Table 3.7. Test for the effect of sequence permutation on species equitability for the final sampling date.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Treatments	0.081	3	0.027	4.61	0.037	4.07
Within Treatments	0.047	8	0.006			
Total	0.128	11				

b. Tukey HSD post-hoc test of differences in pairwise means of species equitability for the final sampling date. For an α=0.05, Q critical (k=4, df=8) = 4.53. Significance differences are underlined.

	Sequence B	Sequence C	Control
Sequence A	2.01	3.37	0.87
Sequence B		5.38	1.13
Sequence C			4.24

Table 3.8. Summary of pairwise treatment comparisons of mean values for four community parameters. An "x" indicates a significant difference.

Treatment Comparison	**Community Attributes**			
	Species Richness	*Total Cell Abundance*	*Shannon Diversity*	*Species Equitability*
Sequence A - Sequence B	x	x	x	
Sequence A - Sequence C	x	x		
Sequence B - Sequence C			x	x
Sequence A - Control	x	x	x	
Sequence B - Control	x			
Sequence C - Control	x		x	

Table 3.9. Test of the effect of sequence permutation and sampling date on mean Hk44 abundance between Sequence A and Sequence C.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Treatment	7.13E+14	1	7.13E+14	26.81	<0.000	4.49
Sampling Date	2.09E+14	3	6.98E+13	2.62	0.086	3.24
Treatment * Date	3.91E+14	3	1.30E+14	4.91	0.013	3.24
Within	4.25E+14	16	2.66E+13			
Total	1.74E+15	23				

b. Tukey HSD post-hoc test: Comparison of mean abundance of HK44 between Sequence A and Sequence C by sampling date. For an α=0.05, Q critical (k=2, df=16) = 2.998. Significant differences are underlined.

		Sequence A			
		Day 14	*Day 35*	*Day 63*	*Day 136*
Sequence C	*Day 14*	7.852			
	Day 35		3.758		
	Day 63			2.675	
	Day 136				0.361

Table 4.1. Experiment schedule indicating dates of introductions, population censusing and media replenishment.

Day	Introduction		Population Census	Media Addition
	Sequence I	*Sequence II*		
0	*Colpidium*	*Colpidium*		
5	*Euplotes*	*Blepharisma*	*	
10				*
15	*Cinetochilum*	*Tetrahymena*	*	
20	*Blepharisma*	*Euplotes*	*	*
30	*Tetrahymena*	*Cinetochilum*	*	*
40			*	*
50			*	*
60				*
64			*	

Table 4.2. Two-factor analysis of variance test of the effect of sequence permutation and nutrient concentration on mean *Colpidium* abundance for the final sampling date. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Nutrient Concentration	5262262.8	4	1315565.7	7.88	<0.000	2.61
Sequence	292904.9	1	292904.9	1.75	0.193	4.08
Media * Sequence	255530.6	4	63882.7	0.38	0.820	2.61
Within	6678297.0	40	166957.4			
Total	12488995.2	49				

Table 4.3. Two-factor analysis of variance test of the effect of sequence permutation and nutrient concentration on mean *Euplotes* abundance for the final sampling date.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Nutrient Concentration	66.8	4	16.7	7.21	<0.000	2.61
Sequence	17.6	1	17.6	7.60	0.009	4.08
Media * Sequence	79.3	4	19.8	8.57	<0.000	2.61
Within	92.6	40	2.3			
Total	256.4	49				

b. Tukey HSD post-hoc comparison between sequences of mean *Euplotes* abundance for the final sampling date by nutrient concentration. For an α=0.05, Q critical (k=2, df=40) = 2.858. Significant differences are underlined.

0.05 g L^{-1}	0.10 g L^{-1}	0.50 g L^{-1}	0.75 g L^{-1}	1.00 g L^{-1}
0.312	0.115	9.14	0.00	0.00

Table 4.4. Two-factor analysis of variance test of the effect of sequence permutation and nutrient concentration on mean *Cinetochilum* abundance for the final sampling date.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Nutrient Concentration	501402.7	4	125350.7	4.33	0.005	2.61
Sequence	5976.3	1	5976.3	0.21	0.652	4.08
Media * Sequence	451216.5	4	112804.1	3.89	0.009	2.61
Within	1158455.5	40	28961.4			
Total	2117051.1	49				

b. Tukey HSD post-hoc comparison between sequences of mean *Cinetochilum* abundance for the final sampling date by nutrient concentration. For an α=0.05, Q critical (k=2, df=40) = 2.858. Significant differences are underlined.

0.05 g L^{-1}	0.10 g L^{-1}	0.50 g L^{-1}	0.75 g L^{-1}	1.00 g L^{-1}
0.070	0.544	3.78	2.82	3.00

Table 4.5. Two-factor analysis of variance test of the effect of sequence permutation and nutrient concentration on mean *Blepharisma* abundance for the final sampling date. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Nutrient Concentration	162713.8	4	40678.4	8.45	<0.000	2.61
Sequence	904.4	1	904.4	0.19	0.667	4.08
Media * Sequence	19388.8	4	4847.2	1.01	0.416	2.61
Within	192614.3	40	4815.4			
Total	375621.4	49				

Table 4.6. Two-factor analysis of variance of the effect of species and nutrient concentration on the mean rank abundance of constituent species among community assembled under Sequence II at the 0.50-1.00 g L^{-1} nutrient concentrations.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Nutrient Concentration	0.00	2	0.00	0.00	1.000	3.26
Species	10.53	2	5.27	18.23	<0.000	3.26
Media * Species	9.07	4	2.27	7.85	<0.000	2.63
Within	10.40	36	0.29			
Total	30.00	44				

b. Tukey HSD post-hoc comparison of mean rank abundance* between species by nutrient concentration. For an α=0.05, Q critical (k=3, df=36) = 3.46. Significant differences are underlined.

		Cinetochilum	*Blepharisma*
0.50 g L^{-1}	*Colpidium*	4.99	0.00
	Cinetochilum		4.99
0.75 g L^{-1}	*Colpidium*	2.50	7.49
	Cinetochilum		4.99
1.00 g L^{-1}	*Colpidium*	3.33	6.66
	Cinetochilum		3.33

*Mean rank by species and nutrient concentration.

	Colpidium	*Cinetochilum*	*Blepharisma*
0.50 g L^{-1}	1.6	2.8	1.6
0.75 g L^{-1}	1.2	1.8	3
1.00 g L^{-1}	1.2	2	2.8

Table 4.7. Summary of community parameters by treatment and replicate for final sampling date.

Nutrient Concentration	Rep	Community Parameter					
		Species Richness		Equitability		Shannon Diversity	
		Seq I	*Seq II*	*Seq I*	*Seq II*	*Seq I*	*Seq II*
1.00 g L^{-1}	**1**	2.00	3.00	0.194	0.866	0.135	0.951
	2	2.00	3.00	0.059	0.534	0.041	0.587
	3	2.00	3.00	0.468	0.254	0.324	0.279
	4	2.00	3.00	0.175	0.857	0.121	0.941
	5	2.00	3.00	0.135	0.666	0.094	0.732
	Avg	2.00	3.00	0.206	0.635	0.143	0.698
	CV	0.00	0.00	0.753	0.400	0.751	0.400
0.75 g L^{-1}	**1**	3.00	3.00	0.887	0.859	0.974	0.943
	2	3.00	3.00	0.408	0.837	0.448	0.919
	3	3.00	3.00	0.844	0.656	0.927	0.721
	4	3.00	3.00	0.206	0.912	0.227	1.002
	5	3.00	3.00	0.873	0.626	0.960	0.687
	Avg	3.00	3.00	0.644	0.778	0.707	0.854
	CV	0.00	0.00	0.491	0.165	0.490	0.165
0.50 g L^{-1}	**1**	4.00	3.00	0.607	0.835	0.842	0.918
	2	4.00	3.00	0.625	0.944	0.866	1.037
	3	4.00	3.00	0.695	0.323	0.963	0.355
	4	4.00	3.00	0.791	0.621	1.096	0.683
	5	4.00	3.00	0.892	0.668	1.237	0.733
	Avg	4.00	3.00	0.722	0.678	1.001	0.745
	CV	0.00	0.00	0.165	0.350	0.165	0.349
0.10 g L^{-1}	**1**	3.00	3.00	0.195	0.463	0.214	0.509
	2	3.00	2.00	0.411	0.246	0.451	0.171
	3	3.00	3.00	0.541	0.870	0.594	0.956
	4	3.00	2.00	0.673	0.396	0.740	0.274
	5	3.00	3.00	0.679	0.459	0.746	0.504
	Avg	3.00	2.60	0.500	0.487	0.549	0.483
	CV	0.00	0.21	0.406	0.476	0.406	0.626
0.05 g L^{-1}	**1**	0.00	3.00	0.000	0.984	0.000	1.081
	2	2.00	2.00	0.918	0.918	0.637	0.637
	3	2.00	2.00	0.619	0.918	0.429	0.637
	4	3.00	2.00	0.670	0.918	0.736	0.637
	5	3.00	2.00	0.818	0.722	0.898	0.500
	Avg	2.00	2.20	0.605	0.892	0.540	0.698
	CV	0.61	0.20	0.592	0.111	0.641	0.318

Table 4.8. Two-factor analysis of variance on the effect of sequence permutation and nutrient concentration on species richness.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Nutrient Concentration	11.08	4	2.77	13.85	<0.000	2.61
Sequence	0.02	1	0.02	0.10	0.753	4.08
Media * Sequence	5.48	4	1.37	6.85	<0.000	2.61
Within	8.00	40	0.20			
Total	24.58	49				

b. Tukey HSD post-hoc pairwise comparison of means.

Comparison of mean species richness between sequence permutations by nutrient concentration. For an α=0.05, Q critical (k=2, df=40) = 2.858. Significant differences are underlined.

0.05 g L^{-1}	**0.10 g L^{-1}**	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
1.0	2.0	5.0	0.0	5.0

Comparison of mean species richness between nutrient concentrations within Sequence I communities. For an α=0.05, Q critical (k=5, df=40) = 4.039. Significant differences are underlined.

	0.10 g L^{-1}	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
0.05 g L^{-1}	5	10	5	0
0.10 g L^{-1}		5	0	5
0.50 g L^{-1}			5	10
0.75 g L^{-1}				5

Comparison of mean species richness between nutrient concentrations within Sequence II communities. For an α=0.05, Q critical (k=5, df=40) = 4.039. Significant differences are underlined.

	0.10 g L^{-1}	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
0.05 g L^{-1}	2	4	4	4
0.10 g L^{-1}		2	2	2
0.50 g L^{-1}			0	0
0.75 g L^{-1}				0

Table 4.9. Two-factor analysis of variance on the effect of sequence permutation and nutrient concentration on Shannon diversity.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Nutrient Concentration	1.288	4	0.322	5.20	0.002	2.61
Sequence	0.117	1	0.117	1.89	0.177	4.08
Media * Sequence	1.022	4	0.255	4.12	0.007	2.61
Within	2.478	40	0.062			
Total	4.905	49				

b. Tukey HSD post-hoc pairwise comparison of means.

Comparison of mean species diversity between sequence permutations by nutrient concentration. For an α=0.05, Q critical (k=2, df=40) = 2.858. Significant differences are underlined.

0.05 g L^{-1}	**0.10 g L^{-1}**	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
1.42	0.59	2.79	1.32	4.99

Comparison of mean species diversity between nutrient concentrations within Sequence I communities. For an α=0.05, Q critical (k=5, df=40) = 4.039. Significant differences are underlined.

	0.10 g L^{-1}	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
0.05 g L^{-1}	0.08	4.20	1.50	3.57
0.10 g L^{-1}		4.12	1.42	3.65
0.50 g L^{-1}			2.70	7.77
0.75 g L^{-1}				5.07

Comparison of mean species diversity between nutrient concentrations within Sequence II communities. For an α=0.05, Q critical (k=5, df=40) = 4.039. Significant differences are underlined.

	0.10 g L^{-1}	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
0.05 g L^{-1}	1.94	0.01	1.40	0.00
0.10 g L^{-1}		1.93	3.34	1.93
0.50 g L^{-1}			1.41	0.01
0.75 g L^{-1}				1.41

Table 4.10. Two-factor analysis of variance on the effect of sequence permutation and nutrient concentration on species equitability.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Nutrient Concentration	0.837	4	0.209	4.26	0.006	2.61
Sequence	0.278	1	0.278	5.66	0.022	4.08
Media * Sequence	0.455	4	0.114	2.31	0.074	2.61
Within	1.967	40	0.049			
Total	3.537	49				

b. Tukey HSD post-hoc pairwise comparison of means.

Comparison of mean species equitability between sequence permutations by nutrient concentration. For an α=0.05, Q critical (k=2, df=40) = 2.858. Significant differences are underlined.

0.05 g L^{-1}	**0.10 g L^{-1}**	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
2.90	0.13	0.92	1.36	4.34

Comparison of mean species equitability between nutrient concentrations within Sequence I communities. For an α=0.05, Q critical (k=5, df=40) = 4.039. Significant differences are underlined.

	0.10 g L^{-1}	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
0.05 g L^{-1}	1.06	1.23	0.39	4.03
0.10 g L^{-1}		2.29	1.45	2.97
0.50 g L^{-1}			0.84	5.25
0.75 g L^{-1}				4.42

Comparison of mean species equitability between nutrient concentrations within Sequence II communities. For an α=0.05, Q critical (k=5, df=40) = 4.039. Significant differences are underlined.

	0.10 g L^{-1}	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
0.05 g L^{-1}	4.09	2.60	1.15	2.59
0.10 g L^{-1}		1.49	2.94	1.50
0.50 g L^{-1}			1.45	0.01
0.75 g L^{-1}				1.44

Table 4.11. Two-factor analysis of variance test of the effect of sequence permutation and nutrient concentration on mean pairwise dissimilarity for the final sampling date. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Sequence	0.000818	1	0.000818	3.402	0.068	3.947
Nutrient Concentration	0.001743	4	0.000436	1.813	0.133	2.473
Sequence * Media	0.002020	4	0.000505	2.101	0.087	2.473
Within	0.021631	90	0.000240			
Total	0.026211	99				

Table 4.12. Two-factor analysis of variance on the effect of sequence permutation and nutrient concentration on mean maximal dissimilarity.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Sequence	0.068692	1	0.068692	20.06	<0.000	3.95
Nutrient Concentration	0.079893	4	0.019973	5.83	<0.000	2.47
Sequence * Media	0.034826	4	0.008706	2.54	0.045	2.47
Within	0.308229	90	0.003425			
Total	0.491640	99				

b. Tukey HSD post-hoc pairwise comparison of means.

Comparison of mean maximal dissimilarity between sequence permutations by nutrient concentration. For an α=0.05, Q critical (k=2, df=90) = 2.81. Significant differences are underlined.

0.05 g L^{-1}	**0.10 g L^{-1}**	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
5.70	2.60	0.49	0.88	4.49

Comparison of mean maximal dissimilarity between nutrient concentrations within Sequence I communities. For an α=0.05, Q critical (k=5, df=90) = 3.95. Significant differences are underlined.

	0.10 g L^{-1}	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
0.05 g L^{-1}	2.74	5.34	4.02	1.15
0.10 g L^{-1}		2.60	1.28	3.88
0.50 g L^{-1}			1.32	6.48
0.75 g L^{-1}				5.16

Comparison of mean maximal dissimilarity between nutrient concentrations within Sequence II communities. For an α=0.05, Q critical (k=5, df=90) = 3.95. Significant differences are underlined.

	0.10 g L^{-1}	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
0.05 g L^{-1}	0.35	0.13	0.80	2.35
0.10 g L^{-1}		0.49	0.45	1.99
0.50 g L^{-1}			0.93	2.48
0.75 g L^{-1}				1.54

Table 4.13. Summary of comparisons between sequence permutations of biotically-related parameters for the final sampling date by media concentration. An "x" indicates a significant difference.

Media Level	Individual Species Abundance[1]					Community Parameters[1]			Sorensen Dissimilarity[1]		Final Species Composition[2]
	Colpidium	*Cinetochilum*	*Tetrahymena*	*Blepharisma*	*Euplotes*	*Richness*	*Diversity*	*Equitability*	*Final*	*Maximal*	
0.05 g L^{-1}										x	
0.10 g L^{-1}											x
0.50 g L^{-1}		x			x	x					x
0.75 g L^{-1}											
1.00 g L^{-1}		x				x	x	x		x	x

[1]Analysis of Variance and Tukey HSD post-hoc tests
[2]MRPP test

Table 4.14. Summary of pH and dissolved oxygen values for protist media for final sampling date by treatment and replicate treatments.

Nutrient Concentration	Rep	pH		DO Concentration (mg L^{-1})	
		Sequence I	*Sequence II*	*Sequence I*	*Sequence II*
1.00 g L^{-1}	1	8.14	8.16	4.48	3.16
	2	8.22	7.98	4.20	3.76
	3	8.11	7.91	3.78	3.34
	4	8.16	7.99	3.79	3.08
	5	8.24	8.12	4.53	3.80
0.75 g L^{-1}	1	8.09	8.22	4.28	4.52
	2	8.03	7.97	4.24	3.65
	3	8.10	7.91	4.24	3.31
	4	8.08	8.21	4.18	4.49
	5	8.23	8.22	4.48	4.24
0.50 g L^{-1}	1	8.22	8.04	4.67	4.62
	2	8.12	8.20	4.90	4.60
	3	8.12	8.19	4.85	5.00
	4	8.12	8.14	5.04	4.69
	5	8.17	8.15	5.00	4.90
0.10 g L^{-1}	1	7.97	7.97	4.94	5.24
	2	8.00	8.00	5.26	5.58
	3	7.96	7.96	5.17	5.52
	4	7.95	7.93	5.00	5.00
	5	7.94	7.96	4.91	5.10
0.05 g L^{-1}	1	7.91	8.04	5.15	4.88
	2	7.89	7.89	5.28	4.41
	3	7.91	7.98	5.14	4.46
	4	7.90	7.89	5.13	5.36
	5	7.86	7.88	5.08	5.10

Table 4.15. Summary of media chemical analysis for final sampling for Sequence I microcosms by media treatment and replicate.

Media Treatment	Rep	Concentration by Element (mg L^{-1})													
		C	*N*	*Ca*	*Fe*	*K*	*Mg*	*Mn*	*Na*	*P*	*S*	*Si*	*Sr*	*Zn*	*Zr*
1.00 g L^{-1}	1	84.79	30.86	48.08	0.081	35.75	5.12	0.004	9.14	4.16	25.26	26.04	0.307	0.011	0.000
	2	81.61	31.89	40.78	0.055	30.21	4.26	0.004	7.64	3.24	25.45	22.73	0.245	0.014	0.000
	3	80.48	28.24	43.84	0.056	34.38	4.72	0.002	8.06	4.33	27.90	24.86	0.273	0.010	0.000
	4	84.80	30.35	42.67	0.072	33.33	4.75	0.002	9.37	4.43	27.25	24.27	0.267	0.036	0.000
	5	82.72	28.49	44.79	0.074	35.42	5.09	0.004	15.53	4.30	28.76	25.79	0.287	0.175	0.000
0.75 g L^{-1}	1	68.78	26.69	35.93	0.052	27.78	4.07	0.002	8.38	3.41	18.91	23.42	0.243	0.000	0.000
	2	61.37	23.57	36.17	0.055	27.78	4.04	0.002	8.60	2.24	19.11	23.59	0.245	0.000	0.000
	3	63.55	22.09	36.53	0.046	28.26	4.11	0.002	8.81	2.75	18.77	24.08	0.247	0.000	0.000
	4	66.70	22.92	35.16	0.050	27.29	4.17	0.002	8.89	2.93	18.03	23.24	0.240	0.000	0.000
	5	64.19	21.48	35.01	0.048	26.33	3.89	0.005	8.17	2.64	17.99	23.12	0.241	0.000	0.000
0.50 g L^{-1}	1	45.50	17.73	26.23	0.030	19.08	3.27	0.002	8.34	2.26	13.51	21.44	0.179	0.000	0.000
	2	47.06	14.65	26.82	0.034	19.08	3.20	0.002	8.39	2.30	13.87	21.66	0.185	0.000	0.000
	3	48.70	16.70	26.56	0.031	18.84	3.41	0.002	8.90	2.15	13.90	21.55	0.184	0.000	0.000
	4	48.59	15.55	26.73	0.028	19.81	3.38	0.002	8.51	2.06	13.71	21.66	0.185	0.000	0.000
	5	47.72	17.48	26.25	0.024	19.08	3.28	0.004	8.41	2.12	13.73	21.56	0.183	0.000	0.000
0.10 g L^{-1}	1	14.01	2.86	8.48	0.010	3.86	1.69	0.002	7.79	0.49	3.10	17.68	0.081	0.000	0.000
	2	18.52	4.65	10.41	0.010	5.07	1.80	0.002	7.65	0.81	4.21	18.17	0.092	0.000	0.000
	3	19.05	5.04	10.41	0.010	5.56	1.91	0.002	7.80	0.86	4.42	17.98	0.091	0.000	0.000
	4	18.84	5.52	10.10	0.010	5.56	1.90	0.002	8.22	0.83	4.31	17.65	0.090	0.000	0.000
	5	19.15	4.84	10.45	0.010	5.07	1.84	0.005	7.94	0.82	4.36	18.12	0.097	0.000	0.000
0.05 g L^{-1}	1	18.14	3.66	10.57	0.010	5.07	1.82	0.002	7.91	0.62	4.24	17.83	0.092	0.000	0.000
	2	14.26	3.33	8.59	0.010	3.38	1.64	0.002	8.08	0.36	3.12	18.10	0.084	0.000	0.000
	3	16.77	4.13	8.84	0.010	3.62	1.80	0.002	8.17	0.72	3.20	18.05	0.084	0.000	0.000
	4	16.05	4.92	8.56	0.010	3.86	1.71	0.002	8.21	0.88	3.18	17.92	0.083	0.000	0.000
	5	14.62	3.87	8.06	0.010	3.62	1.64	0.004	7.48	0.47	2.94	17.37	0.082	0.000	0.000

Table 4.16. Summary of media chemical analysis for final sampling for Sequence II microcosms by media treatment and replicate.

Media Treatment	Rep	Concentration by Element (mg L^{-1})													
		C	*N*	*Ca*	*Fe*	*K*	*Mg*	*Mn*	*Na*	*P*	*S*	*Si*	*Sr*	*Zn*	*Zr*
1.00 g L^{-1}	1	80.90	27.81	46.94	0.073	35.51	4.81	0.002	9.25	3.63	24.52	25.14	0.303	0.000	0.004
	2	74.56	19.31	47.09	0.056	37.20	4.78	0.002	8.71	3.66	23.97	25.43	0.307	0.000	0.006
	3	68.00	17.82	44.99	0.051	33.33	4.56	0.002	8.84	2.81	23.51	24.25	0.291	0.000	0.005
	4	71.19	22.20	46.21	0.061	35.75	4.94	0.002	8.80	3.84	24.32	24.90	0.299	0.000	0.006
	5	81.25	32.00	45.97	0.073	35.51	4.81	0.002	9.37	3.95	24.59	24.98	0.298	0.000	0.003
0.75 g L^{-1}	1	65.47	26.08	36.27	0.049	27.54	3.98	0.002	9.06	2.86	18.87	23.38	0.243	0.000	0.000
	2	65.60	22.65	36.57	0.053	27.29	4.02	0.002	8.98	2.61	19.27	23.48	0.246	0.000	0.000
	3	59.94	19.86	35.52	0.049	28.02	4.19	0.002	8.69	2.69	18.52	22.90	0.240	0.000	0.000
	4	61.81	25.42	35.42	0.056	27.78	4.12	0.002	8.31	2.90	18.25	23.20	0.241	0.000	0.000
	5	68.04	28.02	37.10	0.055	27.29	4.07	0.002	8.87	2.79	19.27	23.82	0.253	0.000	0.000
0.50 g L^{-1}	1	47.97	18.50	25.89	0.033	19.08	3.39	0.002	8.37	2.34	13.88	21.02	0.178	0.000	0.000
	2	45.66	15.45	26.51	0.036	18.60	3.21	0.002	8.38	2.33	13.49	21.42	0.183	0.000	0.000
	3	45.45	17.31	25.94	0.028	18.36	3.25	0.002	8.82	2.36	13.81	21.14	0.182	0.000	0.000
	4	51.04	23.96	25.58	0.038	19.08	3.67	0.002	8.96	3.79	13.58	21.02	0.179	0.000	0.000
	5	47.94	17.64	25.83	0.031	18.36	3.39	0.002	8.63	2.90	13.40	20.87	0.178	0.000	0.000
0.10 g L^{-1}	1	20.22	6.07	10.26	0.010	4.83	1.81	0.002	7.99	0.82	4.30	18.12	0.091	0.000	0.000
	2	17.96	4.63	10.23	0.010	4.59	1.80	0.002	7.63	0.66	4.12	18.03	0.090	0.000	0.000
	3	19.46	5.66	10.04	0.014	5.56	1.88	0.002	7.81	0.78	4.20	17.93	0.089	0.000	0.000
	4	20.25	5.56	10.32	0.010	5.80	1.83	0.002	8.05	0.74	4.39	18.17	0.091	0.000	0.000
	5	21.61	6.70	10.26	0.010	5.31	1.90	0.002	7.62	1.01	4.41	18.11	0.090	0.000	0.000
0.05 g L^{-1}	1	14.98	2.86	8.71	0.010	6.76	1.66	0.002	7.66	0.41	3.12	17.58	0.081	0.000	0.000
	2	14.50	3.23	8.42	0.010	4.11	1.62	0.002	7.66	0.41	3.13	17.40	0.079	0.000	0.000
	3	15.16	3.33	8.57	0.010	5.07	1.70	0.002	8.08	0.48	3.24	17.85	0.081	0.000	0.000
	4	14.15	3.64	8.37	0.010	5.56	1.66	0.002	7.67	0.47	3.18	17.67	0.080	0.000	0.000
	5	16.87	4.72	8.19	0.010	13.29	1.71	0.002	7.90	0.62	3.14	17.82	0.078	0.000	0.000

Table 4.17. Two-factor analysis of variance of the effect of sequence permutation and nutrient concentration on H^+ concentration. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Nutrient Concentration	1.85E-16	4	4.63E-17	20.55	<0.000	2.61
Sequence Treatment	2.32E-18	1	2.32E-18	1.03	0.316	4.08
Media * Treatment	1.99E-17	4	4.98E-18	2.21	0.085	2.61
Within	9.01E-17	40	2.25E-18			
Total	2.98E-16	49				

Table 4.18. Two-factor analysis of variance of the effect of sequence permutation and nutrient concentration on dissolved oxygen concentration.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Nutrient Concentration	13.813	4	3.453	40.18	<0.000	2.61
Sequence Treatment	0.699	1	0.699	8.13	0.007	4.08
Media * Treatment	1.196	4	0.299	3.48	0.016	2.61
Within	3.438	40	0.086			
Total	19.145	49				

b. Tukey HSD post-hoc comparison of mean dissolved oxygen concentration between sequence permutations. Separate comparisons were made by nutrient concentration. For an α=0.05, Q critical (k=2, df=40) = 2.858. Significant differences are underlined.

0.05 g L^{-1}	0.10 g L^{-1}	0.50 g L^{-1}	0.75 g L^{-1}	1.00 g L^{-1}
2.40	1.77	0.99	1.85	5.55

Table 4.19. Two-factor analysis of variance of the effect of sequence permutation and nutrient concentration on total dissolved carbon.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Nutrient Concentration	30946.9	4	7736.7	1046.75	<0.000	2.61
Sequence	25.9	1	25.9	3.50	0.069	4.08
Media * Sequence	135.3	4	33.8	4.58	0.004	2.61
Within	295.6	40	7.4			
Total	31403.8	49				

b. Tukey HSD post-hoc comparison of mean dissolved carbon between sequence permutations. Separate comparisons were made by nutrient concentration. For an α=0.05, Q critical (k=2, df=40) = 2.858. Significant differences are underlined.

0.05 g L^{-1}	0.10 g L^{-1}	0.50 g L^{-1}	0.75 g L^{-1}	1.00 g L^{-1}
0.69	1.63	0.08	0.61	6.33

Table 4.20. Two-factor analysis of variance of the effect of sequence permutation and nutrient concentration on total inorganic nitrogen.

a. Analysis of variance results. For an α=0.05, significant effects are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
Nutrient Concentration	4487.8	4	1121.9	168.35	<0.000	2.61
Sequence	2.5	1	2.5	0.37	0.547	4.08
Media * Sequence	109.8	4	27.5	4.12	0.007	2.61
Within	266.6	40	6.7			
Total	4866.7	49				

b. Tukey HSD post-hoc comparison of total inorganic nitrogen between sequence permutations. Separate comparisons were made by nutrient concentration. For an α=0.05, Q critical (k=2, df=40) = 2.858. Significant differences are underlined.

0.05 g L^{-1}	**0.10 g L^{-1}**	**0.50 g L^{-1}**	**0.75 g L^{-1}**	**1.00 g L^{-1}**
0.35	0.94	1.77	0.87	5.05

Table 4.21. MRPP comparison of anion/cation concentrations between sequences for the final sampling date by nutrient concentration. For an α=0.05, significant differences are underlined.

Nutrient Concentration	p-value
0.05 g L^{-1}	<u>0.024</u>
0.10 g L^{-1}	0.977
0.50 g L^{-1}	<u>0.005</u>
0.75 g L^{-1}	0.531
1.00 g L^{-1}	<u>0.021</u>

Table 4.22. Summary of comparisons between sequence permutations of abiotically-related parameters for the final sampling date by nutrient concentration. An "x" indicates a significant difference..

Media Level	H+ Concentration[1]	Dissolved Oxygen[1]	Dissolved Carbon[1]	Inorganic Nitrogen[1]	Anion/ Cation[2]
0.05 g L^{-1}					x
0.10 g L^{-1}					
0.50 g L^{-1}					x
0.75 g L^{-1}					
1.00 g L^{-1}		x	x	x	x

[1]Analysis of Variance and Tukey HSD post-hoc tests
[2]MRPP test

Table 4.23. Total unique community states that arose from the interaction of sequence effects with productivity level.

Community Composition (rank abundance)	Sequence	Nutrient Concentration
Cinetochilum	Both	0.05 g L^{-1} 0.10 g L^{-1}
Colpidium/Blepharisma, Cinetochilum	II	0.50 g L^{-1}
Colpidium, Cinetochilum/Blepharisma	Both II	0.75 g L^{-1} 1.00 g L^{-1}
Colpidium, Cinetochilum, Blepharisma, Euplotes	I	0.50 g L^{-1}
Colpidium, Blepharisma	I	1.00 g L^{-1}

Table 5.1. Complete list of sampled species classified by taxonomic and functional categories. Species indicated with an asterisk were dropped from species-level analysis.

Taxonomic	Functional	Species
Cyanobacteria	Coccals (<6 µm)	Picoplankton
	Coccals (6-20 µm)	*Chroococcus turgidus*
	Filaments (small)	*Dactylococcopsis sp.*
		Limnothrix sp.
		Phormidium sp.
Bacillariophyta	Coccals (6-20 µm)	*Achnanthes sp.*
		Gomphonema olivaceum
		Stephanodiscus sp.
Chlorophyceae	Coccals (<6 µm)	*Chlorella sp.*
		Monoraphidium minutum *
		Monoraphidium obtusum *
		Oocystis sp.
		*Planktonema sp.**
		Scenedesmus naegelii *
		Unidentified coccoid
	Flagellates (<20 µm)	*Carteria sp.*
		Chlamydomonas sp. (12 µm)
		Collodictyon triciliatum
		Unidentified flagellate
	Flagellates (>20 uµm)	*Chlamydomonas sp.* (20 µm)
Conjugatophyta	Coccals (6-20 µm)	*Cosmarium laeve*
Cryptophyta	Flagellates (>20 µm)	*Cryptomonas marssonii* *
		Cryptomonas phaseolus *
		Ochromonas sp.
Euglenophyta	Flagellates (>20 µm)	*Trachelomonas sp.*
	Flagellates heterotrophs (<20 µm)	*Astasia sp.* (12-20µm)
	Flagellates heterotrophs (>20 µm)	*Astasia sp.* (>20 µm)
		Entosiphon sulcatum (>20 µm)
Dinoflagellata	Flagellates heterotrophs (<20 µm)	*Gymnodinium sp.*
Ciliophora	Ciliates (>20 µm)	*Vorticella* sp. *
		Unidentified ciliate
Rhizopoda	Rhizopods (>20 µm)	*Arcella dentata* *
		Arcella discoides *
		Unidentified rhizopod
Rotifera	Rotifera (20-200 µm)	*Habrotrocha sp.* *
		Lecane sp.
		Lepadella sp. *
		Unidentified rotifer (<40 µm)
		Unidentified rotifer (80-240 µm)
Gastrotricha		*Chaetonotus sp.* *
Tendipedidae		Unidentified midge *

Table 5.2. Species colonization schedule indicating the occurrence (# microcosms) of species by Julian date, the initial occurrence (# microcosms) of species by Julian date, and the total number of microcosms in which each species was found in at least one sampling period.

Species	Occurrence By Date						Initial Occurrence By Date						Total
	130	144	158	172	186	200	130	144	158	172	186	200	
Chlorella sp.	11	9	5	10	7	7	11	2					13
Unidentified ciliate	10	12	10	8	11	9	10	4	1				15
Collodictyon triciliatum	10		1	1			10		1				11
Unidentified flagellate	9	6	8	1	1		9	3	2				14
Gomphonema olivaceum	9	6	3	4	7	7	9			1	1		11
Astasia sp. (12-20µm)	8	11	6	7	6	8	8	3	2	1	1		15
Unidentified rotifer (<40 µm)	6	1	3		5	3	6		1		3		10
Carteria sp.	5	4	8	2	3	1	5	1	6				12
Limnothrix sp.	5	1					5	1					6
Dactylococcopsis sp.	5						5						5
Oocystis	4	2		2	3	1	4	1		1	1	1	8
Ochromonas sp.	4	1	5	1	1	1	4		4	1	1	1	11
Phormidium sp.	4		1	1			4			1			5
Monoraphidium minutum	2		1				2						2
Cosmarium laeve	2				1	1	2						2
Trachelomonas sp.	2				1	1	2				1		3
Chlamydomonas sp.	1	2	4	5	1		1	2	2	3	1		9
Astasia sp. (>20 µm)	1	1					1	1					2
Scenedesmus naegelii	1						1						1
Cryptomonas phaseolus	1			1			1			1			2
Achnanthes sp.		10	7	6		3		10	2			1	13
Unidentified coccoid		3	1	2				3	1	2			6
Unidentified rotifer (80-240 µm)		3	1	2	3	1		3			1	1	5
Monoraphidium obtusum		3						3					3
Planktonema sp.		1	1					1					1
Vorticella sp.		1	1					1	1				2
Chlamydomonas sp.		1	1	6	2	3		1	1	5	1	1	9
Stephanodiscus sp.		1		2		2		1		1		2	4
Arcella discoides		1			1	1		1			1	1	3
Cryptomonas marssonii			5			2			5			2	7
Unidentified rhizopod			4	1		1			4	1			5
Gymnodinium sp.			2	4	7	4			2	4	4	1	11
Entosiphon sulcatum			2	2	2	1			2	1	2		5
Habrotrocha sp.			1	1					1	1			2
Picoplankton				2		6				2		4	6
Chroococcus turgidus				2	2					2	2		4
Lecane sp.				1	2	3				1	1	1	3
Chaetonotus sp.					2						2		2
Lepadella sp.					2						2		2
Arcella dentata					1						1		1
Unidentified midge					1						1		1

Table 5.3. Two-factory analysis of the effect of microcosm and sampling date on community attributes. Results significant for an α=0.05 level are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
			Species Richness			
Microcosm	76.0	14	5.4	1.24	0.270	1.84
Sampling Date	45.9	5	9.2	2.09	0.077	2.35
Error	307.2	70	4.4			
Total	429.1	89				
			Shannon Diversity			
Microcosm	2.7	14	0.2	1.01	0.451	1.84
Sampling Date	1.5	5	0.3	1.65	0.157	2.35
Error	13.1	70	0.2			
Total	17.3	89				
			Species Equitability			
Microcosm	0.667	14	0.047	0.73	0.729	1.83
Sampling Date	0.169	5	0.033	0.52	0.756	2.34
Error	4.519	70	0.064			
Total	5.355	89				
			Species Abundance			
Microcosm	750541.5	14	53610.1	1.03	0.433	1.84
Sampling Date	328542.3	5	65708.5	1.27	0.289	2.35
Error	3635117.2	70	51930.2			
Total	4714201.1	89				
			Community Size			
Microcosm	4347.4	14	310.5	1.41	0.170	1.84
Sampling Date	3803.8	5	760.8	3.46	0.007	2.35
Error	15375.0	70	219.6			
Total	23526.3	89				
			Edible:Nonedible Ratio			
Microcosm	11587.1	14	827.6	1.07	0.399	1.84
Sampling Date	3122.3	5	624.5	0.81	0.548	2.35
Error	54131.4	70	773.3			
Total	68840.8	89				
			Heterotroph:Autotroph Ratio			
Microcosm	4359.1	14	311.4	0.93	0.530	1.84
Sampling Date	2506.6	5	501.3	1.50	0.201	2.35
Error	23377.1	70	334.0			
Total	30242.8	89				

Table 5.4. Two-factor analysis of variance on the effect of spatial clustering and sampling date on community attributes. Results significant for an α=0.05 level are underlined.

Source of Variation	SS	df	MS	F	P-value	F crit
			Species Richness			
Cluster	20.3	4	5.1	1.38	0.251	2.53
Sampling Date	45.9	5	9.2	2.50	0.040	2.37
Cluster * Date	142.9	20	7.1	1.95	0.025	1.75
Error	220.0	60	3.7			
Total	429.1	89				
			Shannon Diversity			
Cluster	0.0	4	0.0	0.02	0.999	2.53
Sampling Date	1.5	5	0.3	1.95	0.100	2.37
Cluster * Date	6.2	20	0.3	1.95	0.024	1.75
Error	9.5	60	0.2			
Total	17.3	89				
			Species Equitability			
Cluster	0.1	4	0.0	0.46	0.763	2.53
Sampling Date	0.2	5	0.0	0.63	0.674	2.37
Cluster * Date	1.9	20	0.1	1.77	0.046	1.75
Error	3.2	60	0.1			
Total	5.4	89				
			Species Abundance			
Cluster	118891.2	4	29722.8	0.56	0.694	2.53
Sampling Date	328542.3	5	65708.5	1.23	0.305	2.37
Cluster * Date	1068062.8	20	53403.1	1.00	0.474	1.75
Error	3198704.7	60	53311.7			
Total	4714201.1	89				
			Community Size			
Cluster	1884.9	4	471.2	2.02	0.103	2.53
Sampling Date	3803.8	5	760.8	3.26	0.011	2.37
Cluster * Date	3818.8	20	190.9	0.82	0.684	1.75
Error	14018.8	60	233.6			
Total	23526.3	89				
			Edible:Nonedible Ratio			
Cluster	5233.6	4	1308.4	1.76	0.148	2.53
Sampling Date	3122.3	5	624.5	0.84	0.526	2.37
Cluster * Date	15930.4	20	796.5	1.07	0.400	1.75
Error	44554.5	60	742.6			
Total	68840.8	89				
			Heterotroph:Autotroph Ratio			
Cluster	1419.0	4	354.8	1.03	0.401	2.53
Sampling Date	2506.6	5	501.3	1.45	0.219	2.37
Cluster * Date	5585.1	20	279.3	0.81	0.694	1.75
Error	20732.1	60	345.5			
Total	30242.8	89				

Table 5.5. T-test results for comparisons of the mean decrease in dissimilarity within all clusters combined and mean decrease in dissimilarity between clusters for selected sampling periods by data type. Reported values include the difference between in means (δ). An "*" indicates a mean increase in mean dissimilarity within clusters for this data type. For an □=0.05 all comparison were insignificant.

Date Comparison	**Test Results**			
	δ	***t***	***df***	***p***
		Species (abundance)		
Day 144 – Day 130	-0.082	-1.51	103	0.066
Day 172 – Day 158	-0.064	-0.82	103	0.205
Day 186 – Day 172	0.017	0.27	103	0.273
		Species (presence/absence)		
Day 144 – Day 130	-0.061	-1.00	103	0.159
Day 172 – Day 158	-0.069	-0.83	103	0.204
Day 186 – Day 172	0.023	0.34	103	0.368
		Taxonomic		
Day 144 – Day 130	*	*	*	*
Day 172 – Day 158	-0.054	-0.81	103	0.209
Day 186 – Day 172	-0.057	-0.75	103	0.226
		Functional		
Day 144 – Day 130	*	*	*	*
Day 172 – Day 158	-0.067	-0.69	103	0.245
Day 186 – Day 172	-0.036	-0.47	103	0.319

Table 5.6. Analysis of variance comparisons of the mean decrease in dissimilarity within individual clusters with the mean decrease in dissimilarity between clusters for selected sampling periods by data type. For an α=0.05, all comparisons were insignificant.

Dates	Source of Variation	SS	df	MS	F	P	F crit
		Species (abundance)					
Day 144 vs 130	Between Groups	0.22	4	0.05	1.44	0.225	2.47
	Within Groups	3.66	97	0.04			
	Total	3.88	101				
Day 172 vs 158	Between Groups	0.15	4	0.04	0.47	0.761	2.47
	Within Groups	7.72	97	0.08			
	Total	7.87	101				
Day 186 vs 172	Between Groups	0.12	3	0.04	0.86	0.465	2.70
	Within Groups	4.51	95	0.05			
	Total	4.64	98				
		Species (presence/absence)					
Day 144 vs 130	Between Groups	0.19	4	0.05	1.00	0.411	2.47
	Within Groups	4.55	97	0.05			
	Total	4.74	101				
Day 172 vs 158	Between Groups	0.19	4	0.05	0.51	0.726	2.47
	Within Groups	8.94	97	0.09			
	Total	9.13	101				
Day 186 vs 172	Between Groups	0.17	3	0.06	1.02	0.387	2.70
	Within Groups	5.33	95	0.06			
	Total	5.50	98				
		Taxonomic					
Day 144 vs 130	Between Groups	0.10	2	0.05	0.97	0.383	3.09
	Within Groups	4.60	93	0.05			
	Total	4.69	95				
Day 172 vs 158	Between Groups	0.23	4	0.06	1.01	0.408	2.47
	Within Groups	5.56	97	0.06			
	Total	5.79	101				
Day 186 vs 172	Between Groups	0.37	3	0.12	1.71	0.170	2.70
	Within Groups	6.82	95	0.07			
	Total	7.19	98				
		Functional					
Day 144 vs 130	Between Groups	0.25	4	0.06	1.23	0.303	2.47
	Within Groups	4.85	97	0.05			
	Total	5.10	101				
Day 172 vs 158	Between Groups	0.48	3	0.16	1.30	0.278	2.70
	Within Groups	11.63	95	0.12			
	Total	12.11	98				
Day 186 vs 172	Between Groups	0.21	3	0.07	0.93	0.431	2.70
	Within Groups	7.13	95	0.08			
	Total	7.34	98				

Table 5.7. Single-factor analysis of variance on the effect of sampling date on mean information loss by data type. For an α=0.05, all comparisons were insignificant. The effect of removing Cluster 3 prior to analysis was tested for all data types. A significant result for this amended data set was found for *Species (abundance)* data. Only test results for this data type are shown.

Source of Variation	SS	df	MS	F	P-value	F crit
			Species (abundance)			
Between Dates	0.214	5	0.043	0.75	0.593	2.62
Within Dates	1.363	24	0.057			
Total	1.577	29				
			Species (abundance) - Less Cluster 3			
Between Dates	0.496	5	0.099	3.05	0.036	2.77
Within Dates	0.585	18	0.032			
Total	1.081	23				
			Species (presence)			
Between Dates	180.0	5	36.0	0.64	0.672	2.62
Within Dates	1351.2	24	56.3			
Total	1531.2	29				
			Taxonomic			
Between Dates	0.132	5	0.026	0.74	0.601	2.62
Within Dates	0.858	24	0.036			
Total	0.990	29				
			Functional			
Between Dates	11969.4	5	2393.9	1.54	0.214	2.62
Within Dates	37252.1	24	1552.2			
Total	49221.5	29				
			Community Features			
Between Dates	0.590	5	0.118	0.60	0.699	2.62
Within Dates	4.703	24	0.196			
Total	5.293	29				

Table 5.8. Analysis of variance comparison of mean dissimilarity for the following: 1) within Group 1, 2) within Group 2, and 3) between Group 1 and Group 2. Initial and final sampling dates are tested. Results significant at an α=0.05 level are underlined.

Date	Analysis of Variance Results						
	Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
			Species (abundance)				
130	Between Groups	0.059	2	0.029	1.43	0.243	3.09
	Within Groups	2.031	99	0.021			
	Total	2.090	101				
200	Between Groups	0.871	2	0.436	20.45	<0.000	3.09
	Within Groups	2.109	99	0.021			
	Total	2.981	101				
			Species (presence)				
130	Between Groups	0.096	2	0.048	1.88	0.159	3.09
	Within Groups	2.530	99	0.026			
	Total	2.626	101				
200	Between Groups	1.179	2	0.590	26.01	<0.000	3.09
	Within Groups	2.244	99	0.023			
	Total	3.424	101				
			Taxonomic				
130	Between Groups	0.030	2	0.015	0.93	0.397	3.09
	Within Groups	1.616	99	0.016			
	Total	1.646	101				
200	Between Groups	0.654	2	0.327	9.61	<0.000	3.09
	Within Groups	3.368	99	0.034			
	Total	4.022	101				
			Functional				
130	Between Groups	0.160	2	0.080	4.18	0.018	3.09
	Within Groups	1.892	99	0.019			
	Total	2.052	101				
200	Between Groups	1.289	2	0.645	15.62	<0.000	3.09
	Within Groups	4.086	99	0.041			
	Total	5.375	101				

Table 5.9. Tukey-Kramer post-hoc pairwise comparisons of mean dissimilarity by data type. Q statistics significant at an α=0.05 are underlined

Comparison	Difference	Pooled Variance	|Q|	Q crit
		Species (abundance)		
Group 1 - Group 2	0.022	0.030	0.75	3.36
Group 1 - Between	-0.178	0.026	6.77	
Group 2 - Between	-0.200	0.024	8.43	
		Species (presence)		
Group 1 - Group 2	0.067	0.031	2.15	3.36
Group 1 - Between	-0.178	0.027	6.49	
Group 2 - Between	-0.245	0.025	9.86	
		Taxonomic		
Group 1 – Group 2	-0.062	0.038	1.65	3.36
Group 1 – Between	-0.191	0.033	5.72	
Group 2 – Between	-0.129	0.030	4.26	
		Functional – Day 130		
Group 1 – Group 2	-0.115	0.028	4.09	3.36
Group 1 – Between	-0.066	0.025	2.63	
Group 2 – Between	0.050	0.023	2.19	
		Functional – Day 200		
Group 1 – Group 2	0.045	0.041	1.08	3.36
Group 1 – Between	-0.208	0.037	5.67	
Group 2 – Between	-0.253	0.033	7.62	

Vita

Craig Richard Zimmermann was born in Tallahassee, Florida on May 25, 1960. At the age of five, he moved to Lafayette, Louisiana where he received most of his elementary education. He graduated from Lafayette High School in 1978. He received a Bachelor's of Science in Forestry at Louisiana State University in 1983. From there, he attended the University of Georgia where he received a Master's of Science in Forestry. After working for three years as a professional forester in Alabama, Craig returned to Athens where he worked for three years as a research technician with the University of Georgia. From there, Craig attended the University of Tennessee where he received a Doctorate of Philosophy in Ecology and Evolutionary Biology in 2002. Craig is currently working with the Warnell School of Forest Resources at the University of Georgia.