

**THE INFLUENCE OF
PROPAGULE PRESSURE ON
COMMUNITY DIVERSITY AND INVASION SUCCESS
IN AN AQUATIC PROTIST SYSTEM**

By

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ABSTRACT OF THE DISSERTATION

The Influence of

Propagule Pressure on

Community Diversity and Invasion Success

in an Aquatic Protist System

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Propagule pressure, the size and frequency with which invaders enter an area, may influence many aspects of invasion. Using a bacterivorous protist model system, this study examines the effect of propagule pressure on the diversity of established communities, the success of invaders, and the effect invaders have on the established community.

Increasing propagule size and frequency significantly increased diversity and species richness and decreased dominance in three experimental protist communities.

Propagule pressure also significantly influenced invasion. Increasing propagule pressure promoted establishment and invasion success, and reduced probability of stochastic extinction, of slow-growing invaders. Increasing propagule pressure also enhanced the growth of fast-growing invaders, but only up to a point. Further increase in propagule pressure diminished growth. Propagule size and frequency interacted in influencing invasion, with the outcome related to the invader's growth

rate. Fast-growing protist invaders achieved the greatest success when introduced in one large inoculation, while the success of the slow-growing invaders was enhanced by high propagule frequency. In this study, high values of propagule pressure enhanced the coexistence of protist species, established and invasive, and might therefore create a positive regional diversity-invasibility relationship in natural protist communities.

Invasion success might be described by a general Gaussian dose-response curve, the placement of specific propagule pressure values on the curve determined by a species' realized growth rate. Slow-growing invaders reach saturation at high values of propagule size and frequency, while fast-growing invaders show saturation at much lower values.

In this system, propagule pressure has a strong indirect influence on the growth of individual established species and the degree to which invasion affects community diversity, richness, and evenness. Invaders have a particularly strong effect on slow-growing established species. Fast-growing invaders have especially powerful effects on established species. The effect the invader has on the specific species composing the community determines species richness and evenness, and ultimately, community diversity. In this way, propagule pressure plays an important role in the complex interaction of factors that influence the effect invasion has on the established community.

DEDICATION

To Sara

May you find challenge, joy, and fulfillment in learning.

And to Eldo

For expecting me to do more than I think I can.

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CHAPTER 1. INTRODUCTION

Background

The invasion of exotic species into a wide variety of communities has increased dramatically during the last few centuries and is now a major factor in biodiversity change and ecosystem stability (Gordon; 1998; Mack *et al.*, 2000; Cassey *et al.*, 2005; Wilson *et al.*, 2009; Eschtruth and Battles, 2009). Biological invasion threatens native species (Wilcove *et al.*, 1998), causes the degradation of terrestrial and aquatic environments (Carlton, 2001; D'Antonio and Kark, 2002), and can even alter biogeochemical cycles (D'Antonio and Vitousek, 1992; Mack and D'Antonio, 1998). Propagule pressure, which is the size of an invading population and frequency with which groups of invaders arrive in a location, appears to be an important factor in many aspects of invasion (Richardson *et al.*, 2000b; von Holle and Simberloff, 2005; Kalwij *et al.*, 2008). Propagule pressure influences the diversity and invasibility of communities (Lonsdale, 1999; Williamson and Fitter, 1996a; Green, 1997; Brown and Peet, 2003; Knight and Reich, 2005), the success of invaders (D'Antonio *et al.*, 2000; Lockwood *et al.*, 2005; and Colautti *et al.*, 2006), and perhaps even the effect an invader has on the native community.

Community-Level Patterns of Diversity and Invasibility

Some communities seem exceptionally vulnerable to invasion, while others appear to be much more resistant. A community's invasibility, the likelihood that an invader will establish a reproducing population or the increase in abundance of the invasive species over time, appears to be closely linked its diversity (Davis

et al., 2000; Eschtruth and Battles, 2009). Community diversity might directly influence invasibility, or it might instead respond to some external factor that influences both diversity and invasibility (Lonsdale, 1999; Levine, 2000; Shea and Chesson, 2002; Brown and Peet, 2003; Jiang and Morin, 2004; Knight and Reich, 2005). Propagule pressure can influence both the diversity and the invasibility of native communities (Brown and Peet, 2003; Knight and Reich, 2005). Therefore, propagule pressure is an important factor to consider when examining the relationship between community diversity and invasibility.

How is a community's diversity related to its invasibility? The classic theory is that highly diverse communities are inherently stable structures, relatively resistant to invasion (Elton, 1958; Fox and Fox, 1986; Holdgate, 1986; Levine and D'Antonio, 1999; Tilman, 1999; Jiang and Morin, 2004). As community diversity, usually measured as species richness, increases, community invasibility decreases. The negative relationship between native species richness and invasibility is strongly supported by theoretical models (Robinson and Valentine, 1979; Post and Pimm, 1983; Drake, 1990) and experimental work in the laboratory and field across a broad range of communities from grasslands and agricultural borders to marine invertebrate communities (McGrady-Steed *et al.*, 1997; Tilman, 1997; Knops *et al.*, 1999; Stachowicz *et al.*, 1999; Levine, 2000; Naeem *et al.*, 2000; Symstad, 2000; Dukes, 2001; Hector *et al.*, 2001; Foster *et al.*, 2002; Kennedy *et al.*, 2002; Troumbis *et al.*, 2002; Stachowicz

et al., 2002, Brown and Peet, 2003; Pfisterer *et al.*, 2004; Stohlgren *et al.*, 2006; Fridley *et al.*, 2007).

In 1999, Lonsdale and Stohlgren independently discovered a seemingly contradictory diversity-invasibility pattern (Lonsdale, 1999; Stohlgren *et al.*, 1999). In their surveys, areas with high native diversity were associated with a large number of invasive species. Since then, a positive relationship between diversity and invasibility has been found in a wide variety of community types, including savanna (Keely *et al.*, 2003), tallgrass prairie (Cleland *et al.*, 2004), chaparral (Keely *et al.*, 2003), desert grassland (Cleland *et al.*, 2004), oak forest (Knight and Reich, 2005), riparian (Levine, 2000; Brown and Peet, 2003), and marine invertebrate (Stachowicz and Byrnes, 2006). The pattern has been found around the globe, in North American states (Stohlgren *et al.*, 2003; Eschtruth and Battles, 2009), central grasslands (Stohlgren *et al.*, 1998), tussocks along a northern California river (Levine, 2000), the Rocky and Appalachian Mountains (Stohlgren *et al.*, 1999; Brown and Peet, 2003), European grasslands (Essl and Dirnbock, 2008); and a forest understory in Poland (Knight *et al.*, 2008).

The negative and positive diversity-invasibility patterns are not mutually exclusive, but instead seem to be associated with the scale at which the community is examined and the methods of study used. Controlled experiments employing small plots of 1 square meter or less typically reveal a negative diversity-invasibility relationship. In contrast, surveys that span large geographic

ranges of 1 square kilometer or more predominantly reveal a positive diversity-invasibility pattern (Fridley *et al.*, 2007). Indeed, negative small-scale and positive large-scale-invasibility relationships have been found simultaneously in the several systems (Levine, 2000; Brown and Peet, 2003; Davies *et al.*, 2005; Knight and Reich, 2005).

Since there is a strong connection between the slope of the diversity-invasibility relationship and the scale at which the community is observed, the characteristics associated with scale may suggest mechanisms that shape diversity-invasibility relationships. Small-scale plots have homogeneous environmental characteristics and are small enough to allow individuals to directly interact with one another (Fridley *et al.*, 2007). In contrast, in large-scale areas contain environmental heterogeneity, and groups of individuals are prohibited from direct interaction by separation across great distances (Fridley, *et al.*, 2007).

At the small scale, species interactions determine community diversity and invasibility. The processes by which competition affects diversity and invasibility are well understood. In species-rich assemblages, individual niches are tightly packed in resource space and all available resources are utilized (MacArthur, 1970; Tilman, 1997; Knops *et al.*, 1999; Stachowicz *et al.*, 1999; Naeem *et al.*, 2000; Dukes, 2001; Hector *et al.*, 2001). Competition among native species that reduces resources to critical levels also reduces the success of invaders

(Theoharides and Battles, 2007). It is also believed that indirect interactions among species in diverse communities are exceptionally strong, further blocking the entrance of invaders (Case, 1990). In contrast, depauperate communities do not completely fill available niche space, leaving resources that invaders can use and room for invaders to enter (MacArthur, 1970; Tilman, 1997; Knops *et al.*, 1999; Stachowicz *et al.*, 1999; Naeem *et al.*, 2000; Dukes, 2001; Hector *et al.*, 2001). With few species, there may also be few indirect interactions among native species, and this allows invaders to enter more easily (Case, 1990).

Theoretical and experimental work on diversity and invasibility has primarily focused on negative interspecies interactions that increase in strength along with native species richness, creating a negative small-scale diversity-invasibility relationship. However, positive interspecies interactions and effects also occur, and these generate a positive diversity-invasibility relationship at the local level. The native community can promote invasion through facilitation and increased structural heterogeneity. The effect of facilitation on invasion may be very important in natural communities, especially those exposed to harsh environmental conditions (Theoharides and Battles, 2007). In plots as small as one square meter, native species have been shown to facilitate the entrance and success of invaders, creating a positive diversity-invasibility pattern (Smith *et al.*, 2004; Bruno *et al.*, 2005; Brooker, 2006; Theoharides and Battles, 2007; Fridley *et al.*, 2007). If an increase in native diversity enhances structural heterogeneity and the number of microenvironments that can be exploited, it can increase

invasibility, allowing more exotic species to enter and persist at the local level (Palmer and Maurer, 1997). Experimental work has confirmed the presence of this process (Stachowicz and Byrnes, 2006). Finally, a positive small-scale diversity-invasibility relationship can arise at the neighborhood level from local environmental conditions. Areas with high-quality conditions have been found to support a rich collection of both native and exotic species, and a positive diversity-invasibility relationship (Levine and D'Antonio, 1999; Stohlgren *et al.*, 1999, Levine, 2000; Brown and Peet, 2003; Stohlgren *et al.*, 2003; Stohlgren *et al.*, 2006.)

The mechanisms that determine the positive community diversity-invasibility relationship at the regional level are not well understood (Davies *et al.*, 2007; Fridley *et al.*, 2007). It is thought that different processes operate at different spatial scales (Levine and D'Antonio, 1999; Stohlgren *et al.*, 1999; Levine, 2000; Shea and Chesson, 2002). As scale increases, there is a shift from biotic to environmental determinants of community diversity and invasibility. Since environmental factors are essentially homogeneous at the local level, strong biotic interactions of competition and facilitation shape small-scale diversity and invasibility. As spatial scale and environmental heterogeneity increase, the effect of biotic interactions weakens and that of environmental determinants increases. Diversity and invasibility at the regional level are shaped primarily by environmental factors that promote diversity and vary in intensity across the range (Fridley *et al.*, 2007). Since community diversity and invasibility covary

across a heterogeneous environment, a positive diversity-invasibility relationship is produced (Levine and D'Antonio, 1999; Lonsdale, 1999; Stohlgren *et al.*, 1999; Stohlgren *et al.*, 2003; Eschtruth and Battles, 2009).

The extent of environmental heterogeneity appears to be the critical factor determining community invisibility (Melbourne *et al.*, 2007) and the positive diversity-invasibility relationship. There are many forms of environmental heterogeneity that allow species to coexist (Davies *et al.*, 2007). Any of these could potentially influence regional diversity and invasibility patterns, creating a positive diversity-invasibility relationship. The positive pattern may arise simply from the extent to which species in the regional pool saturate an area's niche space. At the small scale, the regional pool of species is sufficiently large to utilize all niche conditions. However, as scale and associated heterogeneity increases, the pool of species is less able to utilize all conditions, leaving room for invaders to enter (Fridley *et al.*, 2007). It has also been suggested that positive patterns of native and exotic diversity are largely the result of variation in the quality of environmental conditions. What is good for native species is also good for invasive species, so areas with high-quality conditions support a rich collection of both native and exotic species (Levine and D'Antonio, 1999; Stohlgren *et al.*, 1999, Levine, 2000; Brown and Peet, 2003). As scale increases, the chance of including high-quality areas also increases, creating a positive pattern of diversity and invasibility. The problem with this hypothesis is that beneficial environmental conditions have been found in theory and practice

to increase competitive exclusion, not diversity. This hypothesis also predicts incorrectly that the tropics should be highly invaded and does not explain why they are not (Davies *et al.*, 2005; Chesson, 2000; Fridley *et al.*, 2007).

The underlying pattern of diversity and invasibility is commonly understood to be spatial availability of resources (Stohlgren *et al.*, 1999; Stachowicz *et al.*, 1999; Davis *et al.*, 2000; Wardle, 2001, Eschtruth and Battles, 2009). However, propagule pressure may also be important in determining regional patterns of invasion. Like resource availability, propagule pressure varies spatially and promotes diversity when at high level (Levine and D'Antonio, 1999; Eschtruth and Battles, 2009). Theoretical models that incorporate the effect of propagule pressure more accurately describe regional abundance and distribution patterns of invaders (Rouget and Richardson, 2003, Richardson and Pysek, 2006).

The interaction among diversity, invasibility, and scale is complex. At the regional scale, the diversity-invasibility relationship is predominantly positive, but at the local level, the relationship can be either negative or positive depending of the system (Fridley *et al.*, 2007). A positive diversity-invasibility relationship does not mean that there is no negative effect of community interactions. Resource competition structures communities and blocks invasion at the local level, but the strength of competition, and thus the resistance of the community to invasion, decreases with increasing scale, the result of increasing environmental heterogeneity (Byers and Noonburg, 2003; Davies *et al.*, 2005; Fridley *et al.*,

2007). If the effect of competition is greater than diversity-promoting environmental factors, as is often the case in controlled experiments, the community will show a negative diversity-invasibility relationship (Fridley *et al.*, 2007). However, environmental processes as such rapid short-term increases in resource availability, disturbance, high invader propagule pressure, and the facilitation of invasion by native species can all swamp the negative effects of competition, increase invasion success, and produce a positive diversity-invasibility relationship (Richardson *et al.*, 2000a; Stachowicz, 2001; Bruno *et al.*, 2003). The diversity-invasibility relationship is determined by the net outcome of biotic and abiotic mechanisms operating at the regional scale (Levine, 2000; Tilman, 2004; Stachowicz and Byrnes, 2006; Fridley *et al.*, 2007). Negative diversity-invasibility patterns should be found in communities that have high competitive interactions inhabiting areas with low disturbance and benign environmental conditions (Fridley *et al.*, 2007). In contrast, positive diversity-invasibility relationships should be found in communities shaped by dispersal or immigration, in areas with a high frequency of disturbance, and in areas of great environmental stress. Stressful environmental conditions appear to increase the prevalence and importance of facilitation (Callaway and Walker, 1997). In these areas, positive interspecies interactions, rather than effects arising from species richness, may determine local, fine-scale invasibility. (Smith *et al.*, 2004). Because of the complexity of the diversity, invasibility, and scale relationship, Fridley *et al.* (2007) suggests that it is better to explain invasion in terms of biotic

influences at the neighborhood level and abiotic influences at the regional level, rather than in terms of simple species richness, as has traditionally been done.

It appears then that community diversity and invasibility are both influenced by the interaction between biotic interactions, which may have either a positive or negative effect on species coexistence, and environmental forces, the majority of which promote diversity and invasibility. Scale and research method are important because they determine which interactions and forces are involved in influencing diversity and invasibility and the relative strength of these factors in shaping the resulting diversity-invasibility relationship. The primary role of biotic forces in producing a negative diversity-invasibility relationship at the local level is well understood. Much less is known about how environmental factors shape the positive diversity-invasibility relationship at the regional level. An important area of future research is to quantify the different mechanisms by which species coexist (Davies *et al.*, 2007). To date, most work has examined the role of heterogeneity of resource availability in allowing native and exotic species to coexist, and in so doing influencing community diversity and invasibility.

Although propagule pressure varies in intensity across the landscape and is known to increase diversity, its role in influencing diversity-invasibility relationships in experimental communities is not well tested. In this project, I examine the role of propagule size and propagule frequency on the diversity and invasibility of three aquatic protist communities to determine whether propagule pressure could promote a positive diversity-invasibility relationship.

Species-Level Patterns of Invasion Success

Some species, such as the ship rat (*Rattus rattus*), European rabbit (*Oryctolagus cuniculus*), and water hyacinth (*Eichhornia crassipedes*), are highly successful invaders with near global distributions. Other species are not at all invasive and limited to a small native range. The Caerulean Paradise-flycatcher (*Eutrichomyias rowleyi*), for example, utilizes a total range size of only 6 square kilometers in Sangihe island, Indonesia (BirdLife International, 2009). Some invasion attempts are dramatically successful. For example, the brown treesnake (*Boiga irregularis*) probably arrived on Guam as a very small population of stowaways on U.S. military equipment shipped from the Admiralty Islands in Papua New Guinea immediately after World War II. After a lag of several years, the population grew steadily in range and abundance, until it now inhabits all terrestrial environments and maintains an extremely high density of approximately 13,000 individuals per square mile (Fritts and Leasman-Tanner, 2001). Many introductions, however, end with the disappearance of the invader. Williamson (1996) suggested, as a general rule, that only 10 per cent of introduced species establish a breeding population. A recent study of the introductions of vertebrate species to North America and Europe estimated a much higher success rate of approximately 50% (Jeschke and Strayer, 2005). Even at the higher approximation, the establishment of an introduced species is far from assured.

Invasion success is influenced by several factors. Research has focused on identifying the species characteristics, community properties, environmental factors, and interspecies interactions that promote invasion success. Proficient invaders tend to have high abundance and wide distribution in their native range, high reproductive rate, the tendency to migrate, generalist resource use, and the ability to thrive in human-modified areas (Williamson, 1996; Williamson and Fitter, 1996b; Brook, 2004). Successful invasions tend to be into habitats that have low native diversity, rich resource availability, the absence of predators and pathogens, and high habitat heterogeneity (Shea and Chesson, 2002; Essi and Dirnbock, 2008). The positive interaction between native and exotic species known as facilitation has been shown to enhance invasion (Richardson *et al*, 2000, Richardson and Pysek, 2006). Invasion is also promoted by several human-induced environmental factors including high propagule pressure, habitat degradation, environmental disturbance (Lonsdale, 1999). In contrast, invasion is minimized in areas that have strong negative species interactions including competition, predation, and parasitism (Levine and D'Antonio, 1999; Levine *et al.*, 2004).

Tilman (2004) proposed that invasion arises from the interaction of two processes: the positive effect of increased propagule pressure and the negative effect of biotic resistance. Propagule pressure is important in bringing invaders to an area, allowing them to avoid stochastic extinction, and produce a persistent population. To increase in abundance, invading populations use resources not

exploited by native community members. Invaders with resource requirements that are similar to species already at the site will not find sufficient resources to allow establishment. Therefore, processes that free up resources promote invasion (Tillman, 2004).

Of all the factors influencing invasion, propagule pressure may be the strongest predictor of the establishment and persistence of invasive species (Lockwood *et al.*, 2005; von Holle and Simberloff, 2005). High propagule pressure promotes invasion at both local and regional scales (Tilman, 1997; Lonsdale, 1999; Levine, 2000; Foster and Tilman, 2003; Knight and Reich, 2005), even in communities with strong barriers to invasion (Brown and Kodric Brown, 1977; D'Antonio *et al.*, 2000; Ahlroth, 2003; Lockwood *et al.*, 2005; Rejmanek *et al.*, 2005; Richardson and Pysek, 2006; Hayes and Barry, 2008). In general, the greater an invader's propagule size and frequency, the greater its invasion success (Pimm, 1991). For some species, however, there may be a threshold viable invader population size. For these invaders, successful establishment occurs only when the species is introduced at propagule pressure greater than the threshold value (Brook *et al.*, 2006; Traill *et al.*, 2007; Cassey *et al.*, 2008).

High propagule pressure promotes establishment by weakening several barriers to invasion (Richardson *et al.*, 2000b; von Holle and Simberloff, 2005). Invading populations tend to be quite small. To establish a persistent population, the invader must avoid extinction and achieve positive population growth at low

density (Chesson, 2000; Sakai *et al.*, 2001, Theoharides and Dukes, 2007).

Small populations have a naturally high extinction rate from demographic stochasticity and low genetic variation. Large or multiple releases of invading individuals can overcome the small founding population's high extinction rate (Brown and Kodric Brown, 1977; Lockwood *et al.*, 2005) and increase its genetic variation, possibly improving its ability to adapt to the new environment (Sakai *et al.*, 2001; Ahlroth, 2003; Brook, 2004; Lockwood *et al.*, 2005; Lavergne and Molofsky, 2007; Wilson *et al.*, 2009).

Some small invading populations also exhibit reduced growth rate at low density, the defining characteristic of the Allee effect. The fitness of these species is positively related to their population density. When the species is at low density, individual fitness is also low, and this reduces per capita growth rate (Taylor and Hastings, 2005). Species with a weak Allee effect continue to increase in abundance at low density, but at much lower rate than when community density is greater. In contrast, species with a strong Allee effect actually have negative population growth when their population density is less than some threshold value. These populations inevitably decline to extinction (Taylor and Hastings, 2005). Allee effects would reduce the establishment, increase the lag time, and slow the growth of small invading populations (Taylor and Hastings, 2005). Because it has a direct influence on the size of founding invader populations, propagule pressure may determine whether Allee effects influence a founding population's invasion success (Taylor and Hastings, 2005). Allee effects are

pervasive in many animal and plant groups, and have been found in several invasive plants, birds, insects, mollusks, and pathogens (Taylor and Hastings, 2005). For example, when the well-known invasive species, *Spartina alterniflora*, is at low density, pollen limitation causes lower seed production (Davis *et al.*, 2004a, Davis *et al.*, 2004b). If Allee effects are prevalent among invasive species, propagule pressure directly determines whether these effects influence invasion processes.

The strong positive relationship between high propagule size and invader establishment suggests many invader populations are limited by factors that depend on population size. Demographic stochasticity, low genetic variation, and Allee effects might strongly impede establishment, and this barrier is weakened by high propagule pressure (Grevstad, 1999).

Many theoretical studies have recognized the critical role propagule pressure plays in the establishment and success of invaders (Rouget and Richardson, 2003; Lockwood *et al.*, 2005; Colautti *et al.*, 2006; Pauchard and Shea, 2006; Theoharides and Battles, 2007; Eschtruth and Battles, 2009). Multivariate statistical models of exotic bird invasion suggest that high propagule pressure swamps out other factors that are important when propagule pressure is left out of the analysis. When invader immigration is high, invasion success is no longer influenced by large native range, migration tendency, and similarity between native and introduced conditions (Cassey, 2004; Lockwood *et al.*, 2005). In a

model of a diploid sexual species, increasing propagule pressure initially increases the number of invaders because new invaders introduce additional genetic variation that allows the population to better adapt to the new habitat (Traub *et al.*, 2005).

Empirical research also recognizes the importance of propagule pressure (Rouget and Richardson, 2003; Lockwood *et al.*, 2005; Colautti *et al.*, 2006; Pauchard and Shea, 2006; Theoharides and Battles, 2007; Eschtruth and Battles, 2009). Many experimental studies have identified a positive relationship between immigration rate and invader establishment (Crowell, 1973; Bierne, 1975; Ebenhard, 1989; Hopper and Roush, 1993; Veltman *et al.*, 1996; Duncan, 1997; Green, 1997; Grevstad, 1999; Sol and Lefebvre, 2000; Duncan *et al.*, 2001; Forsyth and Duncan, 2001; Ahlroth *et al.*, 2003; Marchetti *et al.*, 2004; Lockwood *et al.*, 2005; Chadwell and Engelhard, 2008). The greater the number of individuals introduced at one time, the more likely it is that establishment will occur (Lockwood *et al.*, 2005; Cassey *et al.*, 2008). This relationship has been found in groups as diverse as biocontrol agents, grassland and aquatic plants, insects, large mammals, and passerine birds (Daehler and Strong, 1993; Kowarik, 1995; Crooks and Soule, 1999; Richardson, 1999; Rejmanek, 2000; Forsyth and Duncan, 2001; Mulvaney, 2001; Kolar and Lodge, 2002; Rouget and Richardson, 2003; Bown and Peet, 2003; Kuhn *et al.*, 2004; Foxcroft *et al.*, 2004; Tilman, 2004; Wilson *et al.*, 2009). For example, Duncan *et al.* (2001) discovered that invading bird species released in higher numbers had a greater

probability of invasive success. In a study of two chrysomelid beetle species used in the biological control of purple loosestrife in New York state, persistence four years after release increased with increasing propagule size (Grevstad, 1999). Several large-scale studies independently identified propagule pressure as the strongest and most consistent determinant of success (Tilman, 1997; von Holle and Simberloff, 2005; Colautti *et al.*, 2006). A meta-analysis of 49 invasion studies covering seven biological taxa (Hayes and Barry, 2008), a review of more than 600 exotic bird introductions worldwide (Cassey *et al.*, 2004), an analysis of bird invasion on 41 oceanic islands around the globe (Cassey *et al.*, 2005) and three independent studies of Australian bird invasion (Duncan *et al.*, 2001; Duncan *et al.*, 2003; Brook, 2004) all identified propagule pressure as being the critical determinant of invasion success. Plant invasion is also influenced by propagule pressure. In one study, propagule pressure explained 70% of the variance in the number of invading plant species in experimental plots (Tilman, 1997). A recent study of the abundance of an exotic tree, *Cordia alliodora*, along transects radiating from a single source population at a plantation in Tanzania into differentially-disturbed forests found that the distance from the source population was the strongest predictor of invader abundance and even swamped the effect of disturbance, suggesting that propagule pressure controls *Cordia alliodora* invasion patterns even when habitat disturbance is substantial (Edward and Munishi, 2009).

Studies of variance identify the importance of propagule pressure in shaping global invasion patterns. Williamson (1996) believes that much of the variation in the number of invasive species in different regions around the globe is determined by variation in propagule pressure. Lonsdale (1999) agrees that variation in propagule pressure is a major factor in determining invasibility, suggesting that it accounts for up to 56% of the variance between areas.

Although high propagule pressure is a critical determinant of invasion success, it does not guarantee establishment. Propagule pressure may not be an important influence on invasion success if environmental factors are more important or if the invading population has a very high growth rate. Many species, introduced at high propagule size, have dwindled to very small population size or disappeared (Cassey *et al.*, 2008). If density-independent factors, such as unsuitable weather or insufficient patch size, are the primary determinant of invasion success in an area, propagule pressure may not be able to rescue the invading population (Grevstad, 1999). There is also a great deal of variation among the dynamics of invading populations even within the same general site, and this leads to stochasticity in invasion success (Grevstad, 1999). A study of the invasion of two chrysomelid beetle species revealed a wide range of variation in population growth rate and probability of establishment both among patches and across years (Grevstad, 1999).

High propagule pressure may not strongly influence the success of invaders that are able to establish a dynamic population from just a few introduced individuals. If the invader has very high growth rate, it may be able to achieve a large, stable population quickly and not need the hedge against extinction conferred by high propagule pressure (Grevstad, 1999). Invaders that are facilitated by native species may also be able to enter a community easily and not require high propagule pressure (Richardson *et al.*, 2000, Richardson and Pysek, 2006).

Propagule pressure is recognized as being critically important to the establishment success of most species and a large body of work highlights its importance in influencing invasion. However, the details of the relationship between propagule pressure and invasion, and the mechanisms by which propagule pressure actually facilitates establishment, are not well known. Ruiz and Carlton (2003) and Lockwood *et al.* (2005) assert that we also do not yet fully understand the fine-scale relationship between propagule pressure and establishment success. Ruiz and Carlton (2003) depict the relationship between propagule pressure and establishment success with a dose-response curve. What is the shape of a species' dose-response curve? How does the curve vary from species to species? Few experimental or theoretical studies have quantified the role of propagule pressure to the degree needed to create a dose-response curve for an invading species (Williamson, 1996; Travis *et al.*, 2005).

We do not yet understand how the individual effects of propagule size and frequency, functioning together, affect the success of an invader (Lockwood *et al.*, 2005) or how these effects vary from one invader to another (Lockwood *et al.*, 2005). Propagule size and frequency together accounted for 90% of the variation in establishment rate for bird species invading Australia and New Zealand. Of these two factors, propagule frequency was much more important (Brook, 2004). Whether propagule size or propagule frequency plays the most important role in invasion may depend on the degree of environmental stochasticity and Allee effects (Cassey *et al.*, 2008). In a model of a fluctuating environment, high propagule frequency conferred greater invasion success, but this benefit was reduced if initial propagule size was very large (Haccou and Iwasa, 1996). When Allee effects are strong, greater propagule size is needed to ensure the population is large enough to have a positive growth rate (Grevstad, 1999; Taylor and Hastings, 2005; Cassey *et al.*, 2008).

In this project, I examine the role of propagule size and frequency on the establishment and invasion success of four protist invaders. Treatment levels are scaled finely enough to create dose-response curves for each species so that the effect of variation in propagule pressure can be compared both within and among species. The curves are examined to determine whether propagule pressure influences the effect of stochastic extinction. The independent and relative effects of propagule size and frequency are compared to learn how these factors interact.

Invader Effects on the Native Community and its Members

An established invader can impact the native community directly and indirectly, in multiple ways, and at several levels of organization (Melbourne *et al.*, 2007).

Invasion can directly influence the physiology (Kittelson *et al.*, 2008), abundance, distribution, and interspecies interactions of native species. It can affect community characteristics, including species richness, community composition and diversity (Reaser *et al.*, 2007). Invasion has also been implicated in the direct disruption of ecosystem function. For example, the invasion of introduced grasses has altered the fire cycle on many tropical islands (D'Antonio and Vitousek, 1992; D'Antonio, 2000). By changing native habitats, the invader may indirectly stimulate additional effects on native species and ecosystem function (Reaser *et al.*, 2007).

Invasion is a major threat to biodiversity worldwide (Vitousek *et al.*, 1997; Duncan *et al.*, 2003). Evidence suggests that the establishment of exotic species is the primary factor stimulating the decline and extinction of native populations on islands (Reaser *et al.*, 2007). The extinction of native species has been linked to the establishment of exotic predators, parasites, and diseases. For example, the overgrazing of introduced herbivores, such as goats and rabbits, has caused well-documented direct and indirect loss of biodiversity on islands (Reaser *et al.*, 2007). Invaders that cause the extinction of native species are predicted to have strong reproductive potential, the ability to occupy a vacant niche, superior

competitive ability, few predators or pathogens in the new community, and the ability to alter resource availability or disturbance frequency (Elton, 1958; Vitousek, 1990; D'Antonio and Vitousek, 1992; Gordon, 1998). By modifying resource and disturbance conditions, the invader could potentially alter the abundance of native species, reduce species richness, and transform interspecies interactions in ways that would enhance the invader's competitive ability and facilitate even greater invasive success (D'Antonio and Vitousek, 1992; Gordon, 1998).

Although the general role of invasion in extinction is well accepted (Clavero and Garcia-Berthou, 2005; Melbourne *et al.*, 2007), the number of invasions that have caused known extinctions is few (Simberloff, 2001). Data suggest that only invasive predators cause extinction of native species (Clavero and Garcia-Berthou, 2005). Well-documented examples of invasion-caused extinction events are linked to invasive predators (Coote and Loeve, 2003). For example, the brown treesnake (*Boiga irregularis*), introduced to Guam shortly after the end of World War II, has extirpated many of the island's native species, several of which were endemic. Nine of the island's eleven native species of forest-dwelling birds have been extirpated, along with half of the native lizard species. Only three mammals were native to Guam, all species of fruit bats. Only one of these remains, and is currently at very low abundance (Birdlife International, 2009). Few, if any, known extinctions have been caused by invader-driven competitive exclusion (Davis, 2003; Gurevitch and Padilla, 2004).

A great deal of evidence actually suggests many invasion events do not result in the elimination of native species (Tillman, 2004). Indeed, invasion can increase local community diversity (Davis, 2003; Sax and Gaines, 2003; Bruno *et al.*, 2004; Gurevitch and Padilla, 2004; Melbourne *et al.*, 2007). For example, the introduction of exotic plant species to oceanic islands has caused only a few extinctions of native plants. Instead, the native and invasive species coexist, augmenting island diversity (Tillman, 2004). Similarly, the presence of invasive plants has increased floral richness in California by 20% (Tillman, 2004). The New Zealand bird community has been shaped by a historically high extinction rate. However, the recent incorporation of a large number of invasive species has resulted in the highest bird diversity the island has ever experienced (Wilson, 1997; Sax *et al.*, 2002).

By shaping patterns of invader presence and abundance, propagule pressure may influence the effect the invader has on the community. For example, in areas that have been cleared in preparation for a habitat restoration project, the propagule pressure of invasive species may influence the establishment success of native species and might even determine whether the site is eventually dominated by native or exotic species (Matus *et al.*, 2003; Reinhardt Adams and Galatowitsch, 2008). In a study of a native wet meadow in a recently restored wetland, an increase in the propagule pressure of the invader, *Phalaris arundinacea*, from 50 to 100 seeds per square meter reduced native species biomass by 50%, even though native species were seeded at the much

greater density of 3000 seeds per meter square. In this study, the propagule pressure of native species influenced the effect of invader propagule pressure. When a native species was seeded at low density, its biomass decreased with increasing invader propagule pressure. However, when the native species was seeded at higher density, its biomass was suppressed only by the highest invader propagule pressure (Reinhardt Adams and Galatowitsch, 2008). This suggests that the propagule pressures of native and invasive species interact in determining the strength of the suppression. Exotic species may have a threshold propagule pressure that influences the strength of their effect on native species. When introduced at a propagule pressure lower than the threshold, the invader will have little effect on the community. However, when introduced at a propagule pressure that exceeds the threshold, the invader suppresses the growth of native species (Reinhardt Adams and Galatowitsch, 2008). Environmental conditions might modify an invader's threshold level. When conditions are beneficial for the invader, threshold propagule pressure may decline. In contrast, harsh conditions raise the threshold value (Reinhardt Adams and Galatowitsch, 2008).

In this project, I examine the influence of four protist invaders on established species and their community. I quantify the effect of the invaders, at various propagule size and frequency values, on the presence and abundance of individual established species and on the richness, diversity, and dominance of invaded communities. Relationships are examined to determine whether

invasion has positive or negative effects on community richness, evenness, and diversity and to learn how variation in invader propagule pressure influences this effect. The species in this system are linked by competition, not predation, so invasion is not expected to result in the extinction of established species. Nevertheless, data are analyzed to learn if the presence of any of the four invaders promotes extinction in any established species and how invader propagule pressure influences this effect. Finally, data are studied to determine whether there are threshold values for invasion effects.

Specific Aims

In this study, I address four specific aims that examine the role of propagule pressure on community diversity, invasion success, and the effect of invaders on the established community.

1. To examine the role of propagule size and propagule frequency on the richness and diversity of protist communities.
2. To examine the role of propagule size and propagule frequency on the success of protist invaders.
3. To examine how propagule size and propagule frequency interact in influencing invasion success.

4. To examine how the effects invaders have on the established community and on individual community members vary with the invader's propagule size and frequency.

Protist Model System

It is possible to use a bacterivorous aquatic protist system to study the effect of propagule pressure on community diversity and invasibility, invader success, and the effect of invaders on the established community. Propagule size and frequency can be varied independently so that the individual and combined contributions of each might be examined on community diversity and invader success. For invaders, this information can be summarized with the construction of a dose-response curve. Finally, comparing the cell concentration of established species in treatments that differ in invader propagule size and frequency can illustrate the effect of invader propagule pressure on the community.

Aquatic protists that consume bacteria are an exceptionally good model system for studying the effect of propagule pressure on community dynamics in general, and invasion in particular. Protists have minimal culture requirements. They grow well in simple media that can be easily replicated and modified, and in small flasks on the bench top, allowing a myriad of treatments and replicates to be assembled. The species' small size and rapid reproductive rate allow large

communities to develop and numerous generations to elapse over the course of a short six- to eight-week experiment. As a group, aquatic protists utilize a variety of trophic levels and many species can be easily differentiated under 30x magnification. This allows diverse and complex community structures to be assembled in the laboratory and individual species within these assemblages to be differentiated. In this project, all species consume only bacteria and, since they are all known to consume many of the same species of bacteria, their resource niches overlap substantially. Therefore, all of the protist species used in this project compete with one another in all treatments, and there is no effect of predation. Aquatic protists can be easily manipulated with micropipettes and pulled capillary tubes, allowing a specific number of cells to be inoculated into experimental flasks. This permits accurate experimental assemblages to be created in replicate. Finally, the collection of protist cell concentration data is relatively simple and straightforward. Using a micropipette, a sample of exact volume can be removed from each experimental flask, the number of protists of each species counted, and their cell concentration (cells/ml) calculated. If a species' concentration is too dense to allow an accurate count, the sample can be diluted by adding an exact volume of sterile media, individual cells counted in the diluted sample, and a dilution equation used to determine the species' cell concentration in the experimental flask.

All protist species used in this study are members of the phylum Ciliophora. The species vary considerably in structure, falling into three classes: Colpodea,

Oligohymenophorea, and Heterotrichea, and several different families: Colpodidae, Paramecidae, Spirostomidae, Uronematidae, and Tetrahymenidae. The species vary considerably in growth rate. Published intrinsic rates of increase for the species range from 0.204 day^{-1} for *Spirostomum teres* (Finlay, 1977), a close relative of *Spirostomum ambiguum*, to 4.3 day^{-1} for *Uronema sp.* (Ohman and Snyder, 1991) (Figure 1). Growth rates fluctuate considerably with temperature. For example, the intrinsic rate of increase for *Paramecium bursaria* is only 0.464 day^{-1} at 15°C , but 0.818 day^{-1} at 20°C (Finlay, 1977). *Colpidium striatum* rate of increase rises from $0.1 - 0.15 \text{ hour}^{-1}$ as the temperature increases from 15°C to 20°C (Fox and Morin, 2001). Growth rate differs even among clones of the same species. The rate of increase of different clones of *Uronema sp.* has been measured as low as 0.120 hour^{-1} to as high as 0.180 hour^{-1} (Perez-Uz, 1995). In this particular study, there is wide variation in the realized rates of increase when the experimental species are grown in the assemblages used in the community diversity and invasion experiments (Table. 1-1). When communities are initiated with 8 cells of all species, realized growth rate during the period from one to six weeks following inoculation ranges from 0 for *Paramecium sp.* and $0.01 \text{ cell/ml week}^{-1}$ for *Spirostomum ambiguum* to $0.23 \text{ cells/ml week}^{-1}$ for *Tillina magna*. When communities are initiated with 128 cells of all species, realized rates of increase vary from $-0.06 \text{ cells/ml week}^{-1}$ for *Paramecium sp.* to $0.3 \text{ cells/ml week}^{-1}$ for *Colpidium striatum*. The realized rates of increase for *Uronema sp.* and *Colpidium striatum* are relatively low throughout this period because the species are already in lag phase by the time

the first data points are collected one week after inoculation. However, the cell concentration of species, seven days after inoculation, clearly shows that *Uronema sp.* and *Colpidium striatum* have considerably greater realized growth rates than the other species used in this study (Table 1-1). After seven days of growth in the experimental community, *Uronema sp.* concentration is 153 cells/ml and *Colpidium striatum* concentration is 313 cells/ml, while the combined concentration of all other species is less than 3.5 cells/ml. The abundance of the experimental species six weeks after inoculation shows the wide variation in these species' realized growth (Figure 1-1). Cell concentration values range from 0 cells/ml for *Paramecium sp.* and 0.48 cells/ml for *Spirostomum ambiguum* and *Paramecium bursaria*, to 113 cells/ml for *Tillina magna*, 766 cells/ml for *Uronema sp.* and 5069 cells/ml for *Colpidium striatum*.

Terms Used in this Paper

In this study, assemblages of protist species inoculated simultaneously into fresh media and allowed to grow together are referred to as *established communities*. The species comprising these communities are referred to as *established species*. A single protist species introduced to an established community after the community has developed for two or three weeks is referred to as an *invader*. An established community and its invader, together, are referred to as the *whole community*.

The size of the introduced invader population is its *propagule size*. The number of times an invader is inoculated into the established community during the experiment is referred to as *propagule frequency* in this paper. In invasion literature, however, this factor is typically called propagule number. The terms *propagule size* and *propagule frequency number* were developed to describe the characteristics of invasion. However, in Specific Aim #1 of this study *propagule size* and *propagule frequency* are also used to describe the size and number of inoculations of established species used to create the established community.

Species	Mean Abundance (cells/ml)		Mean Realized Rate of Increase (cells/ml week ⁻¹)		Intrinsic Rate of Increase (r _m day ⁻¹)
	Week 1	Week 6	Propagule Size 8	Propagule Size 128	
<i>Paramecium sp.</i>	0	0	0 r ² = 1.0	-0.06 ± 0.01 r ² = 1.0	1.32 (a)
<i>Spirostomum ambiguum</i>	0.48 ± 0.48	0.48 ± 0.48	0.01 ± 0.02 r ² = 0.01	0.04 ± 0.02 r ² = 0.05	0.204 (b)
<i>Paramecium bursaria</i>	0	4.8 ± 1.9	0.21 ± 0.02 r ² = 0.66	0.21 ± 0.01 r ² = 0.85	0.816 (a) 0.818 (b)
<i>Tillina magna</i>	2.9 ± 2.0	113.3 ± 18.5	0.23 ± 0.03 r ² = 0.67	0.21 ± 0.02 r ² = 0.70	Mean daily division rate = 2.63 (c), 2.65(d)
<i>Uronema sp.</i>	152.9 ± 16.7	765.7 ± 192.5	0.10 ± 0.02 r ² = 0.44	0.13 ± 0.02 r ² = 0.57	2.9 (e), 3.3 (f), 3.5 (g), 4.3 (e)
<i>Colpidium striatum</i>	313.5 ± 125.5	5068.6 ± 1423.9	0.15 ± 0.04 r ² = 0.36	0.30 ± 0.03 r ² = 0.80	1.5 (h), 2.4 - 3.6 (i)

Table 1-1. The mean abundance, realized rate of increase, and intrinsic rate of increase of *Paramecium sp.*, *Spirostomum ambiguum*, *Paramecium bursaria*, *Tillina magna*, *Uronema sp.*, and *Colpidium striatum*. The abundance values are from populations grown in communities initiated with 8 cells of all species. The realized rate of increase values are from species grown in communities initiated with 8 and 128 cells of all species. Values are the means of six replications for each species, four for *Colpidium striatum*. Intrinsic rate of increase data are from published literature: (a) Taylor, 1978; (b) Finlay, 1977; (c) Beers, 1944; (d) Beers, 1946. (e) Ohman and Snyder, 1991; (f) Turley *et al.*, 1986; (g) Hamilton and Preslan, 1970, (h) Petchey, 2000; (i) Fox and Morin, 2001.

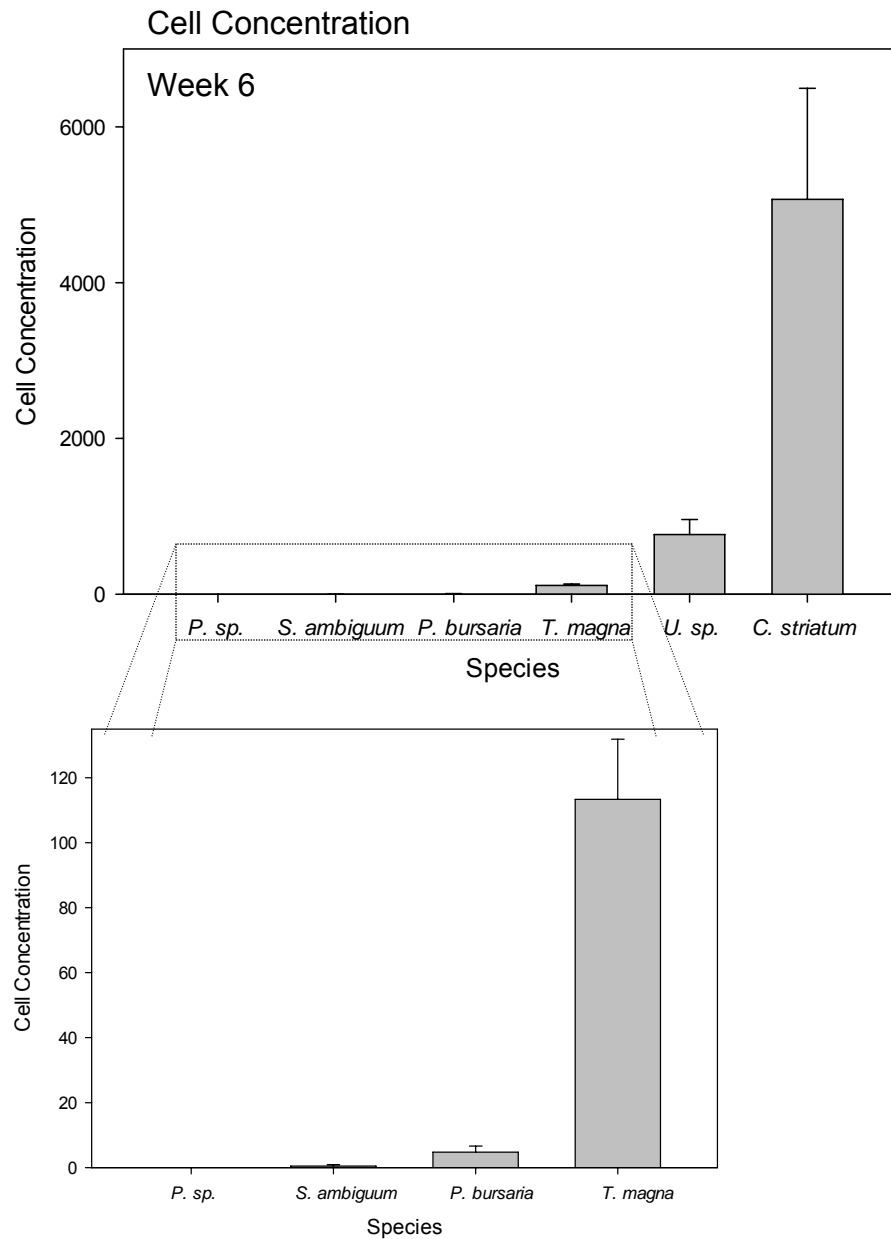


Figure 1-1. The cell concentration, at week 6, of *Paramecium sp.*, *Spirostomum ambiguum*, *Paramecium bursaria*, *Tillina magna*, *Uronema sp.*, and *Colpidium striatum* in communities initiated with 8 cells of each species. Plotted values are the means (± 1 SE) of six replications of each species, four for *Colpidium striatum*.

CHAPTER 2. THE ROLE OF PROPAGULE PRESURE ON THE RICHNESS AND DIVERSITY OF ESTABLISHED PROTIST COMMUNITIES.

Introduction

The way in which environmental factors shape the positive diversity-invasibility relationship at the regional level is not well understood, and quantifying the different mechanisms by which species coexist is considered to be an important area of future research (Davies *et al.*, 2007). To date, most work has examined the role of heterogeneity of resource availability in allowing native and exotic species to coexist, and in so doing influencing community diversity and invasibility. Propagule pressure may also be a diversity-promoting factor. It exhibits the two characteristics necessary to promote a positive diversity-invasibility relationship at the regional level. High levels of propagule pressure are known to increase community diversity and there is heterogeneity in the intensity of propagule pressure across the landscape. Nevertheless, the role of propagule pressure in influencing regional diversity-invasibility relationships in experimental communities is not well tested. High propagule pressure has been found to promote diversity in a riparian plant community (Brown and Peet, 2003), and I expect that it enhances diversity in protist communities as well. In Specific Aim #1, I examine the role of propagule size and propagule frequency on the diversity of three different bacterivorous aquatic protist communities, each with a unique dominant species. I predict that high propagule size and high propagule

frequency will promote coexistence of native community members, resulting in greater species richness and community diversity, and lower dominance. The results of this experiment will be complemented by those of Specific Aim #2 which examines the role of propagule pressure on invasion success. Taken together, the results of these two specific aims will suggest the system's diversity-invasibility relationship.

Materials and Methods

Experimental Treatments

To determine the influence of propagule pressure on community diversity, identical assemblages of protist species were initiated and maintained under several different propagule size and frequency conditions, as illustrated in Figure 2-1. To examine the role of propagule size, communities were initiated with 8, 16, 32, 64, or 128 cells of each species. To examine the role of propagule frequency, *Tillina*-dominated communities were initiated with 16 cells of each species. The treatments were maintained with subsequent additions of 32 or 64 cells of each species for a total of 1, 2, 3, or 6 additions over a period of five weeks, as illustrated in the timeline of figure 2-1. In *Uronema sp.*-dominated communities, the effect of propagule frequency was examined using initial and subsequent additions of 128-cells of each species. In *Colpidium striatum*-dominated communities 16-cell additions were used.

Preparation of Experimental Flasks and Culture Conditions

Resource Base. All treatments utilized a standard resource base that employed a protist media made by autoclaving 1 Carolina Biological protist pellet and 1375 ml well water for 30 minutes. Twenty-four hours later, the media was divided among treatment vessels, 100 ml media in a sterile 250-ml flask containing two sterile wheat seeds, capped and then autoclaved an additional 30 minutes to ensure sterility. After cooling, individual treatment flasks were inoculated with 150 ml volumes from each overnight culture of bacterial species known to be consumed by all protists used in this system: *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Serratia marcescens*. To ensure consistency in bacterial composition across all treatments, each experimental flask was also inoculated with 300 ml of an overnight culture of pooled protist-free stock media made by passing 2 ml stock solution from all species used in the experiment through a 1.2 micron syringe filter. Immediately after inoculation, flasks were refrigerated until needed. Twenty-four hours before protists were to be added, flasks were placed on the bench top and bacterial cultures were allowed to develop at room temperature (20-21° C).

Culture Conditions. During the experiment, experimental flasks were stored on the bench top at room temperature (20-21° C). To reduce the concentration of waste and add fresh nutrients, every seven days 10% (10 ml) of the media from

each flask was removed and replaced with 10 ml sterile media. At this time, one sterile wheat seed was added to each flask to provide a slow release of additional nutrients.

Protist Species. Protists added to experimental flasks came from healthy stock cultures. To ensure consistency, all the cells of each species used in an experiment came from the same stock flask. The number of protist cells required for inoculation was exactly counted for treatments 8, 16, and 32. For treatments requiring a larger number of cells, the volume of stock culture inoculated into experimental flasks was adjusted so that it contained an estimate of the correct number of cells based on an average stock cell concentration determined by the mean of ten independent samples withdrawn immediately prior to inoculation.

Replication. All propagule size and propagule frequency treatments were replicated six times. The only exceptions were the propagule size treatments utilizing *Colpidium striatum*-dominated communities. These were replicated four times.

Data Collection and Analysis

Data Collection. Data were collected on all experimental flasks every seventh day, starting with the seventh day following the inoculation of protists, and continuing for a total of eight weeks as shown in the timeline, figure 2-1. Each experimental flask was swirled and a 350- μ l sample of media removed.

Determining Cell Concentration. The number of cells of each protist species in each sample was counted and then converted to cell concentration (# cells/ml) using the equation:

$$\text{Cell concentration in experimental flask} = \# \text{ cells in } 350\text{-}\mu\text{l sample} / 0.35$$

If the number of cells in a sample was too great to count, as was sometimes the case for *Uronema sp.* and *Colpidium striatum*, the sample was serially diluted and cell concentration determined by:

$$\text{Cell concentration in experimental flask} = (\# \text{ cells in } 350\text{-}\mu\text{l sample} \times 2^{(\# \text{ dilutions})}) / 0.35$$

When log cell concentration was used, for instance in graphs that showed the growth of a population over the course of the experiment, cell concentrations were log-transformed as:

$$\text{Log cell concentration} = \text{Log}_{10}(\text{cell concentration} + 1)$$

Determining Species Richness. Species richness was determined for each flask every week as the number of species recovered in the 350- μ l sample. Mean species richness was calculated for each treatment every week.

Determining Community Diversity. Diversity was calculated for each flask every week using the Shannon Index:

$$H = -\sum_{i=1}^S (p_i \ln p_i), \text{ where } p_i \text{ is the proportion of species } i \text{ in the community of } S \text{ species}$$

Mean diversity was calculated for each treatment every week.

Determining Dominance. The dominance of the most abundant species in each assemblage was calculated for each flask every week:

$$\text{Dominance} = \text{cell concentration of most abundant species} / \text{community cell concentration}$$

Mean dominance was calculated for each treatment every week.

Statistical Analysis.

Propagule Size. To determine whether propagule size influenced cell concentration, diversity, species richness, or species evenness, values of the dependent variable were compared across propagule size treatments with regression analysis using SPSS Statistics 17.0 software (SPSS, Inc., 2008). Propagule size values, transformed as log base 2, and measured dependent variable data were both identified as numeric scale measures. A generalized linear model used propagule size as the predictor and the different variables as the response to fit a linear curve to the data set. A Wald's Chi-Square test determined the significance of the relationship.

Propagule Frequency. To determine whether propagule frequency influenced values of the dependent variable, cell concentration, diversity, species richness, and species evenness data were compared across propagule frequency treatments with ANCOVA using SPSS Statistics 17.0 software (SPSS, Inc., 2008). Values of propagule frequency and measured dependent variable data were both identified as numeric scale measures. The categorical data of addition size was identified as a factor. A generalized linear model used propagule size data as the covariate predictor, addition size as the factor, and the dependent variable data as the response to fit a linear curve. The model considered the main effects of propagule frequency and addition size, as well as the interaction of propagule frequency and addition size, on the values of the dependent variable. A Wald's Chi-Square test determined the significance of the factor and covariate. In one case, figure 2-27, an insignificant interaction term hindered the ability of the model to identify the significance of the main effects. The model was run without the interaction term for this data set.

The chi-square values, degrees of freedom, and probability for all tests included in this chapter are given in Appendix: Results of Statistical Analysis.

Protist Communities

The experimental design described in this section was repeated with three different assemblages of bacterivorous protists. Four of the species grow

relatively slowly: *Spirostomum ambiguum*, *Tillina magna*, *Paramecium sp.*, and *Paramecium bursaria*. Throughout the experiment, these species were always found in numbers that could be easily counted in undiluted samples. Six weeks after inoculation, most of these species had cell concentrations less than 5.0 cells/ml. *Tillina magna* reached the greatest abundance, 113.3 cells/ml (Table 1-1). In contrast, two other species, *Uronema sp.* and *Colpidium striatum*, grow much more rapidly and were often recovered at concentrations too dense to count without serial dilution. Six weeks after inoculation, *Uronema sp.* had attained a concentration of 765.7 cells/ml and *Colpidium striatum* had reached 5068.6 cells/ml (Table 1-1). Since all of the species consume the prokaryotic resource base employed in these experiments, competition is the dominant interspecies interaction structuring all three communities. Although many of the same species are present in all three assemblages, each community has a unique dominant species. Assemblages are listed in columns below, with the dominant species of each shown in bold at the top of the column.

<i>Tillina magna</i>	<i>Uronema sp.</i>	<i>Colpidium striatum</i>
<i>Paramecium bursaria</i>	<i>Tillina magna</i>	<i>Tillina magna</i>
<i>Spirostomum ambiguum</i>	<i>Paramecium bursaria</i>	<i>Paramecium bursaria</i>
<i>Paramecium sp.</i>	<i>Spirostomum ambiguum</i>	<i>Spirostomum ambiguum</i>
	<i>Paramecium sp.</i>	<i>Paramecium sp.</i>

Results

Propagule Size

Tillina magna-dominated Community

Diversity. During the first 4 weeks of culture, low propagule size treatments (8, 16, 32) tend to increase in diversity while high propagule size treatments (64, 128) remain stable (Figure 2-2A). From week 4 to 6, the diversity values of all treatments are essentially stable, making this the best time period to analyze. All propagule size analysis is made from values taken during this period. On week 5, there is a significant increase in community diversity with increasing propagule size ($\chi^2 = 39.706$, $df = 1$, $P < 0.01$) (Figure 2-2B). This pattern is seen every week during the week 4–6 period, up to the end of the experiment (week 8).

Species Richness. The species richness of some treatments increases over the period 4-6 weeks after inoculation, and the richness of other treatments decreases, but this variation is slight and the overall pattern is essentially stable (Figure 2-3A). During this time, there is a trend of increasing richness with increasing propagule size. This trend is most pronounced, and strongly significant, at week 6 ($\chi^2 = 19.109$, $df = 1$, $P < 0.01$) (Figure 2-3B).

Dominance. *Tillina magna* is the dominant species in this system, but its dominance of the assemblage decreases with increasing propagule size. During weeks 4-8, this species accounts for less than 50% of the total cell concentration in all flasks in treatments 8, 16, and 32. However, as propagule size increases at the higher treatments of 64 and 128, the second dominant species, *Paramecium bursaria*, accounts for greater than 50% of the total cell concentration in an increasing number of flasks (2 at 64; 4 at 128). The decrease in *Tillina magna* dominance with increasing propagule size is even more evident when the actual proportion of *Tillina magna* in communities is examined. During each week, 4 through 6, there is a significant pattern of decreasing *Tillina magna* proportion within the assemblage as propagule size increases ($\chi^2 = 35.007$, $df = 1$, $P < 0.01$) (Figure 2-4).

Total Cell Concentration. The cell concentration of all treatments increases during the 4 to 6 week period following inoculation. During this time, there is also a significant trend of increased total cell concentration with increasing propagule size ($\chi^2 = 18.804$, $df = 1$, $P < 0.01$) (Figure 2-5). This pattern is seen every week during the period.

Diversity and Richness when Cell Concentration is Held Constant. During weeks 4-6, the cell concentration of treatments covaries with propagule size. Are the positive relationships of increased community diversity and increased species richness associated with increased propagule size the result of propagule size

itself or are they associated instead with the increase in cell concentration? To test, diversity and richness trends were analyzed for treatments that have similar cell concentrations. There are two cell concentrations during the general period 4-6 weeks after inoculation that are repeated, albeit loosely, in all treatments:

1. “65” cells/ml – Seen at Week 3 for treatments 8 (50 cells/ml), 32 (70 cells/ml) and 128 (68 cells/ml); Week 4 for treatments 16 (64 cells/ml) and 64 (61 cells/ml).
2. “100” cells/ml – Seen at Week 4 for 32 (95 cells/ml) and 128 (134 cells/ml); Week 5 for 16 (95 cells/ml) and 64 (119 cells/ml); and Week 6 for 8 (119 cells/ml).

Diversity at Constant Cell Concentration. There is a strong significant trend of increasing community diversity with increasing propagule size at both 65 ($\chi^2 = 41.704$, $df = 1$, $P < 0.01$) (Figure 2-6A) and 100 cells/ml ($\chi^2 = 41.366$, $df = 1$, $P < 0.01$) (Figure 2-6B).

Species Richness at Constant Cell Concentration. Although this pattern is not as pronounced as that of diversity, there is still a significant increase in species richness with increased propagule size at 65 cells/ml ($\chi^2 = 13.158$, $df = 1$, $P < 0.01$) (Figure 2-7A) and 100 cells/ml ($\chi^2 = 7.009$, $df = 1$, $P < 0.01$) (Figure 2-7B).

Dominance at Constant Cell Concentration. The pattern of decreased dominance with increasing propagule size is found when all treatments have similar cell concentration. At both 65 and 100 cells/ml, the

proportion of *Tillina magna* decreases sharply and significantly as propagule size increases (65 cells/ml: $\chi^2 = 35.237$, df = 1, $P < 0.01$, Figure 2-8A) (100 cells/ml: $\chi^2 = 4.362$, df = 1, $P < 0.01$, Figure 2-8B).

Effect of Propagule Size on the Growth of the Dominant Species. The population growth of *Tillina magna* is strongly influenced by propagule size. The species' cell concentration increases significantly as propagule size increases ($\chi^2 = 6.642$, df = 1, $P = 0.010$) (Figure 2-9). The pattern of increasing community diversity, increasing species richness, and decreased dominance with increasing propagule size is found even though the dominant species' growth is enhanced at higher propagule size. Therefore, propagule size must have a very strong influence on the non-dominant species in the assemblage.

Effect of Propagule Size on the Growth of Non-dominant Species.

Paramecium bursaria is the second dominant species in this system. In all cases where *Tillina magna* accounted for less than 50% of total community cell concentration, *Paramecium bursaria* was the dominant species. *Paramecium bursaria* is strongly influenced by propagule size. During the week 4-6 period, *Paramecium bursaria* cell concentration increased from approximately 5 cells/ml in flasks initiated with 8 cells to 50-80+ cells/ml in treatments initiated with 128 cells. There is a smooth and significant trend of increasing cell concentration with increasing propagule size at all weeks, especially week 6 ($\chi^2 = 76.905$, df = 1,

$P < 0.01$) (Figure 2-10).

Spirostomum ambiguum grows weakly in this assemblage, but its growth is significantly affected by propagule size. At every week during the experiment, except one, treatments initiated with 128 cells have much greater cell concentration than those started with 8 cells. During the weeks 4-6 period, cell concentrations of treatment 8 are essentially no different than 0, while those of treatment 128 are 2-5 cells/ml. At week 6, there is a strong and significant trend of increased *Spirostomum* cell concentration with increased propagule size 6 ($\chi^2 = 15.105$, $df = 1$, $P < 0.01$) (Figure 2-11). Propagule size also affects the number of flasks in which *Spirostomum ambiguum* is recovered in data collection samples. Six weeks after inoculation, there is a strong pattern of an increased *Spirostomum ambiguum* presence in flasks.

Paramecium sp. is the weakest competitor in the assemblage. It is never found in any data collection sample taken from any propagule size treatment. The species clearly grows very poorly, if at all, as an established species in this assemblage and is not affected by propagule size in any way that can be measured by the data collection method used in this experiment.

Summary. Propagule size is a diversity promoting-factor in this protist community. Large propagule size promotes coexistence among established species, creating significant trends of increased diversity and species richness, and decreased dominance. These trends are the result of increased propagule size, and not simply associated with the greater cell concentration found at higher propagule size. The growth of the dominant species in this assemblage, *Tillina magna*, is positively affected by propagule size. The species attains significantly greater cell concentration at high propagule size. Despite this, the dominance of the species declines with increasing propagule size. This is because the positive effect of increased propagule size is extremely strong for two of the non-dominant species, *Paramecium bursaria* and *Spirostomum ambiguum*.

Uronema sp.-dominated Community

Diversity. The diversity of all treatments increases through week 4, and then becomes essentially stable from week 4 through week 6 (except for the diversity of treatment 128 on week 5 which is higher than that on week 4). All analysis of propagule size is made during the week 4-6 period. During weeks 4, 5, and 6, there is a clear pattern of increased diversity with increasing propagule size. On week 5, the pattern is highly significant ($\chi^2 = 11.677$, $df = 1$, $P = 0.001$) (Figure 2-12).

Species Richness. Throughout weeks 4-6, the species richness of low propagule size treatments (8, 16, and 32) is nearly perfectly stable. The richness of higher propagule size treatments (64 and 128) increases only slightly during this period. During weeks 4-6, there is a strong and significant pattern of increased species richness with increasing propagule size ($\chi^2 = 67.431$, $df = 1$, $P < 0.01$) (Figure 2-13).

Dominance. Propagule size does not influence dominance in this community. The mean proportion of *Uronema sp.* is nearly identical across all treatments, and differences are not significant.

Total Cell Concentration. The cell concentration of this community increases steadily from week 1 through week 6. There is a trend of increasing cell concentration with increasing propagule size, but it is not significant.

Effect of Propagule Size on the Growth of the Dominant Species. *Uronema sp.* is the dominant species in this system, accounting for the vast majority of the community. During week 6, *Uronema sp.* accounts for approximately 80% of the total cell concentration in all treatments, 8 to 128. *Uronema* cell concentration throughout much of this 8-week experiment ranges from 500-1500 cells/ml. In contrast, the combined cell concentration of the four non-dominant species never exceeds 250 cells/ml. Propagule size does not influence the growth of *Uronema sp.* The species' cell concentration is similar across all treatments.

Summary. Many characteristics of the *Uronema*-dominated community are positively affected by increasing propagule size. Large propagule size significantly increases this community's diversity and richness, but does not affect dominance. The growth of *Uronema* sp. is not influenced by propagule size, and the species comprises the vast majority of the community (80%), so slight changes in the proportion of non-dominant species are likely not apparent.

Colpidium striatum-dominated Community

Diversity. The diversity of all treatments increases until reaching maximum value on week 4, followed by a decline. From week 3 until week 5, the diversity of all treatments is essentially stable. During this period, there is a pattern of increasing diversity with increased propagule size. On week 3, the increase in diversity between treatments 8 and 128 is significant ($\chi^2 = 7.732$, $df = 1$, $P = 0.005$) (Figure 2-14).

Species Richness. Propagule size influences species richness in this community. All five species and all twenty introduced populations are present at the end of the experiment in high propagule size treatments 32, 64, and 128. Species and populations are missing from treatments with lower propagule size, 8 (1 *Tillina* population missing, richness = 4.75) and 16 (3 *Tillina* populations missing, richness = 4.25). On week 5, there is a significant trend of increased

species richness with increasing propagule size ($\chi^2 = 18.621$, $df = 1$, $P < 0.01$) (Figure 2-15).

Total Cell Concentration. The growth of this community is nearly identical across all treatments throughout the entire experiment. Propagule size does not influence cell concentration in this system. This is probably because propagule size has minimal effect on the dominant species in the assemblage. With cell concentrations over 5,000 cells/ml, compared to combined cell concentrations less than 180 cells/ml for the four non-dominant species, *Colpidium striatum* accounts for approximately 97% of all cells in the flask. Propagule size does have a positive affect on the growth and combined cell concentration of the four non-dominant species. Throughout the experiment, with the exception only of week 6, the combined cell concentration of the established species in treatment 128 is greater than that of treatment 8. However, this result is not apparent at the community level because the vast majority of the community is *Uronema sp.*, a species that does not respond to propagule size.

Summary. This community shows a significant increase in diversity and species richness with increasing propagule size. Propagule size does not influence total cell concentration, however. This is because *Colpidium striatum* accounts for approximately 97% of the cells in the experimental flasks, and the growth of *Colpidium striatum* is not influenced by the propagule size values used in this experiment. An inoculum as small as 8 cells allows maximum *Colpidium striatum*

growth. Although the growth of the non-dominant species in the community is promoted by high propagule size, this effect is swamped by the sheer dominance and stability of *Colpidium striatum* across all treatments.

Propagule Frequency

Tillina magna-dominated Community

All the communities in this experiment, regardless of treatment level, are initiated with a sixteen-cell inoculum. A previous experiment showed that this propagule size is not large enough to maintain all species throughout the course of the experiment. Since some species are naturally lost, a positive effect from high propagule frequency is visible. To investigate whether the size of additions influences the effect of propagule frequency, subsequent additions are of two sizes. Either 32- or 64-cell additions of each species are added to flasks until a total of 1, 2, 3, or 6 inoculations are made during the first five weeks of culture (Figure 2-1). Since the final addition of cells is not made until week 5, all analysis is done on data taken from weeks 6 to 8.

Diversity. The diversity of this community remains stable from week 2 until the end of the experiment on week 8. Over the week 6-8 period, the diversity of all treatments at both addition sizes, 32 and 64, is essentially constant

(Figure 2-16). During this time, a strong and significant pattern of increasing diversity with increased propagule frequency is evident in both 32-cell addition and 64-cell addition treatments. However, at every propagule frequency, the diversity values of treatments 32 and 64 are very similar. In this assemblage, propagule frequency has a significant effect on diversity ($\chi^2 = 31.360$, $df = 1$, $P < 0.01$), but the size of additions does not have an influence (Figure 2-17).

Species Richness. Propagule frequency influences species richness, but the size of additions has a negligible effect. The species richness of all treatments is essentially stable over the week 4-6 period. During this time, there is a clear and significant pattern of increased richness with increased propagule frequency for both 32-cell addition and 64-cell addition treatment series ($\chi^2 = 36.414$, $df = 1$, $P < 0.01$) (Figure 2-18). There is no real difference between the richness of 32- and 64-cell addition treatments (Figure 2-18).

Dominance. The dominance of *Tillina magna* in this community decreases with increasing propagule frequency. The proportion of *Tillina magna* in all treatments is essentially stable through the week 6-8 period, and a significant trend of decreasing proportion with increased propagule frequency is evident in both the 32- and 64-cell addition treatments ($\chi^2 = 20.391$, $df = 1$, $P < 0.01$) (Figure 2-19). In contrast, the size of additions has no effect on *Tillina magna* dominance. The decrease in *Tillina magna* proportion with increasing propagule frequency is strong enough to influence the number of flasks in which *Tillina magna* accounts

for greater than 50% of the total cell concentration. *Tillina magna* is the dominant species in all 1x flasks from weeks 6, 7, and 8. However, the number of flasks in which *Tillina magna* is the dominant species decreases steadily with increasing propagule frequency for both 32- and 64-cell additions.

Community Cell Concentration. The cell concentration of all treatments increases during weeks 4-6 and there is a pattern of increasing total cell concentration with increasing propagule frequency. During week 8, the increase in cell concentration with propagule frequency is significant ($\chi^2 = 9.745$, $df = 1$, $P = 0.002$) (Figure 2-20). In contrast, the size of additions made in the propagule frequency series does affect community concentration.

Diversity and Richness when Cell Concentration Held Constant. During weeks 6-8, the cell concentration of treatments covaries with propagule frequency. It is necessary to determine whether diversity, richness, and dominance are responding to propagule frequency and not simply associated with total community cell concentration. To do this, diversity, richness, and dominance trends are compared across treatments that have similar cell concentration. There is one cell concentration during the general period 6-8 weeks after inoculation that is loosely repeated in all treatments:

“200” cells/ml – Seen at Week 6 for 1x (233 cells/ml) and

6x64 (197 cells/ml); Week 7 for 2x32 (218 cells/ml), 3x32 (225 cells/ml), 6x32 (223 cells/ml), 3x64 (216 cells/ml); and Week 8 for 2x64 (231 cells/ml).

Diversity at Constant Cell Concentration. When all treatments have a cell concentration of approximately 200 cells/ml, there is a strong significant pattern of increased community diversity with increasing propagule frequency for both 16-cell addition and 32-cell addition series ($\chi^2 = 27.826$, $df = 1$, $P < 0.01$) (Figure 2-21A). The size of additions does not influence diversity.

Species Richness at Constant Cell Concentration. When treatments have approximately the same cell concentration, there is a strong significant trend of increased richness with increased propagule frequency for both 32-cell addition and 64-cell addition series ($\chi^2 = 15.022$, $df = 1$, $P < 0.01$) (Figure 2-21 B). The size of additions, however, does not influence community concentration.

Dominance at Constant Cell Concentration. When all treatments have a total cell concentration of approximately 200 cells/ml, the proportion of *Tillina magna* shows a strong significant pattern of decreased value as propagule frequency increases for both the 32-cell and 64-cell addition series ($\chi^2 = 10.276$, $df = 1$, $P = 0.001$). Addition size has no effect

(Figure 2-22). When all treatments have a cell concentration of 200 cells/ml, the number of flasks in which *Tillina magna* accounts for greater than 50% of the total cell concentration decreases steadily and significantly with increasing propagule frequency ($\chi^2 = 8.544$, $df = 1$, $P = 0.003$) (Figure 2-23). Although this trend is much stronger for the 64-cell addition series, the effect of addition size does not significantly influence *Tillina magna* presence.

These results suggest that the increased diversity and species richness, and decreased dominance, associated with increased propagule frequency is actually the result of increases in propagule frequency itself, and not simply the result of increased cell concentration in higher propagule frequency flasks.

Effect of Propagule Frequency on the Growth of the Dominant Species.

Propagule frequency does not influence the growth of the dominant species in the community. The cell concentration of *Tillina magna* is essentially the same in all treatments during the week 6-8 period (Figure 20-24). Since propagule frequency does significantly affect community diversity, species richness, and dominance, it must have a very strong influence on the non-dominant species in community.

Effect of Propagule Frequency on the Growth of Non-dominant Species.

Paramecium bursaria growth is strongly affected by propagule frequency but not

by the size of additions. During weeks 6, 7, and 8, there is a significant trend of increasing cell concentration with increasing propagule frequency for both the 32-cell and 64-cell addition series ($\chi^2 = 11.790$, $df = 1$, $P = 0.001$) (Figure 2-25). The substantial increase in *Paramecium bursaria* concentration at higher propagule frequency probably plays a critical role in creating the diversity and species richness patterns associated with increasing propagule frequency in this community.

Spirostomum ambiguum grows poorly in this assemblage, but is strongly affected by propagule frequency and the size of additions. When additions are of 32 cells, *Spirostomum ambiguum* has a lag phase during the first five weeks of the experiment. When additions are of 64 cells, the lag phase is reduced to four weeks. During this time, all propagule frequency treatments have nearly identical cell concentration. Once lag phase ends, the cell concentrations of the 6x(32) and 6x(64) treatments increase tremendously. From week 5 until the end of the experiment, the cell concentration of 6x(64) is higher than that of 6x(32). On week 8, the pattern of increasing *Spirostomum ambiguum* cell concentration with increasing propagule frequency is significant for both the 32-cell and 64-cell addition series ($\chi^2 = 4.009$, $df = 1$, $P = 0.045$) (Figure 2-26). However, the size of additions does not have a significant effect on *Spirostomum ambiguum* concentration. The influence of propagule frequency on *Spirostomum ambiguum* growth also affects the number of

flasks in which the species is found. Eight weeks after inoculation, the species is recovered from an increasingly greater proportion of flasks as propagule frequency rises.

Paramecium sp. is a very weak competitor in this assemblage. However, propagule frequency significantly affects *Paramecium sp.* growth. During the first weeks of culture, the species is not recovered in any treatment. Starting at week 5, the species begins to show exponential growth in treatments 6x(32) and 6x(64) while the concentration of *Paramecium sp.* continues to remain at nearly 0 cells/ml in all lower propagule frequency treatments. On week 7, there is a significant increase in *Paramecium sp.* cell concentration with increasing propagule frequency in both the 32-cell and 64-cell addition series ($\chi^2 = 19.426$, $df = 1$, $P < 0.01$) (Figure 2-27). The size of additions does not significantly influence *Paramecium sp.* growth. The growth pattern of 6x(32) and 6x(64) are nearly identical throughout the entire experiment. Propagule frequency influences the presence of *Paramecium sp.* in flasks. The species is not found in any one-addition flask, regardless of propagule frequency. It is found in one two-addition flask, one three-addition flask, and seven six-addition flasks.

Summary. Propagule frequency is a diversity-promoting factor in the *Tillina magna*-dominated community. Increasing propagule frequency is associated with significant increases in diversity and richness, and a significant

decrease in dominance. The pattern is found when treatments of the same maturity are compared and also when treatments of the same concentration are compared, regardless of maturity. This suggests that these patterns do not arise simply as a natural consequence of higher community concentration at higher propagule frequency. The patterns instead are directly promoted by propagule frequency itself. In contrast, the size of additions does not appear to influence community diversity, species richness, or cell concentration. Propagule frequency does not influence the growth of the dominant species in this community. *Tillina magna* concentration is essentially equivalent across propagule frequency treatments. Since the community shows strong diversity and dominance patterns related to propagule frequency, propagule frequency must have a strong effect on the non-dominant species. In fact, all three of the non-dominant species show a strong, significant positive relationship with propagule frequency.

Uronema sp.-dominated Community

Diversity. The diversity of all treatments increases to a maximum at week 5, then declines. Following week 3, the rate of increase declines. During the period from week 3 to 6, diversity is essentially stable and there is a clear trend of increased diversity with increasing propagule frequency. On week 5, this trend is significant ($\chi^2 = 11.236$, $df = 1$, $P = 0.001$) (Figure 2-28). On week 6, the trend just misses significance ($\chi^2 = 3.428$, $df = 1$, $P = 0.064$).

Species Richness. Species richness increases slightly from week 1 to 6. During the first weeks, there is considerable variation among treatments. However, by the time the final cell additions are made on week 5, all treatments have similar species richness and any difference is not significant.

Dominance. The proportion of *Uronema sp.* declines weakly with increasing propagule frequency. The decline is most pronounced on week 6, but this pattern is still not quite significant ($\chi^2 = 3.428$, $df = 1$, $P = 0.064$).

Total Cell Concentration. Cell concentration increases steadily throughout the experiment. After the final cell additions are made, there is no significant difference in the cell concentration of different treatments.

Cell Concentration of the Dominant Species. On week 6, *Uronema sp.* cell concentration is essentially identical across all treatments.

Summary. Increasing propagule frequency significantly increases diversity in the *Uronema sp.*-dominated community. Although there is a pattern of decreasing dominance with increasing propagule frequency, the trend just misses significance. Propagule frequency has a negligible effect on species richness and cell concentration. The total cell concentration of this community is probably not affected by propagule frequency because the dominant species in the

assemblage is not affected by propagule frequency. *Uronema* is a rapidly-growing species that quickly dominates the community, comprising approximately 80% of the total cell concentration. One addition of 128 cells is sufficient to give maximum *Uronema sp.* growth. Additional inoculations do not provide benefit. If the size of the initial addition had been much smaller, an effect of propagule frequency might have been observed.

Colpidium striatum.-dominated Community

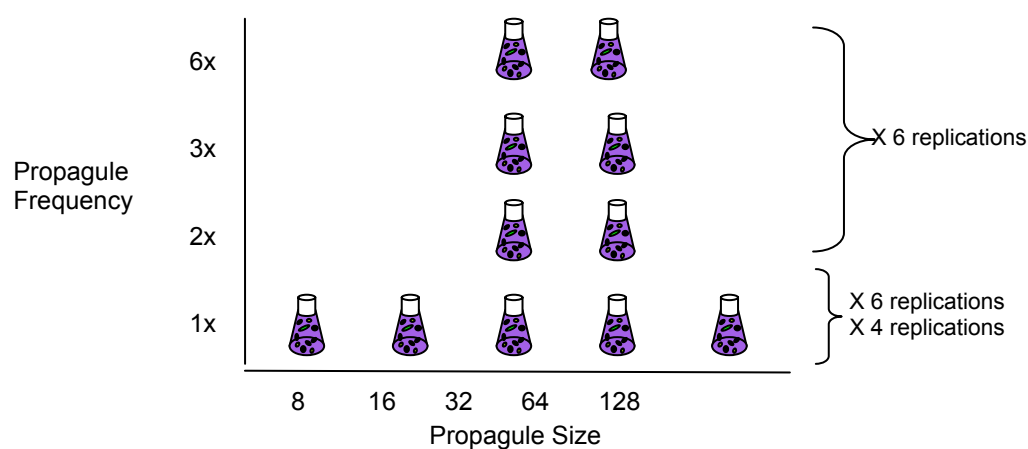
Diversity. On week 6, there is small amount of variation in diversity across propagule frequency treatments, but no trend and the differences are not significant.

Species Richness. Species and populations are lost from the experiment in all treatments. There is no pattern to this loss that can be related to propagule frequency.

Total Cell Concentration. Propagule frequency influences the combined cell concentration of the non-dominant established species, but not total community concentration. For treatments 1x, 3, and 6x, there is a pattern of increased cell concentration with increasing propagule frequency but it is not significant. Surprisingly, the two-addition treatment has significantly lower cell concentration than the rest. This unusual pattern is probably linked entirely to

Colpidium striatum growth, because the expected trend of increased cell concentration with increased propagule frequency is seen for the non-dominant species. The combined cell concentration of the four non-dominant species increases with increasing cell concentration, with treatments 2x and 3x essentially identical and the six-addition treatment substantially greater.

Summary. Increasing propagule frequency does not increase diversity, species richness, or total cell concentration in the *Colpidium striatum*-dominated community. This may be because additions are only of 16-cell inoculations, an addition size that might not have been large enough to provide significant benefit. In addition, *Colpidium striatum* is a very strong competitor that dominates the community, comprising 97% of the total community concentration. Since the growth of *Uronema sp.* is not significantly affected by propagule frequency and the species is found at such high proportion, the positive effect of propagule frequency on the concentration of the non-dominant species is not apparent when total community concentration is examined.



Timeline:

Week:	0	1	2	3	4	5	6	7	8
Addition:	all	6x	3,6x	2,6x	3,6x	6x			
Data:		✓	✓	✓	✓	✓	✓	✓	✓

Figure 2-1. Experimental design for Specific Aim #1: To examine the role of propagule size and propagule frequency on the richness and diversity of protist communities. The influence of propagule size was studied by initiating identical assemblages of protist species with 8, 16, 32, 64, or 128 cells of each species. The effect of propagule frequency was studied by initiating communities with 16 cells of each species, then maintaining them with subsequent additions of 32 or 64 cells of each species for a total of 1, 2, 3, or 6 additions over a period of five weeks. All treatments were maintained for eight weeks. The cell concentration of all species in every experimental flask was determined every seven days for eight weeks.

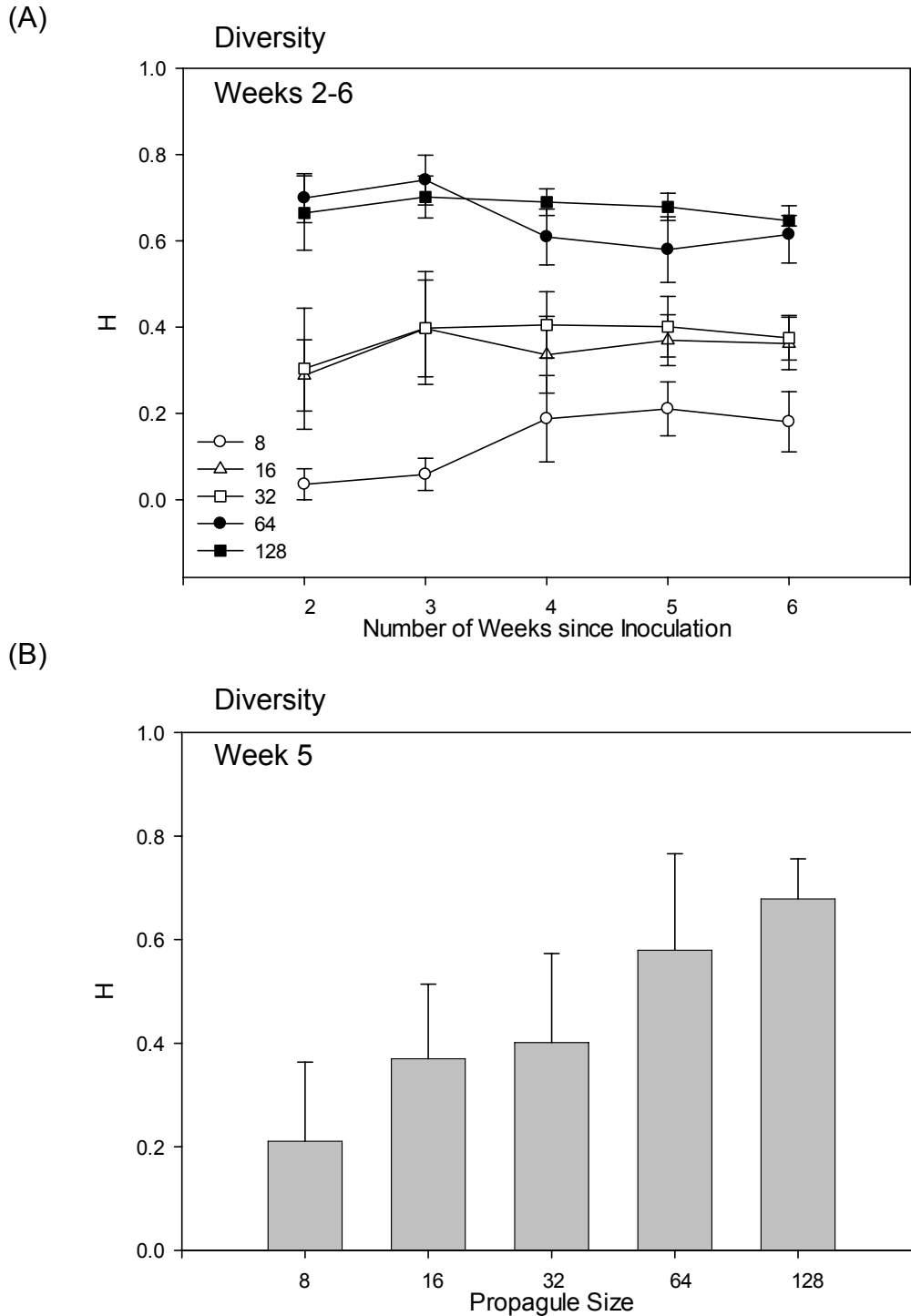


Figure 2-2. The diversity of communities initiated on Day 0 with 8, 16, 32, 64, or 128 cells of *Spirostomum ambiguum*, *Tillina magna*, *Paramecium* sp., and *Paramecium bursaria* over the course of the experiment (A) and on week 5 (B). Diversity is determined by Shannon Index. Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule size on diversity is significant on week 5 (B) ($\chi^2 = 39.706$, $df = 1$, $P < 0.01$).

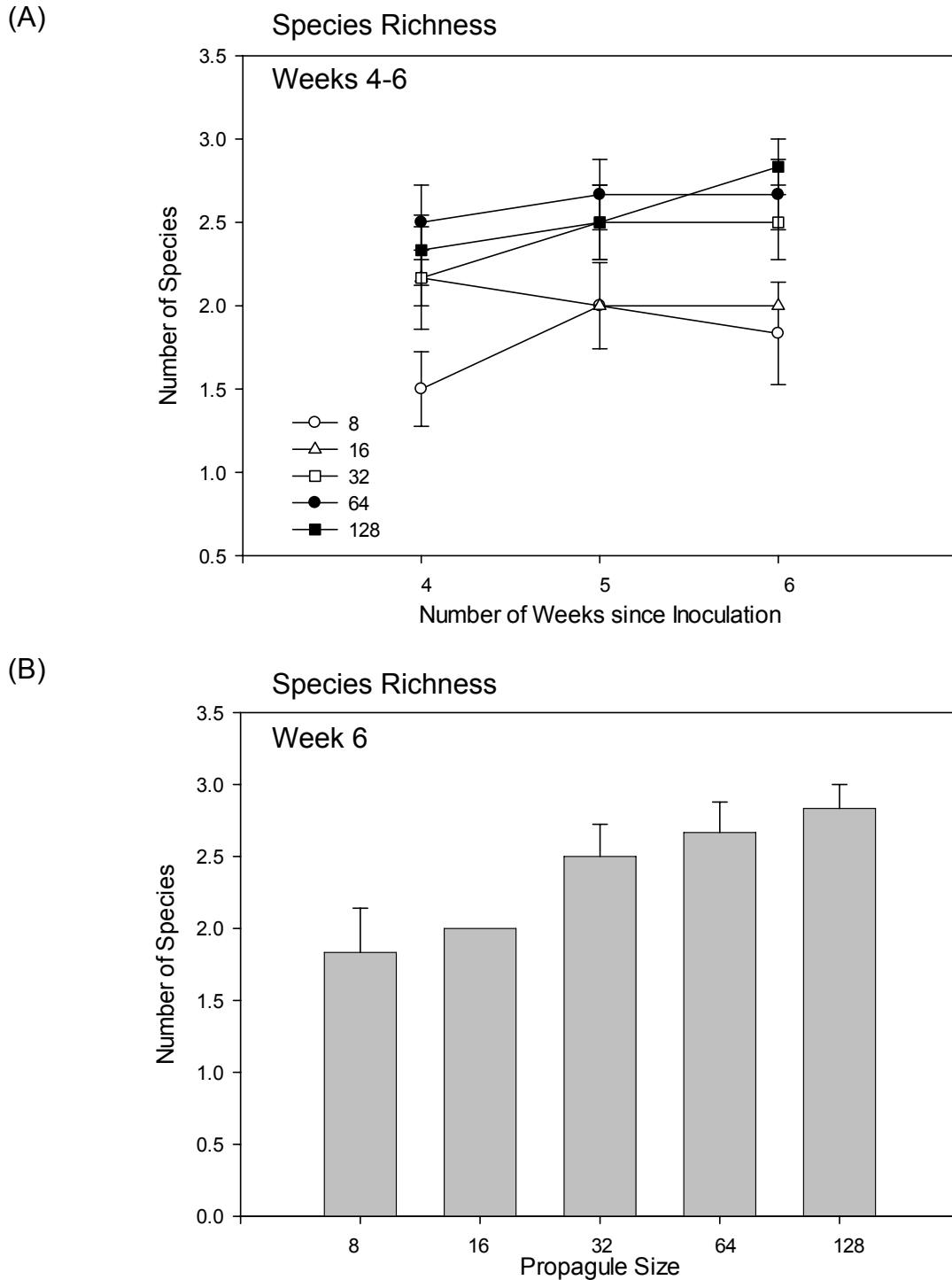


Figure 2-3. The species richness of communities initiated on Day 0 with 8, 16, 32, 64, or 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, *Paramecium* sp., and *Paramecium bursaria* during the week 4-6 period (A) and on week 6 (B). Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule size on species richness is significant on week 6 (B) ($\chi^2 = 19.104$, $df = 1$, $P < 0.01$).

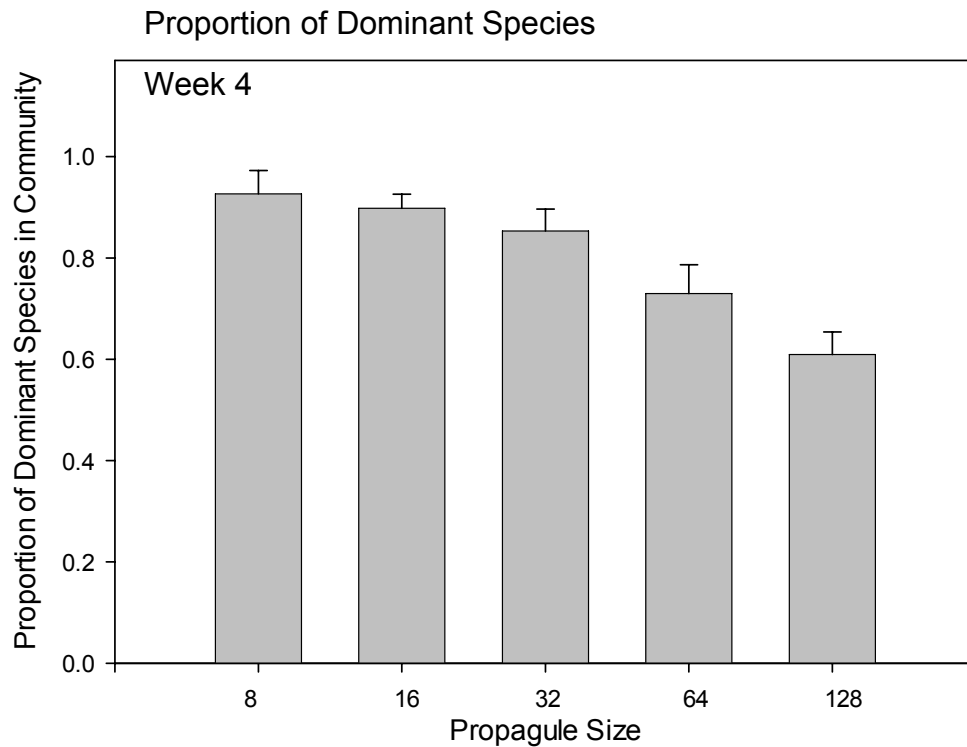


Figure 2-4. The proportion, four weeks after inoculation, of the dominant species, *Tillina magna*, in communities initiated with 8, 16, 32, 64, or 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, *Paramecium* sp., and *Paramecium bursaria*. Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule size on the proportion of *Tillina magna* is significant ($\chi^2 = 35.007$, $df = 1$, $P < 0.01$).

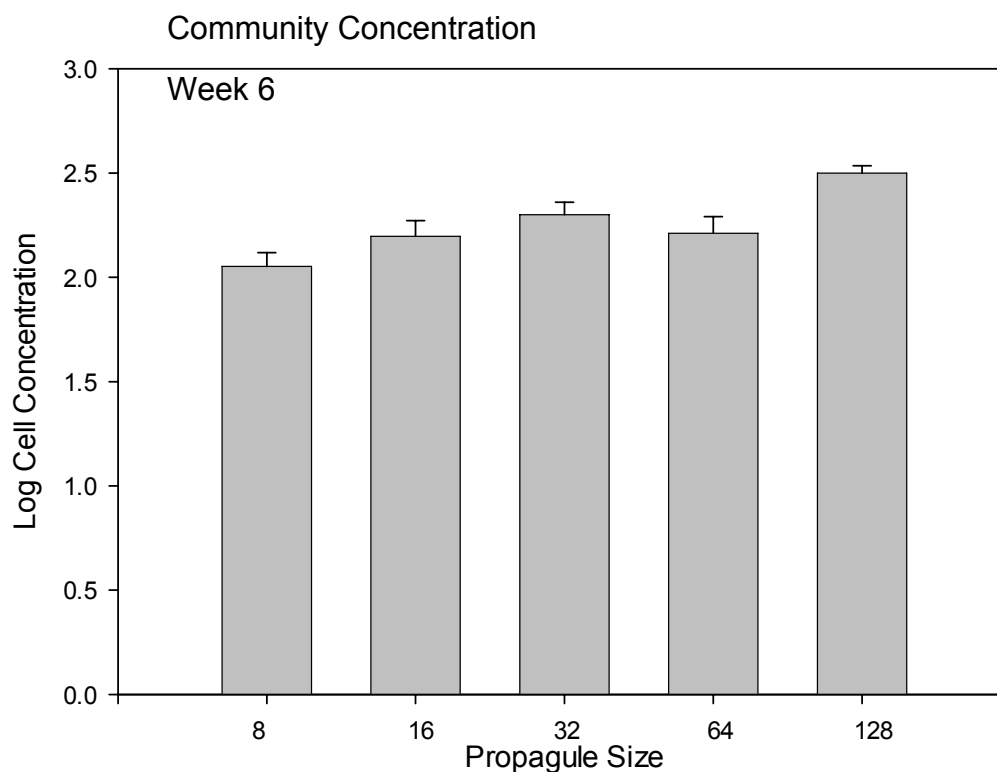
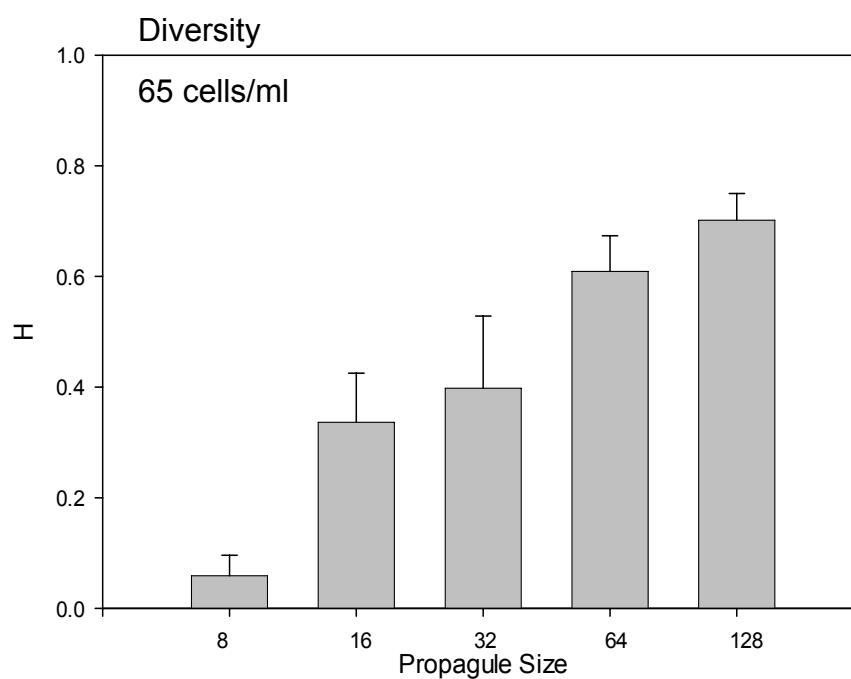


Figure 2-5. The cell concentration, six weeks after inoculation, of communities initiated on Day 0 with 8, 16, 32, 64, or 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, *Paramecium* sp., and *Paramecium bursaria*. Cell concentration data are log-transformed as $\log(N+1)$. Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule size on community cell concentration is significant ($\chi^2 = 18.804$, $df = 1$, $P < 0.01$).

(A)



(B)

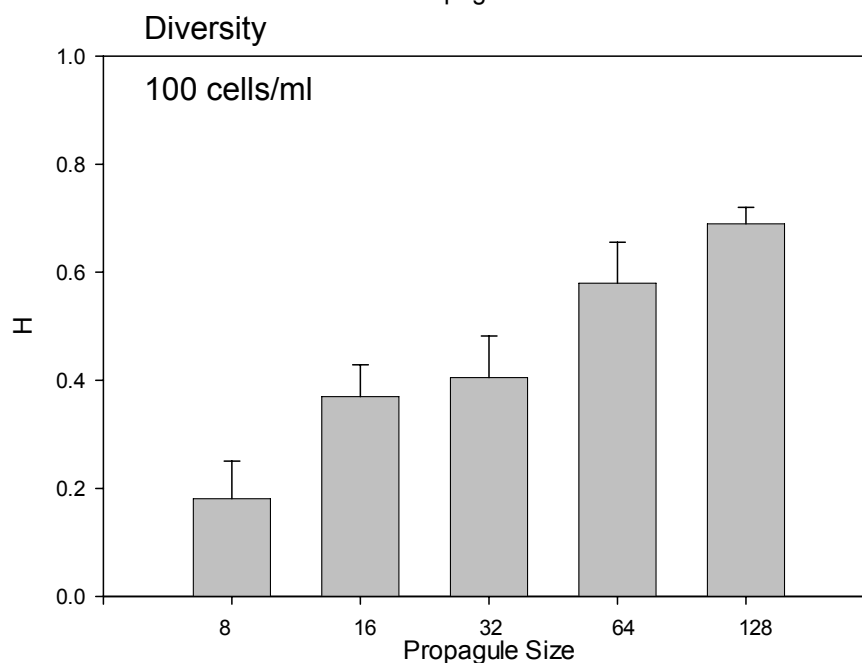
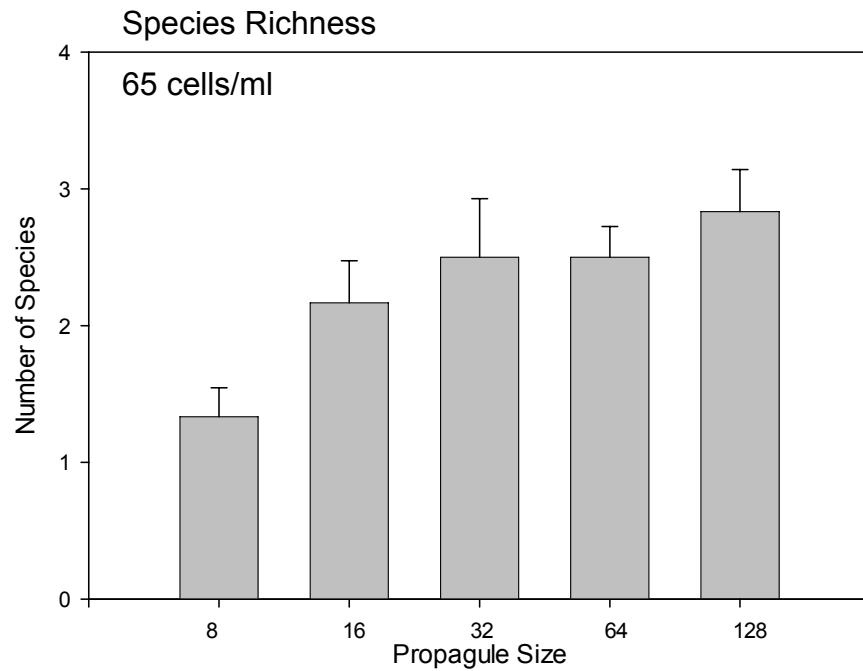


Figure 2-6. The diversity of communities initiated on Day 0 with 8, 16, 32, 64, or 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, *Paramecium sp.*, and *Paramecium bursaria* when the mean total cell concentration of all treatments is approximately 65 cells/ml (A) or 100 cells/ml (B). Values are the mean (± 1 SE) of six replications of each treatment. The effect of propagule size on community diversity is significant at both 65 cells/ml (A) ($\chi^2 = 41.704$, $df = 1$, $P < 0.01$) and 100 cells/ml (B) ($\chi^2 = 41.366$, $df = 1$, $P < 0.01$).

(A)



(B)

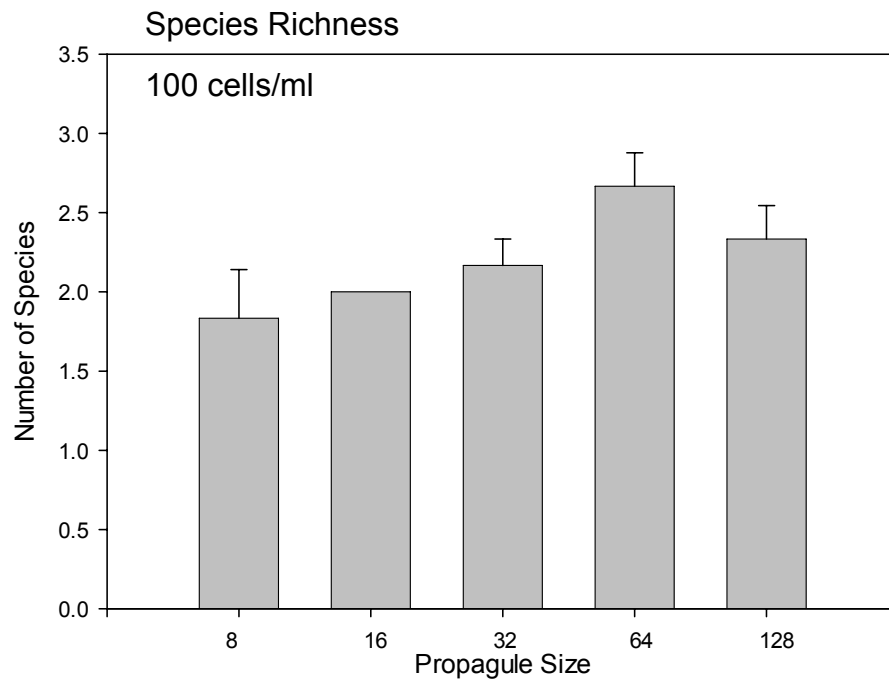
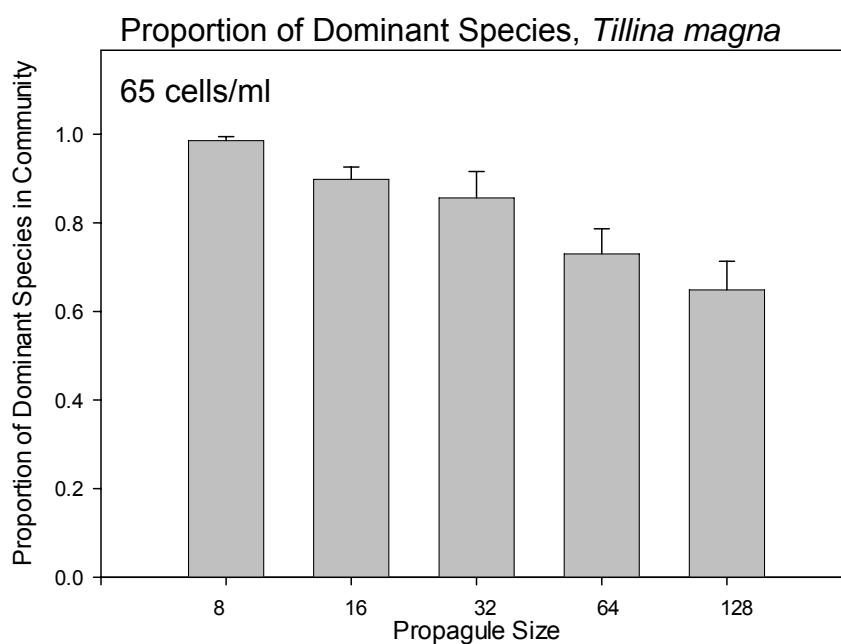


Figure 2-7. The species richness of communities initiated with 8, 16, 32, 64, and 128 cells each of *Paramecium bursaria*, *Tillina magna*, *Spirostomum ambiguum*, and *Paramecium* sp. when all treatments have a mean total cell concentration of approximately 65 cells/ml (A) or 100 cells/ml (B). Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule size on species richness is significant at both 65 cells/ml (A) ($\chi^2 = 13.158$, $df = 1$, $P < 0.01$) and 100 cells/ml (B) ($\chi^2 = 7.009$, $df = 1$, $P < 0.01$).

(A)



(B)

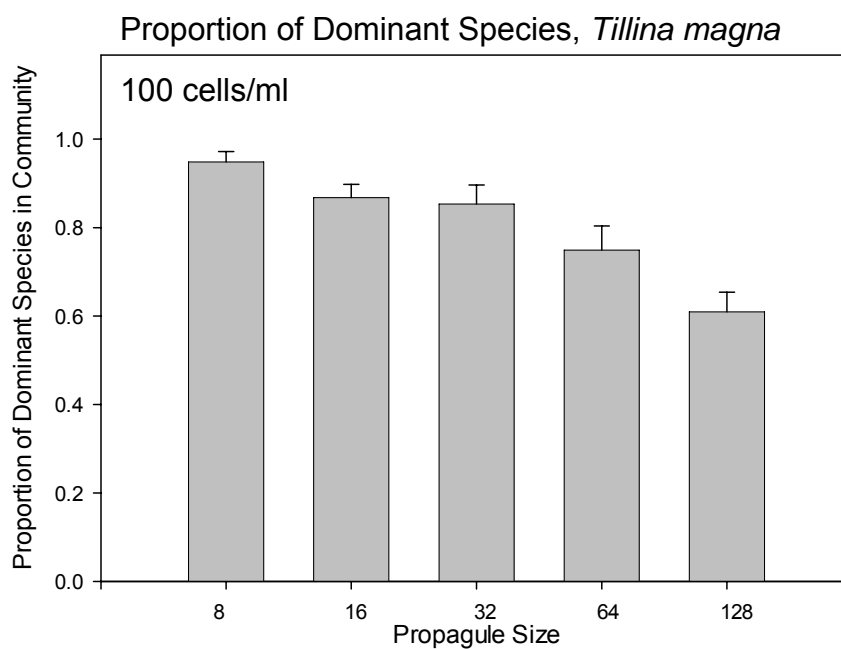


Figure 2-8. The proportion of the dominant species, *Tillina magna*, in communities initiated on Day 0 with 8, 16, 32, 64 or 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, *Paramecium* sp., and *Paramecium bursaria* when the mean total cell concentration of all treatments is approximately 65 cells/ml (A) or 100 cells/ml (B). Values are the means (± 1 SE) of six replications of each treatment. The effect of propagule size on the proportion of *Tillina magna* is significant at both 65 cells/ml (A) ($\chi^2 = 35.237$, $df = 1$, $P < 0.01$) and 100 cells/ml (B) ($\chi^2 = 4.362$, $df = 1$, $P < 0.01$).

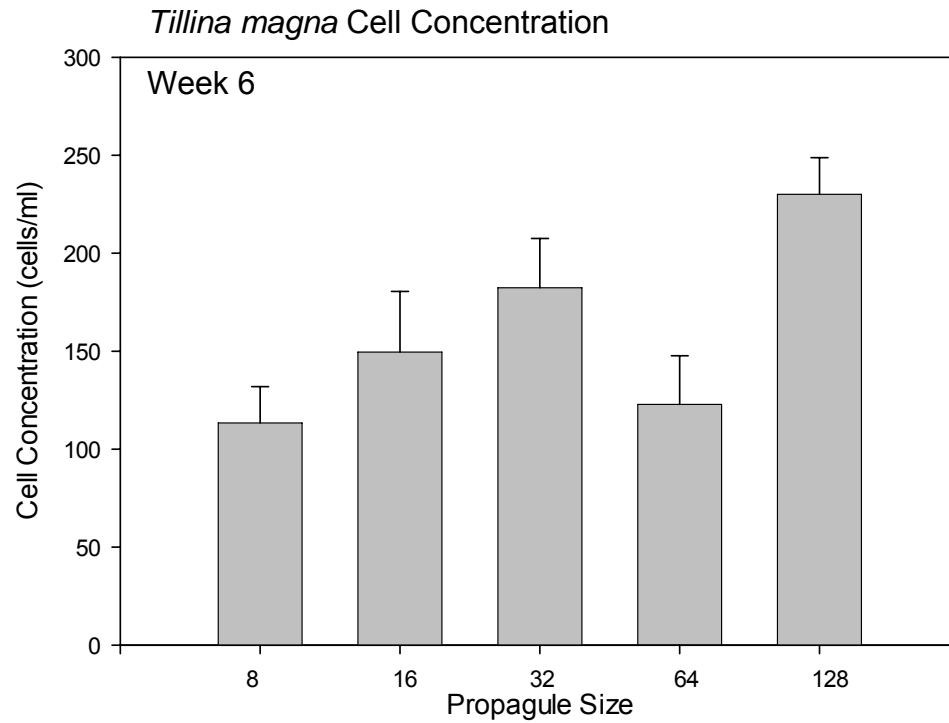


Figure 2-9. *Tillina magna* cell concentration, six weeks after inoculation, into communities initiated on Day 0 with 8, 16, 32, 64, or 128 cells of *Spirostomum ambiguum*, *Tillina magna*, *Paramecium* sp., and *Paramecium bursaria*. Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule size on *Tillina magna* cell concentration is significant ($\chi^2 = 6.649$, $df = 1$, $P = 0.010$).

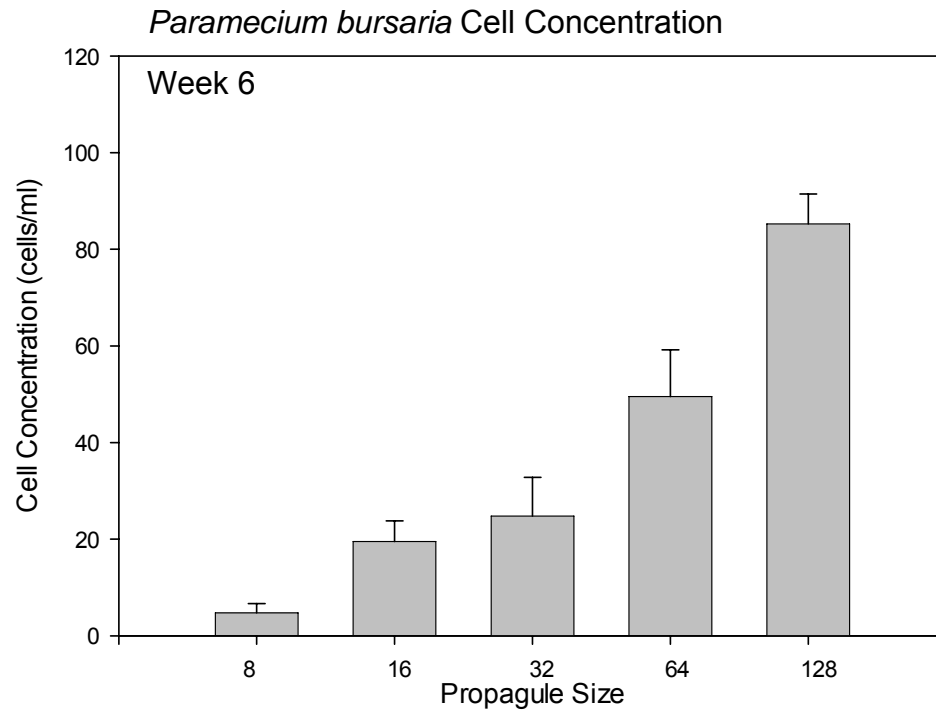


Figure 2-10. *Paramecium bursaria* cell concentration, six weeks after inoculation into communities initiated with 8, 16, 32, 64, and 128 cells each of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule size on *Paramecium bursaria* cell concentration is significant ($\chi^2 = 76.905$, $df = 1$, $P < 0.01$).

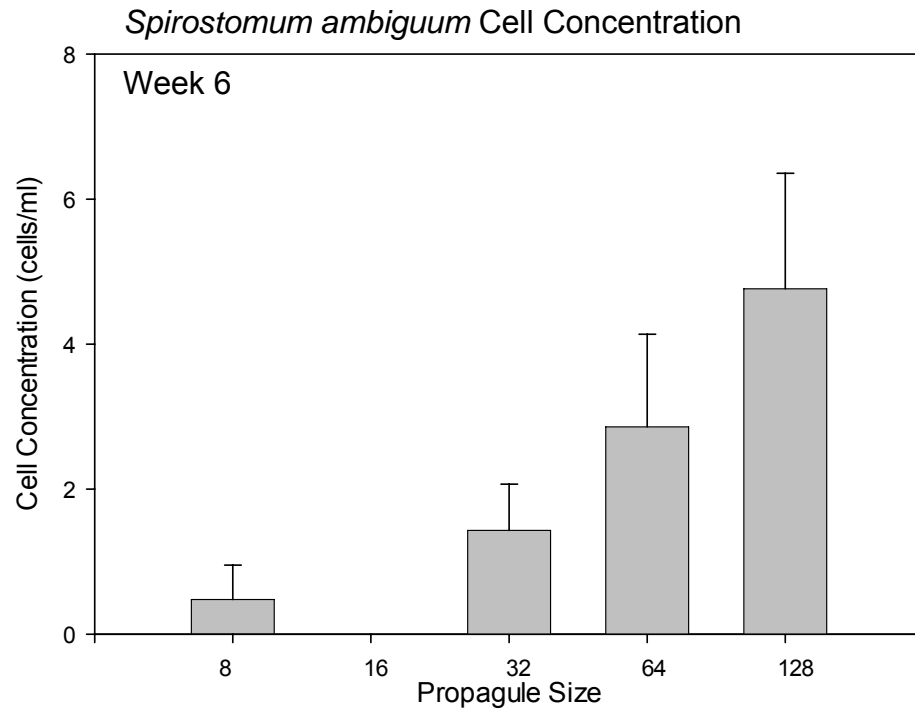


Figure 2-11. *Spirostomum ambiguum* cell concentration six weeks after inoculation into communities initiated with 8, 16, 32, 64, and 128 cells each of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule size on *Spirostomum ambiguum* cell concentration is significant ($\chi^2 = 15.105$, $df = 1$, $P < 0.01$).

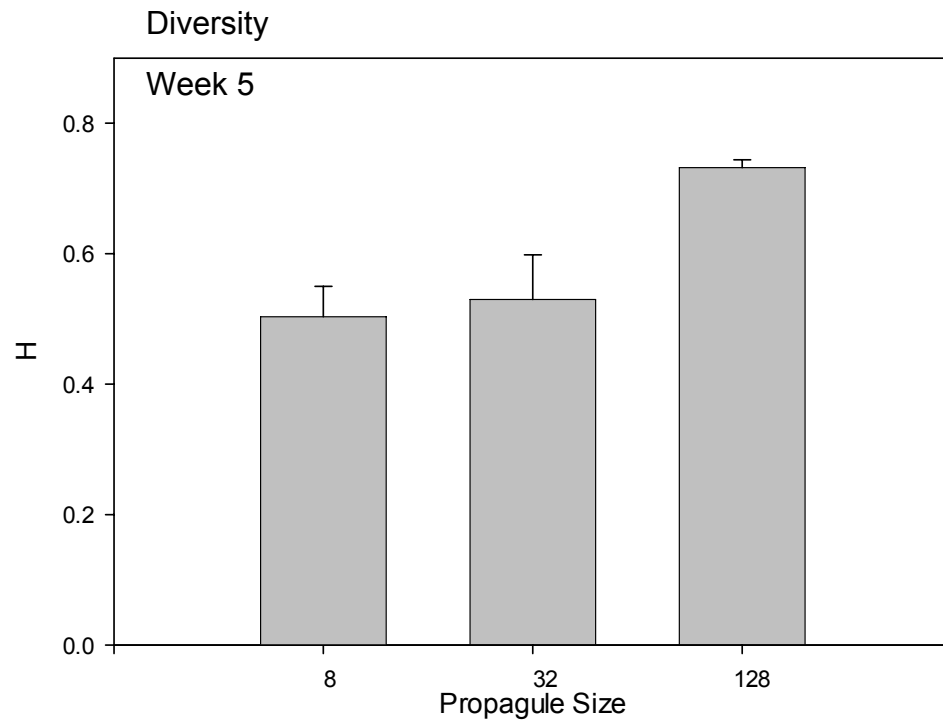


Figure 2-12. The diversity of communities initiated with 8, 16, 32, 64, and 128 cells each of *Uronema* sp., *Paramecium bursaria*, *Tillina magna*, *Spirostomum ambiguum*, and *Paramecium* sp. Plotted values are the means of six replications of each treatment. The effect of propagule size on community diversity is significant ($\chi^2 = 11.677$, $df = 1$, $P = 0.001$).

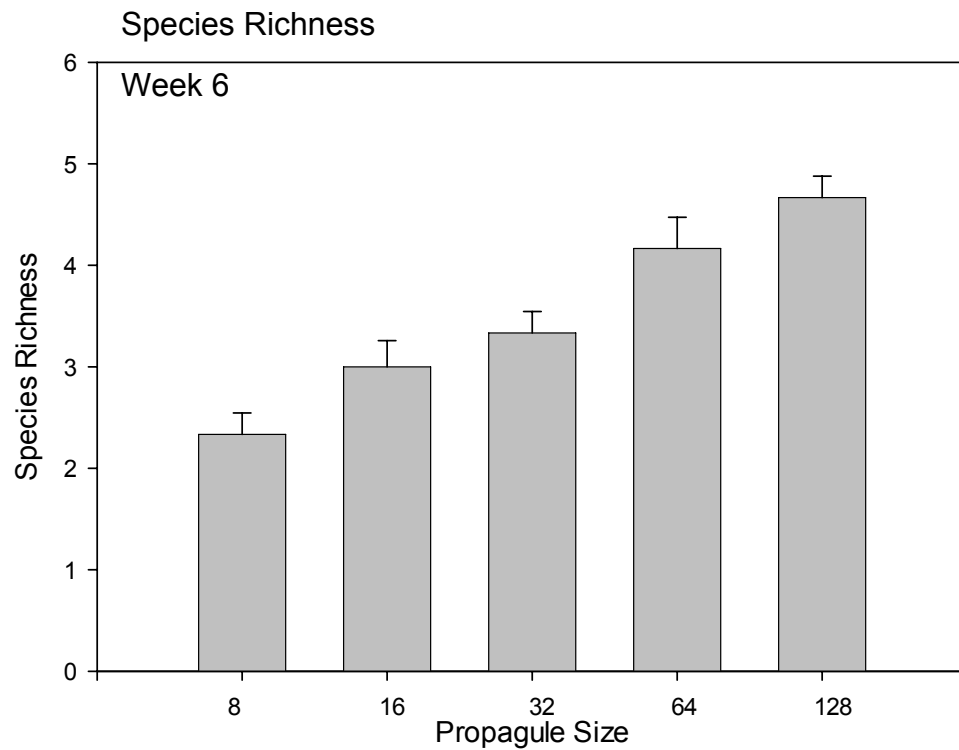


Figure 2-13. The species richness of communities initiated with 8, 16, 32, 64, and 128 cells each of *Uronema* sp., *Paramecium bursaria*, *Tillina magna*, *Spirostomum ambiguum*, and *Paramecium* sp. Plotted values are the means of six replications of each treatment. The effect of propagule size on species richness is significant ($\chi^2 = 67.431$, $df = 1$, $P < 0.01$).

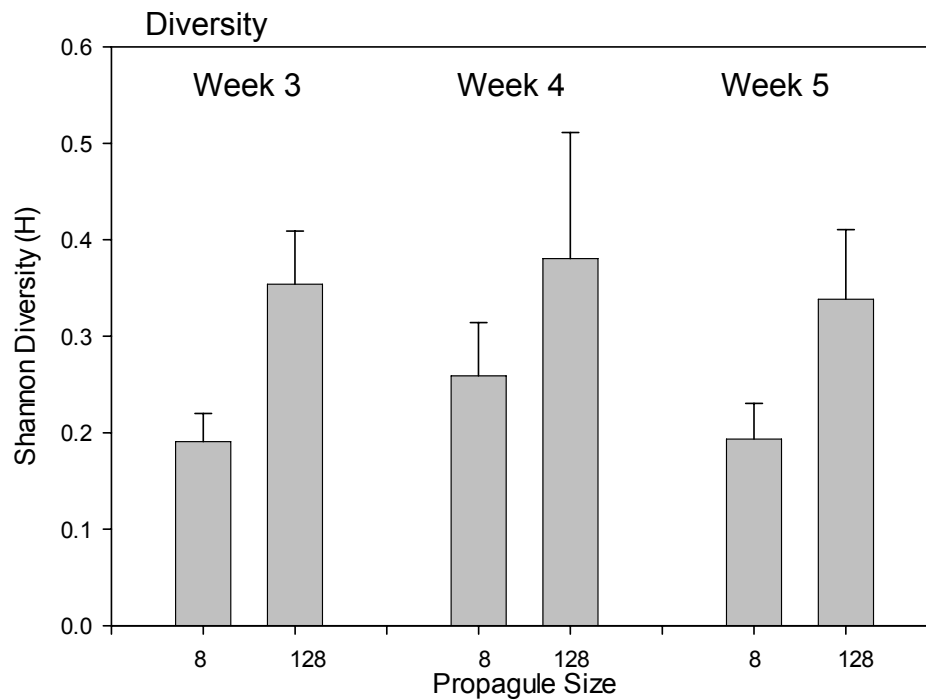


Figure 2-14. The diversity of communities initiated with 8, 16, 32, 64, and 128 cells each of *Colpidium striatum*, *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* Plotted values are the means (± 1 SE) of four replications of each treatment. The effect of propagule size on community diversity is significant ($\chi^2 = 7.732$, $df = 1$, $P = 0.005$).

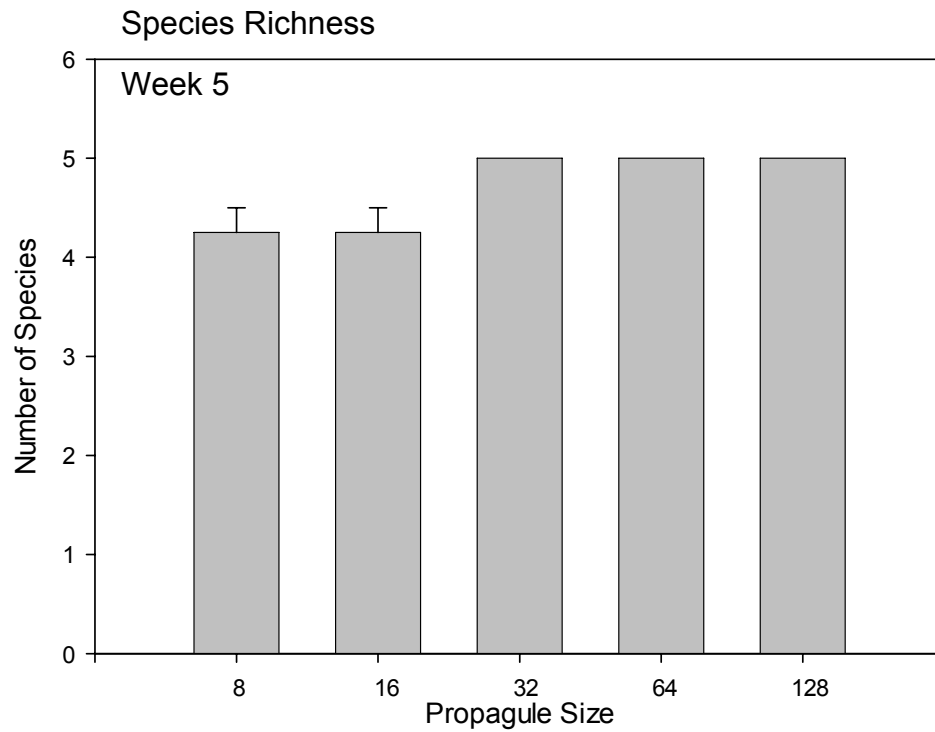
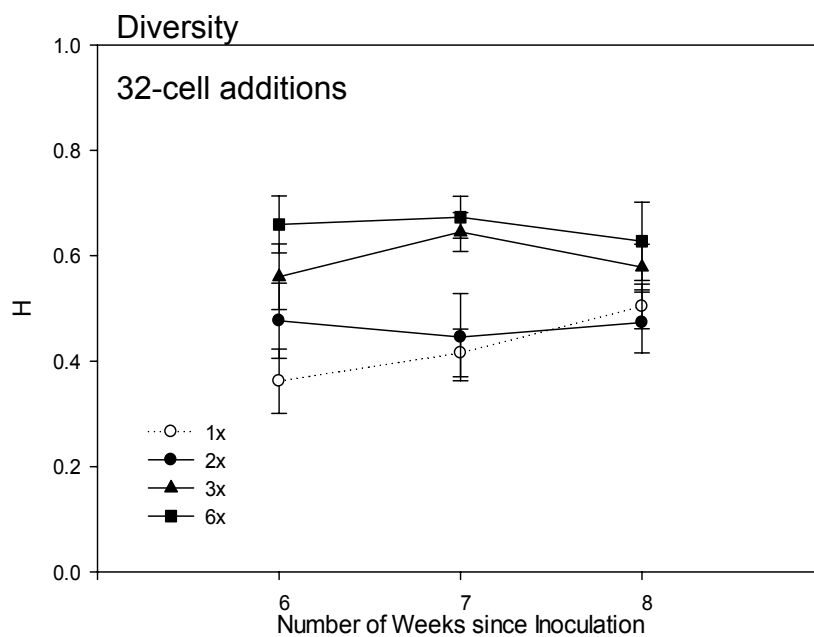


Figure 2-15. The species richness of communities initiated with 8, 16, 32, 64, and 128 cells each of *Colpidium striatum*, *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* Plotted values are the means (± 1 SE) of four replications of each treatment. The effect of propagule size on species richness is significant ($\chi^2 = 18.621$, $df = 1$, $P < 0.01$).

(A)



(B)

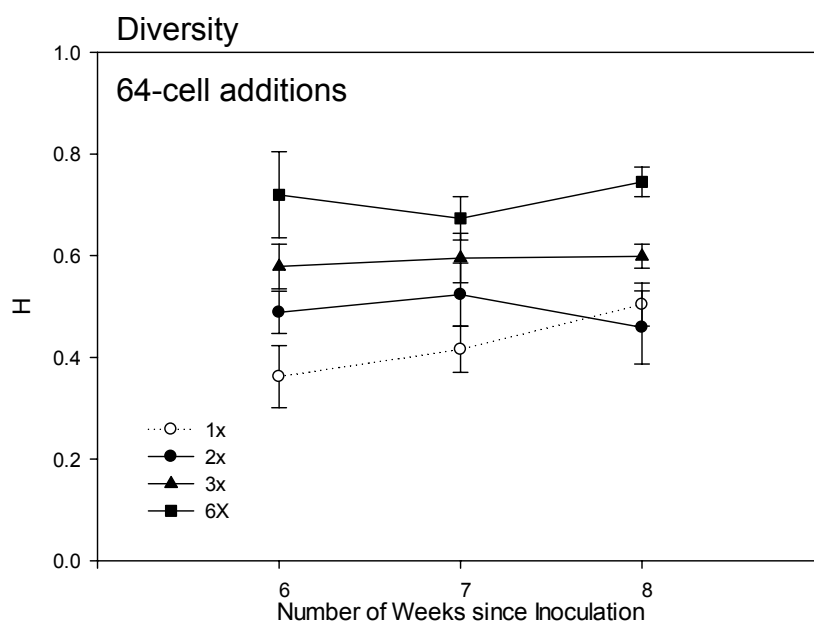


Figure 2-16. The diversity of communities maintained with one addition of 16 cells and 2, 3, and 6 additions of 32 (A) or 64 (B) cells each of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* Plotted values are the means (± 1 SE) of six replications of each treatment.

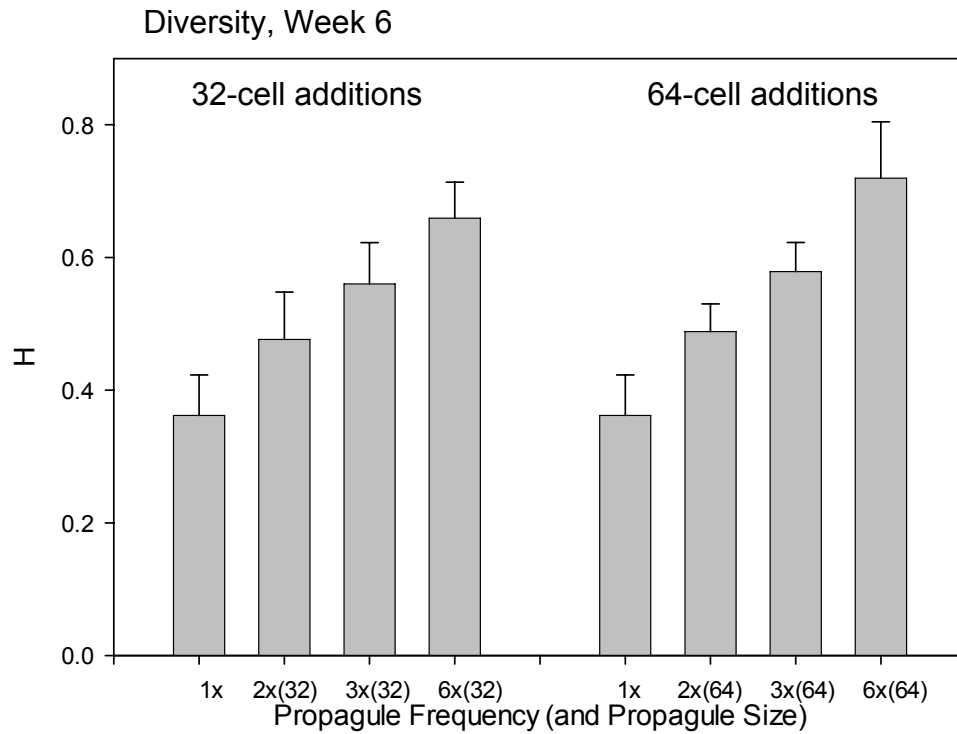


Figure 2-17. The diversity of communities maintained with one addition of 16 cells and 2, 3, and 6 additions of 32 (left) or 64 (right) cells each of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* Diversity is measured with the Shannon Index. Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule frequency on community diversity is significant ($\chi^2 = 31.360$, $df = 1$, $P < 0.01$).

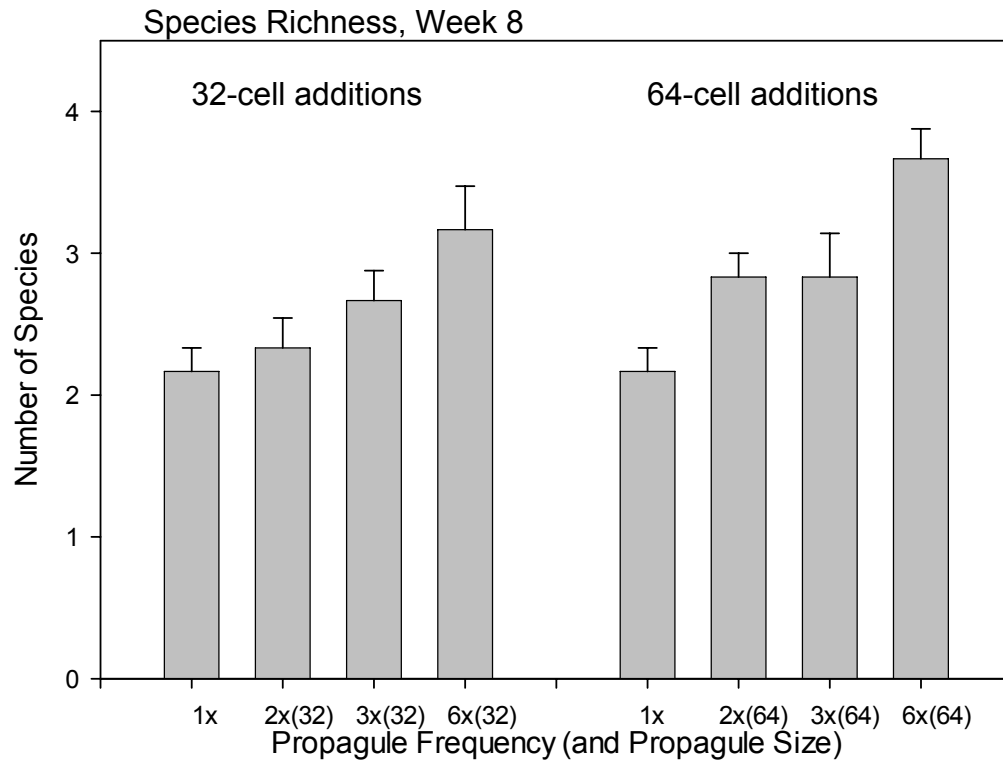


Figure 2-18. The species richness of communities maintained with one addition of 16 cells and 2, 3, and 6 additions of 32 (left) or 64 (right) cells each of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule frequency on species richness is significant ($\chi^2 = 36.414$, $df = 1$, $P < 0.01$).

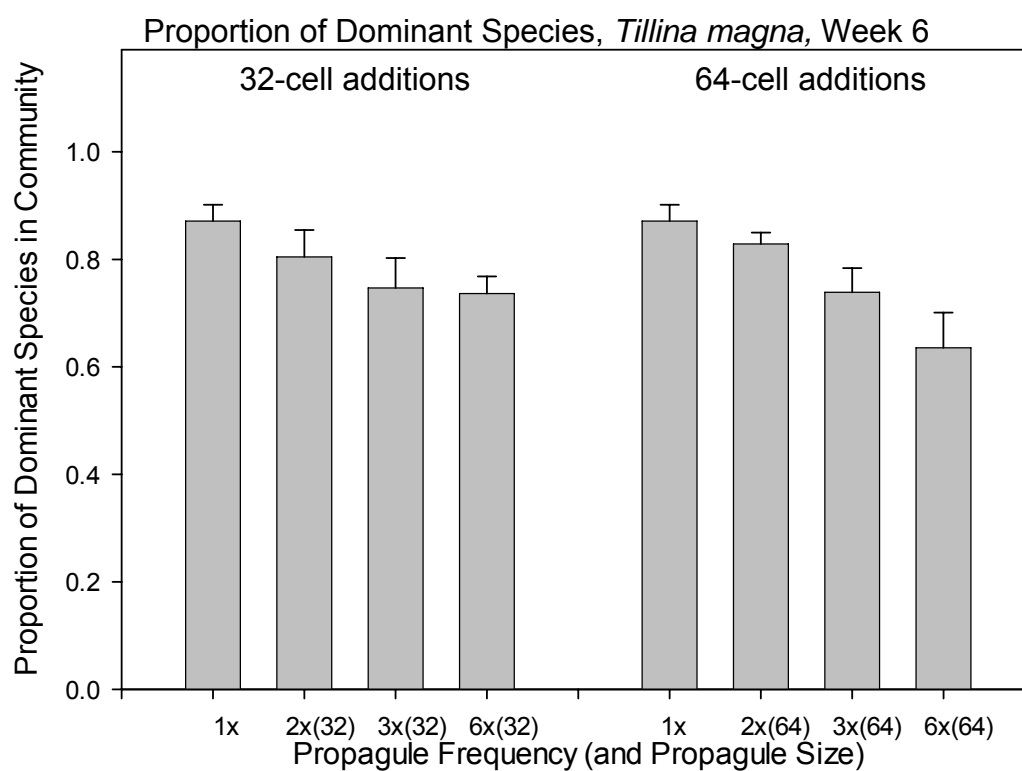


Figure 2-19. The mean proportion of *Tillina magna*, during week 6, in communities maintained with one addition of 16 cells and 2, 3, and 6 additions of 32 (left) or 64 (right) cells each of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule frequency on the proportion of *Tillina magna* is significant ($\chi^2 = 20.391$, $df = 1$, $P < 0.01$).

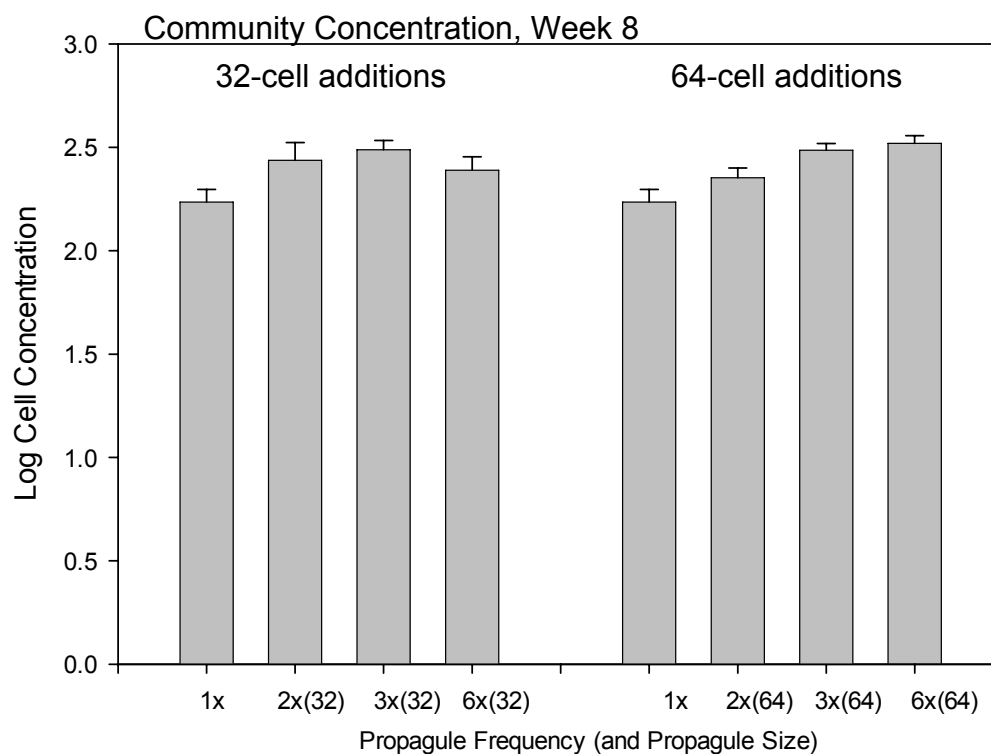
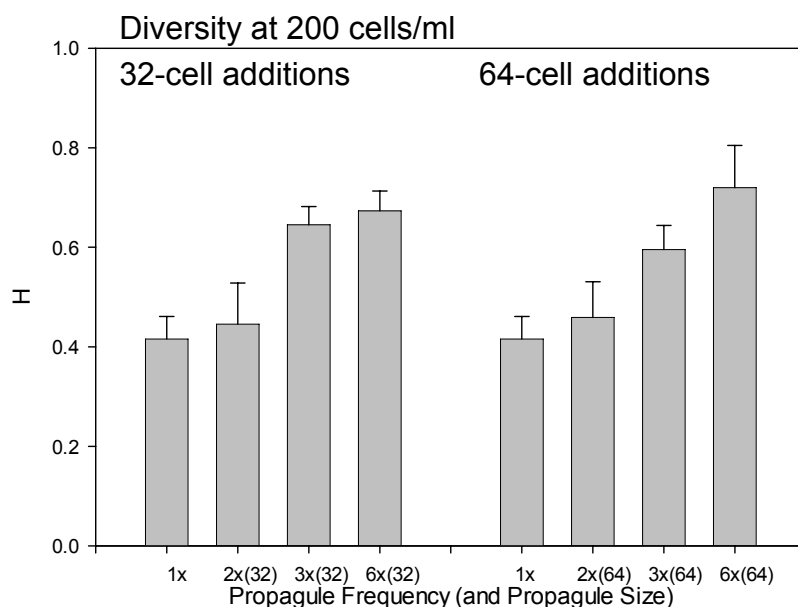


Figure 2-20. Cell concentration, at week 8, of communities maintained with one addition of 16 cells and 2, 3, and 6 additions of 32 (left) or 64 (right) cells each of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule frequency on community concentration is significant ($\chi^2 = 9.745$, $df = 1$, $P = 0.002$).

(A)



(B)

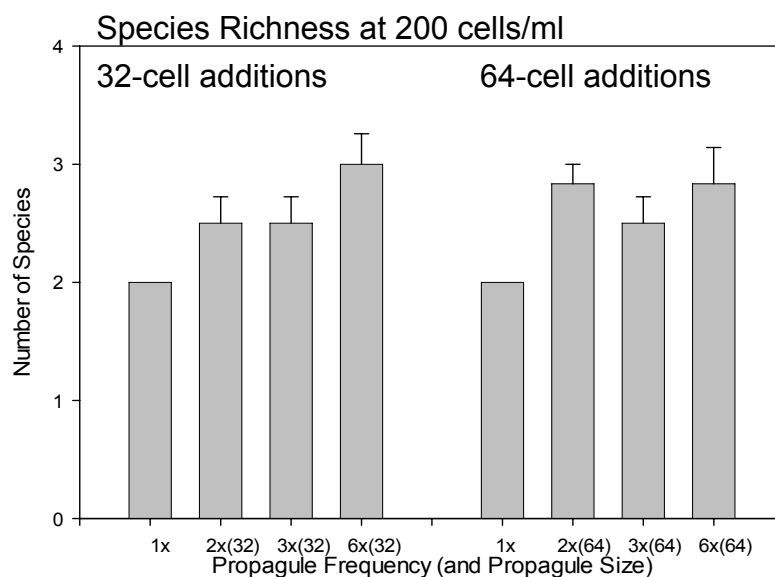


Figure 2-21. The diversity (A) and species richness (B) of communities maintained with one addition of 16 cells and 2, 3, and 6 additions of 32 (left) and 64 (right) cells each of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* when all treatments have a mean cell concentration of approximately 200 cells/ml. Diversity is measured with the Shannon index. Plotted values are the means (± 1 SE) of six replications of each treatment. There is a significant effect of propagule frequency on community diversity (A) ($\chi^2 = 27.826$, $df = 1$, $P < 0.01$) and species richness (B) ($\chi^2 = 15.022$, $df = 1$, $P < 0.01$).

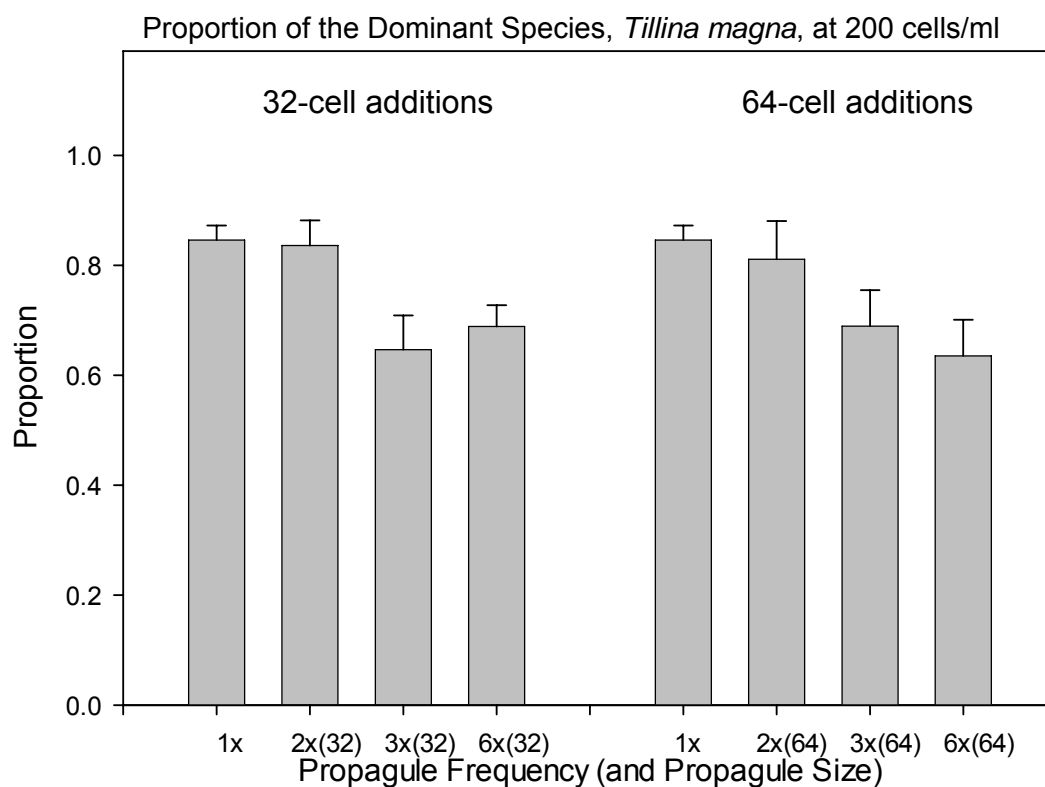


Figure 2-22. The proportion of the dominant species, *Tillina magna*, in communities maintained with one addition of 16 cells and 2, 3, and 6 additions of 32 (left) and 64 (right) cells each of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* when all treatments have a mean cell concentration of approximately 200 cells/ml. Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule frequency on the proportion of *Tillina magna* is significant ($\chi^2 = 10.276$, $df = 1$, $P = 0.001$).

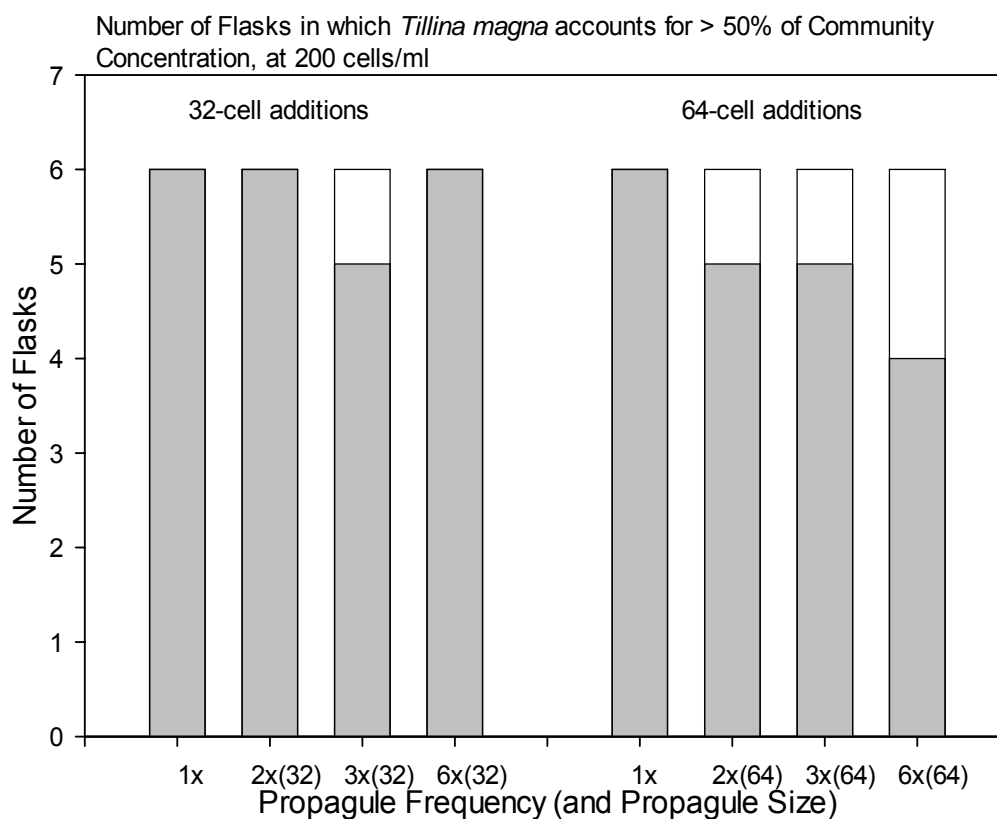


Figure 2-23. The number of flasks, out of six total, in which *Tillina magna* accounts for greater than 50% of the total cell concentration of communities maintained with one addition of 16 cells and 2, 3, and 6 additions of 32 and 64 cells of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. when all treatments have a mean cell concentration of approximately 200 cells/ml. The effect of propagule frequency on the number of flasks in which *Tillina magna* accounts for greater than 50% of the total cell concentration significant ($\chi^2 = 8.544$, df = 1, P = 0.003).

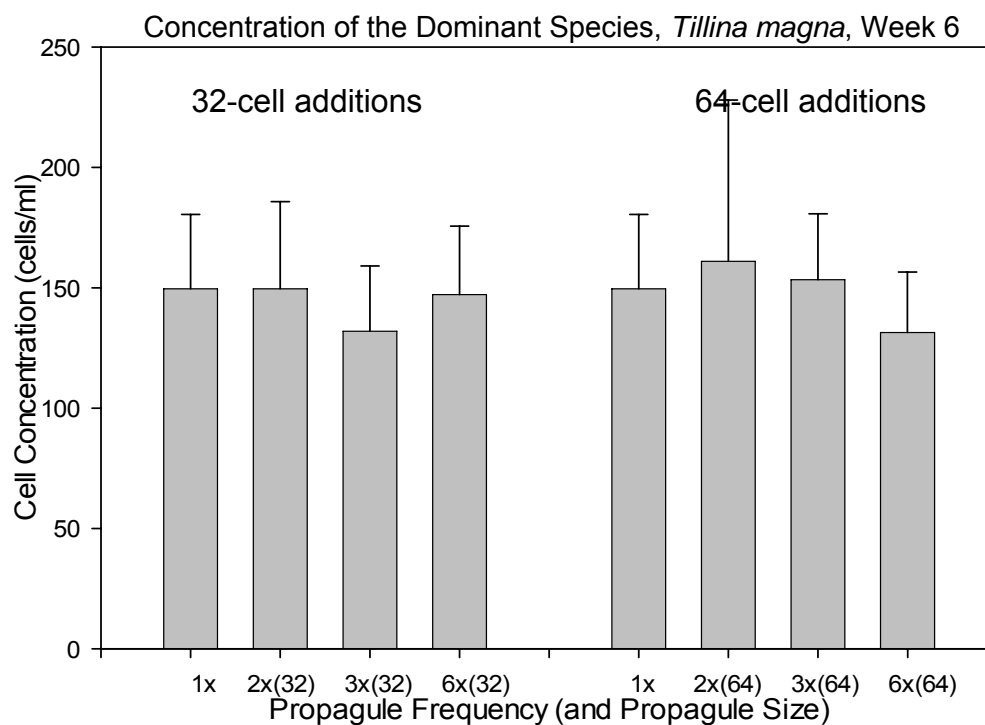


Figure 20-24. *Tillina magna* cell concentration, at week 6, in communities maintained with one addition of 16 cells and 2, 3, and 6 additions of 32 and 64 cells of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. during the first five weeks of culture. Plotted values are the means (± 1 SE) of six replications of each treatment.

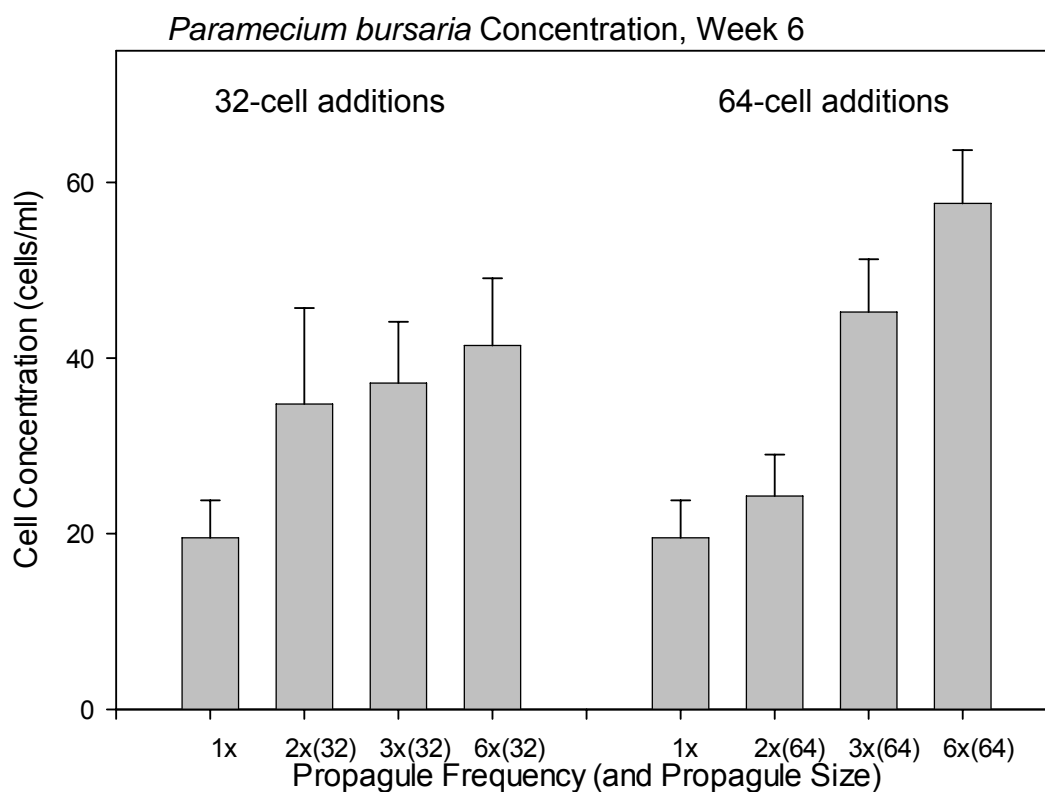


Figure 20-25. *Paramecium bursaria* cell concentration, at week 6, in communities maintained with one addition of 16 cells and 2, 3, and 6 additions of 32 (left) and 64 (right) cells each of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule frequency on *Paramecium bursaria* concentration is significant ($\chi^2 = 11.790$, $df = 1$, $P = 0.001$).

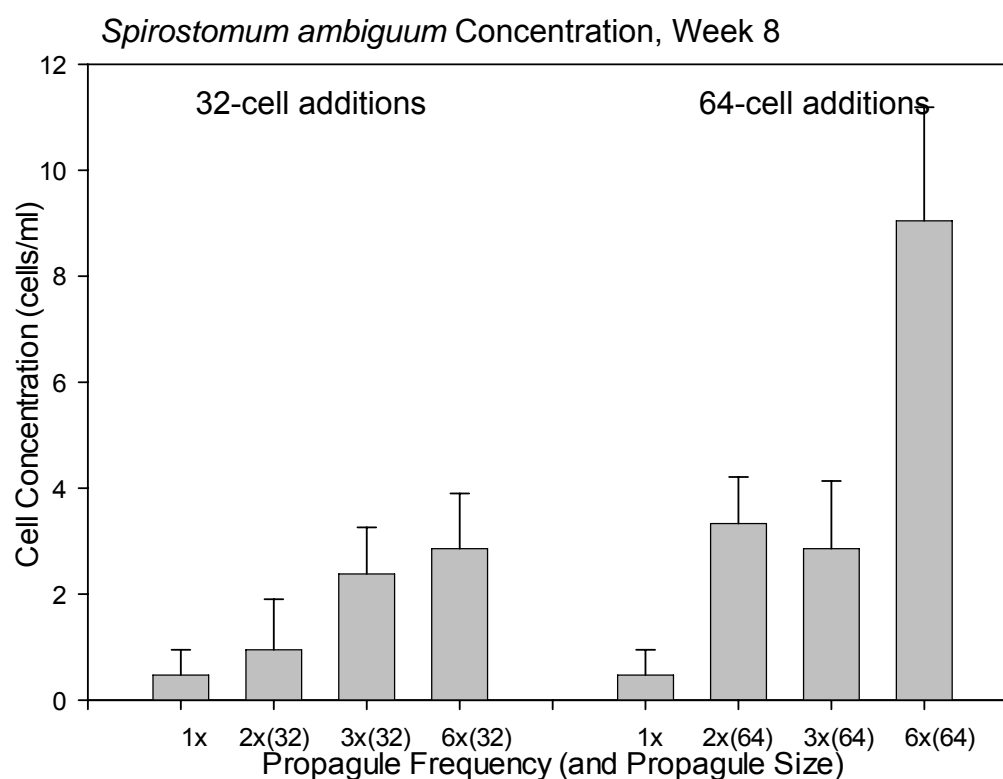


Figure 20-26. *Spirostomum ambiguum* cell concentration, at week 8, in communities maintained with one addition of 16 cells and 2, 3, and 6 additions of 32 (left) and 64 (right) cells each of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* Plotted values are the means (± 1 SE) of six replications of each treatment. The effect propagule frequency on *Spirostomum ambiguum* concentration is significant ($\chi^2 = 4.009$, $df = 1$, $P = 0.045$).

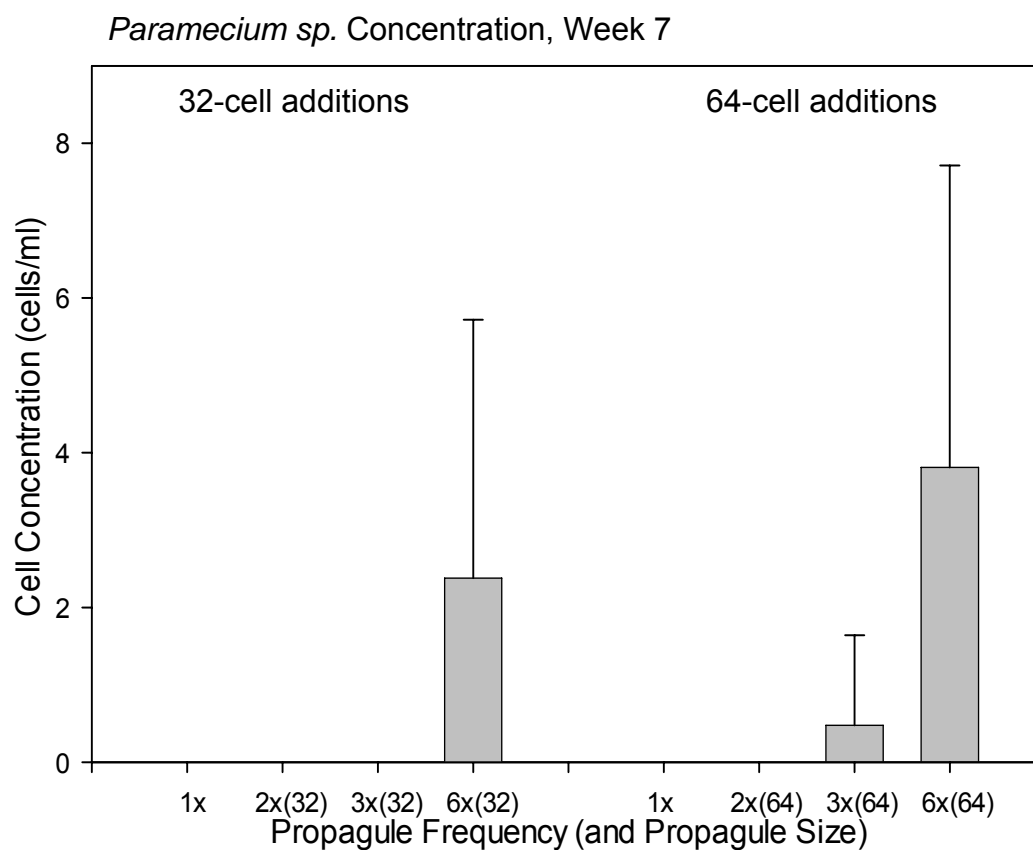


Figure 2-27. *Paramecium* sp. cell concentration, at week 7, in communities maintained with one addition of 16 cells and 2, 3, and 6 additions of 32 (left) and 64 (right) cells each of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. Plotted values are the means (± 1 SE) of six replications of each treatment. The effect of propagule size on cell concentration is significant ($\chi^2 = 19.426$, $df = 1$, $P < 0.01$).

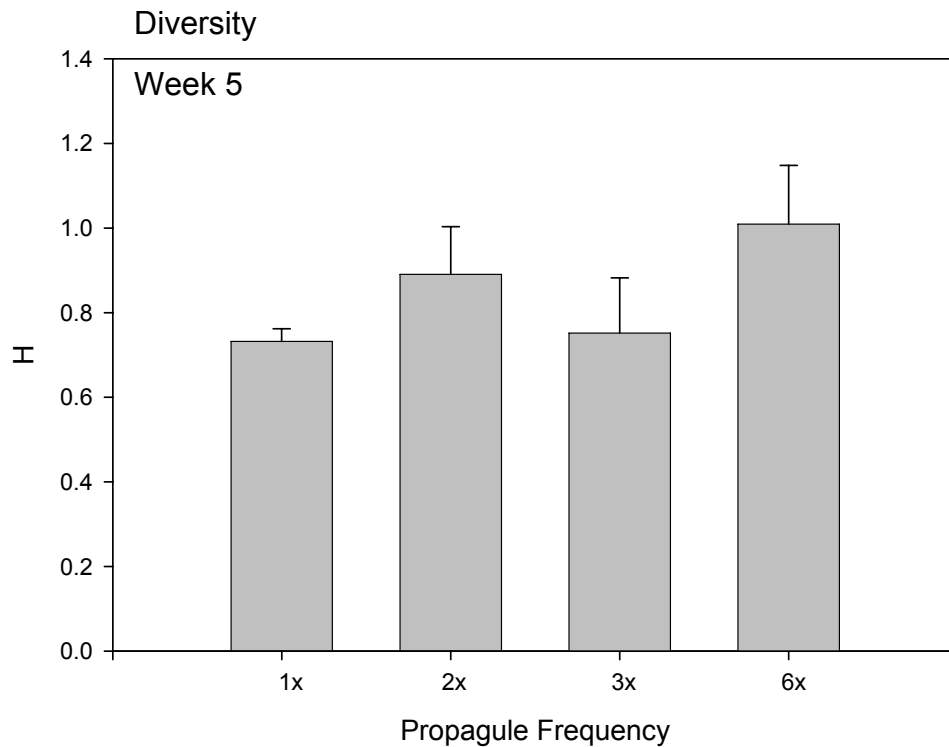


Figure 2-28. The diversity of communities maintained with 1, 2, 3, and 6 additions of 128 cells each of *Uronema* sp., *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. Diversity is measured with the Shannon Index. Plotted values are the means (± 1 SE) of six replications of each treatment, five for the six-addition treatment. The effect of propagule frequency on community diversity is significant ($\chi^2 = 11.236$, $df = 1$, $P = 0.001$).

Discussion

To date, most of the work on the diversity-invasibility relationship has examined the strong influence of heterogeneity in resource availability. The role of propagule pressure in influencing regional diversity and invasibility patterns is not well tested in experimental communities. Propagule pressure exhibits the two characteristics thought to be important in promoting a positive diversity-invasibility relationship at the regional level: 1) the ability to promote diversity and 2) spatial heterogeneity. High levels of propagule pressure are known to increase community diversity and there is heterogeneity in the intensity of propagule pressure across the landscape. High propagule pressure has been linked to a positive diversity-invasibility relationship in a riparian plant community (Brown and Peet, 2003). I predict that propagule pressure also influences the diversity and invasibility of experimental bacterivorous protist communities.

Effect of Propagule Pressure on the Three Experimental Communities

In the *Tillina magna*-dominated community, increasing propagule size and frequency significantly enhances diversity and richness and diminishes dominance. These patterns are found when treatments of the same maturity are compared, as well as when treatments of the same cell concentration are compared, regardless of maturity. This suggests that these patterns are the direct result of increased propagule pressure, and not simply related to increased community concentration in high propagule pressure treatments. Interestingly,

the negative relationship between propagule pressure and dominance is found even though the growth of the dominant species, *Tillina magna*, is increased by high propagule size and not affected by high propagule frequency. This suggests that propagule pressure has an exceptionally strong positive influence on the growth of the non-dominant species in this community. In fact, propagule size and propagule frequency do have strong positive effects on the growth of *Paramecium bursaria* and *Spirostomum ambiguum* growth in this community. The third species in the community, *Paramecium sp.*, grows very weakly and is found only at the highest values of propagule frequency. Increasing propagule size does not benefit *Paramecium sp.* growth, but propagule frequency does have a strong, significant effect on the species' cell concentration. Although propagule size and propagule frequency independently have significant effects on community characteristics, the size of additions in propagule frequency experiments has no real effect on community concentration, diversity, richness, or dominance.

Propagule size significantly influences diversity and species richness in the *Uronema sp.*-dominated community, but does not shape dominance. Propagule frequency significantly influences community diversity and its effect on dominance is nearly significant. However, propagule frequency does not influence species richness. Propagule size and frequency may have limited effect on the *Uronema sp.*-dominated community because the assemblage is so strongly dominated by *Uronema sp.*, and *Uronema sp.* growth is not significantly

affected by either propagule size or propagule frequency. The positive effect propagule size and frequency has on the 20% of the community composed of non-dominant species appears to be diminished at the community level by the 80% of the community, composed of *Uronema sp.*, that is not affected.

In the *Colpidium striatum*-dominated community, propagule size significantly, influences diversity and species richness, but not dominance. Propagule frequency does not influence diversity, richness, or dominance. The effect of propagule frequency is negligible in this community perhaps because additions are only of 16 cells. This addition size might have been too small to affect community characteristics. Also, *Colpidium striatum* completely dominates the community, accounting for approximately 97% of the total cell concentration. *Colpidium striatum* is not significantly affected by the propagule size and frequency values used in the experiment, and has similar cell concentration in all treatments. Although propagule pressure positively influences the growth of the four non-dominant species in the community, this effect may be hidden by the sheer abundance of *Colpidium striatum*. Perhaps the combination of low propagule frequency and a dominant species that accounts for the vast majority of the community and is itself not affected by propagule pressure negates any benefit of increased propagule frequency. If the addition size had been larger, a positive effect of propagule frequency may have been found.

Effect of Propagule Size on Community Diversity, Species Richness, and

Dominance. I predicted that high propagule size would promote community diversity and species richness, and diminish dominance, in bacterivorous protist communities. Increasing propagule size does significantly increase community diversity and species richness in all three communities. However, high propagule size reduces dominance only in the *Tillina magna*-dominated community. The other two communities show no effect of propagule size on dominance.

Effect of Propagule Frequency on Community Diversity, Species Richness, and

Dominance. I predicted that the bacterivorous protist communities would show patterns of increased community diversity and species richness, and decreased dominance, when propagule frequency is high. Increasing propagule frequency does significantly increase community diversity in two of the three experimental communities. However, propagule frequency significantly raises species richness values and lowers dominance only in the *Tillina magna*-dominated community. The other two communities do not show significant effects on species richness or dominance.

The Possible Role of the Dominant Species on the Effect of Propagule Pressure

on Community Characteristics. In this system, a community shows strong significant trends of increased diversity and richness, and a decrease in dominance, with increasing propagule size and frequency when all species in an

assemblage have fairly similar growth rate and proportion, and the growth of the dominant species is influenced by propagule pressure. The effect of propagule size and propagule frequency on species richness and dominance is curtailed in experimental communities with a dominant species that comprises a very large proportion of total cell concentration. This occurs even if propagule pressure has a positive effect on the growth of non-dominant community members. The reduction in the range of effects attributed to propagule pressure as the proportion of the dominant species increases may occur for two reasons that are related and function together. First, the dominant species in a community is dominant because its realized growth rate in the community is much greater than that of the non-dominant species. When a species grows rapidly, even a small inoculum may be enough to allow maximum population growth. If this is the case, increasing propagule size or frequency gives little benefit. Therefore, the greater a dominant species' growth rate, and associated dominance, the less likely it is that experimental values of propagule frequency will influence its growth. Second, the greater the proportion of the dominant species in the community, the greater its influence. When the majority of a community is unaffected by propagule frequency, the positive effect exhibited by non-dominant species is less apparent when viewed at the community level. As a dominant species' relative growth rate and its associated proportion within the community increases, the more likely it is that the community will show weakened or even no positive effect from increased propagule pressure.

This hypothesis assumes that propagule pressure positively affects the growth of most species that attain moderate realized growth rate and proportion in the community, as it did in the experimental species. It may be valid to assume that propagule pressure does generally influence these species. Increasing propagule size might promote growth by overcoming Allee effects and minimizing stochasticity. Increasing propagule frequency may provide rescue effect.

The Possible Role of Realized Growth on the Effect of Propagule Size and Frequency. In this experimental system, the individual effects of propagule size and frequency appear to vary with the species' realized growth in the assemblage. The two species with realized growth rates considerably greater than those of all other species are unaffected by propagule pressure. The cell concentration of *Uronema sp.* and *Colpidium striatum* are consistent across all propagule size and propagule frequency treatments. In contrast, the species in this experiment with a rapid growth rate, *Tillina magna*, is positively affected by propagule size, but not propagule frequency. The species with moderate growth rate, *Paramecium bursaria* and *Spirostomum ambiguum*, exhibit a strong positive relationship with both propagule size and frequency. Finally, the species that grows most poorly in these experiments, *Paramecium sp.*, is positively influenced only by the highest values of propagule frequency. It appears that the benefit conferred by high propagule size and frequency varies with a species' growth rate in the community. Species with strong growth rate are not influenced by propagule pressure or influenced only by propagule size. Since the population

grows quickly, inoculating relatively small additions of cells later has no effect. In contrast, species that grow poorly in the protist community are influenced strongly, but only at the highest level of propagule frequency, perhaps the consequence of rescue effect.

*The Possible Effect of Propagule Pressure on
Natural Protist Communities*

Propagule pressure is a diversity-promoting factor in all three experimental communities. Increasing propagule size raises diversity in three communities, and propagule frequency increases diversity in two of the assemblages.

Propagule pressure may generally increase diversity in wild bacterivorous protist communities as well. However, the degree to which high propagule pressure increases diversity and species richness and decreases dominance may depend on the actual values of propagule size and frequency and the growth characteristics of the dominant species in the assemblage, its growth rate relative to other species and how strongly it is itself influenced by propagule pressure.

The positive effect of propagule pressure in influencing community characteristics of species richness and dominance might best be seen when values of propagule size and frequency are relatively large and in communities with members that grow at approximately the same rate with a dominant species whose abundance is influenced by propagule pressure. Communities dominated

by a rapidly-growing species that are themselves not influenced by propagule pressure may show only a weak influence of propagule pressure.

CHAPTER 3. THE ROLE OF PROPAGULE PRESSURE ON THE SUCCESS OF PROTIST INVADERS.

Introduction

A large body of theoretical and empirical work highlights the important influence of propagule pressure on the establishment of invaders. Nevertheless, the details of the relationship between propagule pressure and invasion, and the mechanisms by which propagule pressure actually facilitates establishment, are not well known. We do not yet fully understand the fine-scale relationship between propagule pressure and establishment success (Ruiz and Carlton, 2003; Lockwood *et al.*, 2005). Few experimental studies have quantified the role of propagule pressure in enough detail to create a dose-response curve that depicts the fine-scale relationship between the propagule pressure of an invading species and the probability it will establish a persistent population (Williamson, 1996; Travis *et al.*, 2005). To establish a persistent population, an invader must avoid extinction and achieve population growth at low density (Chesson, 2000; Sakai *et al.*, 2001, Theoharides and Dukes, 2007). Demographic stochasticity may strongly impede the establishment of some invaders (Grevstad, 1999). Few studies have examined how this barrier is weakened by high propagule pressure. Finally, many studies quantify the influence of propagule pressure as the probability of establishment, the likelihood that an invader will establish a reproducing population. This measure may not completely capture all the

aspects of invasion. Perhaps the influence of propagule pressure should also be quantified as invasion success, the proportion of the invasive species in the community.

In Specific Aim #2, I examine the role of propagule size and frequency on the establishment and invasion success of four bacterivorous protist invaders that have slow to moderate growth rate. Treatment levels are finely scaled so that dose-response curves for each invader can be created, allowing the effects of propagule pressure to be compared both within and among species. The dose-response curves are examined to determine whether propagule pressure influences the effect of stochastic extinction. I predict that high propagule size and high propagule frequency will weaken the negative influence of demographic stochasticity and promote establishment and invasion success in all four invaders. However, I also expect that there will be minor differences in the way propagule pressure affects each species, seen as variation in the shape of the species' dose-response curves.

The community diversity experiments of Specific Aim #1, described in chapter 2, demonstrated that high propagule pressure promotes coexistence among established species in bacterivorous protist communities, resulting in greater species richness and community diversity, and lower dominance. Those conclusions will be complemented by this study of the role of propagule pressure on invasion success. I expect this study will show that high propagule pressure

promotes coexistence among native and invasive species in this system, promoting establishment and invasion success. Taken together, the results of these two specific aims will suggest the direction of the system's diversity-invasibility relationship.

Materials and Methods

Experimental Treatments

To determine the influence of propagule pressure on invasion, protist invaders were inoculated into identical established communities at a range of propagule size and frequency values, as illustrated in Figure 3-1. To examine the role of propagule size on a species' invasion success, inoculations of 25, 50, 100, and 300 invader cells were added to replicate established communities. To examine the role of propagule frequency, replicate established communities were maintained with one, two, three, or five 25-cell additions and one, two, three, or five 50-cell additions of the invader during the first four weeks of culture, as illustrated in the timeline (Figure 3-1).

Preparation of Experimental Flasks and Culture Conditions

Resource Base. All treatments utilized a standard resource base that employed a protist media made by autoclaving one Carolina Biological protist pellet and

1375 ml well water for 30 minutes. Twenty-four hours later, the media was divided among treatment vessels, 100 ml media in a sterile 250-ml flask containing two sterile wheat seeds, capped and then autoclaved an additional 30 minutes to ensure sterility. After cooling, individual treatment flasks were inoculated with 150 ml volumes from each overnight culture of bacterial species known to be consumed by all protists used in this system: *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Serratia marcescens*. To ensure consistency in bacterial composition across all treatments, each experimental flask was also inoculated with 300 ml of an overnight culture of pooled protist-free stock media made by passing 2 ml stock solution from all species used in the experiment through a 1.2 micron syringe filter. Immediately after inoculation, flasks were refrigerated until needed. Twenty-four hours before protists were to be added, flasks were placed on the bench top and bacterial cultures were allowed to develop at room temperature (20-21° C).

Established Communities. Established communities were prepared in an identical way in all experimental flasks to minimize variation. Flasks containing 100 ml sterile protist media and inoculated with identical volumes of bacterial stock cultures, as described in the Resource Base section above, were allowed to develop on the bench top at room temperature for 24 hours prior to the inoculation of native protist species. Then 128 cells of each established species were added to each flask. This was done by adjusting the volume of the

inoculated stock culture so that it contained an estimated 128 protist cells based on an average stock cell concentration determined by the mean of ten independent samples withdrawn immediately prior to inoculation. All the cells added to experimental flasks came from the same healthy stock culture.

Established communities were allowed to develop on the bench top at room temperature for three weeks before invaders were added. To minimize variation among established communities, experimental flasks were pooled and re-aliquoted immediately prior to invasion. The media from sets of ten experimental flasks were pooled in sterile 2000 ml flasks, swirled, and then 100 ml volumes were transferred using aseptic technique to sterile 250 ml flasks containing sterile wheat seeds to replace those that were present before pooling.

Culture Conditions. To reduce the concentration of waste and add fresh nutrients, every seven days 10% (10ml) of the media from each flask was removed and replaced with 10 ml sterile media. At this time, one sterile wheat seed was added to each flask to provide a slow release of additional nutrients. During the experiment, flasks were stored on the bench top at room temperature. The entire range of propagule size and frequency treatments for an invader were run simultaneously and all experimental flasks were stored in the same location, exposing them to comparable environmental conditions. However, experiments for different invaders were conducted in different laboratories, and there was variation in the temperature of these rooms. The experiment involving one invader (*Paramecium bursaria*) was run in a laboratory with a room temperature

of 20-21° C. The experiments involving three other invaders (*Tillina magna*, *Spirostomum ambiguum*, and *Paramecium sp.*) were conducted in a different laboratory that had considerably higher ambient temperature (25.6° C).

Replication. All propagule size and propagule frequency treatments were replicated five times. Some experimental communities crashed during the experiment, particularly those maintained at high temperature. When a population crashed, that flask was removed from all analysis over the entire experiment, reducing the number of replications for these treatments to four.

Invaders and Protist Communities

Invaders. Invaders came from healthy stock cultures. To ensure consistency, all invader cells inoculated during the same week came from the same stock flask. The volume of stock culture inoculated into experimental flasks was adjusted so that it contained the correct number of cells based on an average stock cell concentration determined by the mean of ten independent samples withdrawn immediately prior to inoculation.

Protist Communities. The experimental design described in this section was repeated with four different bacterivorous invaders. Each invader was introduced into a unique established community. In total, four protist species were used in these experiments: *Spirostomum ambiguum*, *Tillina magna*, *Paramecium sp.*,

and *Paramecium bursaria*. These are the same four species that comprised the *Tillina magna*-dominated community found to respond so positively to the effects of propagule pressure in the community diversity experiments described in chapter 2. Since all of the species consume the prokaryotic resource base employed in these experiments, competition is the dominant interspecies interaction structuring the communities. The four established community/invaser assemblages are listed in columns below, with the invader shown in bold at the top of each column.

Tillina magna

Paramecium bursaria

Spirostomum ambiguum

Paramecium sp.

Paramecium bursaria

Tillina magna

Spirostomum ambiguum

Paramecium sp.

Spirostomum ambiguum

Tillina magna

Paramecium bursaria

Paramecium sp.

Paramecium sp.

Tillina magna

Paramecium bursaria

Spirostomum ambiguum

Rationale for Choice of Experimental Species

Four of the six protist species employed in the community diversity experiments described in chapter 2 are used in the four invasion assemblages studied here.

Tillina magna, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. were chosen for three reasons. First, when cultured together at certain values of propagule pressure, they form a fairly stable assemblage in which all four species are present at comparable cell concentrations. Second, the community composed of all four species shows a strong positive effect of propagule pressure on diversity, richness, and dominance. High propagule size and high propagule frequency promote coexistence among these species when they are established members of a community. The experiments here will determine whether propagule pressure promotes their coexistence as combinations of invader and established species. Finally, propagule pressure affects the four species differently when they are established species in a community (Figure 3-2). The species vary in the percent change in cell concentration as a response to increasing propagule size and propagule frequency.

Effect of Temperature on Population Dynamics and Associated Data Analysis

The invasion experiments of different protist species had to be conducted in different laboratories that unavoidably varied in temperature. The temperature of the laboratory affected the dynamics of the established communities. The *Paramecium bursaria*-invaded community maintained at 20-21°C continued to increase in cell concentration at a moderate rate throughout the entire experiment. In contrast, the three communities maintained at 25.6° C grew

rapidly reaching a maximum cell concentration, typically on week 2, and then the populations in most flasks declined. Invasion success was analyzed at the time communities were at maximum concentration. This was week 2 for communities invaded by *Tillina magna*, *Spirostomum ambiguum*, and *Paramecium sp.*, and week 5 for the community invaded by *Paramecium bursaria*.

Data Collection and Analysis

Data Collection. Data were collected on all experimental flasks every seventh day, starting with the day the invader was introduced and continuing for a total of six weeks as shown in the timeline, figure 3-1. Each experimental flask was swirled and a 350- μ l sample of media removed.

Determining Cell Concentration. The number of cells of each protist species in each sample was counted and then converted to cell concentration (# cells/ml) using the equation:

$$\text{Cell concentration in experimental flask} = \# \text{ cells in } 350\text{-}\mu\text{l sample} / 0.35$$

Determining the Invader's Probability of Establishment. The probability of establishment was calculated for the invader in every treatment at the time of maximum community concentration as:

$$\text{Probability of Establishment} = \# \text{ flasks containing invader} / \text{total number of flasks in treatment}$$

Determining Invasion Success. Invasion success was calculated for every treatment at the time of maximum community concentration as the proportion of the invader in the community:

$$\text{Invasion success} = \text{Invader cell concentration} / \text{community cell concentration}$$

Statistical Analysis.

Propagule Size. To determine whether propagule size influenced invader cell concentration, probability of establishment, or invasion success, values of the dependent variable were compared across propagule size treatments with regression analysis using SPSS Statistics 17.0 software (SPSS, Inc., 2008). Propagule size values and measured dependent variable data were both identified as numeric scale measures. A generalized linear model used propagule size as the predictor and the different variables as the response to fit a linear curve to the data set. A Wald's Chi-Square test determined the significance of the relationship. In one case, Figure 3-27, the y-intercept of the linear curve was negative. A generalized linear model using propagule size as the predictor and probability of stochastic extinction as the response fit an exponential curve with Normal errors to the data set. For two experimental species, *Tillina magna* and *Paramecium bursaria*, the relationship between increasing invader propagule size and cell concentration, and between propagule size and proportion, was unexpectedly Gaussian. Regression analysis could not be used on these data sets. Treatments were instead compared with one-way

ANOVA followed by Tukey post-hoc comparisons using SigmaPlot for Windows 11.00 (Systat Software, 2008).

Propagule Frequency. To determine whether propagule frequency influenced values of the dependent variable, invader cell concentration, probability of establishment, and invasion success data were compared across propagule frequency treatments with ANCOVA using SPSS Statistics 17.0 software (SPSS, Inc., 2008). Values of propagule frequency and measured dependent variable data were both identified as numeric scale measures. The categorical data of addition size was identified as a factor. A generalized linear model used propagule size data as the covariate predictor, addition size as the factor, and the dependent variable data as the response to fit a linear curve. The model considered the main effects of propagule frequency and addition size, as well as the interaction of propagule frequency and addition size, on the values of the dependent variable. A Wald's Chi-Square test determined the significance of the factor and covariate.

The chi-square values, degrees of freedom, and probability for all tests included in this chapter are given in Appendix: Results of Statistical Analysis.

Results

Uniformity of the Established Communities

To be sure variation in the probability of cell concentration, establishment, and proportion of the invaders is caused by differences in propagule pressure, and not differences among established communities, it is important that the established communities are a standard condition at the time of invasion. To minimize variation, the resource base and established communities of all experimental flasks were prepared in an identical way. Even so, some variation among the established communities developed as the cultures grew for three weeks prior to invasion. In an attempt to further improve consistency among the established communities, the experimental flasks were pooled and re-aliquoted immediately prior to invasion. After pooling, all flasks were analyzed for total cell concentration, species richness, and diversity. At the time of invasion, there was some variation, but the vast majority of established communities were statistically equivalent in terms of cell concentration, species richness, and diversity. These established communities can be considered a standard condition, and differences in invasion success attributed to invader propagule size and frequency. At the time of invasion, a handful of experimental flasks in the *Spirostomum ambiguum* and *Paramecium sp.* invasion experiments had significantly different cell concentration, richness, or diversity values than other communities. In all cases, this initial variation disappeared by week 1, one week

before the communities reached maximum concentration and invasion success was analyzed. For this reason, I felt that it was valid to include these treatments in analysis. Nevertheless, I took care in the analysis of these flasks and would have deleted them from consideration if they exhibited unusual patterns of community characteristics or invasion success.

Propagule Size

Tillina magna Invader

Previous experiments have shown that *Tillina magna* is the dominant species in this assemblage, with cell concentrations slightly higher than that of the other strong competitor, *Paramecium bursaria*.

Probability of Establishment. Propagule size does not influence the probability of *Tillina magna* establishment (Figure 3-3). *Tillina magna* establishes a persistent population in all flasks at all propagule sizes.

Cell Concentration. Propagule size does not significantly influence *Tillina magna* abundance at the time of maximum community concentration, but it does appear to influence the length of time the species maintains a stable population. During the first two weeks of culture, all propagule size treatments grow rapidly, reaching a peak cell concentration at week 2 (Figure 3-4). Propagule size 100

grows extremely rapidly. After one week of growth, the cell concentration of treatment 100 (48 cells/ml) is already much greater than that of all other treatments (18-24 cells/ml). When the majority of *Tillina magna* populations reach their maximum at week 2, there is a pattern of increasing *Tillina magna* cell concentration with increasing propagule size through treatment 100 (Figure 3-5A), but the trend is not significant (ANOVA df 3, 16, $F = 2.563$, $P = 0.091$). After week 2, the cell concentration of all treatments declines rapidly. By week 4, *Tillina magna* is not recovered in samples from most flasks. Propagule size influences the length of time the *Tillina magna* population maintains maximum cell concentration before declining. Low propagule size allows the invader to sustain maximum cell concentration longer than other treatments before crashing. The propagule size treatment associated with the highest maximum cell concentration (100) produces a population that declines very rapidly, and the species disappears from culture one week earlier than other treatments (Figure 3-4).

Proportion of Invader in Community. Propagule size significantly influences the proportion of *Tillina magna* in the community, affecting 1) the maximum proportion achieved by the invader, 2) when this maximum occurs, and 3) how long a stable population is maintained in the community. At week 1, the propagule size 100 treatment has considerably greater proportion of the community (0.22) than all other treatments, which have similar proportion to one another (0.1 - 0.12). The proportion of *Tillina magna* in the community increases

in all treatments from week 1 to week 2, with treatments 50, 100, and 300 increasing at a similar rate much greater than that of treatment 25. In treatments 50, 100, and 300, the invader is at the maximum cell concentration and maximum proportion at week 2. Treatment 25 reaches maximum concentration and proportion at week 3, but this increase is not significantly greater than that of week 2. At week 2, the effect of propagule size on *Tillina magna* proportion is significant (ANOVA df 3, 16, $F = 6.942$, $P = 0.003$) (Figure 3-5B). The proportion of propagule size 100 (0.48) is significantly greater than that of treatment 25 (Tukey $P = 0.002$) (Figure 3-5B). Treatments 50 and 100 have similar proportion (0.38) that is greater than that of treatment 25 (0.24), but not significantly so. When the proportion of *Tillina magna* in the community on week 2 is compared to the species' cell concentration on week 2, a significant positive relationship is seen ($\chi^2 = 13.175$, $df = 1$, $P < 0.01$) (Figure 3-6A). The greater the cell concentration of *Tillina magna*, the greater its proportion in the community. By influencing cell concentration, propagule size indirectly influences *Tillina magna* invasion success, as measured by the species' proportion within the community. When maximum proportion is compared across treatments, regardless of when maxima are achieved, there is still a pattern of increasing concentration with increasing propagule size through treatment 100, but the trend is not significant (Figure 3-6B). Propagule size affects the pattern of decline in *Tillina* proportion over time. Higher propagule size treatments (100, 300) decrease in proportion immediately after week 2, while lower propagule size treatments remain constant (50) or even increase slightly (25) from week 2 through week 3, and begin to

decline only after week 3. After week 3, the proportion of all treatments decreases rapidly. By week 5, the proportion of all treatments is essentially zero.

Summary. Propagule size does not affect the probability of *Tillina magna* establishment because the species is able to form a persistent population even at the lowest propagule size. However, propagule size affects the invasion success of *Tillina magna* in this system in three ways – by influencing the proportion achieved on week 2, the time at which maximum proportion is reached, and the length of time the invader maintains a viable population in the community. There is a clear and significant pattern of increasing *Tillina magna* invasion success, as measured by proportion, with increasing propagule size from treatment 25 through treatment 100. When 100 *Tillina magna* cells are added to established communities, the invader achieves significantly higher proportion in the community than when only 25 invader cells are added. Increasing propagule size over 100 does not provide benefit, and actually leads to a slight, but not significant, decrease in invasion success. When *Tillina magna* is inoculated at low propagule size (25, 50), it grows well and maintains a stable concentration or even continues to increase in proportion and ratio one week longer than that of treatment 100. When introduced at high propagule size (300), *Tillina magna* cell concentration and proportion are reduced to levels similar to those of treatment 50.

Paramecium bursaria Invader

Paramecium bursaria is a robust competitor in this community, with cell concentrations slightly lower than that of dominant species, *Tillina magna*. In established community experiments, *Paramecium bursaria* concentration is strongly influenced by propagule size.

Probability of Establishment. Propagule size does not influence the probability of *Paramecium bursaria* establishment (Figure 3-7). The species establishes a persistent population in all flasks, regardless of propagule size.

Cell Concentration. Propagule size significantly influences the invasion success of *Paramecium bursaria* five weeks after inoculation, with treatments 50 and 100 associated with the greatest success (ANOVA df 4, 19, $F = 4.860$, $P = 0.007$) (Figure 3-8A). During the first five weeks of culture, all propagule size treatments grow exponentially, but at different rates. The intermediate treatments (50,100) grow similarly and at a rate that gives them significantly greater cell concentration (110 cells/ml) than that of the propagule size 25 treatment by week 5 (Tukey $P \leq 0.027$) (Figure 3-8A). The highest propagule size treatments (200, 300) grow similarly to each other, at a rate similar to the lowest treatment level (25). At week 5, the cell concentrations of all three of these treatments are essentially equivalent (42-60 cells/ml) (Figure 3-8A).

Proportion of Invader in Community. Propagule size strongly influences the proportion of *Paramecium bursaria* in the community, beginning as early as the second week after inoculation. At week 2, *Paramecium bursaria* populations in high propagule size treatments (200, 300) have similar large proportion (0.11). However, the proportion of these treatments is basically stable from weeks 2 through 5. In contrast, propagule size treatments 25, 50, and 100 have similar low proportion on week 2 (0.01-0.05), but all three treatments increase in proportion from week 2 through week 5. Treatments 50 and 100 increase at similar high rate, reaching nearly identical large proportion (0.28) on week 5 (Figure 3-8B). Treatment 25 increases more slowly, so that its proportion on week 5 is similar to that of treatments 100 and 200. At week 5, the effect of propagule size on the proportion of *Paramecium bursaria* is significant (ANOVA df 4, 19, $F = 4.156$, $P = 0.014$) (Figure 3-8B). When *Paramecium bursaria* proportion is compared to its cell concentration on week 5, there is a significant trend of increasing invader proportion with increasing invader N ($\chi^2 = 20.898$, df = 1, $P < 0.01$). (Figure 3-9). This suggests that propagule size influences invader cell concentration on week 5, which in turn influences invader proportion in the community.

Summary. Propagule size does not influence the probability of establishment of *Paramecium bursaria*. The species forms persistent populations in all communities at all propagule sizes. Propagule size does significantly affect the invasion success of *Paramecium bursaria* in this system, as measured by both

concentration and proportion. When 50 or 100 *Paramecium bursaria* cells are added to 3-week communities of *Spirostomum ambiguum*, *Tillina magna*, and *Paramecium sp.*, the invader achieves significantly greater cell concentration and proportion in the community than other treatments. When *Paramecium bursaria* is added at low propagule size (25), it grows slowly, but not well enough to produce good invasion success. When introduced at high propagule size, *Paramecium bursaria* initially has a high concentration and high proportion in the community, but these measures remain stable throughout the week 2-5 period and are surpassed by the intermediate propagule size treatments (50, 100).

Spirostomum ambiguum Invader

Previous experiments have shown that *Spirostomum ambiguum* is a weak competitor in this assemblage, with cell concentrations much lower than those of *Tillina magna* and *Paramecium bursaria*.

Probability of Establishment. There is variation in the probability of *Spirostomum ambiguum* establishment. On week 2, when probability levels are at maximum values, increasing propagule size is associated with increasing probability of establishment through propagule size 100 (Figure 3-10). At this time, the probability of *Spirostomum ambiguum* establishment increases from 0.4 for the 25-cell inoculation, to 0.6 for the 50-cell inoculation, reaching a maximum probability of 1.0 at propagule size

100. Probability declines slightly with propagule size greater than 100. The probability of propagule size 300 is 0.8. The effect of propagule size on variation in *Spirostomum ambiguum* establishment success is not significant when all propagule sizes, 25, 50, 100, and 300, are compared ($\chi^2 = 1.867$, $df = 3$, $P = 0.600$). However, there is a significant trend of increasing establishment with increasing propagule size for treatments 25, 50, and 100 ($\chi^2 = 279.101$, $df = 1$, $P < 0.01$) (Figure 3-10).

Cell Concentration. Propagule size influences 1) the maximum cell concentration attained by *Spirostomum ambiguum*, 2) the time at which maximum cell concentration is achieved, and 3) the time at which population decline begins. There is a significant trend of increasing maximum *Spirostomum ambiguum* cell concentration with increasing propagule size, 1.4 cells/ml for propagule size 25 to 7.4 cells/ml for propagule size 300 ($\chi^2 = 7.004$, $df = 1$, $P = 0.008$) (Figure 3-11A). As propagule size increases, the time at which the maximum concentration is achieved decreases. Propagule size 300 reaches its maximum concentration at week 1, propagule size 100 reaches maximum at week 2, and propagule sizes 25 and 50 take until week 3 to reach their maxima. After achieving maximum cell concentration, all cultures decline. The higher the propagule size, the earlier this decline begins. The propagule size 300 population begins to decline after week 1. The propagule size 100 treatment declines after week 2. The propagule size 50 treatment declines after week 3, and the propagule size 25 treatment remains

fairly stable through week 4 (declining minimally after week 3 and more sharply after week 4) (Figure 3-12).

Proportion of Invader in Community. Propagule size influences the proportion of *Spirostomum ambiguum* in the community, affecting 1) the maximum proportion achieved by the invader, 2) when this maximum occurs, and 3) the pattern of change in proportion over time for the population. There is a significant trend of increasing maximum *Spirostomum ambiguum* proportion with increasing propagule size ($\chi^2 = 7.744$, $df = 1$, $P = 0.005$) (Figure 3-11B). When *Spirostomum ambiguum* proportion on week 2 is compared to the species' cell concentration at the same time, there is a significant trend of increasing proportion with increasing cell concentration ($\chi^2 = 12.277$, $df = 1$, $P < 0.01$) (Figure 3-13). This suggests that propagule size significantly influences *Spirostomum* cell concentration, which significantly influences the proportion of the invader in the community. Propagule size also influences when maximum proportion is attained. *Spirostomum ambiguum* populations in high propagule sizes reach maximum proportion earlier than those inoculated at lower propagule size. Propagule size 300 is at maximum proportion at week 1, size 100 reaches maximum at week 2, and treatment 25 is at maximum at week 3. Propagule size treatment 50 reaches maximum on week 1, but remains essentially stable through week 3. The greater the propagule size, the less time the population spends increasing in proportion and the more time is spent in decline. Propagule size 300 is at maximum on week 1 and begins to decline in proportion

immediately. Treatment 100 increases in proportion from week 1 to week 2 and then declines. Treatment 50 has stable proportion through week 3 and then declines, while treatment 25 increases in proportion through week 3 and declines immediately after.

Summary. There is variation in the establishment success of

Spirostomum ambiguum in this system. Increasing propagule size is associated with a significant increase in probability of establishment with until reaching a maximum at propagule size 100. Propagule size also influences

Spirostomum ambiguum cell concentration and proportion, the time at which maximum concentration and proportion are achieved, and the pattern of change in concentration and proportion. There are significant trends of increasing

maximum *Spirostomum ambiguum* cell concentration and proportion with increasing propagule size. The relationship between propagule size and

Spirostomum ambiguum cell concentration is significant, as is the relationship between *Spirostomum ambiguum* cell concentration and proportion. This

suggests that propagule size influences *Spirostomum ambiguum* cell concentration, which in turn influences that species' proportion in the community.

The greater the propagule size, the earlier the maximum concentration and proportion are achieved, from week 1 for propagule size 300 to week 3 for propagule size 25. The greater the propagule size, the more time the population spends in a decline in concentration and proportion – from 4 weeks for propagule size 300 to 2 weeks for propagule size 25. The lower the propagule size, the

more time is spent in increasing concentration and proportion – from 3 weeks for propagule size 25 to 0 for propagule size 300.

Paramecium sp. Invader

Previous experiments have shown that *Paramecium sp.* is a weak competitor in this assemblage, with cell concentrations much lower than those of all other species.

Probability of Establishment. In general, *Paramecium sp.* does not successfully invade this community. The species is found only when inoculated at high or relatively high propagule size. At week 2, when the community is at maximum concentration, *Paramecium sp.* has a probability of establishment of 0.2 at propagule size 50 and 0.4 at propagule size 300. The relationship between propagule size and probability of *Paramecium sp.* establishment is significant ($\chi^2 = 8.181$, $df = 1$, $P = 0.004$) (Figure 3-14). There is variation in the time at which *Paramecium sp.* is first found in samples. *Paramecium sp.* is found earlier when inoculated at high propagule size. It is first found in the propagule size 300 treatment on week 1, and is first found in the propagule size 50 treatment at week 2.

Cell Concentration. Over the course of the experiment, there is variation in *Paramecium sp.* growth at different propagule sizes. All treatments remain at low

concentration throughout the experiment (< 5 cells/ml), but the species' cell concentration is substantially greater than zero only at the highest propagule size (300). Invading *Paramecium* sp. populations increase to an early maximum cell concentration at week 2 (Figure 3-15A). At the week 2 maximum, there is a significant trend of increasing *Paramecium* sp. cell concentration with increasing propagule size, from 0 cells/ml for propagule size 25, to 0.5 cells/ml for propagule size 50, to 2.2 cells/ml for propagule size 300 ($\chi^2 = 5.767$, $df = 1$, $P = 0.016$) (Figure 3-15B). *Paramecium* sp. populations decline after week 2 to concentrations so low that they are not recovered in samples. When propagule size is high (300), the population is able to increase again at the end of the experiment to 4.5 cells/ml (Figure 3-15A).

Proportion of Invader in Community. Propagule size significantly effects the maximum proportion of *Paramecium* sp. in the community ($\chi^2 = 5.934$, $df = 1$, $P = 0.015$) (Figure 3-16). The species is found only in two treatments. Of these, maximum *Paramecium* sp. proportion is substantially greater at higher propagule size (0.002 for propagule size 50, 0.22 for propagule size 300) (Figure 3-16).

Summary. *Paramecium* sp. is not a strong invader in this community. However, increasing propagule size significantly improves the species' ability to invade the system. Increasing propagule size significantly increases the probability of *Paramecium* sp. establishment, its cell concentration, and its proportion within the community. The species' establishment and invasion success is greater, and

the species is recovered earlier, at the highest propagule size (300). At propagule size 300, *Paramecium* sp. invaders are able to establish populations in 40% of the experimental flasks, a probability twice as high as that of the only other treatment in which establishment occurs (propagule size 50). When *Paramecium* sp. invaders reach an early maximum cell concentration on week 2, the populations at propagule size 300 have cell concentrations over two times greater than that of a lower propagule size. After this maximum, all invader populations decline, but the population at propagule size 300 is able to grow again at the end of the experiment. The maximum proportion the *Paramecium* sp. invader achieves in the community at propagule size 300 is significantly greater than that achieved at lower propagule size.

Propagule Frequency

Tillina magna Invader

Probability of Establishment. Propagule frequency does not influence the probability with which *Tillina magna* establishes a persistent population in the community. *Tillina magna* invaders successfully establish populations in all flasks in all treatments, regardless of propagule frequency (Figure 3-17).

Cell Concentration. Since the temperature in the lab in which this experiment was conducted was very high, the cultures grew much more rapidly than in

previous experiments. For propagule frequency experiments, treatments can only be compared after all invader additions are made and the cultures have had at least one week of growth after the last addition. The earliest the full series of five additions can be checked is week 5. By this time, the *Tillina magna* cultures had reached maximum propagule size and crashed. On week 5, the invader was at very low concentration and was found in samples taken from only 1/16th of the experimental flasks. However, some propagule frequency treatments could be compared on week 2, at the time of maximum cell concentration. There are two valid propagule frequency comparisons at this time – one-addition and two-addition treatments at two different propagule addition sizes, 25 and 50. This design is certainly sub par. The range of propagule frequency treatments is too narrow, and there is little time for the populations in different treatments to react to dissimilar conditions. Nevertheless, the treatments show significant effects of propagule frequency, addition size, and the interaction of frequency and addition size on *Tillina magna* cell concentration (3-18A). There is a significant difference in the way addition size influences the affect of increasing propagule frequency on *Tillina magna* cell concentration ($\chi^2 = 5.544$, df = 1, P = 0.019). The interaction between addition size and frequency also significantly affects *Tillina magna* cell concentration ($\chi^2 = 6.154$, df = 1, P = 0.013). When addition size is low (25 cells), increasing propagule frequency from one- to two-additions significantly increases *Tillina magna* cell concentration ($\chi^2 = 4.134$, df = 1, P = 0.042). The 2x(25) treatment has a cell concentration (112 cells/ml) significantly greater than that of treatment 1x(25) (73 cells/ml). However, when

addition size is larger (50 cells), increasing propagule frequency from one to two additions does not significantly affect *Tillina magna* cell concentration (Figure 3-18A). Treatment 1x(50) has a cell concentration of 99 cells/ml, while the 2x(50) treatment has a slightly, but not significantly, lower cell concentration of 77 cells/ml. The maximum cell concentration attained influences the pattern of growth and decline of the invader population. Propagule frequency treatments that result in low invader cell concentration allow the population to maintain stable abundance longer than populations that reach higher maximum cell concentration. In both the 25-cell and 50-cell addition series, the propagule frequency treatment associated with the highest cell concentration begins to decline immediately after week 2. In contrast, the propagule frequency treatment associated with the lowest cell concentration is stable through week 3. Low cell concentration gives these treatments one extra week of population stability.

Proportion of Invader in Community. The effect of propagule frequency and addition size on the proportion of *Tillina magna* in the community is similar to that on cell concentration. The experimental treatments show significant effects of propagule frequency, addition size, and the interaction of frequency and addition size on *Tillina magna* proportion (3-18B). There is a significant difference in the way addition size influences the affect of increasing propagule frequency on *Tillina magna* proportion ($\chi^2 = 8.367$, df = 1, P = 0.004). The interaction between addition size and frequency also significantly affects *Tillina magna* proportion

($\chi^2 = 5.242$, $df = 1$, $P = 0.022$). When addition size is low (25 cells), increasing propagule frequency from one- to two-additions slightly increases *Tillina magna* proportion, from 0.24 to 0.3, but the trend is not significant. However, when addition size is larger (50 cells), increasing propagule frequency from one to two additions significantly decreases *Tillina magna* proportion from 0.38 to 0.30 ($\chi^2 = 4.481$, $df = 1$, $P = 0.034$) (Figure 3-18B).

Summary. *Tillina magna* is capable of founding persistent populations in all flasks at all treatment levels, so propagule frequency has no effect on the species' probability of establishment. In contrast, propagule frequency, the size of additions, and the interaction between frequency and addition size do significantly influence *Tillina magna* cell concentration and proportion. The effect of increasing propagule frequency when the size of additions is low is very different from the effect when the size of additions is large. Increasing propagule frequency in the 25-cell addition treatments enhances *Tillina magna* cell concentration and proportion. In contrast, increasing propagule frequency in the 50-cell addition treatments decreases the invader's cell concentration and proportion. Maximum cell concentration is related to the length of time the invading population maintains a stable cell concentration. *Tillina magna* populations with low maximum cell concentration on week 2 persist one week longer before declining than do populations that reach a higher maximum cell concentration.

Paramecium bursaria Invader

Probability of Establishment. *Paramecium bursaria* is able to establish a persistent population in all flasks in all treatments, regardless of propagule frequency (Figure 3-19). Therefore, propagule frequency does not influence the species' probability of establishment.

Cell Concentration. Propagule frequency, addition size, and the interaction between frequency and addition size significantly affect *Paramecium bursaria* growth and cell concentration. On week 5, there is a significant difference in the way addition size influences the affect of increasing propagule frequency on *Paramecium bursaria* cell concentration ($\chi^2 = 0.741$, df = 1, P = 0.002). The interaction between addition size and frequency also significantly affects *Paramecium bursaria* concentration ($\chi^2 = 4.471$, df = 1, P = 0.034). When addition size is low (25 cells), increasing propagule frequency has no effect on *Paramecium bursaria* cell concentration. During the first five weeks of culture, all 25-cell addition treatments grow at similar rate, producing populations of nearly identical cell concentration (42-50 cells/ml) on week 5. However, when addition size is larger (50 cells), increasing propagule frequency significantly decreases *Paramecium bursaria* concentration ($\chi^2 = 5.422$, df = 1, P = 0.020) (Figure 3-20A). The 50-cell addition treatments receiving multiple additions over the first four weeks of culture (2x(50), 3x(50), and 6x(50)) grow similarly, producing comparable cell concentrations on week 5 (55-62 cells/ml). In contrast,

treatment 1x(50) grows at considerably greater rate than other treatments, and this results in substantially greater cell concentration on week 5 (109 cells/ml). The propagule size experiments described earlier show that 25-cell additions of *Paramecium bursaria* do not grow well. This experiment suggests that additional 25-cell inoculations do not improve the probability of this invader's establishment. In contrast, when *Paramecium bursaria* has an initial inoculum of 50 cells, it grows very well, forming a dense population by week 5. Adding additional 50-cell inoculations of *Paramecium bursaria* is associated with decreased cell concentration.

Proportion of Invader in Community. The effect of propagule frequency and addition size on the proportion of *Paramecium bursaria* in the community is similar to the effect on cell concentration. The experimental treatments show significant effects of addition size, and the interaction of frequency and addition size on *Paramecium bursaria* proportion (3-20B). There is a significant difference in the way addition size affects the influence of increasing propagule frequency on *Paramecium bursaria* proportion ($\chi^2 = 5.580$, df = 1, P = 0.0018). The interaction between addition size and frequency also significantly affects *Paramecium bursaria* proportion ($\chi^2 = 3.973$, df = 1, P = 0.046). When addition size is low (25 cells), increasing propagule frequency slightly increases *Paramecium bursaria* proportion, from 0.15 to 0.22, but the trend is not significant. When addition size is larger (50 cells), increasing propagule frequency slightly decreases *Paramecium bursaria* proportion from 2.7 to 1.6.

This trend is also not significant (Figure 3-20B). There are significant trends of increasing *Paramecium bursaria* proportion with increasing *Paramecium bursaria* cell concentration for both the 25-cell and 50-cell addition series ($\chi^2 = 6.135$, $df = 1$, $P = 0.013$) treatments (Figure 3-21).

Summary. Propagule frequency does not influence the probability of *Paramecium bursaria* establishment because the species is able to form persistent populations in all flasks at all treatments, regardless of propagule frequency. Propagule frequency, addition size, and the interaction between frequency and addition size does significantly affect *Paramecium bursaria* cell concentration and proportion on week 5. When small 25-cell additions of *Paramecium bursaria* are inoculated into established communities at a range of propagule frequency values, invading populations grow at similar, slow rate, regardless of the number of additions. They form populations with nearly equivalent cell concentration and proportion on week 5. However, when *Paramecium bursaria* additions are larger (50-cell), multiple additions of the invader are associated with reduced population growth, resulting in significantly lower cell concentration and slightly lower proportion in high propagule frequency treatments.

Spirostomum ambiguum Invader

Probability of Establishment. Propagule frequency significantly influences the probability of *Spirostomum ambiguum* establishment ($\chi^2 = 24.011$, $df = 1$, $P < 0.01$). Increasing propagule frequency increases the probability of establishment, and a larger size of additions significantly amplifies this effect ($\chi^2 = 4.013$, $df = 1$, $P = 0.045$) (Figure 3-22). When 25-cell additions are inoculated into communities, the probability of *Spirostomum ambiguum* establishment increases from 0.2 at 1x(25) to 0.6 at 3x(25). When additions are larger (50-cell), the increase is more pronounced, and the probability of establishment rises from 0 at 1x(50) to 0.8 at 5x(50).

Cell Concentration. Neither addition size nor the interaction between frequency and addition size influence *Spirostomum ambiguum* cell concentration. However, increasing propagule frequency does significantly increase *Spirostomum ambiguum* cell concentration in the 25-cell addition series ($\chi^2 = 4.358$, $df = 1$, $P = 0.037$). Propagule frequency has no effect on cell concentration in the 50-cell addition series (Figure 3-23A). This experiment is unusual in that the established communities of the two highest propagule frequency treatments (3x, 5x) had substantially greater cell concentration, species richness, and diversity at the time of invasion than the two treatments with lower propagule frequency (1x, 2x). The higher propagule frequency treatments should, therefore, be more difficult to invade, and I would expect

Spirostomum ambiguum cell concentration to be lower in these treatments. Since *Spirostomum ambiguum* cell concentration in the higher propagule frequency treatments is similar to or slightly higher than that in the lower propagule size treatments, perhaps high propagule frequency actually does promote *Spirostomum ambiguum* growth. This positive effect is just not evident in the 50-cell addition series because established communities did not have equivalent characteristics at the time of invasion.

Proportion of Invader in Community. On week 5, there is a significant trend of increasing *Spirostomum ambiguum* proportion with increasing propagule frequency in both the 25-cell and 50-cell addition series ($\chi^2 = 7.796$, $df = 1$, $P = 0.005$) (Figure 3-23B). Addition size, however, has no effect. From week 2 through week 4, all treatments have fairly similar proportion and this proportion remains relatively stable over time (0.005-0.01). There is variation, but the error bars are large, so treatments are statistically equivalent, both within and between cell-addition series. After week 4, the lower propagule frequency treatments (1x, 2x) continue to remain stable, while the higher propagule frequency treatments (3x, 5x) increase tremendously in proportion. The 25-cell addition treatments increase to approximately 0.15, the 50-cell addition treatments to 0.27-0.55.

Summary. Increasing propagule frequency significantly increases the probability of *Spirostomum ambiguum* establishment, and a larger size of additions

significantly amplifies this effect. Propagule frequency, but not addition size, significantly affects the cell concentration and proportion of this invader. On week 5, there is a significant trend of increasing *Spirostomum ambiguum* cell concentration with increasing propagule frequency for the 25-cell addition series. There is also a significant increase in the proportion of *Spirostomum ambiguum* in the community with increasing propagule frequency in both the 25- and 50-cell addition series.

Paramecium sp. Invader

Probability of Establishment. Propagule frequency has a significant positive effect on the probability of *Paramecium sp.* establishment ($\chi^2 = 87.584$, $df = 1$, $P < 0.01$) (Figure 3-24). Increasing propagule frequency from one addition to six additions of 25 cells raises *Paramecium sp.* establishment from a probability of 0 to 0.6. The effect of addition size is not significant, so the increase in probability of establishment is similar in the 50-cell addition series. When 50-cell additions are made, the probability of *Paramecium sp.* establishment rises from 0 to 0.8.

Cell Concentration. Propagule frequency influences *Paramecium sp.* cell concentration in several ways over the course of the experiment (Figure 3-25).
1) High propagule frequency fosters relatively high cell concentration during the period of maximum community concentration on week 2, resulting in a density

great enough for the species to be recovered in samples. 2) High propagule frequency minimizes or eliminates the period of decline after week 2, promoting a second period of invader growth late in the experiment after the established community has declined. 3) Larger addition size amplifies propagule frequency effects. The *Paramecium sp.* invader is not recovered in samples until week 2, the time of maximum community cell concentration. At this time, *Paramecium sp.* is recovered at low concentrations under 1.25 cells/ml mainly from high propagule frequency treatments (5x(25), 1x(50) and 5x(50)). The concentration of *Paramecium sp.* in most treatments appears to decline immediately thereafter. The invader is never again recovered from treatment 1x(50), and is not recovered from 5x(25) until three weeks later. High propagule frequency appears to minimize or eliminate the period of decline after week 2, and large addition size intensifies this effect. Starting as early as week 3, and continuing through the end of the experiment, *Paramecium sp.* is recovered in the highest propagule frequency/addition size treatments 5x(50) and 3x(50). Two weeks later, the invader is recovered in the three highest 25-cell addition treatments (2x(25), 3x(25), and 5x(25)). On week 5, the trend of increasing *Paramecium sp.* cell concentration with increasing propagule frequency is significant for both the 25-cell and 50-cell addition series ($\chi^2 = 8.286$, $df = 1$, $P = 0.004$). The interaction between frequency and addition size also has a significant effect on cell concentration ($\chi^2 = 3.734$, $df = 1$, $P = 0.053$) (Figure 3-26A). These patterns suggest that high propagule frequency promotes greater invader cell concentration during week 2 and minimizes the decline that occurs in most

treatments after week 2, thereby allowing a second period of growth toward the end of the experiment. Larger addition size intensifies the effect of increasing propagule frequency on cell concentration, promoting greater invader cell concentration at high propagule frequency compared to that of lower addition size, and stimulating an earlier second period of growth which permits the species to be recovered as much as three weeks earlier than treatments with lower propagule size additions.

Proportion of Invader in Community. Propagule frequency and addition size do not influence the proportion of *Paramecium sp.* There is variation in the proportion of the *Paramecium sp.* invader in the community at various values of propagule frequency and addition size, but no trends (Figure 3-26B).

Summary. Increasing propagule frequency significantly facilitates *Paramecium sp.* establishment. Propagule frequency and the interaction between propagule frequency and addition size also significantly affect *Paramecium sp.* cell concentration. On week 5, there is a significant trend of increasing *Paramecium sp.* cell concentration with increasing propagule frequency. The effect is amplified when addition size is large. Propagule frequency affects *Paramecium sp.* growth throughout the experiment. The invader's cell concentration is greater at high propagule frequency during the period of maximum community concentration on week 2, facilitating densities high enough for the species to be recovered in samples. High propagule

frequency also decreases or eliminates the period of decline following week 2 and promotes a second period of growth late in the experiment. The second period of growth begins earlier when propagule frequency is high and the size of additions is great. *Paramecium sp.* is recovered as much as three weeks earlier in 50-cell addition treatments than in treatments with smaller 25-cell additions.

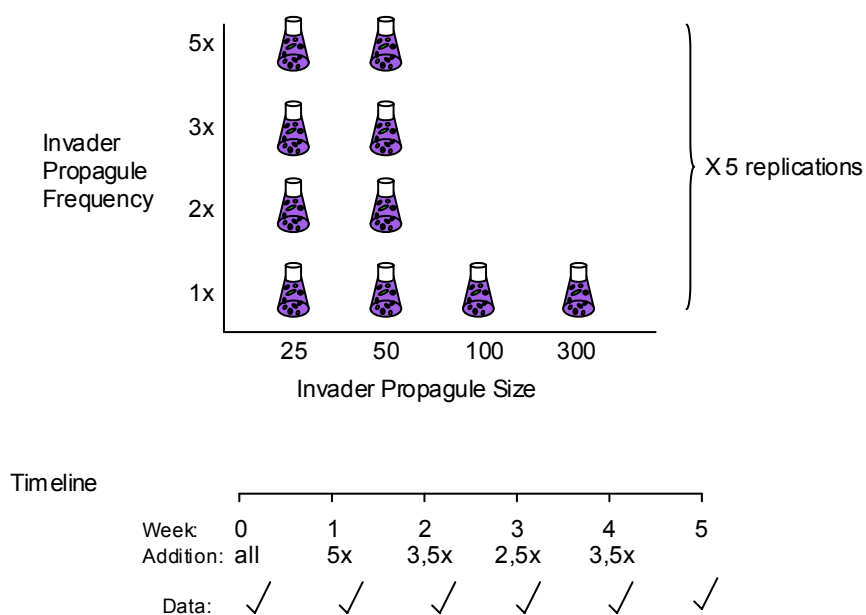
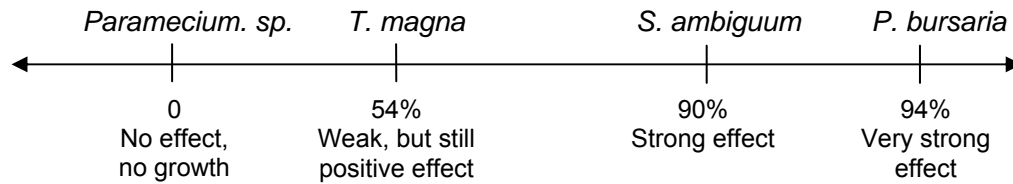


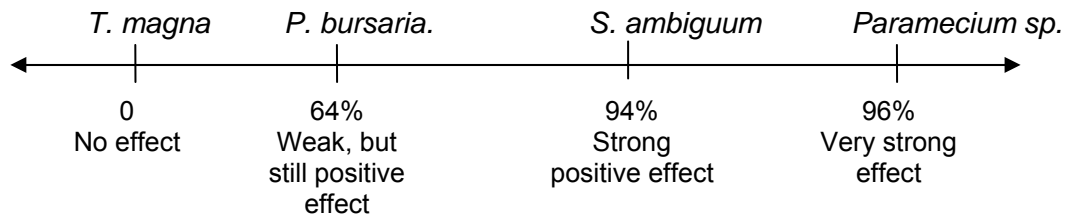
Figure 3-1. Experimental design for Specific Aim #2: To examine the role of propagule size and propagule frequency on the success of protist invaders. The influence of propagule size was studied by inoculating replicate established communities with 25, 50, 100, or 300 cells of an invader species. The effect of propagule frequency was studied by maintaining replicate established communities with 1, 2, 3, or 5 25- or 50-cell additions of the invading species during the first four weeks of culture. All treatments were maintained for five weeks. The cell concentration of all species in every experimental flask was determined every seven days for five weeks.

(A)



Effect of Propagule Size

(B)



Effect of Propagule Frequency

Figure 3-2. The effect of propagule size (A) and propagule frequency (B) on the growth of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* when they are cultured together as a community. The effect of propagule pressure is measured as the species' percent change in cell concentration between the highest and lowest propagule pressure treatments, 8 and 128 for propagule size (A) and 1x(50) and 6x(50) for propagule frequency (B).

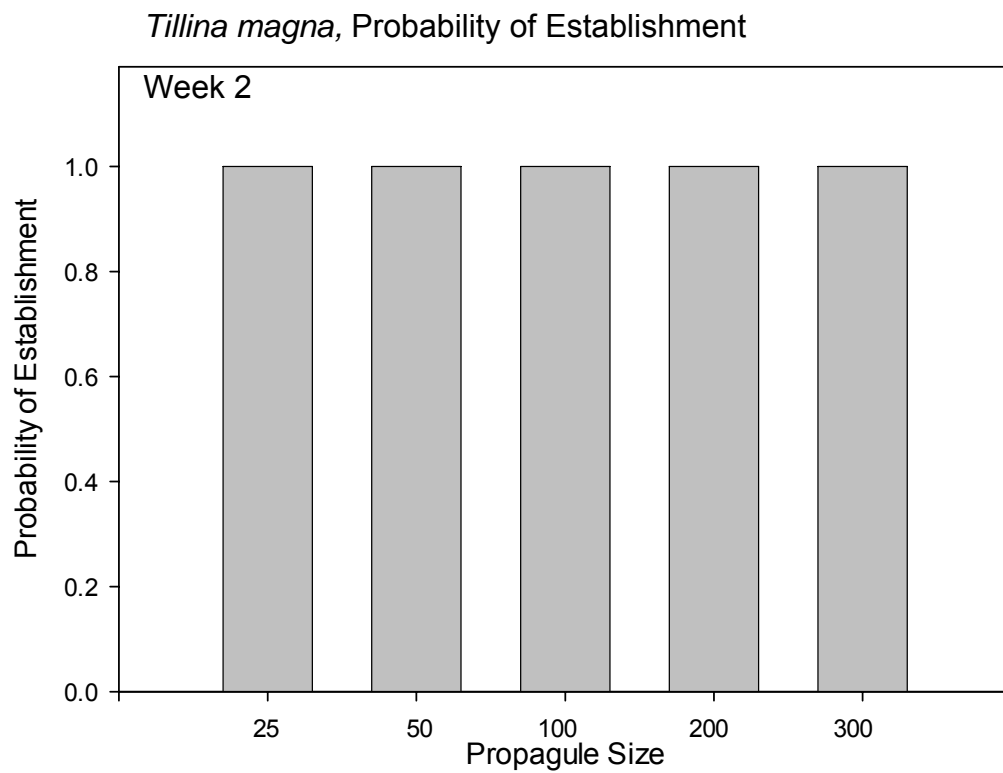


Figure 3-3. The probability of establishment of the invader, *Tillina magna*, two weeks after 25, 50, 100, or 300 cells of the species are introduced into established communities. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. and cultured three weeks before the invader was added.

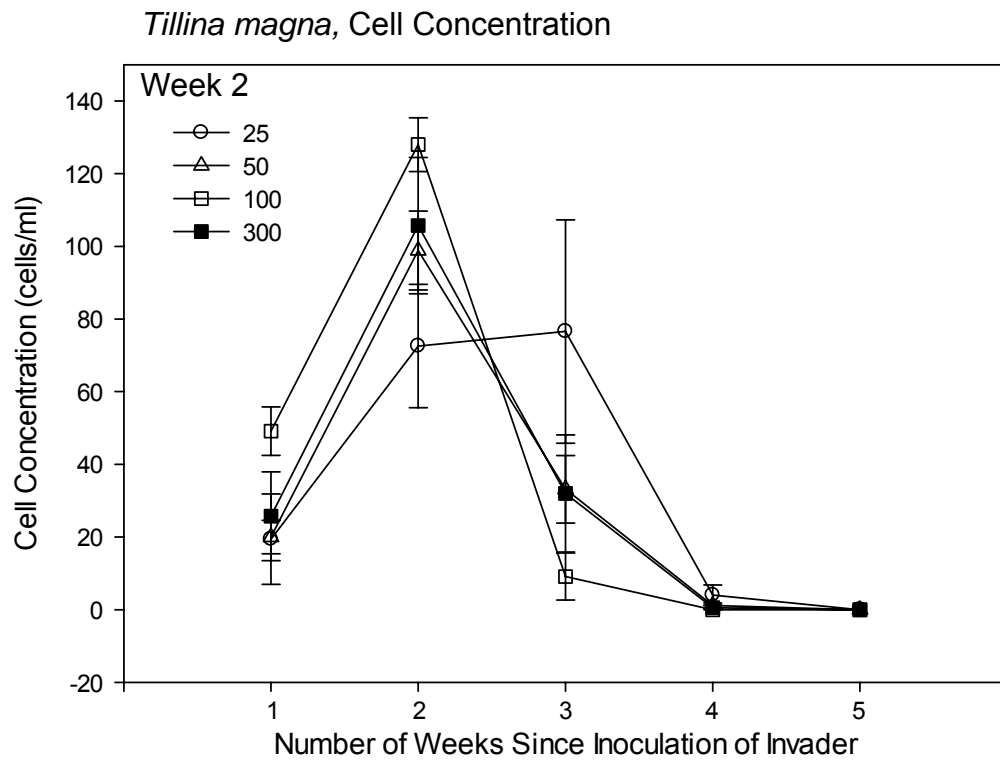
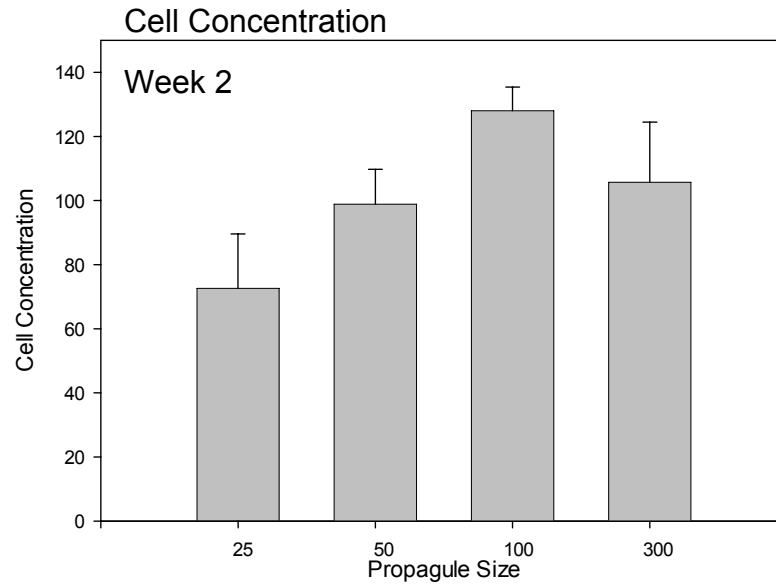


Figure 3-4. Cell concentration of the invader, *Tillina magna*, when 25, 50, 100 or 300 cells of the species are introduced into established communities. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment.

(A)



(B)

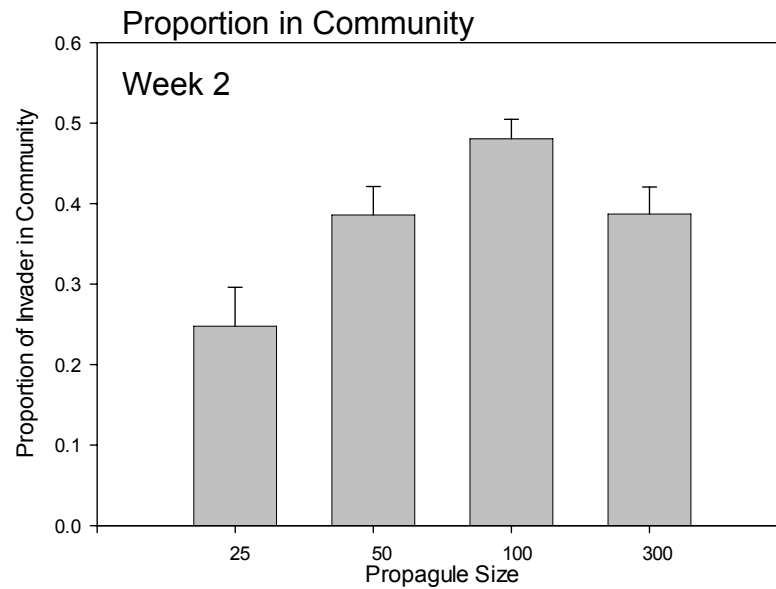
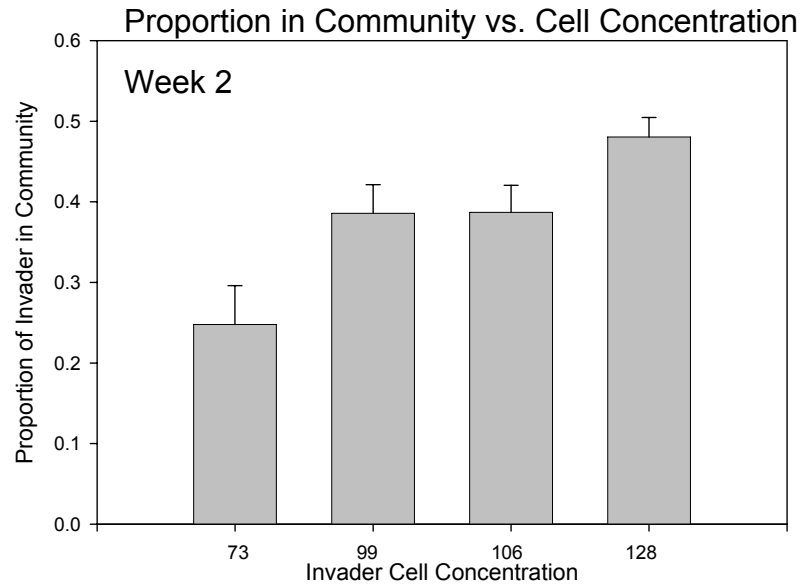


Figure 3-5. The cell concentration (A) and proportion (B) of the invader, *Tillina magna*, two weeks after 25, 50, 100, or 300 cells of the species are introduced into established communities. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment. The effect of propagule size on proportion (B) is significant, 1-way ANOVA (df 3, 16; $F = 6.942$; $P = 0.003$); Tukey post-hoc comparison (propagule size 25 < 100, $P = 0.002$).

(A)



(B)

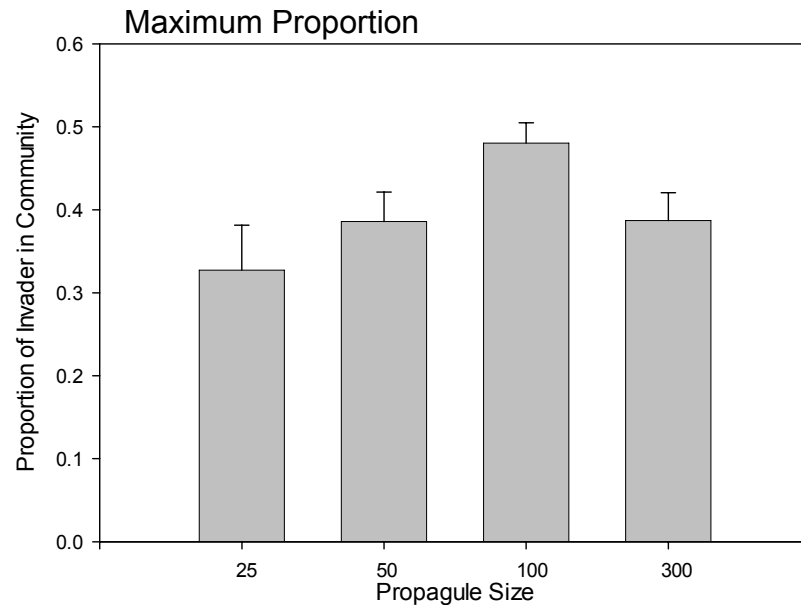


Figure 3-6. The proportion of the invader *Tillina magna* at associated cell concentration (A) and the maximum proportion of *Tillina magna* (B) when 25, 50, 100, or 300 cells of the species are introduced into established communities. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment. The effect of cell concentration on proportion is significant (A) ($\chi^2 = 13.173$, $df = 1$, $P < 0.01$).

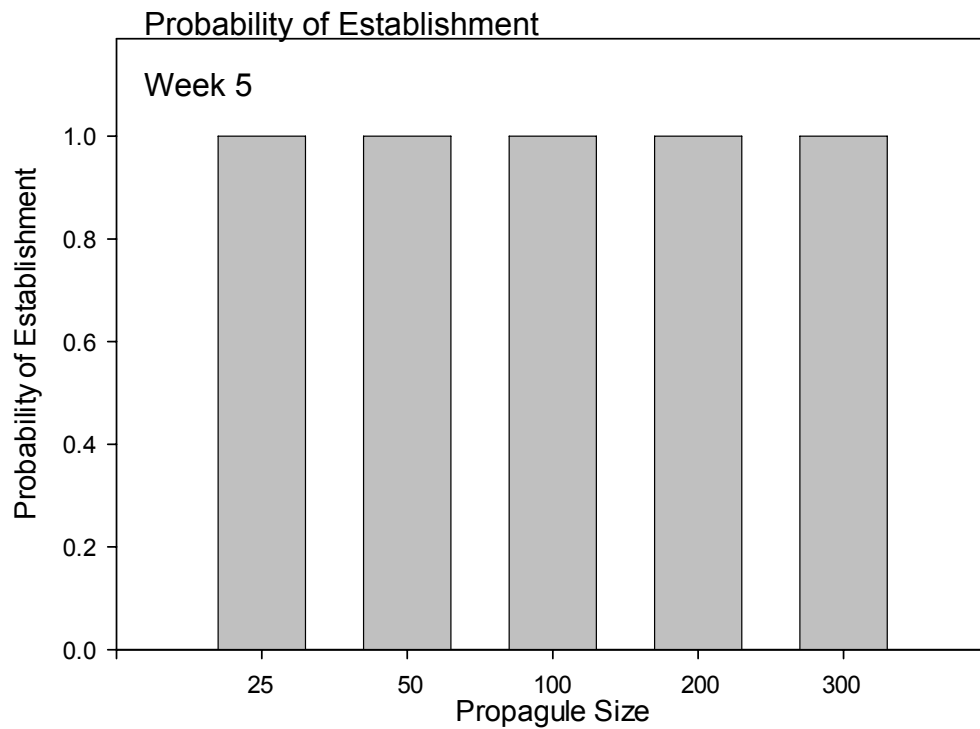
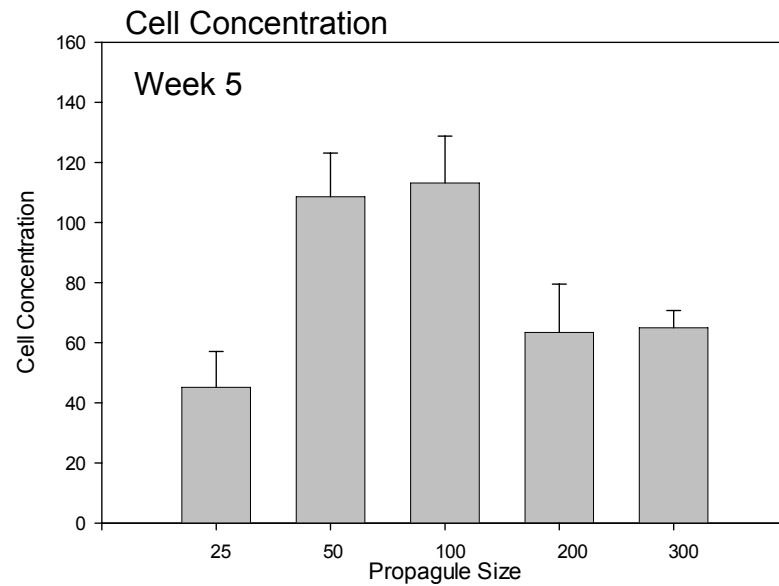


Figure 3-7. The probability of establishment of the invader *Paramecium bursaria* five weeks after 25, 50, 100, or 300 cells of the species are introduced into established communities. The established communities were initiated with 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, and *Paramecium sp.* and cultured three weeks before the invader was added.

(A)



(B)

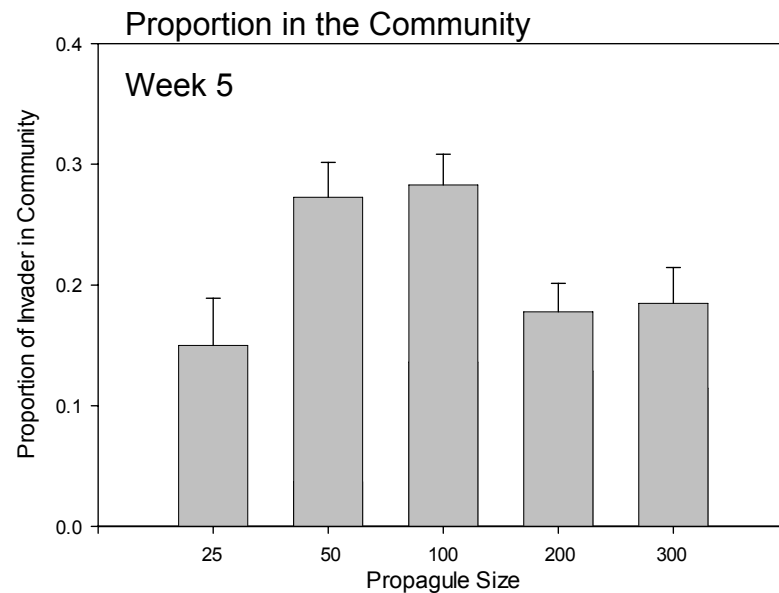


Figure 3-8. The cell concentration (A) and proportion (B) of the invader, *Paramecium bursaria*, five weeks after 25, 50, 100, or 300 cells of the species are introduced into established communities. The established communities were initiated with 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, and *Paramecium sp.* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for propagule size 300. The effect of propagule size on cell concentration (A) is significant, 1-way ANOVA (df 4, 19; $F = 4.860$; $P = 0.007$); Tukey post-hoc comparison (propagule size 25 < 50 and 100; $P \leq 0.027$). The effect of propagule size on proportion (B) is also significant, 1-way ANOVA (df 4, 19; $F = 4.156$, $P = 0.014$); Tukey post-hoc comparison (propagule size 25 < 100; $P = 0.033$).

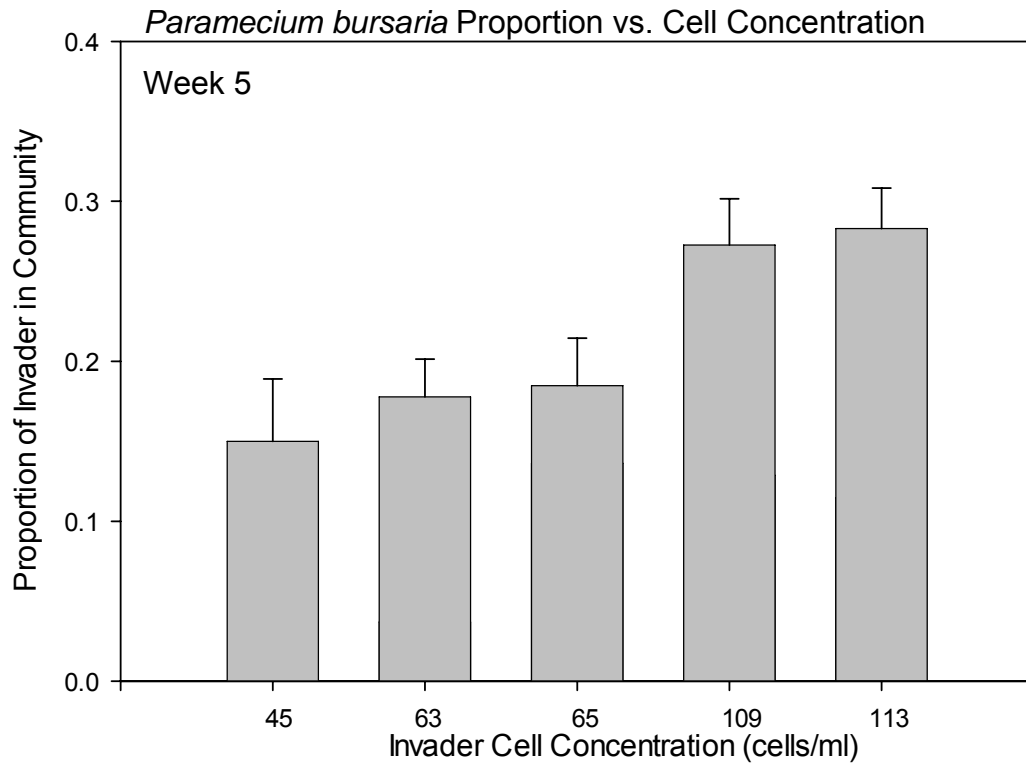


Figure 3-9. The proportion, at associated cell concentration, of the invader *Paramecium bursaria* five weeks after 25, 50, 100, or 300 cells of the species are introduced into established communities. The established communities were initiated with 128 cells each of *Tillina magna*, *Spirostomum ambiguum*, and *Paramecium sp.* and cultured for three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for propagule size 300. The effect of cell concentration on proportion is significant ($\chi^2 = 20.898$, $df = 1$, $P < 0.01$).

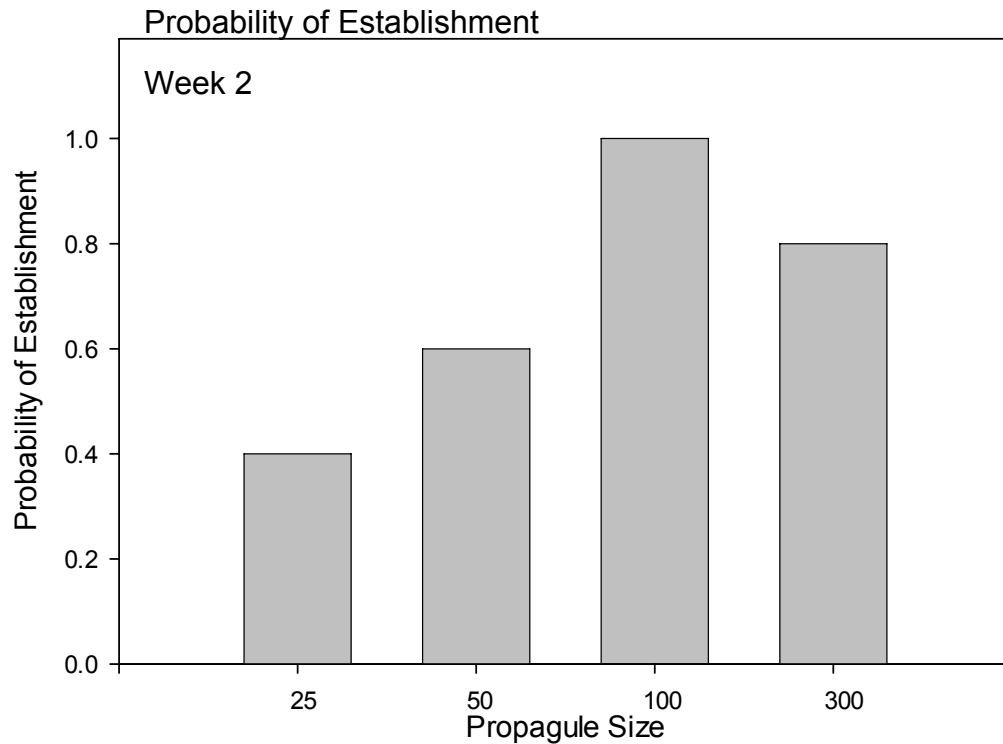


Figure 3-10. The probability of establishment of the invader, *Spirostomum ambiguum*, two weeks after 0, 25, 50, 100, or 300 cells of the species are introduced into established communities. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Tillina magna*, and *Paramecium sp.* and cultured for three weeks before the invader was added. The relationship between probability of establishment and *Spirostomum ambiguum* propagule sizes 25, 50, and 100 is significant ($\chi^2 = 279.101$, $df = 1$, $P < 0.01$)

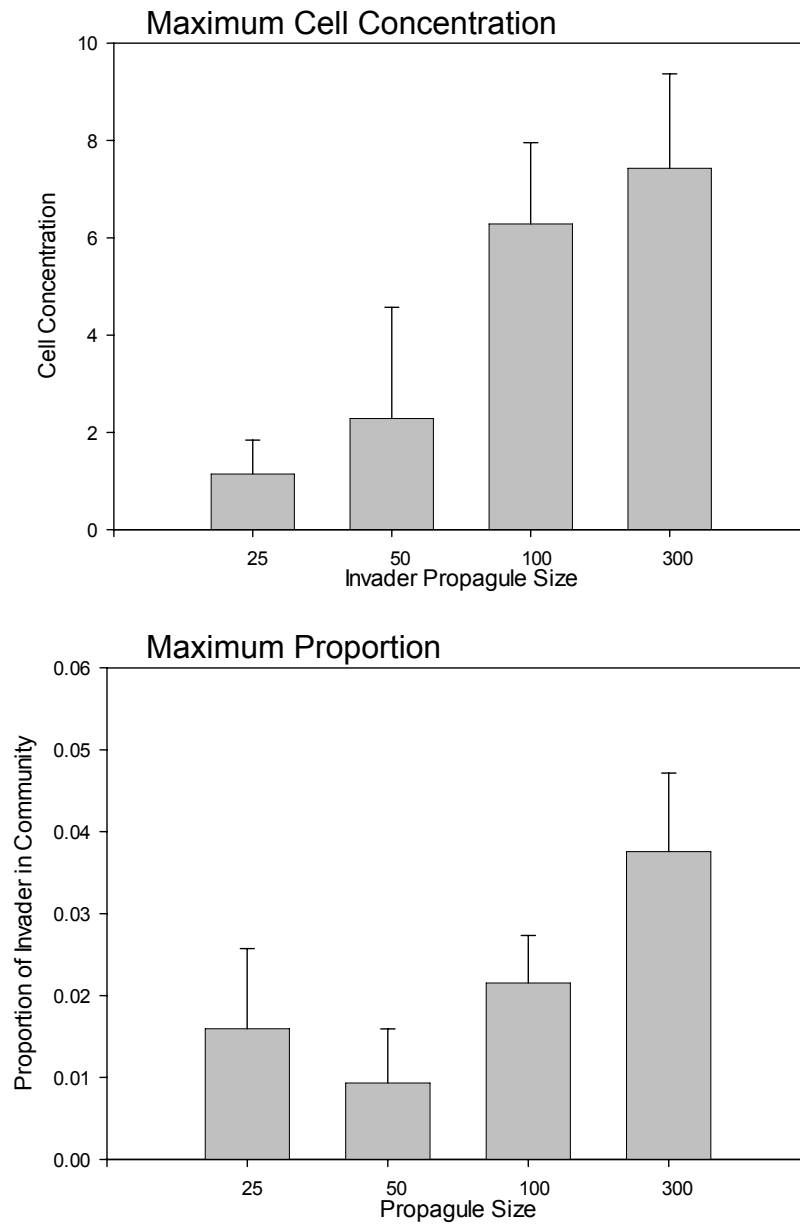


Figure 3-11. The maximum cell concentration (A) and proportion (B) of the invader, *Spirostomum ambiguum*, after the introduction of 25, 50, 100, or 300 cells of the species into established communities. The established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium sp.* and cultured for three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment. The effects of propagule size on maximum cell concentration (A) ($\chi^2 = 7.004$, $df = 1$, $P = 0.008$) and proportion (B) ($\chi^2 = 7.744$, $df = 1$, $P = 0.005$) are significant.

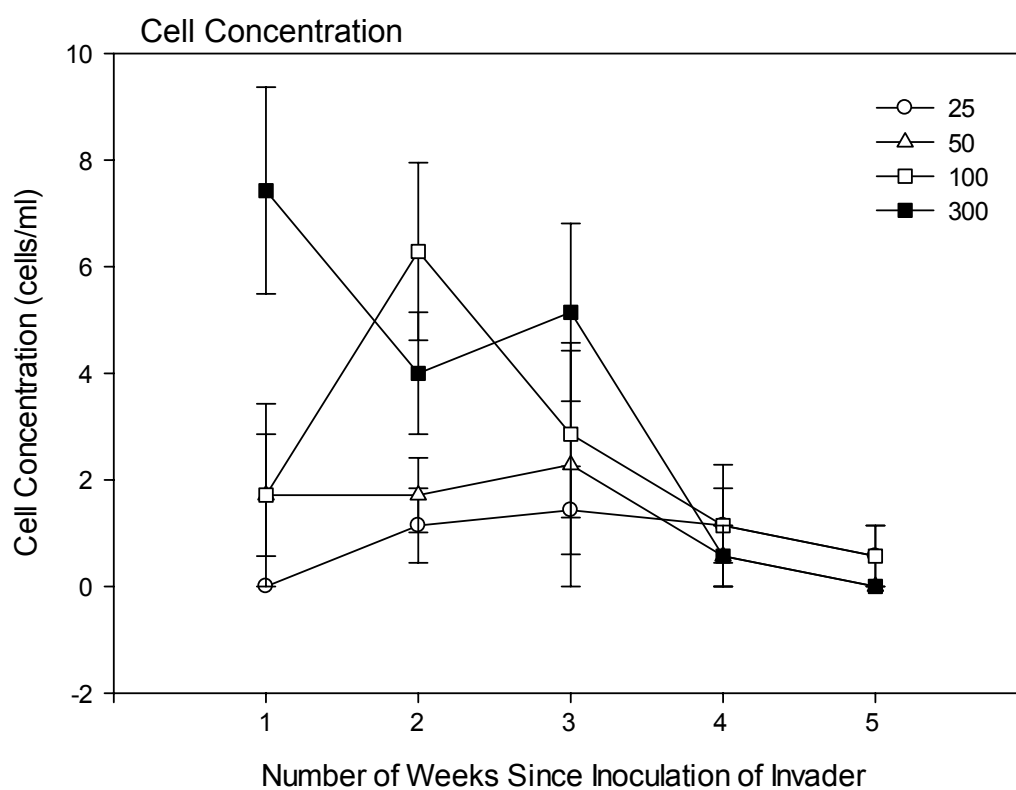


Figure 3-12. Cell concentration of the invader, *Spirostomum ambiguum*, when 25, 50, 100 or 300 cells of the species are introduced into established communities. The established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium sp.* and cultured for three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for propagule size 25, week 3.

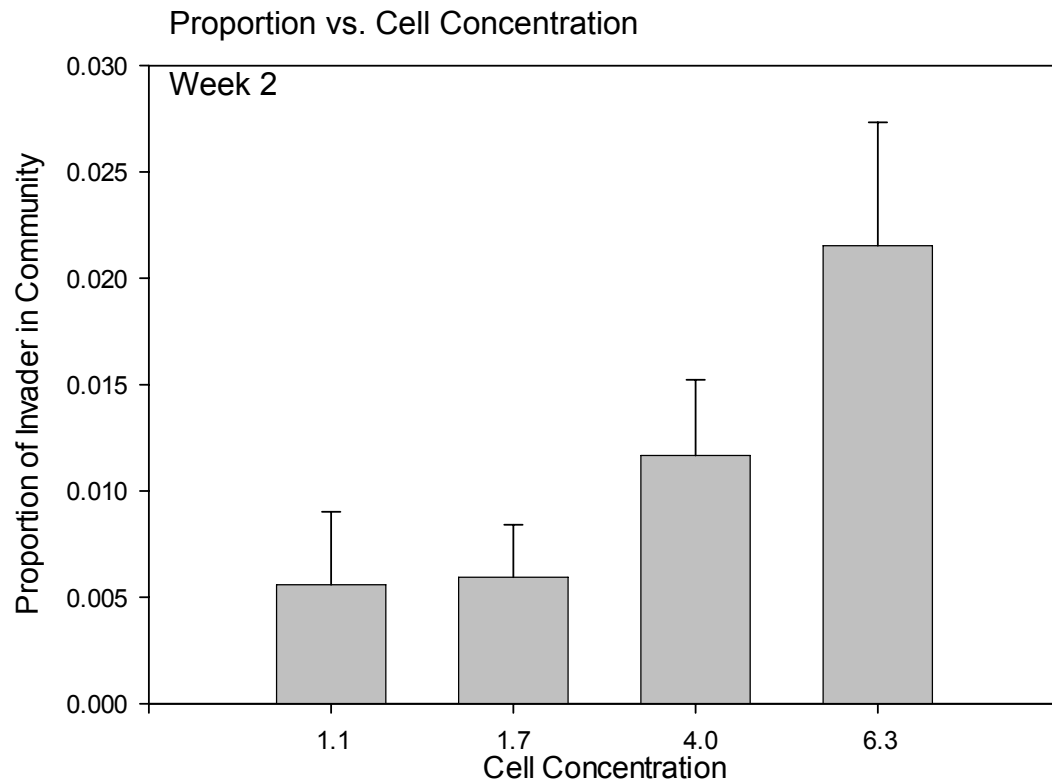


Figure 3-13. The proportion, at associated cell concentration, of the invader *Spirostomum ambiguum* two weeks after 25, 50, 100, or 300 cells of the species are introduced into established communities. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Tillina magna*, and *Paramecium sp.* and cultured for three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment. The effect of cell concentration on proportion is significant ($\chi^2 = 12.277$, $df = 1$, $P < 0.01$).

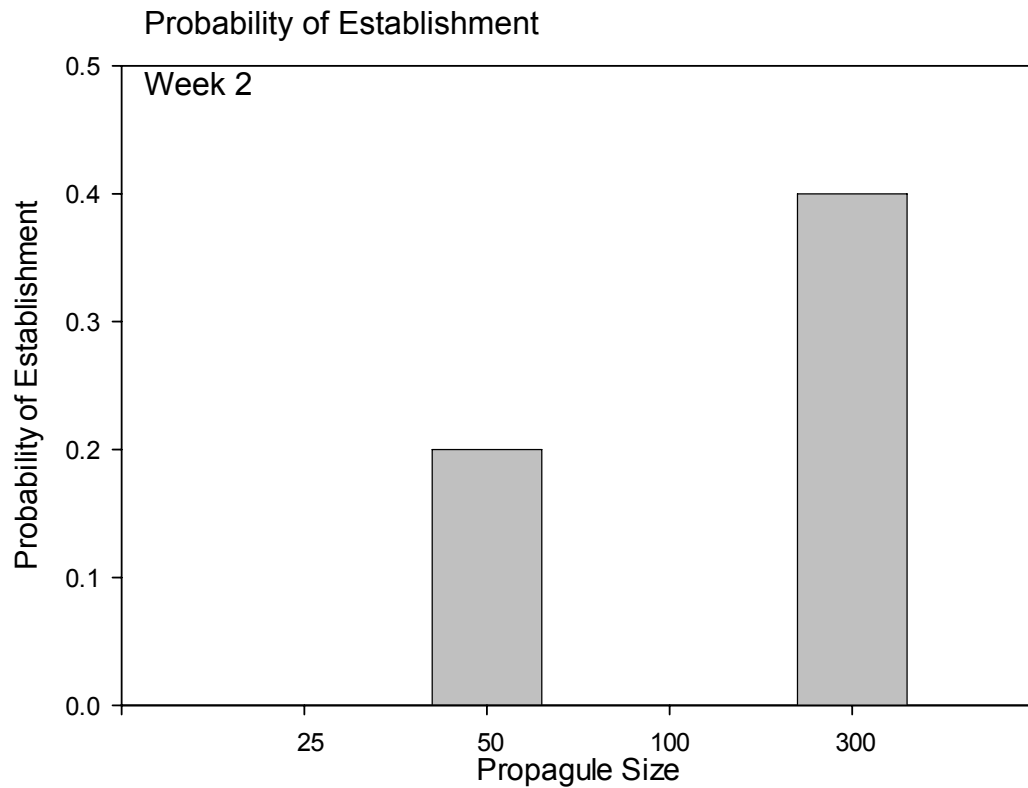


Figure 3-14. The probability of establishment of the invader, *Paramecium sp.*, two weeks after 25, 50, 100, and 300 cells of the species are introduced into established communities. The established communities were initiated three weeks earlier with the inoculation of with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Tillina magna* and cultured three weeks before the invader was added. The effect of propagule size on probability of establishment is significant ($\chi^2 = 8.181$, $df = 1$, $P = 0.004$).

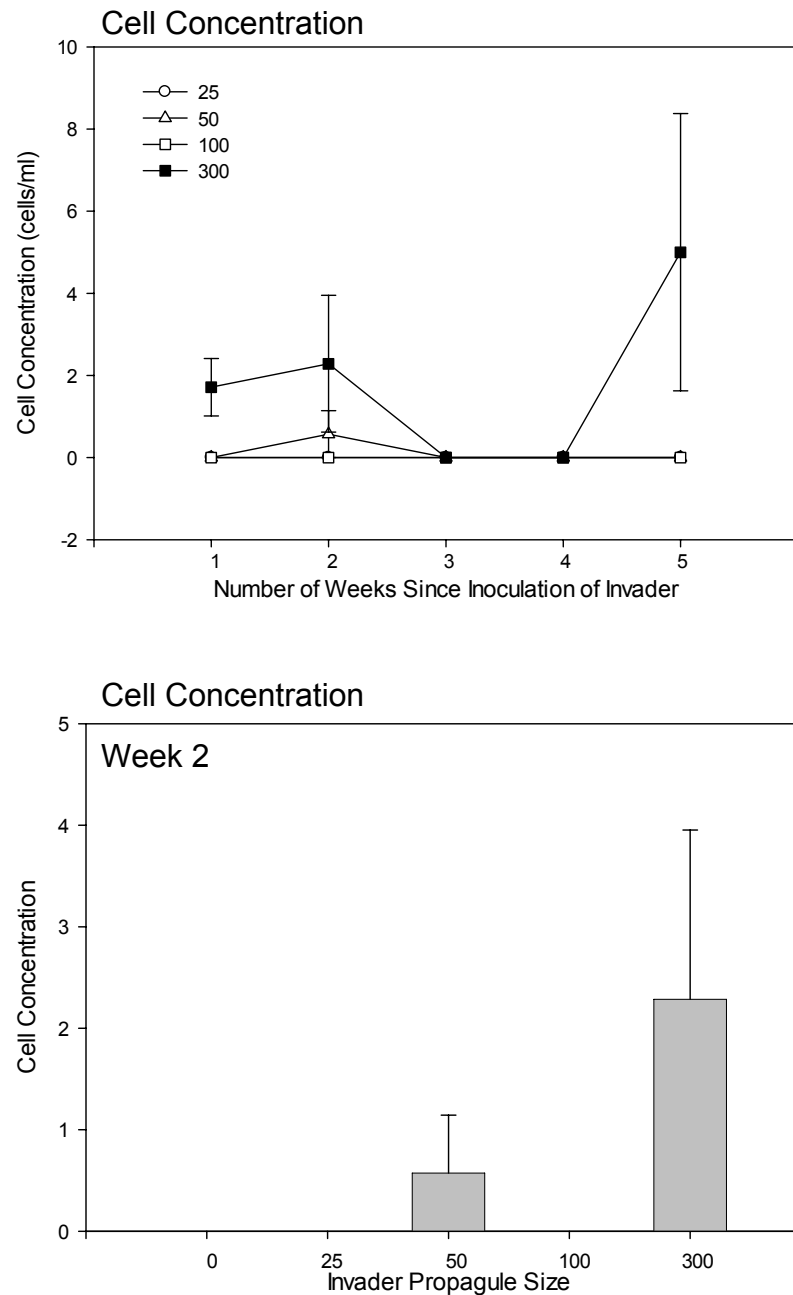


Figure 3-15. Cell concentration of the invader, *Paramecium sp.*, over the course of the experiment (A) and on week 2 (B) when 25, 50, 100 or 300 cells of the species are introduced into established communities. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Tillina magna* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, 4 for propagule size 25 and 300 (wk 5), and three for 300 (wk 4). On week 2, the effect of propagule size on cell concentration (B) is significant ($\chi^2 = 5.767$, df = 1, $P = 0.016$).

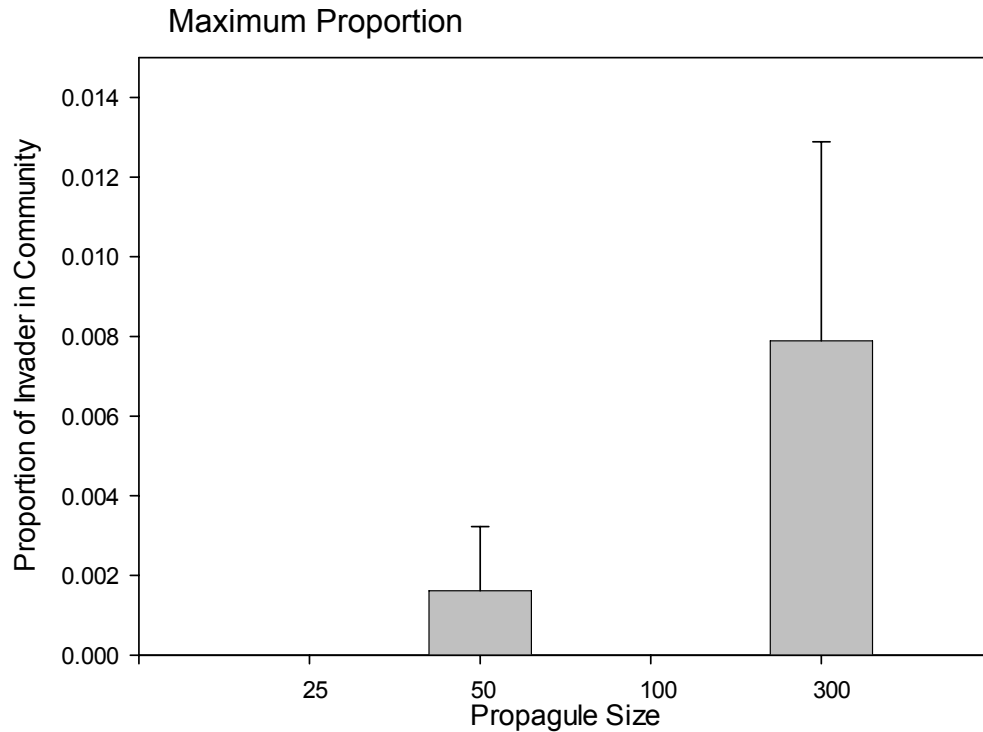


Figure 3-16. The maximum proportion of the invader, *Paramecium sp.*, when 25, 50, 100, or 300 cells of the species are introduced into established communities. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Tillina magna* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment. The effect of propagule size on maximum proportion is significant ($\chi^2 = 5.934$, $df = 1$, $P = 0.015$).

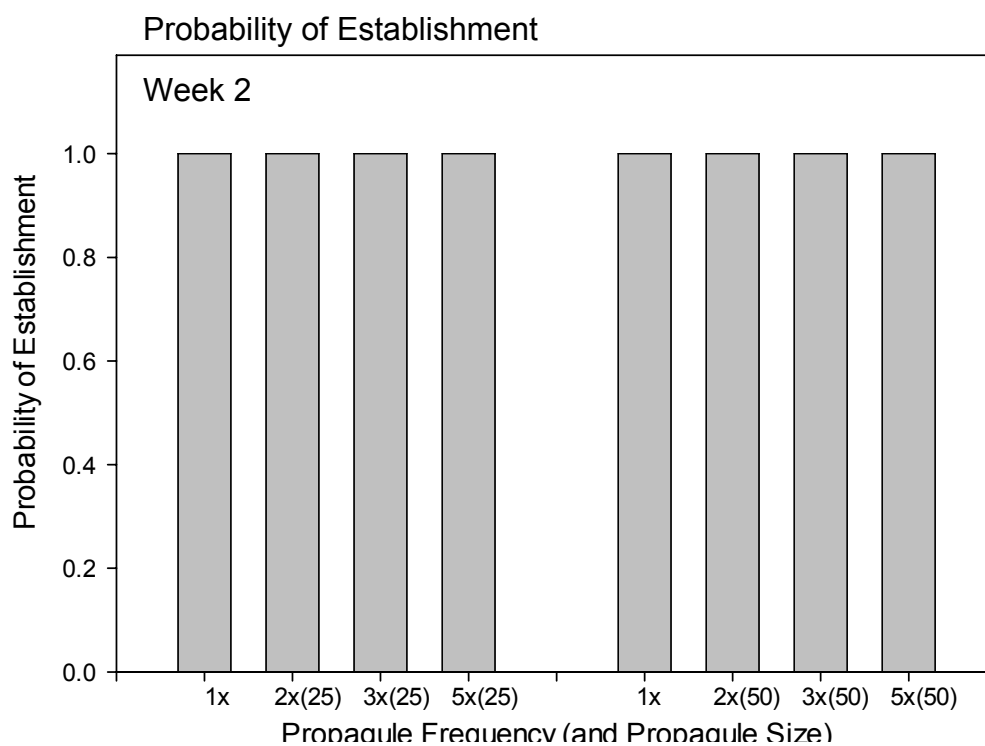


Figure 3-17. The probability of invader *Tillina magna* establishment two weeks after its introduction into established communities when the species is maintained with 1, 2, 3, or 5 25-cell or 50-cell additions during the first four weeks of culture. Established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* and cultured three weeks before the invader was added.

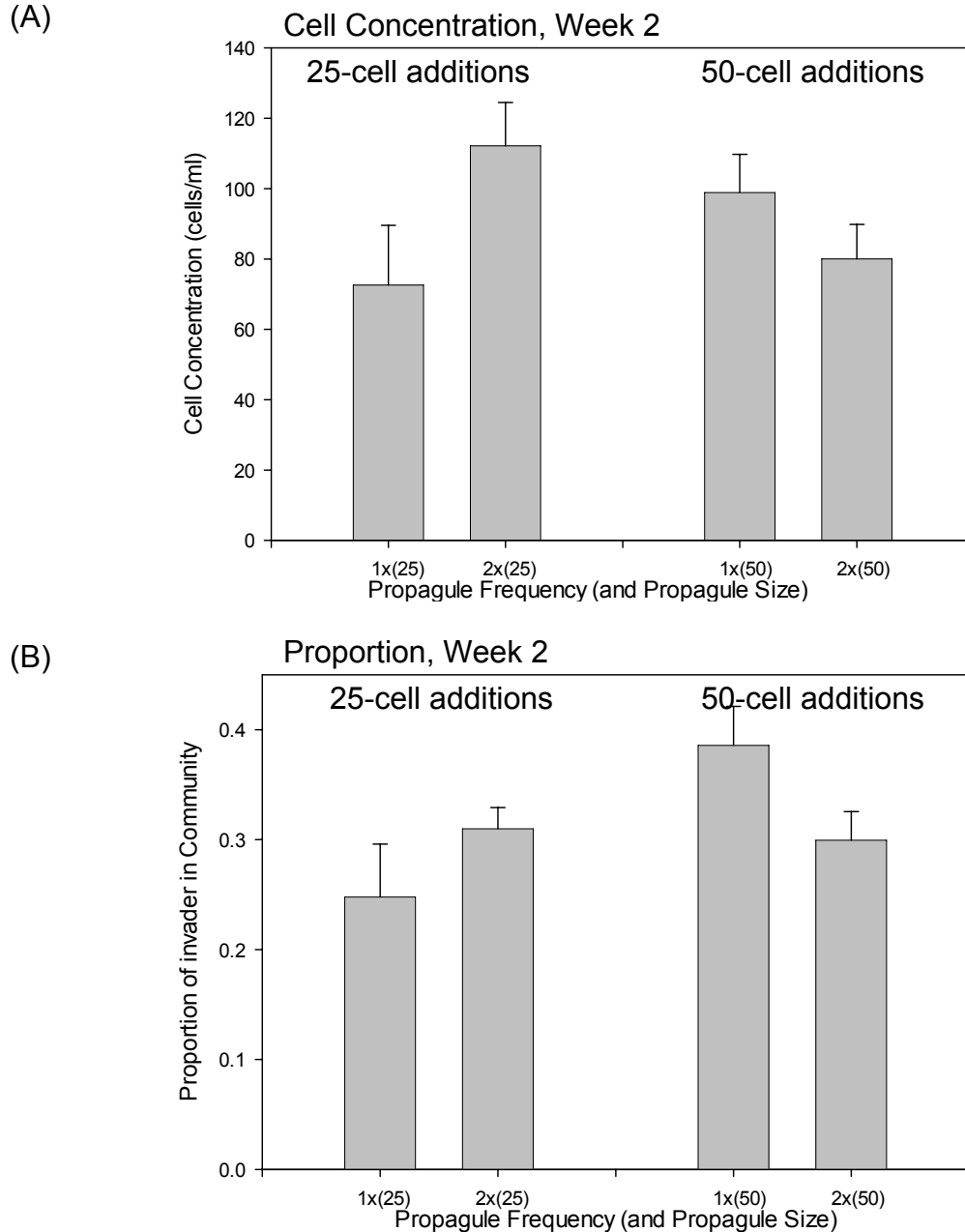


Figure 3-18. The cell concentration (A) and proportion (B), of the invader *Tillina magna* two weeks after its introduction into established communities when the species is maintained with one and two 25-cell or 50-cell additions during the first week of culture. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for 2x(50). The size of additions and the interaction between addition size and propagule frequency have significant effects on the cell concentration (A) and proportion (B) of *Tillina magna*. Propagule frequency has a significant effect on concentration (A) for the 25-cell addition treatments and a significant effect on proportion (B) for the 50-cell addition treatments. See appendix for specific statistical results.

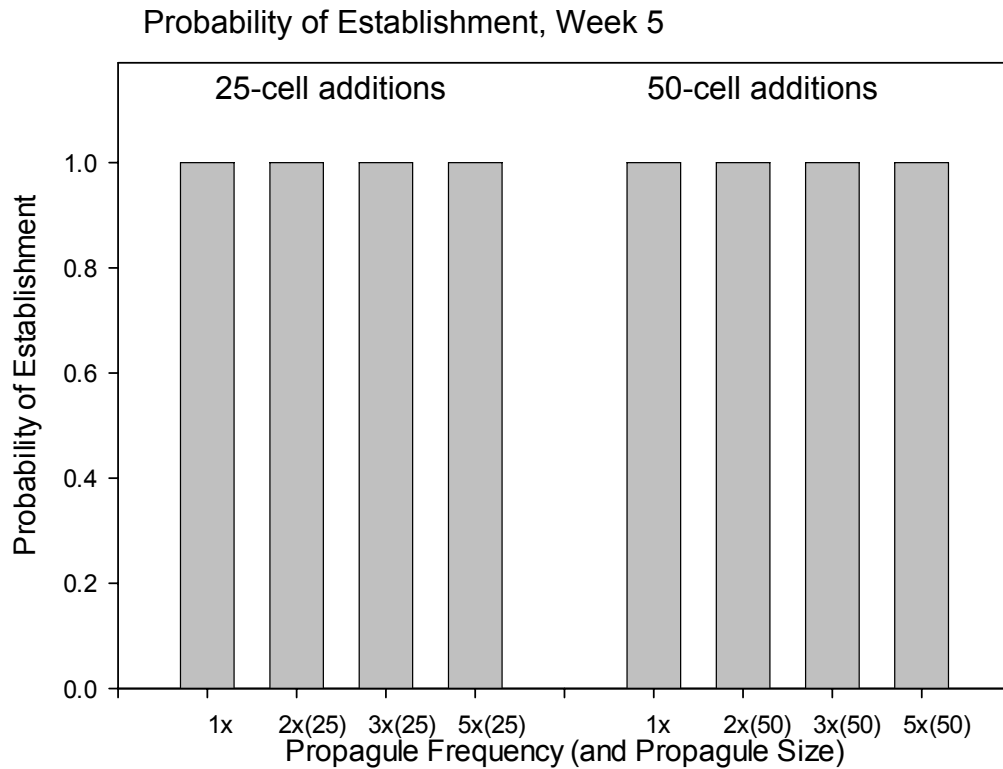


Figure 3-19. The probability of establishment of the invader, *Paramecium bursaria*, five weeks after its introduction into established communities when the species is maintained with 1, 2, 3, or 5 25-cell or 50-cell additions during the first four weeks of culture. The established communities were initiated with 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, and *Paramecium sp.* and cultured three weeks before the invader was added. Plotted values are the means of five replications of each treatment.

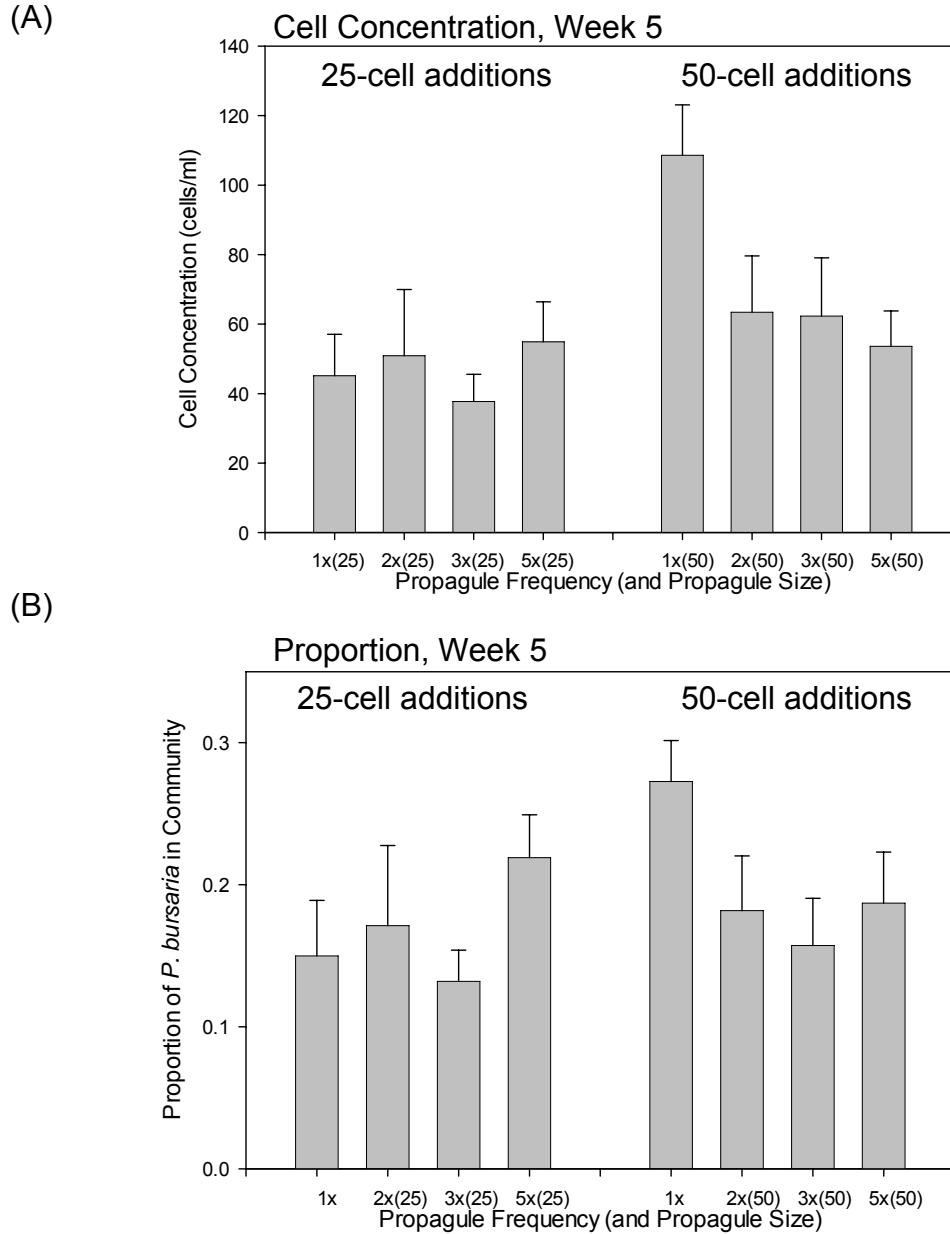


Figure 3-20. The cell concentration (A) and proportion (B) of the invader, *Paramecium bursaria*, five weeks after its introduction into established communities when the species is maintained with 1, 2, 3, or 5 25-cell or 50-cell additions during the first four weeks of culture. The established communities were initiated with 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, and *Paramecium* sp. and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for 5x(50). The size of additions significantly affects cell concentration (A) ($\chi^2 = 9.741$, df = 1, $P = 0.002$) and proportion (B) ($\chi^2 = 5.580$, df = 1, $P = 0.018$). The interaction between propagule frequency and addition size significantly affects cell concentration (A) ($\chi^2 = 4.471$, df = 1, $P = 0.034$) and proportion (B) ($\chi^2 = 3.973$, df = 1, $P = 0.046$).

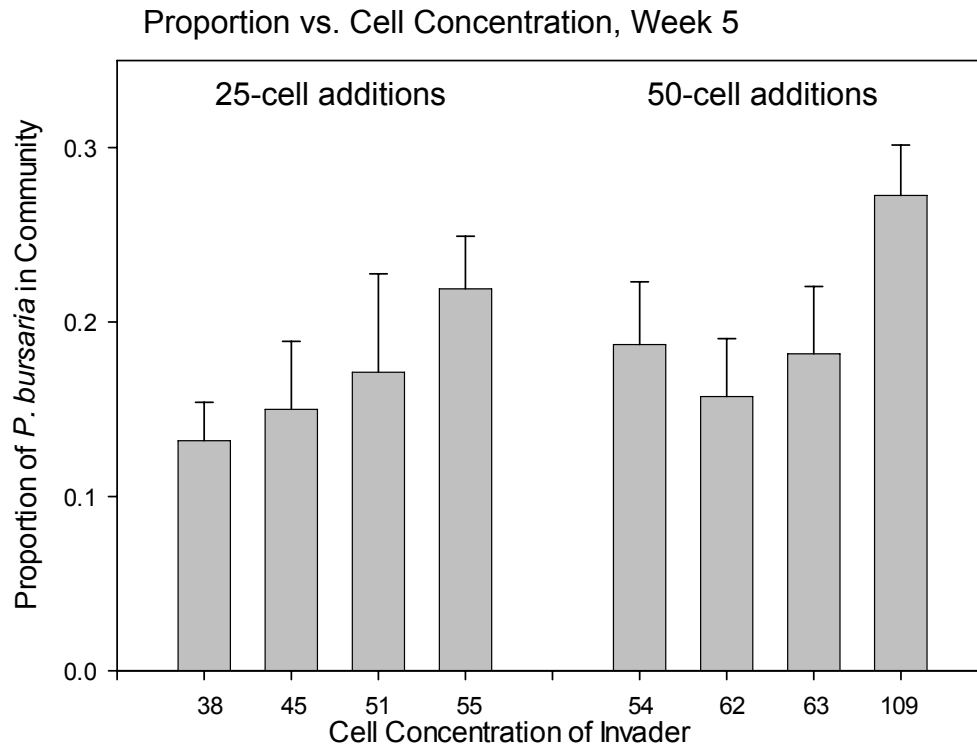


Figure 3-21. The proportion, at associated cell concentration, of the invader *Paramecium bursaria* five weeks after its introduction into established communities when the species is maintained with 1, 2, 3, and 6 25-cell or 50-cell additions during the first four weeks of culture. Established communities were initiated with 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, and *Paramecium sp.* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of 5 replications of each treatment, 4 for 5x(50). The effect of cell concentration on proportion is significant ($\chi^2 = 6.145$, $df = 1$, $P = 0.013$).

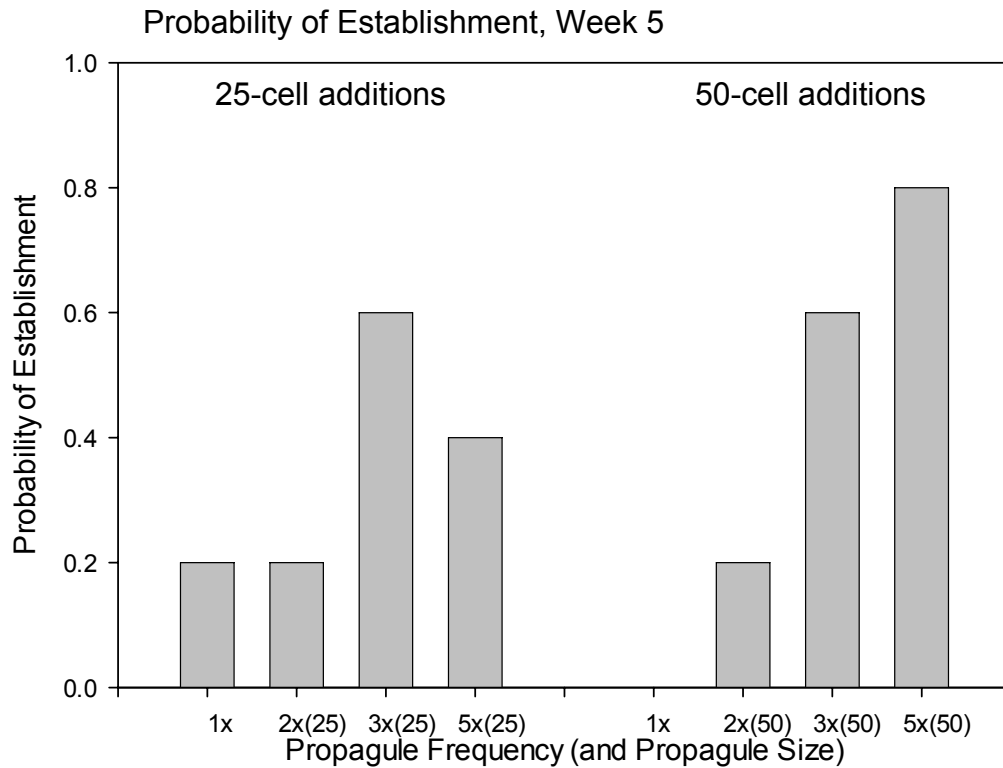
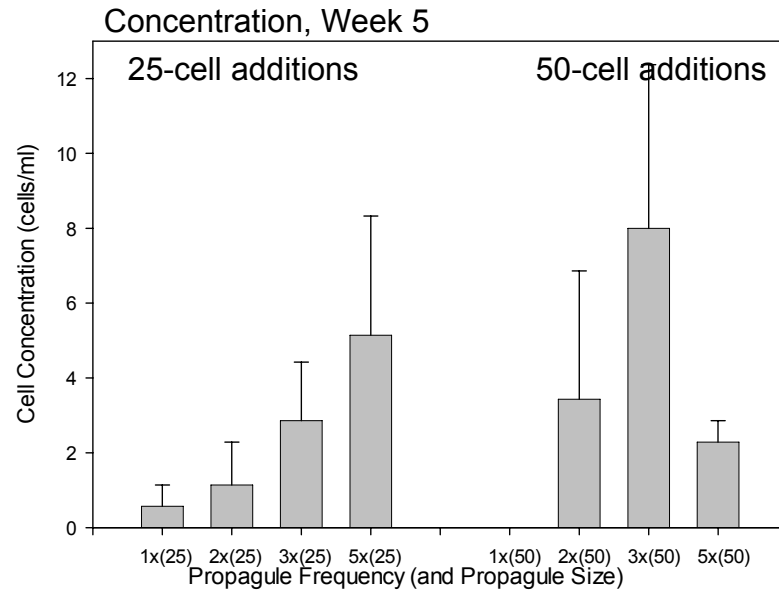


Figure 3-22. The probability of establishment of the invader, *Spirostomum ambiguum*, five weeks after its introduction into established communities when the species is maintained with 1, 2, 3, or 5 25-cell or 50-cell additions during the first four weeks of culture. Established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium sp.* and cultured three weeks before the invader was added. The effect of propagule frequency on probability of establishment is significant ($\chi^2 = 24.011$, $df = 1$, $P < 0.01$). The size of additions ($\chi^2 = 4.013$, $df = 1$, $P = 0.045$) and the interaction between propagule frequency and addition size ($\chi^2 = 6.793$, $df = 1$, $P = 0.009$) also have significant effects on probability of establishment.

(A)



(B)

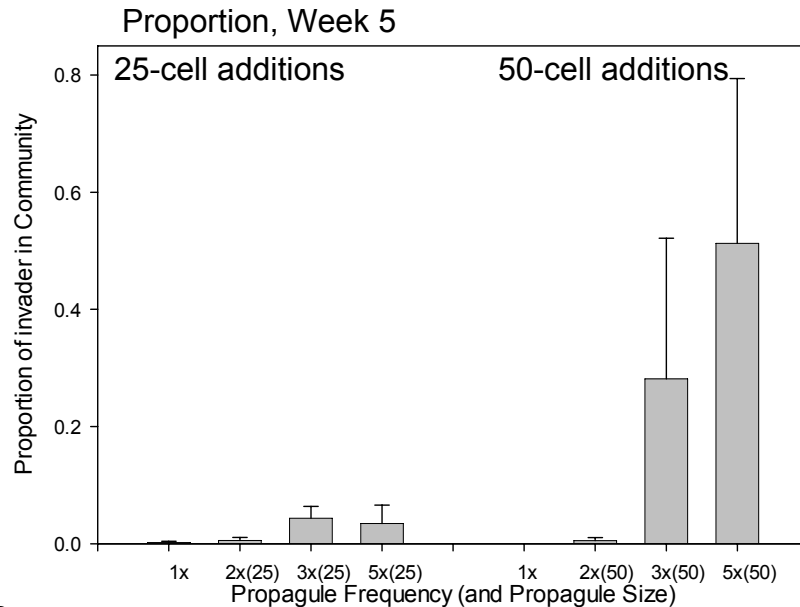


Figure 3-23. The cell concentration (A) and proportion (B) of the invader, *Spirostomum ambiguum*, five weeks after its introduction into established communities when the species is maintained with 1, 2, 3, or 5 25-cell or 50-cell additions during the first four weeks of culture. The established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium sp.* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment. Propagule frequency significantly affects cell concentration in the 25-cell addition series ($\chi^2 = 4.358$, $df = 1$, $P = 0.037$) (A). Propagule frequency ($\chi^2 = 7.796$, $df = 1$, $P = 0.005$) and the interaction between propagule frequency and addition size ($\chi^2 = 6.055$, $df = 1$, $P = 0.014$) have a significant effect on proportion (B).

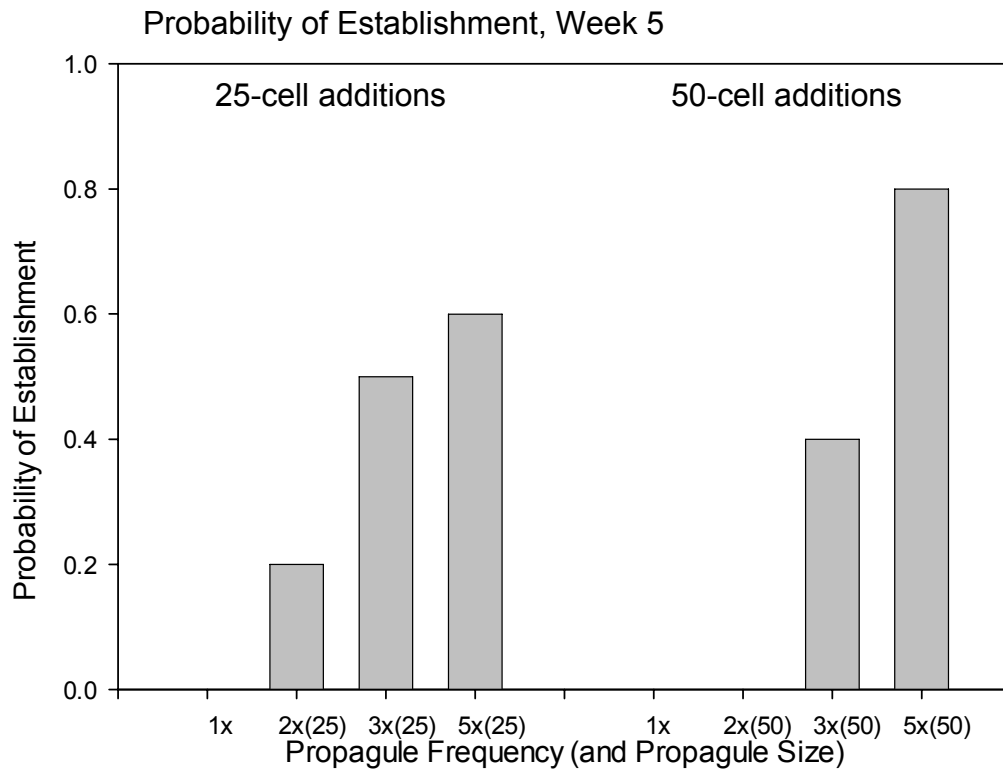
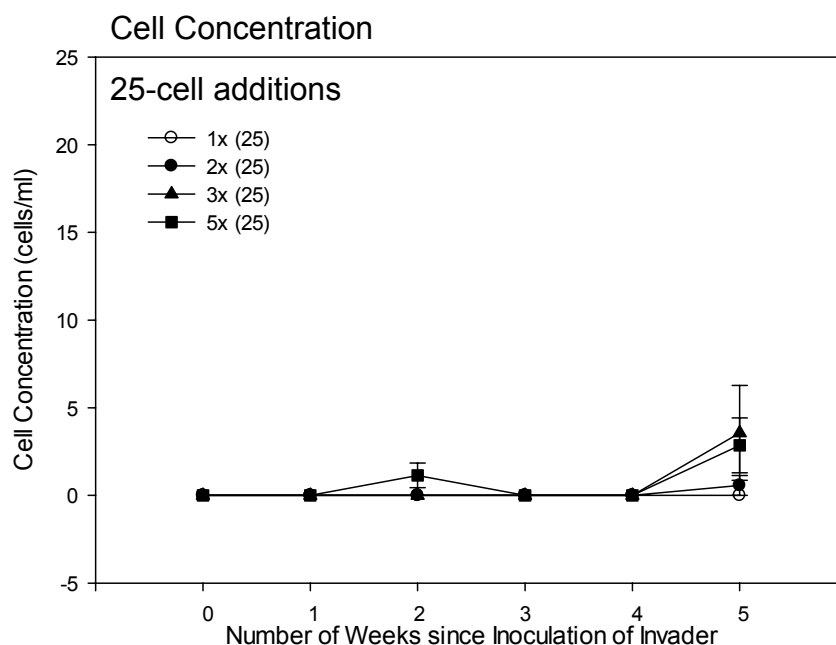


Figure 2-24. The probability of establishment the invader, *Paramecium sp.*, five weeks after its introduction into established communities when the species is maintained with 1, 2, 3, or 5 25-cell or 50-cell additions during the first four weeks of culture. Established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Spirostomum ambiguum* and cultured for three weeks before the invader was added. The effect of propagule frequency on probability of establishment is significant ($\chi^2 = 87.584$, $df = 1$, $P < 0.01$).

(A)



(B)

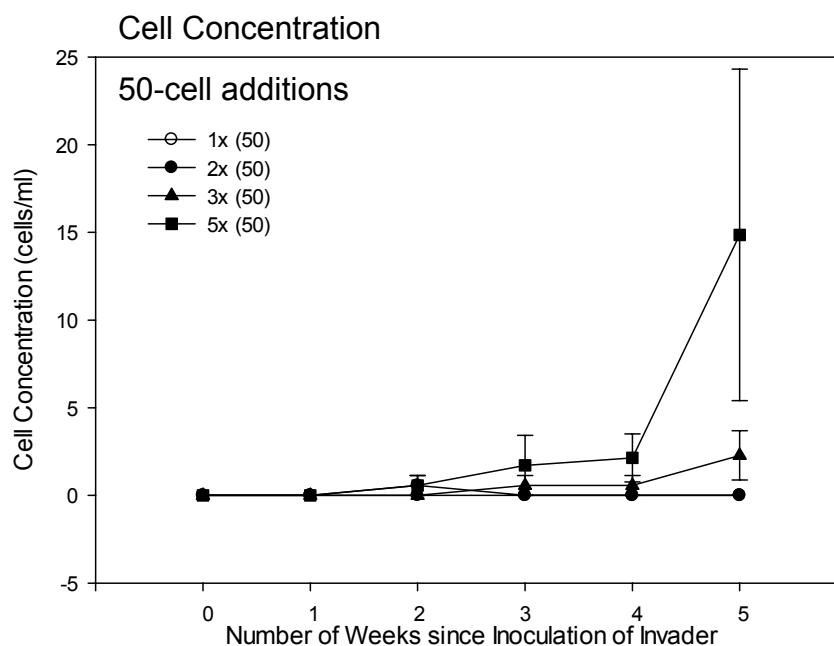
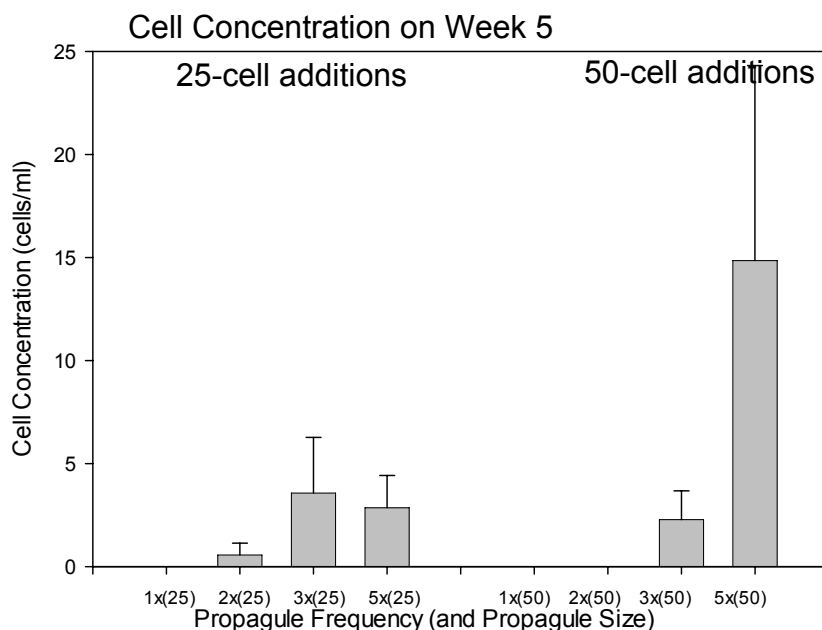


Figure 3-25. Cell concentration of the invader, *Paramecium* sp., when the species is maintained with 0, 1, 2, 3, or 6 25-cell (A) or 50-cell (B) additions during the first four weeks of culture. Established communities were initiated with 128 cells each of *Paramecium. bursaria*, *Spirostomum ambiguum*, and *Tillina magna* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for 1x(25), 3x(25) (weeks 4 and 5), 2x(50) (week 0), and 6x(50) (week 4).

(A)



(B)

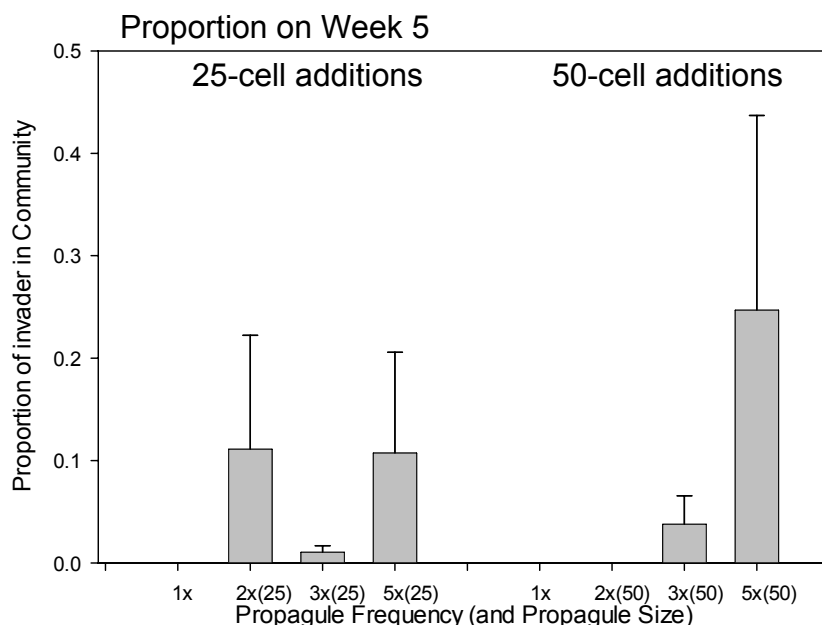
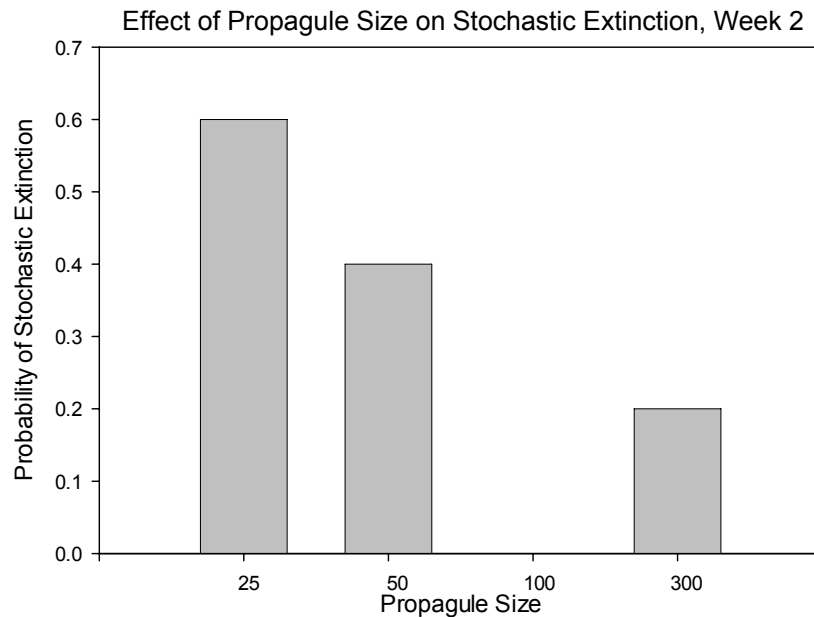


Figure 3-26. The cell concentration (A) and proportion (B) of the invader *Paramecium* sp. five weeks after its introduction into established communities when the species is maintained with 1, 2, 3, or 6 25-cell or 50-cell additions during the first four weeks of culture. Established communities were initiated with 128 cells each of *Paramecium. bursaria*, *Spirostomum ambiguum*, and *Tillina magna* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for 1x(25), 3x(25). Propagule frequency ($\chi^2 = 8.286$, $df = 1$, $P = 0.004$) and the interaction between frequency and addition size ($\chi^2 = 3.734$, $df = 1$, $P = 0.053$) significantly affect cell concentration (A).

(A)



(B)

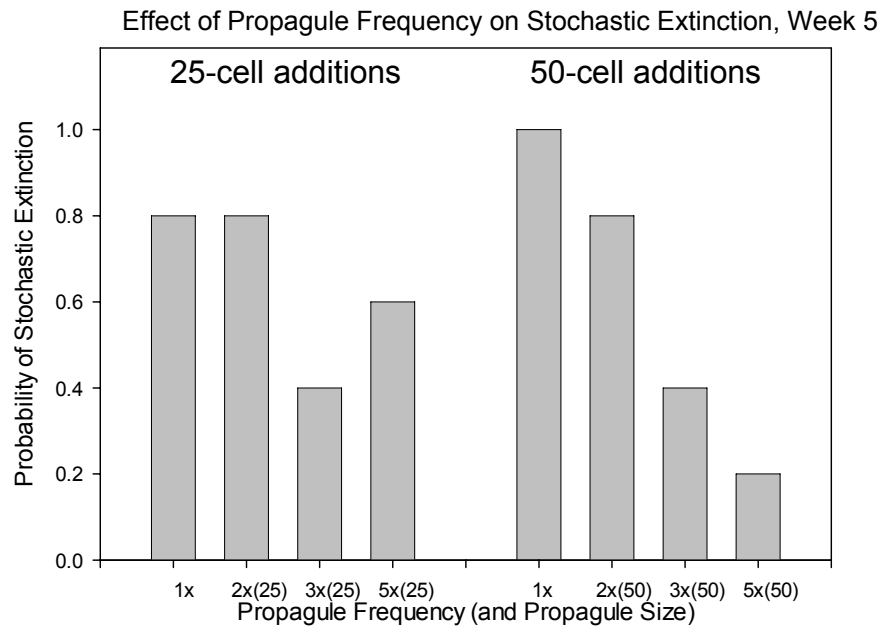
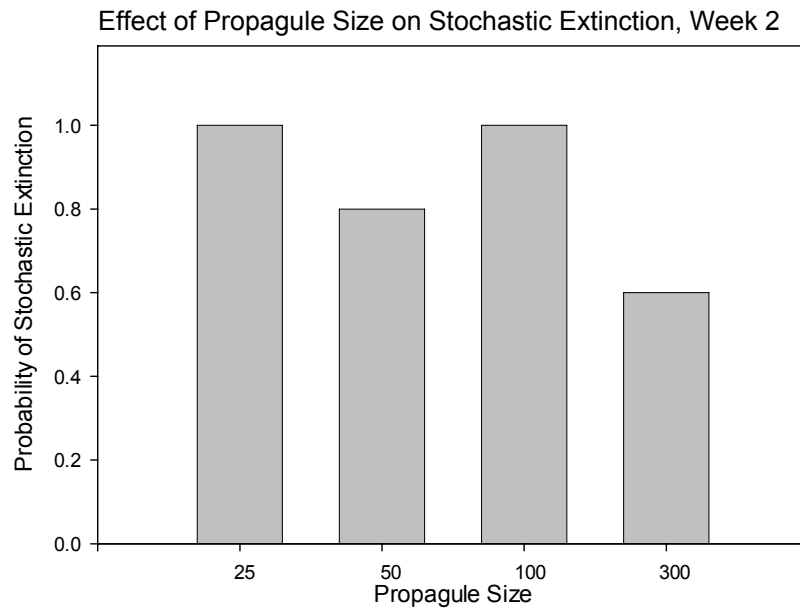


Figure 3-27. The probability of stochastic extinction of the invader, *Spirostomum ambiguum*, at various values of propagule size (A) and propagule frequency (B). The established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium sp.* and cultured three weeks before the invader was added. Propagule size (A) ($\chi^2 = 5.694$, $df = 1$, $P = 0.017$), propagule frequency (B) ($\chi^2 = 24.011$, $df = 1$, $P < 0.01$), and the interaction between frequency and addition size (B) ($\chi^2 = 6.793$, $df = 1$, $P = 0.009$) significantly affect stochastic extinction.

(A)



(B)

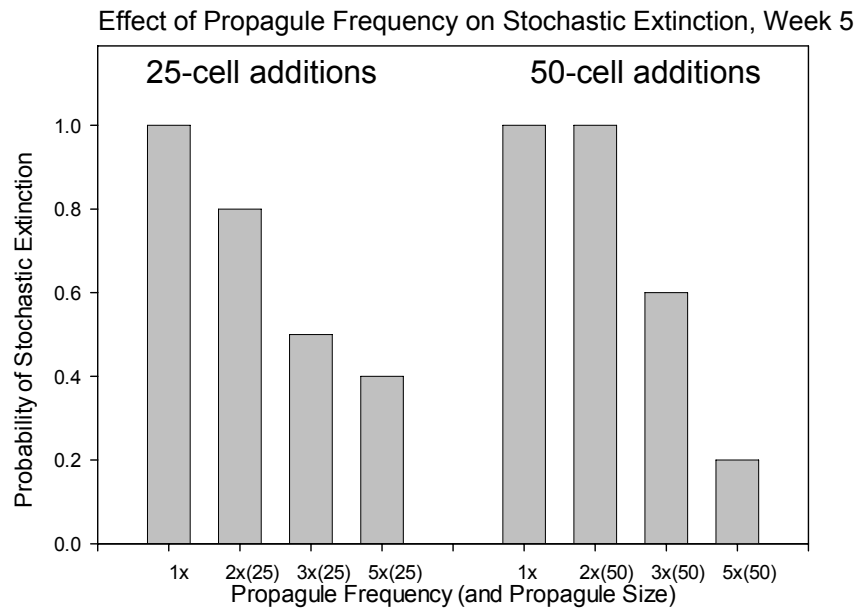


Figure 3-28. The probability of stochastic extinction of the invader, *Paramecium* sp., at various values of propagule size (A) and propagule frequency (B). The established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Spirostomum ambiguum* and cultured three weeks before the invader was added. Propagule size (A) ($\chi^2 = 8.181$, $df = 1$, $P = 0.004$) and propagule frequency (B) ($\chi^2 = 87.584$, $df = 1$, $P < 0.01$) both significantly affect stochastic extinction.

Discussion

The Effect of Propagule Size on Invasion

The results of these experiments show that propagule size can influence invasion in this protist system in several ways. High propagule size can enhance an invader's probability of establishment and increase its cell concentration and proportion. Propagule size can also influence an invader's population dynamics, affecting the length of time required to reach maximum invader concentration, the length of time the population remains viable, and whether a second period of invader growth occurs later. Although all of these effects can be promoted by high propagule size, and are seen in these experiments, not all effects occur in all species. Characteristics of the invader appear to determine how propagule size influences its establishment and invasion success. The four invaders used in these experiments differ in their realized growth rate within the established community and the associated size of the resulting population (Table 1-1). The specific influence of propagule size on a species may be associated with its realized growth. Two of the invaders, *Tillina magna* and *Paramecium bursaria*, have relatively high realized growth rate in the experimental community (Table 1-1). There are many similarities in the way increasing propagule size affects their invasion. Both species show saturation in their response to propagule size, evident in the species' probability of establishment, cell concentration, and invasion success. Both strong invaders are able to establish

viable populations in all experimental flasks, regardless of propagule size. These species are capable of establishing a persistent population from even an extremely small founding propagule. For both of the species, increasing propagule size has a significant effect on invader success, as measured by invader cell concentration and/or proportion, but only up to a point. Both species reach maximum cell concentration and proportion at intermediate propagule size (50, 100). Larger propagule size either has no effect or decreases cell concentration and proportion. Increasing propagule size has very different effects on the remaining two invaders used in these experiments. *Spirostomum ambiguum* and *Paramecium sp.* are much weaker invaders, grow at substantially slower rate within the experimental community and form much smaller invader populations. Neither of these species shows saturation in its response to increasing propagule size. For these species, increasing propagule size enhances the probability of establishment. One species shows a trend of increasing establishment with increasing propagule frequency through propagule size 100. The other species is capable of establishing a viable population only at the highest propagule size values. Unlike the strong invaders which have maximum invasion success at intermediate propagule size, the weaker invaders have increased concentration and proportion with increasing propagule size. At no time does increasing propagule size result in decreased invasive success for these species, as it does for the stronger invaders. One weak invader, *Spirostomum ambiguum*, shows a clear trend of increasing cell concentration and proportion as propagule size increases. The other invader, *Paramecium sp.*,

forms a recoverable population and constitutes a sizable proportion of the community only when inoculated at the highest propagule size. Propagule size clearly affects *Paramecium sp.* growth dynamics. The species grows slowly, reaching an early maximum concentration on week 2. At this time, there is a pattern of increasing cell concentration and proportion with increasing propagule size. Immediately after reaching maximum, populations decline. High propagule size minimizes this decline and promotes a second period of much greater growth at the end the experiment.

Three related, general growth patterns are found in the results of these propagule size experiments. The patterns are seen repeatedly in multiple experiments throughout this study, so bear mention here. First, the proportion of all four of the invaders used in this study is closely related to the species' cell concentration. Since propagule pressure can influence invader cell concentration, it can also indirectly influence the invader's proportion in the community. Second, in general, the higher an invader's growth rate in the community, the more rapidly it reaches maximum cell concentration and the greater the maximum cell concentration. Species with high growth rate reach maximum cell concentration earlier and have higher maximum cell concentration than species with lower growth rate. Finally, the higher an invader's maximum cell concentration, the sooner the population begins to decline.

The Effect of Propagule Frequency on Invasion

These results show that propagule frequency can influence invasion in several ways. High propagule frequency can affect an invader's probability of establishment, cell concentration, and proportion one week after the final invader addition is made. Propagule frequency can also influence an invader's population dynamics, affecting whether a second period of invader growth occurs late in the experiment. The size of additions in propagule frequency experiments can influence invader cell concentration and proportion. Although all of these effects can be promoted by high propagule frequency and are seen in these experiments, only a subset of effects occurs in any one species. The invader's realized growth rate may determine how propagule frequency influences establishment and invasion success. The two invaders with high realized growth, *Tillina magna* and *Paramecium bursaria*, are affected by increasing propagule frequency and the size of additions in a similar way. Both species show saturation in their response to propagule frequency and addition size. This saturation is evident in the species' probability of establishment, cell concentration, and invasion success. Both strong invaders are able to establish viable populations in all experimental flasks, regardless of propagule frequency or the size of additions. These species are capable of establishing a population from an extremely small founding propagule inoculated in one addition. For both of the species, the size of additions changes the effect of increasing propagule frequency in a way that supports the hypothesis of saturation. When the size of

additions is small, increasing propagule size increases *Tillina magna* cell concentration and proportion and has no effect on *Paramecium bursaria*. In contrast, when the size of additions is larger, increasing propagule frequency decreases the cell concentration and proportion of both *Tillina magna* and *Paramecium bursaria*. Increasing propagule frequency and the size of additions affects weak invaders very differently. Neither of the two weak invaders in this system, *Spirostomum ambiguum* and *Paramecium sp.*, shows saturation in its response to increasing propagule frequency or addition size. Both species exhibit a strong trend of increasing probability of establishment with increasing propagule frequency. For *Tillina magna*, larger addition size amplifies this effect. Propagule frequency and the size of additions strongly influence the cell concentration and proportion of the two weak invaders. Increasing propagule frequency is associated with patterns of increased concentration and proportion for both weak competitors. These trends are significant in many cases. Propagule frequency strongly influences the pattern of *Paramecium sp.* growth. This invader grows weakly in this system. After reaching an early maximum cell concentration at week 2, the population declines. High propagule frequency minimizes this decline and promotes a second period of growth at the end of the experiment. When the size of additions is larger, *Paramecium sp.* reaches substantially greater the cell concentration and proportion during this second period of growth.

Relating Propagule Pressure Effects to Invader Growth

The realized growth of an invader in the established community appears to determine the influence that propagule pressure has on its subsequent growth. In this study, invaders that have naturally higher growth rate are influenced similarly by propagule size and frequency, and these effects are quite different than those experienced by slower-growing invaders. Strong, fast-growing invaders show saturation in their response to both propagule size and propagule frequency. These species are capable of establishing viable populations in all flasks at all treatments, regardless of propagule size. Increasing propagule pressure does not confer benefit. Increasing both propagule size and propagule frequency increases the invasion success of the strong invaders, but only up to a point and the effect is always very weak. Increasing propagule pressure beyond the point of maximum invasion success confers no benefit and, in some cases, results in decreased success. Increasing propagule frequency, when the size of additions is small, increases invasion success or has no effect. However, increasing propagule frequency when the size of additions is large has a negligible or even negative effect on invader cell concentration and proportion. In contrast, weak invaders never show saturation in their response to propagule pressure. Increasing propagule pressure always has a positive effect on weak invaders. Both weak invaders in this study show clear trends of increasing establishment, cell concentration, and proportion with increasing propagule size and frequency. In one species, high propagule pressure allows a second period

of growth at the end of the experiment, after the established community has declined. High propagule frequency has an especially strong influence on weak invaders, minimizing the population decline that follows maximum cell concentration.

A Proposed General Shape for the Dose-Response Curve

The fine-scale relationship between propagule pressure and establishment success is not yet well understood (Ruiz and Carlton, 2003; Lockwood *et al.*, 2005). Few experimental studies have quantified the role of propagule pressure in enough detail to create a dose-response curve that depicts the fine-scale relationship between the propagule pressure of an invading species and its probability of establishment (Williamson, 1996; Travis *et al.*, 2005). This study attempted to characterize the dose-response characteristics of four protist invaders. I examined the effect of increasing propagule size and frequency on the probability of invader establishment, as suggested by Ruiz and Carlton (2003). I also examined the effect of increasing propagule pressure on invasion success, defined by cell concentration and proportion of the invader in the community. These results suggest that dose-response curves for invader establishment and invasion success may have a general Gaussian distribution, the particular characteristics of which vary across systems. Increasing propagule size or frequency increases establishment and invasion success up to a point after which increasing propagule pressure confers no benefit. An invader's

realized growth determines the way specific values of propagule size and frequency fall along the x-axis. For strong fast-growing invaders, saturation is reached at lower propagule size and frequency values. For weak slow-growing invaders, the curve levels at much greater values of propagule size and frequency. This suggests that the responses seen in this study depend on the experimental values of propagule size and frequency used. If the study had utilized lower propagule size values, even the fast growing invaders might have shown continuous benefit from increasing propagule size and frequency. If the study had used larger propagule size values, even the slow-growing invaders might have shown saturation in their response to propagule size and frequency.

Effect of Propagule Pressure on Demographic Stochasticity

To establish a persistent population, an invader must avoid extinction and achieve positive population growth at low density (Chesson, 2000; Sakai *et al.*, 2001, Theoharides and Dukes, 2007). Demographic stochasticity may strongly impede the establishment of some invaders (Grevstad, 1999). Few studies have examined how this barrier is weakened by high propagule pressure. Propagule size and propagule frequency influence the size of the founding invader population and the degree of rescue effect available. Therefore, propagule pressure can influence the effect of demographic stochasticity on establishment. The results of this study suggest that both propagule size and propagule frequency influence the degree of stochastic extinction, but only for the weak

invaders. Stochastic extinction is an important factor for the weak invaders in some treatments in this study. It can be seen in the probability of establishment graphs, in the treatments that have probability of establishment values between 0 and 1.0. Stochastic extinction can be quantified as:

$$\text{Stochastic extinction} = 1 - \text{probability of establishment}$$

When the effect of propagule size and frequency on the probability of stochastic extinction is examined for *Spirostomum ambiguum* (Figure 3-27) and *Paramecium sp.* (Figure 3-28), both species show significant patterns of decreased stochastic extinction with increasing propagule size and frequency. For *Spirostomum ambiguum*, the probability of stochastic extinction decreases from 0.6 to 0.2 with increasing propagule size ($\chi^2 = 5.694$, $df = 1$, $P = 0.017$), and from 1.0 to 0.2 with increasing propagule frequency ($\chi^2 = 24.011$, $df = 1$, $P < 0.01$) (Figure 3-27). For *Paramecium sp.*, the probability of stochastic extinction decreases from 1.0 to 0.6 with increasing propagule size ($\chi^2 = 8.181$, $df = 1$, $P = 0.004$) and from 1.0 to 0.2 with increasing propagule frequency ($\chi^2 = 87.584$, $df = 1$, $P < 0.01$) (Figure 3-28).

By increasing the size of the founding population, high values of propagule size minimize the effect of stochastic extinction on the establishment of the slow-growing invaders examined in this study. In addition, high values of propagule frequency provide rescue effect. The effect of propagule pressure on

demographic stochasticity may generally influence invading populations of many different taxa.

Diversity-Invasibility Relationship in the Bacterivorous Protist Communities

Specific Aim # 1 examined whether propagule pressure acts as a diversifying factor in the model protist communities used in this study. The experimental community composed of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* display particularly strong positive effects of both propagule size and propagule frequency. Increasing propagule size and frequency increase the diversity and richness of this community and decrease the proportion of the dominant species. In Specific Aim #2, these four species were used to examine the role of propagule pressure on invasion success. High propagule pressure, in the form of large propagule size, frequency and addition size, can promote establishment and invasion success in the four protist invaders. The intensity and type of this effect varies from species to species, and appears to be related to the species' realized growth rate. In this protist system, propagule size and propagule frequency promote coexistence among species, established and invasive, and in so doing, increase community diversity and invasibility. This study suggests that propagule size and propagule frequency could potentially create a positive diversity-invasibility relationship in this and other bacterivorous protist communities, experimental and natural. Of course, natural protist communities are much more complex than the simple assemblage

studied here. Natural protist communities typically include a diverse group of species, functioning at various trophic levels, exposed to top-down as well as bottom-up regulation, environmental variation, and disturbance. Therefore, the results of this simple study cannot be extended to complex natural systems.

However, this study does show that within the experimental system, high values of propagule size and propagule frequency promote coexistence among established species and between established and invader species. This characteristic is the underlying factor responsible for the positive diversity-invasibility relationship.

CHAPTER 4. THE RELATIVE EFFECTS OF PROPAGULE SIZE AND PROPAGULE FREQUENCY IN INFLUENCING INVASION SUCCESS.

Introduction

Propagule pressure is an important factor in invasion, influencing probability of establishment and invasion success. Nevertheless, the details of the relationship between propagule pressure and invasion are not well known. We do not yet understand how the individual effects of propagule size and frequency, functioning together, affect the success of an invader (Lockwood *et al.*, 2005) or how these effects vary from one invader to another (Lockwood *et al.*, 2005). In Specific Aim #3, I examine how propagule size and propagule frequency interact to influence the establishment and invasion success of the four bacterivorous protist invaders used in the experiments of Specific Aim #2 described in chapter 3.

Specific Aim #2 examined the independent effects of propagule size and frequency on establishment and invasion success, but the results of some treatments hinted at the importance of the interaction between these two factors. In the experiments of Specific Aim #2, the series of propagule frequency treatments was replicated with 25-cell and 50-cell additions. The size of additions often strongly influenced the way propagule frequency affected invader cell concentration and proportion. The type of influence contributed by the size

of additions varied depending on the realized growth rate of the invader. For fast-growing invaders, the size of additions determined whether the effect of increasing propagule frequency had a negligible, positive, or negative effect. For slow-growing invaders, larger additions amplified the positive effect of increasing propagule frequency. Although these treatments demonstrated that propagule size and frequency interact in influencing invasion success, the design of this experiment did not allow the true relative effects of propagule size and frequency to be observed. In these treatments, increasing propagule frequency also increased the total number of cells inoculated. It is not possible to know if the observed results reflect the effect of variation in propagule frequency, the size of additions, or the total number of invader cells inoculated.

In Specific Aim #3, treatments are designed to accurately show whether the way invader cells are introduced to a community influences probability of establishment or invasion success. These treatments also reveal how propagule size and frequency, functioning together in different combinations, affect invasion. In these experiments, a total of three-hundred invader cells are divided among different propagule size and frequency combinations. Some combinations emphasize propagule size, dividing the 300 invader cells into one or two large additions. Other treatments emphasize propagule frequency, dividing the 300 invader cells into many small additions. Since the treatments have tradeoffs between propagule size and frequency, the true relative effects can be discerned.

I predict that propagule size and frequency will affect establishment and invasion success in this experiment, but that the type of effect will vary depending on the invader's growth rate. The experiments of Specific Aim #2 show that increasing propagule size and frequency promotes the success of fast-growing invaders only up to a point after which the species show saturation in their response. Therefore, I predict that fast-growing invaders will exhibit the greatest invasion success in this experiment in treatments with intermediate propagule size and frequency values. In contrast, the success of weak invaders is increases continually with increasing propagule pressure, so I predict that these invaders will have greatest success in treatments that have either high propagule size or high propagule frequency.

Materials and Methods

Experimental Treatments

To examine how propagule size and frequency interact to influence establishment and invasion success, protist invaders are inoculated into identical established communities at four different propagule size-frequency combinations, as illustrated in Figure 4-1. All treatments utilize a total of 300 invader cells, but vary in propagule size and frequency so that there is a tradeoff between these two aspects of propagule pressure. One treatment emphasizes propagule size.

In this treatment, the 300 invader cells are introduced in one inoculation (1x(300)). Another treatment emphasizes propagule frequency. In this treatment, the 300 cells are introduced in six inoculations, each of 50 cells (6x(50)). Two final treatments have intermediate values of propagule size and frequency. In these treatments the 300 cells are inoculated in two additions of 150 cells (2x(150)) and three additions of 100 cells (3x(100)).

Preparation of Experimental Flasks and Culture Conditions

The four treatments analyzed in this specific aim were run along with the experiments conducted for Specific Aim #2 described in chapter 3. The flasks for this experiment were prepared, maintained, and analyzed at the same time as those for Specific Aim #2, using the same materials and methods. The flasks for this experiment were exposed to the same conditions. For information on resource base, established communities, culture conditions, and replication of the treatments used in this experiment, refer to the materials and methods section of chapter 3..

Invaders. Invaders came from healthy stock cultures. To ensure consistency, all invader cells inoculated during the same week came from the same stock flask. The volume of stock culture inoculated into experimental flasks was adjusted so that it contained the correct number of cells based on an average stock cell

concentration determined by the mean of ten independent samples withdrawn immediately prior to inoculation.

Protist Communities. The experimental design described in this section is repeated with four different bacterivorous invaders, each introduced into a unique established community. The invaders and established communities used in these experiments are the ones used in the experiments of Specific Aim #2. The four established community/invader assemblages are listed in columns below, with the invader shown in bold at the top of each column.

Tillina magna

Paramecium bursaria

Spirostomum ambiguum

Paramecium sp.

Paramecium bursaria

Tillina magna

Spirostomum ambiguum

Paramecium sp.

Spirostomum ambiguum

Tillina magna

Paramecium bursaria

Paramecium sp.

Paramecium sp.

Tillina magna

Paramecium bursaria

Spirostomum ambiguum

Effect of Temperature on Population Dynamics and Associated Data Analysis

These relative effects experiments of different invaders had to be conducted in different laboratories that unavoidably varied in temperature. The temperature of the laboratory affected the dynamics of the established communities. The *Paramecium bursaria*-invaded community maintained at 20-21° C continued to increase in cell concentration at a moderate rate throughout the entire experiment. In contrast, the three communities maintained at 25.6° C grew rapidly reaching a maximum cell concentration, typically on week 2, and then the populations in most flasks declined. For this experiment, invasion success had to be analyzed one week after the last invader addition was made. Therefore, analysis had to be done on week 6, even though this meant that three experimental communities were in decline at the time.

Data Collection and Analysis

Data Collection. Data were collected on all experimental flasks every seventh day, starting with the day the invader was introduced and continuing for a total of six weeks as shown in the timeline, figure 4-1. Each experimental flask was swirled and a 350- μ l sample of media removed.

Determining Cell Concentration. The number of cells of each protist species in each sample was counted and then converted to cell concentration (# cells/ml) using the equation:

$$\text{Cell concentration in experimental flask} = \# \text{ cells in } 350\text{-}\mu\text{l sample} / 0.35$$

Determining the Invader's Probability of Establishment. The probability of establishment was calculated for the invader in every treatment at the time of maximum community concentration as:

$$\text{Probability of Establishment} = \# \text{ flasks containing invader} / \text{total number of flasks in treatment}$$

Determining Invasion Success. Invasion success was calculated for every treatment at the time of maximum community concentration as the proportion of the invader in the community:

$$\text{Invasion success} = \text{Invader cell concentration} / \text{community cell concentration}$$

Statistical Analysis.

Propagule Size. To determine whether propagule size influenced invader cell concentration, probability of establishment, or invasion success, values of the dependent variable were compared across propagule size treatments with regression analysis using SPSS Statistics 17.0 software (SPSS, Inc., 2008). Propagule size values and measured dependent variable data were both

identified as numeric scale measures. A generalized linear model used propagule size as the predictor and the different variables as the response to fit a linear curve to the data set. A Wald's Chi-Square test determined the significance of the relationship.

Propagule Frequency. To determine whether propagule frequency influenced values of the dependent variable, invader cell concentration, probability of establishment, and invasion success data were compared across propagule frequency treatments with ANCOVA using SPSS Statistics 17.0 software (SPSS, Inc., 2008). Values of propagule frequency and measured dependent variable data were both identified as numeric scale measures. The categorical data of addition size was identified as a factor. A generalized linear model used propagule size data as the covariate predictor, addition size as the factor, and the dependent variable data as the response to fit a linear curve. The model considered the main effects of propagule frequency and addition size, as well as the interaction of propagule frequency and addition size, on the values of the dependent variable. A Wald's Chi-Square test determined the significance of the factor and covariate.

The chi-square values, degrees of freedom, and probability for all tests included in this chapter are given in Appendix: Results of Statistical Analysis.

Results

Uniformity of the Established Communities

To be sure variations in the probability of establishment, cell concentration, and proportion of the invaders are caused by differences in propagule pressure, and not differences among established communities, established communities must be a standard condition at the time of invasion. To minimize variation, the resource base and established communities of all experimental flasks were prepared in an identical way. To further improve consistency among the established communities, the experimental flasks were pooled and re-aliquoted immediately prior to invasion. After pooling, all flasks were analyzed for total cell concentration, species richness, and diversity. At the time of invasion, all established communities were statistically equivalent in terms of cell concentration, species richness, and diversity. These established communities can be considered a standard condition, and differences in invasion success attributed to propagule size and frequency.

Relative Effects of Propagule Size and Frequency

Tillina magna Invader

By week 6, *Tillina magna* communities had reached maximum cell concentration and crashed. Invading *Tillina magna* populations did not survive long enough to analyze. However, several treatments from the experiments of Specific Aim #2 could be compared for relative effects earlier during culture, when the invading *Tillina magna* populations were still robust. The first relative effects comparison was of 50 cells inoculated as two additions of 25 cells and one addition of 50 cells. The second comparison was of 100 cells inoculated as two additions of 50 cells and one addition of 100 cells. All inoculations were completed by week 1, so these treatments could be analyzed on week 2, the time of maximum community cell concentration. There are drawbacks to using these treatments to study relative effects. The range of propagule size and frequency values is very narrow, and populations are compared very early in their growth. Treatments limited to this degree may not reveal the full effect of the interaction between propagule size and frequency.

Probability of Establishment. The way 50 or 100 *Tillina magna* invader cells are inoculated does not affect the species' probability of establishment on week 2.

Tillina magna is able to establish persistent populations in all treatments (Figure 4-2).

Cell Concentration. The way *Tillina magna* cells are introduced into the established community influences invader cell concentration on week 2, but only when the total number of cells inoculated is large (Figure 4-3A). Total cell number, whether 50 or 100 cells, has a significant effect on *Tillina magna* cell concentration ($\chi^2 = 6.635$, $df = 1$, $P = 0.010$), as does the interaction between total cell number and propagule frequency ($\chi^2 = 7.639$, $df = 1$, $P = 0.006$). The 50-cell relative effects treatments have statistically equivalent cell concentrations of 99 and 112 cells/ml. This suggests that the way 50 *Tillina magna* cells are introduced into the community does not influence invasion success. In contrast, the cell concentration of the 1x(100) treatment (127 cells/ml) is substantially greater than that of the 2x(50) treatment (78 cells/ml). Increasing propagule frequency, while simultaneously decreasing propagule size, in the 100-cell relative effects comparison significantly decreases *Tillina magna* concentration ($\chi^2 = 8.743$, $df = 1$, $P = 0.003$).

Proportion of Invader in Community. The proportion of *Tillina magna* in the relative effects treatments on week 2 shows the same pattern of invasion success as the cell concentration data (Figure 4-3B). Total cell number, whether 50 or 100 cells, has a significant effect on the proportion of *Tillina magna* ($\chi^2 = 6.626$, $df = 1$, $P = 0.010$), as does the interaction between total cell number and propagule frequency ($\chi^2 = 7.707$, $df = 1$, $P = 0.006$). The way 50 *Tillina*

magna cells is inoculated into the established community does not affect the species' invasion success, as measured by its proportion in the community ($\chi^2 = 0.151$, $df = 1$, $P = 0.698$). The two 50-cell treatments have nearly identical proportion on week 2 (0.38-0.42). In contrast, when 100 invader cells are introduced in one inoculation, the resulting population has significantly larger proportion in the community (0.42) than when the cells are inoculated in two small additions (0.28). When 100 *Tillina magna* cells inoculated, increasing propagule frequency, at the expense of propagule size, has a significant negative effect on the species' proportion on week 2 ($\chi^2 = 13.051$, $df = 1$, $P < 0.01$).

Summary. The individual effects of propagule size and frequency seen in Specific Aim #2 are also evident in the results of these relative effects comparisons. Individually, increasing propagule size increases *Tillina magna* cell concentration and proportion through propagule size 100. In the relative effects comparisons, treatment 1x(100) has greater cell concentration and proportion than treatment 1x(50). Individually, the size of additions determines the effect of propagule frequency. When additions are small (25-cell), increasing propagule frequency enhances *Tillina magna* cell concentration and proportion. When additions are larger (50-cell), however, increasing propagule frequency leads to decreased invasion success. Much of this pattern is also found in the relative effects treatments. In the 50-cell relative effects comparison, invasion success is similar regardless of whether one 50-cell or two 25-cell inoculations of invader cells were made. However, the larger addition in the 100-cell relative effects

comparison is associated with a significant decrease in invader concentration and proportion. Invasion success is reduced when two 50-cell inoculations are made, rather than one 100-cell inoculation. These results suggest that propagule size and frequency interact to influence *Tillina magna* invasion. The total number of cells inoculated determines the effect produced by different propagule size-frequency combinations and the strength of propagule frequency in shaping the population's growth. When a small total number of cells is added (50), the effect of propagule frequency is weak and the way invader cells are inoculated does not affect the concentration or proportion of the resulting population. In contrast, when the total number of invader cells is greater (100), propagule frequency has a stronger negative effect. Adding the invader cells in multiple large additions significantly reduces the cell concentration and proportion of the resulting population.

Paramecium bursaria Invader

The way propagule size and propagule frequency interact to influence the invasion success of *Paramecium bursaria* is studied with three different relative effects series. The species' cell concentration and proportion are compared across the 300-cell treatments outlined in Figure 4-1. Additional pairs of treatments that were part of the experiments of Specific Aim #2 are also included. These pairs of treatments share the same number of invader cells, but differ in the way the cells are divided among propagule size and frequency

values. In one pair of treatments, a total of 50 invader cells is inoculated as one addition of 50 cells (1x(50)) and two additions of 25 cells (2x(25)). In another pair of treatments, a total of 100 invader cells is inoculated as one addition of 100 cells (1x(100)) and two additions of 50 cells (2x(50)).

Probability of Establishment. The way *Paramecium bursaria* invader cells are introduced to the community does not influence the species' probability of establishment on week 6. *Paramecium bursaria* is able to found persistent populations in all treatments (Figure 4-4).

Cell Concentration. There is only slight variation in cell concentration among the 300-cell relative effects treatments on week 6 (65-110 cells/ml), and differences are not significant (Figure 4-5A). In contrast, there is significant difference in the cell concentration of treatments in the 50- and 100-cell relative effects comparisons on week 2 (Figure 4-6A). In both of these comparisons, the cell concentration of the single large addition treatment is greater than that of the multiple small additions treatment. Increasing propagule frequency, while simultaneously decreasing propagule size, has a significant negative effect on *Paramecium bursaria* cell concentration in both the 25- and 50-cell comparisons ($\chi^2 = 13.353$, df = 1, $P < 0.01$).

Proportion of Invader in Community. There is a slight pattern of decreased *Paramecium bursaria* proportion with increasing propagule frequency in the 300-

cell relative effects comparison. However, the proportion values are very similar (0.2-0.275), and the trend is not significant (Figure 4-5B). However, the 50-cell and 100-cell relative effects comparisons both show a significant pattern of reduced invasion success with increased propagule frequency (Figure 4-6B). In both of these comparisons, the proportion of the single large addition treatment is substantially greater than that of the multiple small additions treatment. Increasing propagule frequency, while simultaneously decreasing propagule size, has a significant negative effect on *Paramecium bursaria* proportion in both the 25- and 50-cell comparisons ($\chi^2 = 8.355$, $df = 1$, $P = 0.004$).

Summary. The individual effect of propagule frequency seen in Specific Aim #2 is also evident in the results of these relative effects comparisons. Individually, increasing propagule frequency is associated with decreasing *Paramecium bursaria* cell concentration and proportion. This pattern is evident in the 50- and 100-cell relative effect comparisons. The effect of propagule size is not apparent, but almost all treatments have propagule sizes of 50-100, values that generate nearly identical *Paramecium bursaria* growth. The individual effect of propagule size is also seen in the relative effects comparisons, but the influence is subtle. In the 300-cell relative effects comparison, treatment 1x(300) has substantially, although not significantly, lower cell concentration than treatment 2x(150). Since propagule frequency is very low, one might expect the concentration of this treatment to be much greater than the two-addition treatment 2x(150). However, very high propagule size independently results in

low concentration. Treatment 1x(300) has lower cell concentration than treatment 2x(150) because the negative effect of high propagule size is more influential than the positive effect of low propagule frequency. In all three relative effects comparisons, increasing propagule frequency, while simultaneously decreasing propagule size, reduces *Paramecium bursaria* cell concentration and proportion.

Spirostomum ambiguum Invader

Probability of Establishment. On week 6, there is clear pattern of increasing probability of *Spirostomum ambiguum* establishment with increasing propagule frequency, at the expense of propagule size, from 0 for treatment 1x(300) to 0.6 for treatment 6x(50) (Figure 4-7). The trend is significant ($\chi^2 = 11.200$, $df = 1$, $P = 0.001$).

Cell Concentration. Propagule frequency does not influence the cell concentration of *Spirostomum ambiguum* populations on week 6. The two-addition, three-addition, and six-addition treatments have statistically equivalent cell concentration (5-6 cells/ml) (Figure 4-8A).

Proportion of Invader in Community. On week 6, there is a pattern of increasing *Spirostomum ambiguum* proportion with increasing propagule frequency, from 0 for the single-addition treatment, 0.025 for two- and three-addition treatments, to

0.13 for the six-addition treatment. However, this pattern is not significant (Figure 4-8B).

Summary. The individual effect of propagule frequency on establishment success seen in Specific Aim #2 is also evident in the results of the relative effects comparison. In both experiments, increasing propagule frequency enhances *Spirostomum ambiguum* establishment, cell concentration, and proportion. However, only the effect of increasing propagule frequency is significant in the relative effects comparison. The individual effect of propagule size is not found in the relative effects comparison. In the experiments of Specific Aim #2, increasing propagule size was found to facilitate *Spirostomum ambiguum* establishment, cell concentration and proportion on week 2. In the 300-cell relative effects comparison, however, the reverse pattern is found although it is significant only for establishment. In fact, the relative effects treatment with the highest propagule size (1x(300)) declines and is lost from the all treatments by week 6. This suggests that only propagule frequency affects the establishment and invasion success of *Spirostomum ambiguum* six weeks after its introduction. This is because the stability of *Spirostomum ambiguum* populations is associated with maximum cell concentration and propagule size. When large propagule size generates high *Spirostomum ambiguum* cell concentration, maximum abundance is reached on week 2 and then the population declines rapidly. Since the relative effects treatments were compared on week 6, populations influenced by high propagule

size had already disappeared. In contrast, high propagule frequency allows *Spirostomum ambiguum* populations to maintain fairly stable cell concentration throughout the experiment. The relative effects comparisons show that propagule frequency is the major determinant of *Spirostomum ambiguum* invasion success because the treatments are compared many weeks after the species is first introduced.

Paramecium sp. Invader

Probability of Establishment. On week 6, there is a pattern of increasing *Paramecium sp.* establishment with increasing propagule frequency up to a maximum probability at treatment 3x(100), followed by a decline in establishment as propagule frequency continues to increase (Figure 4-9).. This pattern is not significant, which suggests that the way 300 *Paramecium sp.* cells are introduced to the established community does not affect the species' establishment.

Cell Concentration. Multiple large inoculations of *Paramecium sp.* promote the greatest invasion success, as measured by cell concentration. On week 6, the two treatments with intermediate values of propagule size and frequency (2x(150) and 3x(100)) have nearly equivalent cell concentration (125-130 cells/ml) that is substantially greater than the concentration of the other two treatments in the experiment. Those treatments, 1x(300) and 6x(50), have

nearly identical low cell concentration (24-26 cells/ml). However, this pattern is not significant (Figure 4-10A).

Proportion of Invader in Community. The proportion of the *Paramecium sp.* invader in the community is significantly affected by the way invader cells are introduced into the established community (1-way ANOVA (df 3, 14; $G = 7.055$; $P = 0.004$) (Figure 4-10B). Inoculating the *Paramecium sp.* invader in a few large additions (2x(150) and 3x(100)) significantly increases the proportion of the invader in the community on week 6. There is a substantial difference between the proportions of the two- and three-addition treatments (0.65-0.75) and those of the single- and six-addition treatments (0.6-0.2) (Tukey post-hoc comparisons (propagule frequency 2x(150) > 1x(300) and 6x(50), $P \leq 0.078$; propagule frequency 3x(100) > 1x(300), $P = 0.026$)).

Summary. The individual effects of propagule size and frequency are evident in the results of the relative effects comparison, and the influence of both factors plays an important role in shaping *Paramecium sp.* invasion success.

Individually, increasing propagule size and increasing propagule frequency are both associated with significant increases in *Paramecium sp.* establishment, cell concentration, and proportion. In the relative effects comparison, high propagule size and frequency are instead associated with lower invasion. The treatments with intermediate propagule size and frequency, 2x(150) and 3x(100), have significantly greater proportion than the treatments with either high propagule

size or high propagule frequency. This pattern may reflect the simultaneous strong negative effects of low propagule size and low propagule frequency. The simultaneous suppression of growth at low propagule size and frequency allows treatments with intermediate values to have maximum concentration and proportion. The results of the relative effects comparisons suggest that the negative effects of low propagule size and low propagule frequency shape *Paramecium sp.* invasion success.

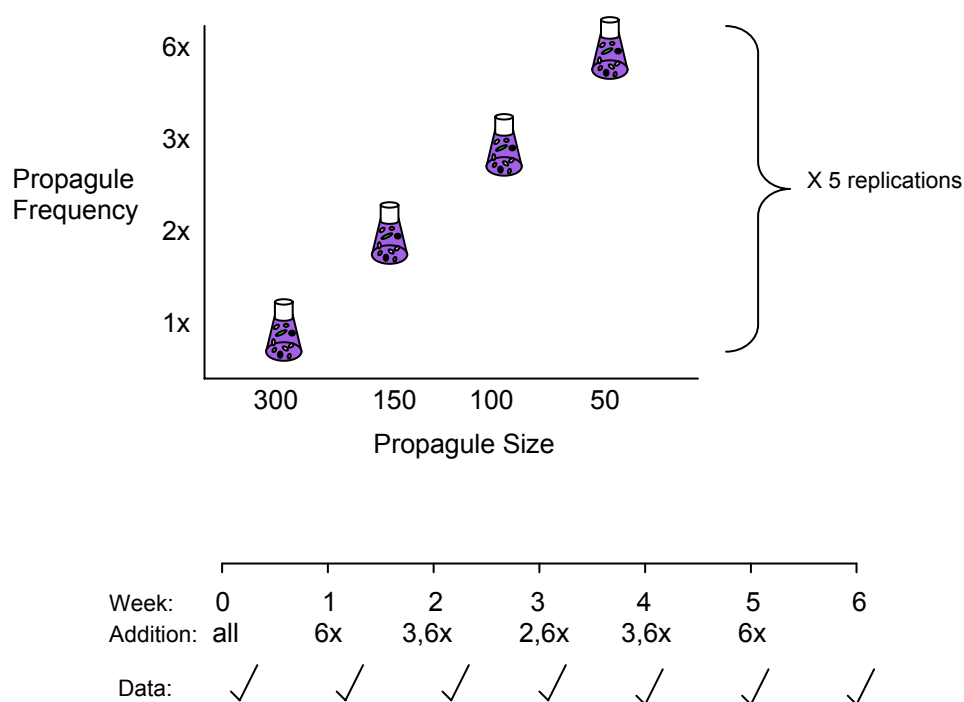


Figure 4-1. Experimental design for Specific Aim #3: To examine how propagule size and propagule frequency interact in influencing invasion success. The relative effects of propagule size and frequency were studied by inoculating replicate established communities with six 50-cell additions, three 100-cell additions, two 150-cell additions, and one 300-cell addition of the invader species during the first five weeks of culture. All treatments were maintained for six weeks. The cell concentration of all species in every experimental flask was determined every seven days, starting with the day the invader was introduced.

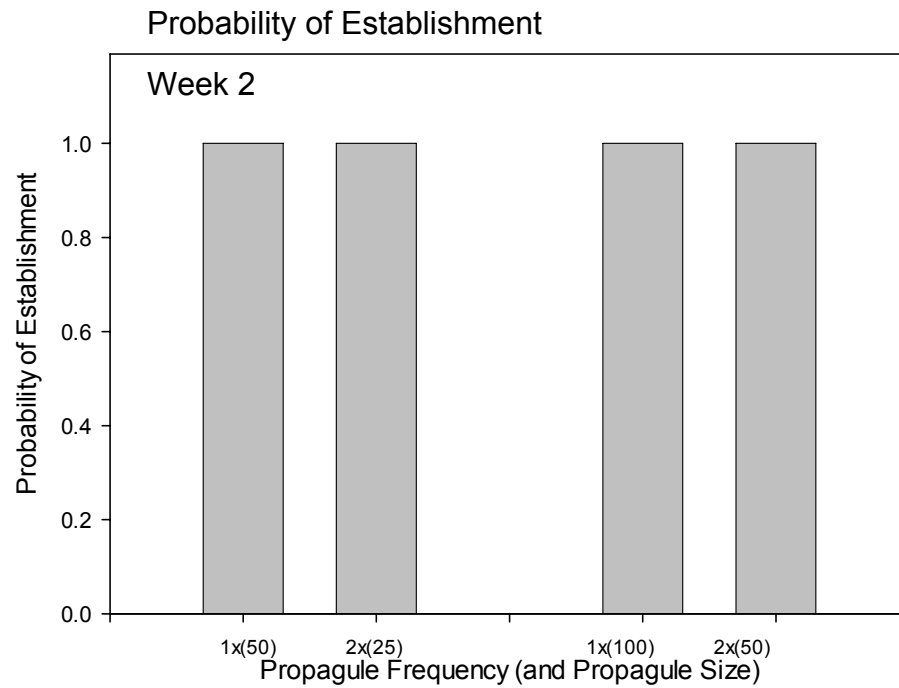
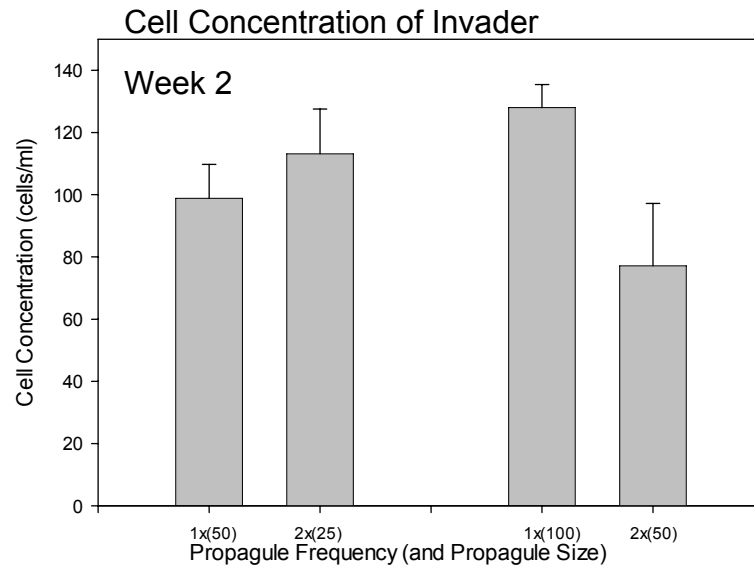


Figure 4-2. The probability of establishment, two weeks after introduction, of the invader, *Tillina magna*, when the species is maintained with one addition of 50 cells, two additions of 25 cells, one addition of 100 cells, and two additions of 50 cells during the first week of culture. Established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. and cultured three weeks before the invader was introduced. Plotted values are the means of five replications of each treatment, four for 2x(50).

(A)



(B)

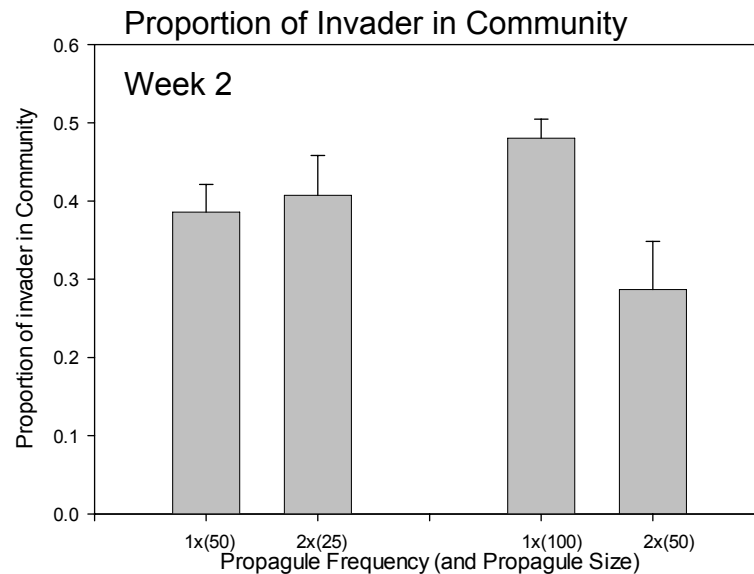


Figure 4-3. The cell concentration (A) and proportion (B), two weeks after introduction, of the invader, *Tillina magna*, when the species is maintained with one addition of 50 cells, two additions of 25 cells, one addition of 100 cells, and two additions of 50 cells during the first week of culture. Established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium sp.* and cultured three weeks before the invader was introduced. Plotted values are the means (± 1 SE) of 5 replications of each treatment, four for 2x(50). Total cell number and the interaction between frequency/size treatments and total cell number significantly affect concentration (A) and proportion (B). In the 100-cell comparison, frequency/size significantly affects concentration (A). See appendix for specific statistical results.

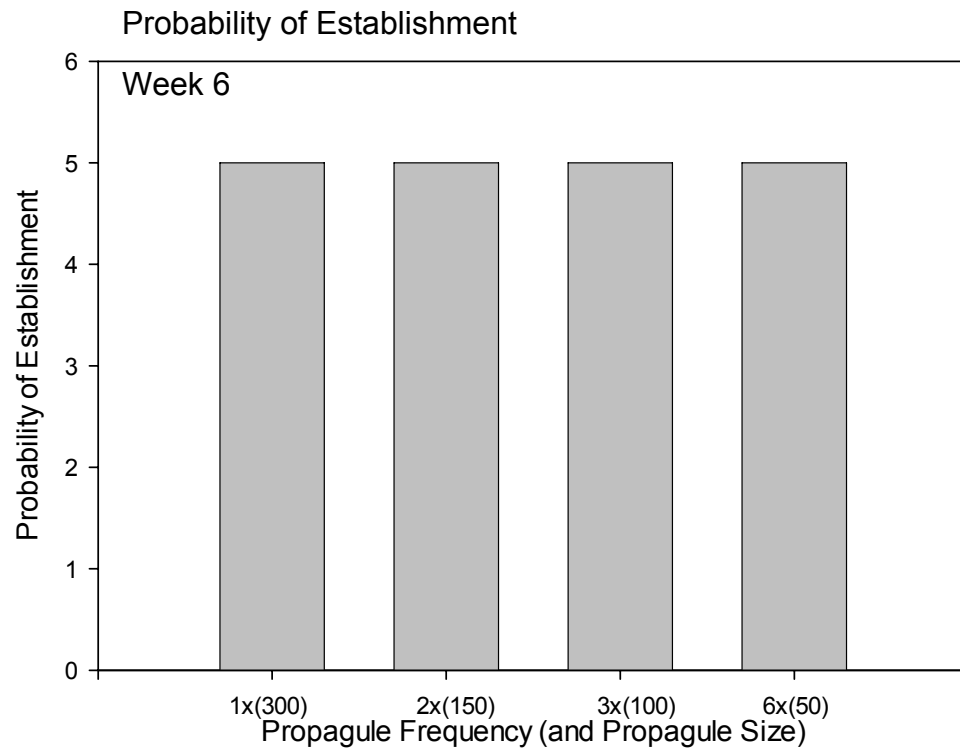
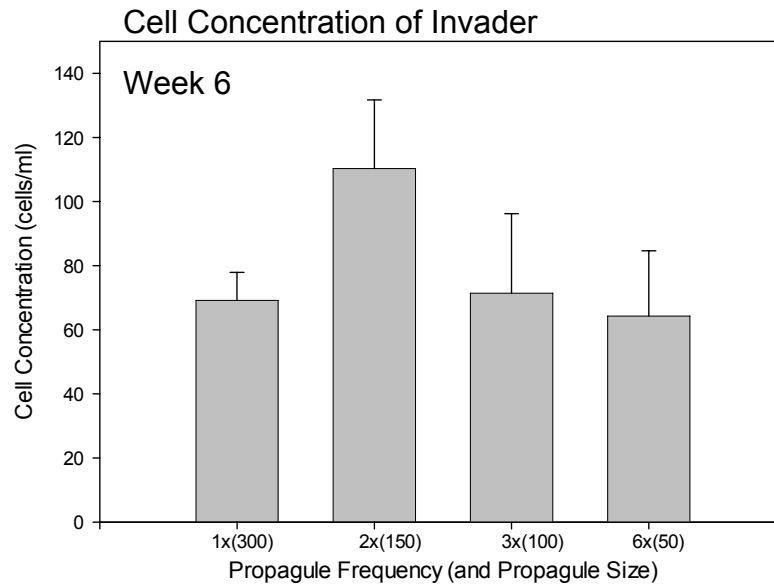


Figure 4-4. The probability of establishment, six weeks after introduction, of the invader, *Paramecium bursaria*, when the species is maintained with one addition of 300 cells, two additions of 150 cells, three additions of 100 cells, or six additions of 50 cells during the first five weeks of culture. The established communities were initiated with 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, and *Paramecium sp.* and cultured three weeks before the invader was added. Values are the means of five replications of each treatment, four for 1x(300).

(A)



(B)

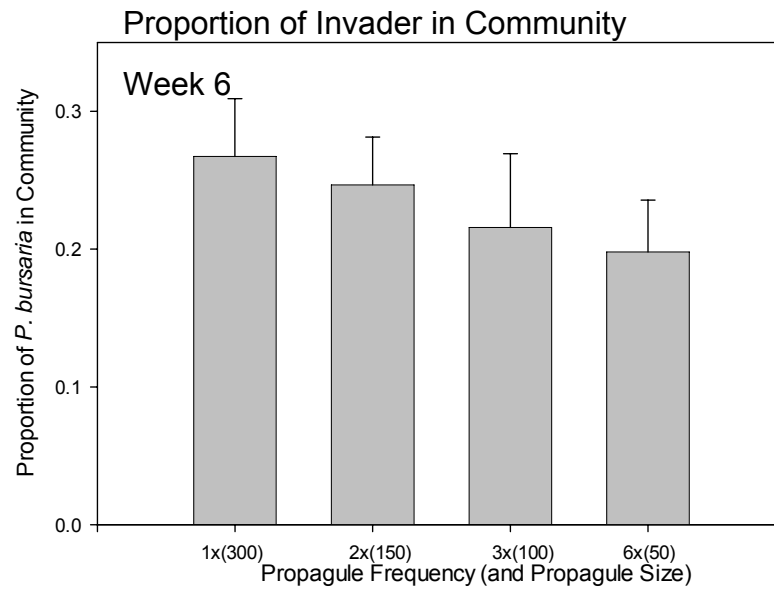
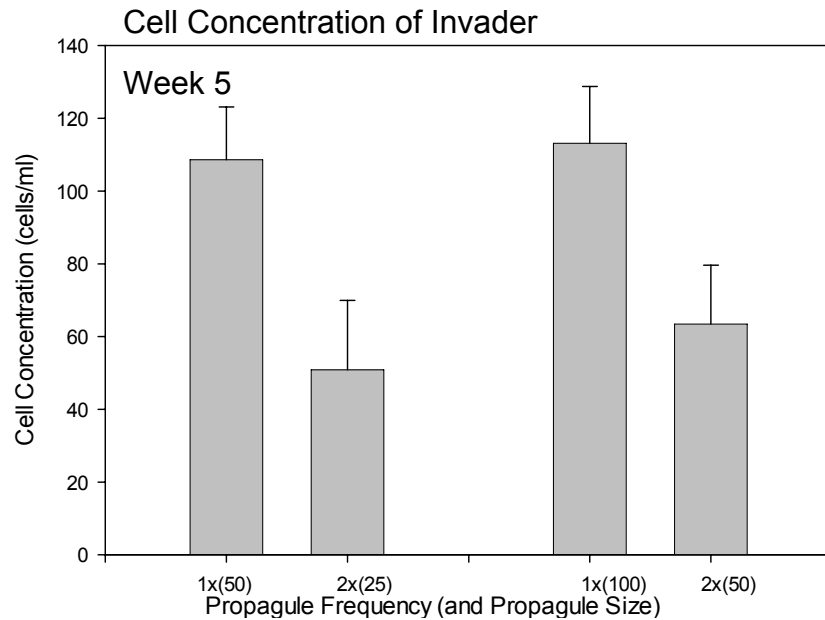


Figure 4-5. The cell concentration (A) and proportion (B), six weeks after introduction, of the invader, *Paramecium bursaria*, when the species is maintained with one addition of 300 cells, two additions of 150 cells, three additions of 100 cells, or six additions of 50 cells during the first five weeks of culture. The established communities were initiated with 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, and *Paramecium sp.* and cultured three weeks before the invader was added. Values are the means (± 1 SE) of five replications of each treatment, four for 1x(300).

(A)



(B)

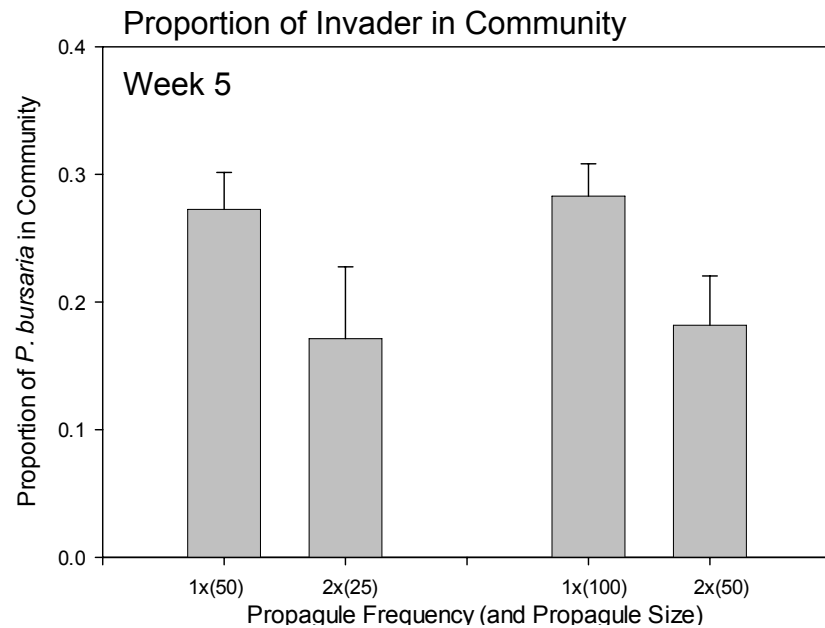


Figure 4-6. The cell concentration (A) and proportion (B), five weeks after introduction, of the invader, *Paramecium bursaria*, when the species is maintained with two additions of 25 cells or one addition of 50 cells and two additions of 50 cells or one addition of 100 cells during the first four weeks of culture. The established communities were initiated with 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, and *Paramecium bursaria* and cultured three weeks before the invader was added. Values are the means (± 1 SE) of five replications of each treatment. Propagule frequency/size significantly affects cell concentration (A) ($\chi^2 = 13.353$, $df = 1$, $P < 0.01$) and proportion (B) ($\chi^2 = 8.355$, $df = 1$, $P = 0.004$).

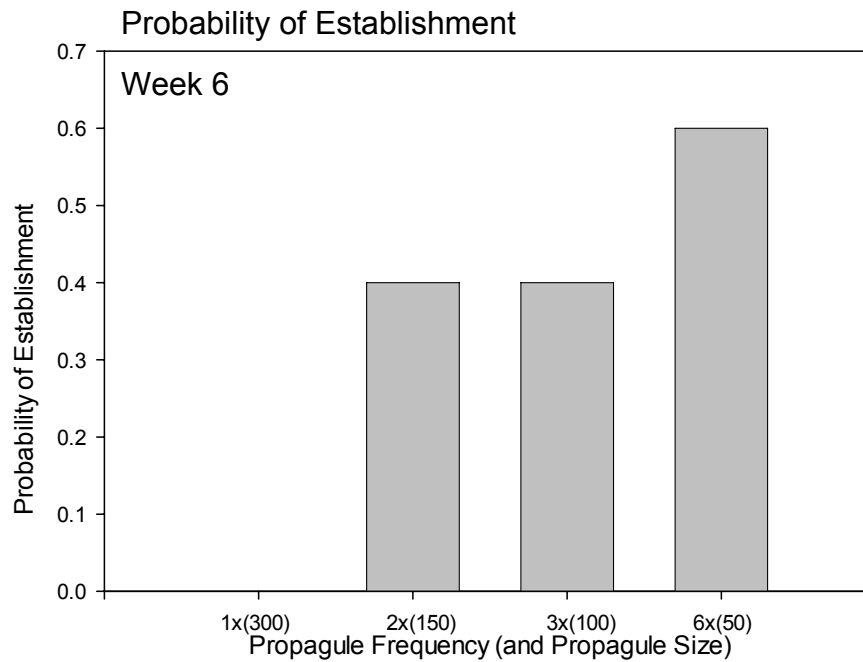
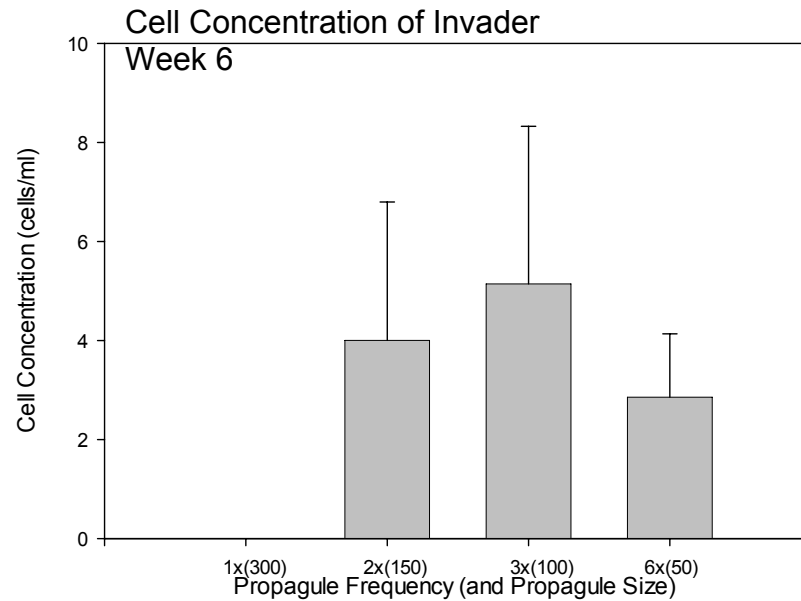


Figure 4-7. The probability of establishment, six weeks after introduction, of the invader *Spirostomum ambiguum*, when the species is maintained with one addition of 300 cells, two additions of 150 cells, three additions of 100 cells, or six additions of 50 cells during the first five weeks of culture. Established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium sp.* and cultured three weeks before the invader was added. Frequency/size significantly affects probability of establishment ($\chi^2 = 11.200$, $df = 1$, $P = 0.001$).

(A)



(B)

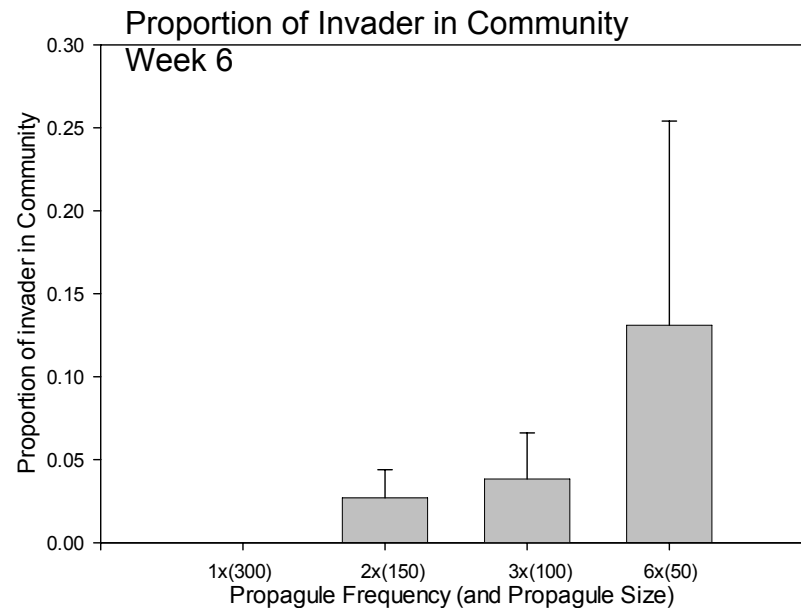


Figure 4-8. The cell concentration (A) and proportion (B), six weeks after introduction, of the invader *Spirostomum ambiguum* when the species is maintained with one addition of 300 cells, two additions of 150 cells, three additions of 100 cells, or six additions of 50 cells during the first five weeks of culture. The established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium sp.* and cultured three weeks before the invader was added. Values are the means (± 1 SE) of five replications of each treatment.

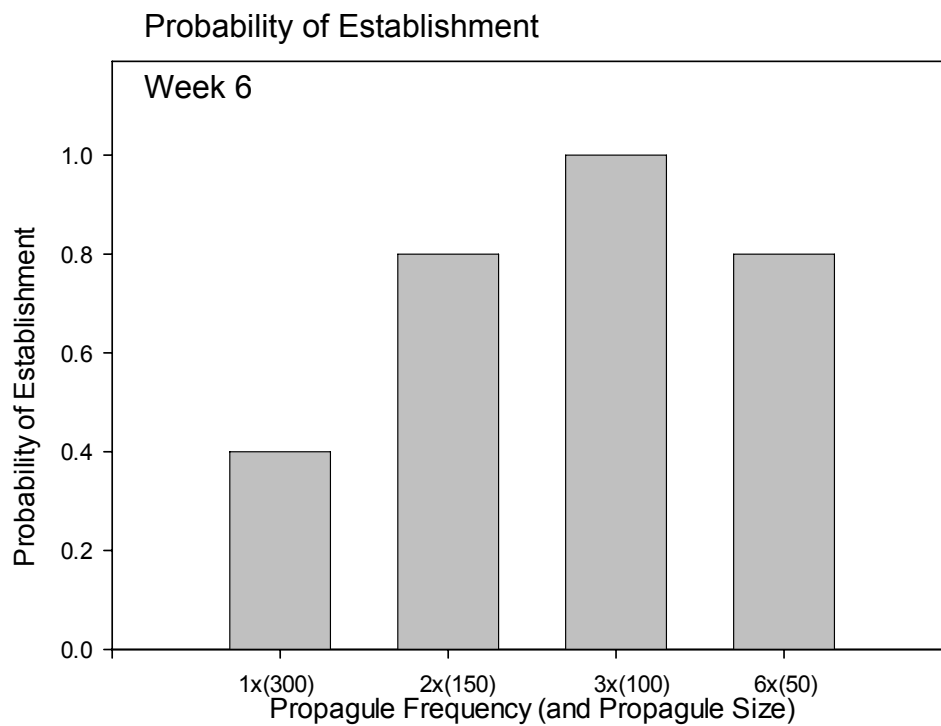
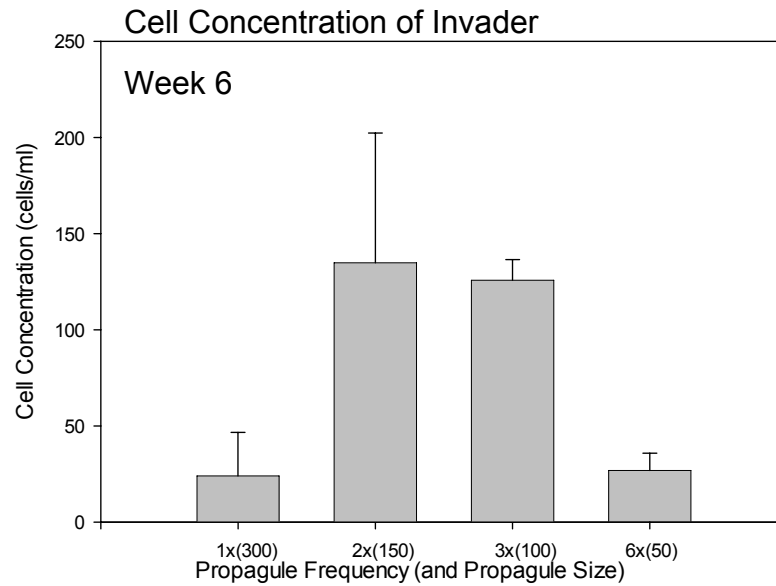


Figure 4-9. The probability of establishment, six weeks after introduction, of the invader, *Paramecium* sp., when the species is maintained with one addition of 300 cells, two additions of 150 cells, three additions of 100 cells, or six additions of 50 cells during the first five weeks of culture. Established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Tillina magna* and cultured 3 weeks before the invader was introduced.

(A)



(B)

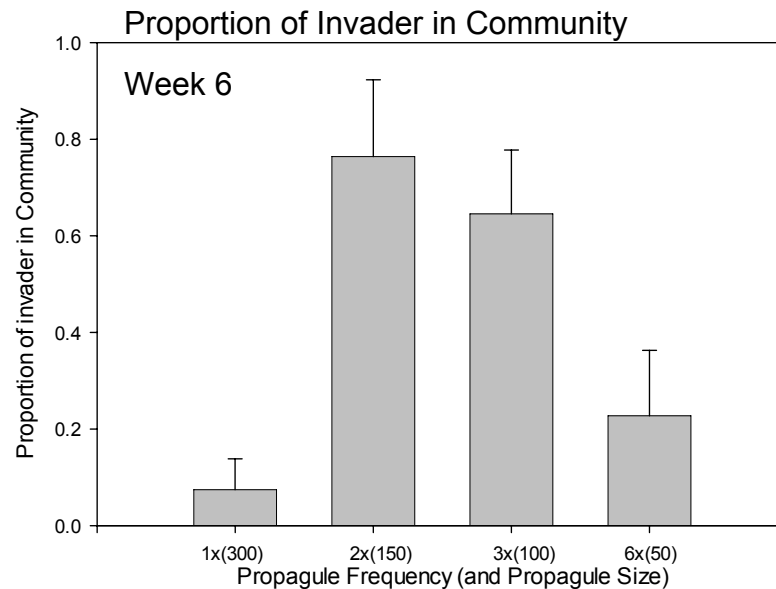


Figure 4-10. The cell concentration (A) and proportion (B), six weeks after introduction, of the invader, *Paramecium* sp., when the species is maintained with one addition of 300 cells, two additions of 150 cells, three additions of 100 cells, or six additions of 50 cells during the first five weeks of culture. Established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Tillina magna* and cultured three weeks before the invader was added. Values are the means (± 1 SE) of five replications of each treatment, four for 3x(100). Frequency/size treatments significantly affect proportion (B), 1-way ANOVA (df 3, 14; $G = 7.055$; $P = 0.004$); Tukey post-hoc comparisons (propagule frequency 2x(150) > 1x(300) and 6x(50), $P \leq 0.078$; propagule frequency 3x(100) > 1x(300), $P = 0.026$).

Discussion

It is not yet known how the effects of propagule size and frequency, functioning together, affect the success of an invader or how these effects vary from one invader to another (Lockwood *et al.*, 2005). In Specific Aim #2, the individual effects of propagule size and frequency were determined for four bacterivorous protist species. The experiments described here expand on that work by showing how propagule size and frequency interact to influence the species' establishment and invasion success. I had predicted that propagule size and frequency would affect establishment and invasion success, but that the type of effect would vary depending on the invader's realized growth rate in the community. These predictions accurately describe the results of this study. The four protist invaders are affected by propagule size and frequency, but the way these factors interact in determining invader establishment and success vary from species to species.

The Relative Effect of Propagule Size and Propagule Frequency on the Probability of Invader Establishment

In these experiments, an invader's realized growth rate influences the strength of propagule size and frequency in influencing establishment, just as it did in the experiments of Specific Aim #2. The two fast-growing invaders, *Tillina magna* and *Paramecium bursaria*, establish populations in all flasks in all treatments regardless of propagule size or frequency of inoculation. In contrast, propagule

size and frequency do influence the establishment of the two slower-growing invaders, *Spirostomum ambiguum* and *Paramecium sp.* In the experiments of Specific Aim #2, independently increasing size and propagule frequency significantly promotes the establishment of both slow-growing invaders. In those experiments, *Spirostomum ambiguum* and *Paramecium sp.* both achieve the greatest probability of establishment at higher propagule size (100 for *Spirostomum ambiguum*, 300 for *Paramecium sp.*) In the relative effects treatments, there is variation the way the two slow-growing species' probability of establishment varies across treatments. The establishment of *Spirostomum ambiguum* is influenced only by propagule frequency. Increasing propagule frequency increases *Spirostomum ambiguum* establishment through propagule size 100. In contrast, both propagule size and propagule frequency appear to influence *Paramecium sp.* establishment. The negative effects of low propagule size and low propagule frequency both suppress *Paramecium sp.* establishment. Maximum establishment is achieved in treatments that have intermediate values of propagule pressure.

*The Relative Effect of Propagule Size and Propagule Frequency
on Invasion Success*

The way propagule size and frequency interact to influence invasion success varies from species to species. For three protist species, the effects of both propagule size and propagule frequency influence invasion when the two factors

interact. Saturation of propagule pressure effects also plays a role in determining invasion success. The invasion of the final species is shaped primarily by propagule frequency. Although the four protist invaders can be grouped based on the number of factors that influence invasion and whether the species shows saturation, the way propagule size and frequency interact to influence invasion is unique for each species.

*Propagule size and frequency interact in determining the success of *Tillina magna* invasion. Propagule size is like a switch, regulating whether the negative effect of increasing propagule frequency will influence invasion. When propagule size is small, the effect of propagule frequency is negligible. However, when propagule size is larger, the negative effect of increasing propagule frequency significantly decreases *Tillina magna* cell concentration and proportion.

*Propagule size and frequency also both influence *Paramecium sp.* invasion, but in a very different way. Low propagule size and low propagule frequency both suppress *Paramecium sp.* growth. Therefore, maximum *Paramecium sp.* invasion occurs when moderate propagule size and frequency values co-occur. Both propagule size and propagule frequency interact to influence *Paramecium sp.* growth, but in every case the negative effect prevails. This suggests that negative effects are more

influential than positive ones, regardless of whether the effect is from propagule size or frequency.

*The cell concentration and proportion of *Paramecium bursaria* in the relative effects experiments can be explained by the simultaneous influence of propagule size and propagule frequency effects. Even though both aspects of propagule pressure affect *Paramecium bursaria* growth, negative propagule size effects hide positive propagule frequency effects. Increasing propagule frequency, at the expense of propagule size, is associated with reduced *Paramecium bursaria* cell concentration and proportion in the 50- and 100-cell relative effects comparisons and three of the four treatments in the 300-cell comparison. This pattern seems to be explained by the sole influence of propagule frequency, since increasing propagule frequency causes a decrease in *Paramecium bursaria* growth when addition size is at least 50. An effect of propagule size is not expected since almost all additions are of 50 or 100 cells. These values of propagule size produced nearly identical *Paramecium bursaria* cell concentration and proportion in the invasion experiments. However, two treatments show the effect of propagule size. The 2x(25) treatment has significantly lower cell concentration than the 1x(50) treatment. In the invasion studies of Specific Aim #2, the effect of increasing propagule frequency has a negligible effect when addition size is low (25-cell). Given this pattern, the 2x(25) treatment is expected to have similar cell

concentration as the 1x(50) treatment. However, the invasion studies also show that the cell concentration produced by an initial inoculation of 25 cells is significantly less than that produced by 50 cells. This suggests that 2x(25) treatment is lower due to the effect of propagule size. The 1x(300) treatment is also unexpectedly lower than the 2x(150) treatment. Given only the negative effect of propagule frequency, the 1x(300) treatment ought to be greater than the 2x(150) treatment. However, an initial inoculation of 300 cells in the invasion studies produced significantly lower cell concentration than that of 100 cells. The unexpectedly low 1x(300) relative effects treatment reflects the negative effect of high propagule size, despite the positive influence that must be exerted by the associated low propagule frequency.

*The establishment of *Spirostomum ambiguum* in the relative effects comparison is influenced solely by propagule frequency. Individually, increasing propagule size and increasing propagule frequency enhance *Spirostomum ambiguum* establishment, cell concentration, and proportion. In the relative effects comparison, there is a significant trend of increasing establishment with increasing propagule frequency at the expense of propagule size. *Spirostomum ambiguum* proportion shows the same pattern, but it is not significant. Although propagule size has a strong effect on *Spirostomum ambiguum* growth, high propagule size treatments reach maximum concentration by week 2 and then decline. In contrast,

high propagule frequency treatments maintain invader cell concentration throughout the experiment. The relative effects treatments show a strong trend of increasing invasion with increasing propagule frequency because the treatments are compared on week 6.

General Relative Effects Patterns

The combined results from the four relative effects experiments suggest that the individual effects of propagule size and propagule frequency simultaneously influence establishment and invasion success. When the two effects vary, the negative effect appears to more powerfully shape the resulting pattern of invasion. When a species shows saturation in its response to propagule size or frequency, its effect may be equivalent across treatments and invasion success is therefore shaped only by the remaining factor. The time at which relative effects are compared may also influence whether propagule size, propagule frequency or both factors most strongly influence invasion success. Propagule size effects occur early in culture, and then populations with high concentration decline. In contrast, high propagule frequency maintains a population's concentration for many weeks. If relative effects are compared very early in culture, they may show a stronger effect from propagule size. When compared many weeks after invasion, they may show an effect primarily from propagule frequency. Cultures compared three to four weeks after invasion may show strong effects of both propagule size and frequency.

CHAPTER 5. THE INFLUENCE OF PROPAGULE PRESSURE ON THE EFFECT OF INVADERS ON THE ESTABLISHED COMMUNITY.

Introduction

Invaders can impact the native community directly and indirectly, in multiple ways, and at several levels of organization (Melbourne *et al.*, 2007). Invasion can directly influence the physiology (Kittelson *et al.*, 2008), abundance, and distribution of native species. It can affect community characteristics, including species richness, community composition and diversity (Reaser *et al.*, 2007). Invasion can also disrupt ecosystem function (D'Antonio and Vitousek, 1992; D'Antonio, 2000; Reaser *et al.*, 2007). Although the general role of invasion in extinction is well accepted (Clavero and Garcia-Berthou, 2005; Melbourne *et al.*, 2007), few invasions have caused known extinctions (Simberloff, 2001) and data suggest that only invasive predators cause extinction (Clavero and Garcia-Berthou, 2005). Many invasion events do not cause the extinction of native species (Tillman, 2004). Indeed, invasion can increase local community diversity (Davis, 2003; Sax and Gaines, 2003; Bruno *et al.*, 2004; Gurevitch and Padilla, 2004; Melbourne *et al.*, 2007).

By shaping patterns of invader presence and abundance, propagule pressure may influence the effect the invader has on the community. Recent work by Reinhardt Adams and Galatowitsch (2008) suggests that the independent

propagule pressures of native and invasive species interact in determining whether the invader suppresses the growth of native community members and the strength of the suppression. Exotic species may have a threshold propagule pressure that influences the strength of their effect on native species. When introduced at a propagule pressure lower than the threshold, the invader will have little effect on the community. However, when introduced at propagule pressure that exceeds the threshold, the invader suppresses the growth of native species (Reinhardt Adams and Galatowitsch, 2008).

The experiments of Specific Aim #4 examine the influence of four protist invaders on established species and their community. These experiments are designed to reveal the effect of the invaders, at various propagule size and frequency values, on the presence and abundance of individual established species and on the concentration, richness, evenness, and diversity of invaded communities. I predict that propagule pressure will influence the effect the four invaders have on established species and the community. I expect invaders to have a negative effect on the growth of individual established species and on community concentration, richness, evenness, and diversity. I assume that propagule pressure will influence the strength of the invader's negative effect by determining the abundance of the invader in the community. Values of propagule pressure that promote invader growth will result in strong negative effects on the community, while treatments that allow only limited invader growth will be associated with minimal negative impact.

Materials and Methods

The results analyzed in this specific aim come from the experiments used in Specific Aim #2. For that specific aim, invader cell concentration was compared across a range of propagule size and frequency values to determine how propagule pressure influences invasion success. For this specific aim, the abundance of established species and the species richness, evenness, and diversity of established communities are compared to invader propagule size, frequency, and cell concentration across the range of propagule pressure treatments to determine how propagule pressure influences the invader's effect on established species and the established community.

Experimental Treatments

Data analyzed in this section come from the experimental treatments used in Specific Aim #2. In addition, this analysis includes a control treatment that shows the effect of the invader on the established community (Figure 5-1). The established communities in this treatment are prepared in the same way as those in other treatments, but the invader is never introduced.

Preparation of Experimental Flasks and Culture Conditions

For information on the preparation of the resource base and established communities, culture conditions, replication, and invasion see the materials and methods section of chapter 3.

Protist Communities

For this specific aim, the effect of four protist species on their unique established communities was compared across a range of propagule size and frequency values. The four invader/established community assemblages are listed in columns below, with the invader shown in bold at the top of each column.

<i>Tillina magna</i>	<i>Paramecium bursaria</i>
<i>Paramecium bursaria</i>	<i>Tillina magna</i>
<i>Spirostomum ambiguum</i>	<i>Spirostomum ambiguum</i>
<i>Paramecium sp.</i>	<i>Paramecium sp.</i>
<i>Spirostomum ambiguum</i>	<i>Paramecium sp.</i>
<i>Tillina magna</i>	<i>Tillina magna</i>
<i>Paramecium bursaria</i>	<i>Paramecium bursaria</i>
<i>Paramecium sp.</i>	<i>Spirostomum ambiguum</i>

Data Collection and Analysis

Data Collection. Data were collected on all experimental flasks every seventh day, starting with the seventh day following the inoculation of protists, and continuing for a total of eight weeks as shown in the timeline, figure 5-1. Each experimental flask was swirled and a 350- μ l sample of media removed.

Determining Cell Concentration. The number of cells of each protist species in each sample was counted and then converted to cell concentration (# cells/ml) using the equation:

$$\text{Cell concentration in experimental flask} = \# \text{ cells in } 350\text{-}\mu\text{l sample} / 0.35$$

Determining Community Diversity. Diversity was calculated for each flask every week using the Shannon Index:

$$H = -\sum_{i=1}^S (p_i \ln p_i), \text{ where } p_i \text{ is the proportion of species } i \text{ in the community of } S \text{ species}$$

Mean diversity was calculated for each treatment every week.

Determining Species Richness. Species richness was determined for each flask every week as the number of species recovered in the 350- μ l sample. Mean species richness was calculated for each treatment every week. Species

richness was compared across control and experimental treatments each week using the proportion of maximum species richness attained:

$$\text{Proportion of Species Richness} = \text{mean \# species in flask} / \text{\# species inoculated into flask}$$

Determining Species Evenness. Evenness was calculated for each flask every week using Pielou's Evenness Index:

$$J' = (H'/H_{\max})$$

H' = Shannon index of diversity value for the community

$H_{\max} = \ln S$, where S is the number of species in the community

Mean species evenness was calculated for each treatment every week:

Determining Species Presence. The presence of each species in each treatment was calculated each week as:

$$\text{Presence of Species } i = \text{\# flasks in treatment in which species } i \text{ was recovered} / \text{total \# flasks in treatment}$$

Determining the Percent Change in Established Species' Cell Concentration. To quantify the effect of an invader on each established species, the percent change in the established species' cell concentration as a result of invasion was

calculated as:

$$\text{Percent change in cell concentration} = ((\text{cells/ml}_1 - \text{cells/ml}_2) / \text{cells/ml}_1) \times 100$$

Cells/ml₁ = cell concentration of established species in uninvaded treatment at week of maximum community concentration

Cells/ml₂ = cell concentration of established species in treatment with highest invader concentration at week of maximum community concentration

Statistical Analysis.

Propagule Size. To determine whether propagule size influenced cell concentration, diversity, species richness, or species evenness, values of the dependent variable were compared across propagule size treatments with regression analysis using SPSS Statistics 17.0 software (SPSS, Inc., 2008). Propagule size values, transformed as log base 2, and measured dependent variable data were both identified as numeric scale measures. A generalized linear model used propagule size as the predictor and the different variables as the response to fit a linear curve to the data set. A Wald's Chi-Square test determined the significance of the relationship. To determine whether the specific treatment values were significantly different from one another in one data set, treatments were compared with one-way ANOVA followed by Tukey post-

hoc comparisons using SigmaPlot for Windows 11.00 (Systat Software, 2008) (Figure 5-6).

Propagule Frequency. To determine whether propagule frequency influenced values of the dependent variable, cell concentration, diversity, species richness, and species evenness data were compared across propagule frequency treatments with ANCOVA using SPSS Statistics 17.0 software (SPSS, Inc., 2008). Values of propagule frequency and measured dependent variable data were both identified as numeric scale measures. The categorical data of addition size was identified as a factor. A generalized linear model used propagule size data as the covariate predictor, addition size as the factor, and the dependent variable data as the response to fit a linear curve. The model considered the main effects of propagule frequency and addition size, as well as the interaction of propagule frequency and addition size, on the values of the dependent variable. A Wald's Chi-Square test determined the significance of the factor and covariate. In one case, figure 2-27, an insignificant interaction term hindered the ability of the model to identify the significance of the main effects. The model was run without the interaction term for this data set.

The chi-square values, degrees of freedom, and probability for all tests included in this chapter are given in Appendix: Results of Statistical Analysis.

Results

Uniformity of the Established Communities

It is important that the established communities are a standard condition at the time of invasion, so that any variation among treatments can be attributed to differences in invader propagule pressure and concentration, and not to initial variation among established communities. To minimize variation, the resource base and established communities of all experimental flasks were prepared in an identical way and then pooled and re-aliquoted immediately prior to invasion. After pooling, all flasks were analyzed for total cell concentration, species richness, and diversity. At the time of invasion, there was some variation, but the vast majority of established communities were statistically equivalent in terms of cell concentration, species richness, and diversity. These established communities can be considered a standard condition, and differences in invasion success attributed to propagule size and frequency. At the time of invasion, a handful of experimental flasks in the *Spirostomum ambiguum* and *Paramecium sp.* invasion experiments had significantly different cell concentration, richness, or diversity values than other communities. In all cases, this initial variation disappeared by week 1, a week before the communities reached maximum concentration. The effect of invasion on the community was generally not analyzed until week 5. For this reason, I felt that it was valid to include these treatments in analysis. Nevertheless, I took care in the analysis of

these flasks and would have deleted them from consideration if they showed unusual patterns of invasion success.

Effect of the Four Invaders on Community Diversity

There is variation in the effect of different invaders on the diversity of the established community, as illustrated in the diagram (Figure 5-2). The invasion of *Paramecium bursaria*, *Tillina magna*, and *Paramecium sp.* increases the diversity of the established community. As propagule pressure and the associated concentration of the invader increases, community diversity, species richness, and species evenness also tend to increase. In contrast, the presence of *Spirostomum ambiguum* has a negative effect on community diversity, species richness, and concentration. Increasing the propagule size of this invader amplifies the negative effect.

Invaders that Increase Community Diversity

Effect of *Paramecium bursaria* Invader

Effect on the Diversity of the Whole Community. There is a significant trend of increasing whole community diversity with increasing *Paramecium bursaria* cell concentration ($\chi^2 = 105.242$, $df = 1$, $P < 0.01$) (Figure 5-3). The presence of the *Paramecium bursaria* invader increases the diversity of the whole community,

from 0.16 in the uninvaded treatment on week 5 to 0.45-0.70 for invaded treatments.

Effect on the Species Richness of the Whole Community. By affecting the concentration of the invader, propagule pressure indirectly affects the species richness of the whole community. On week 5, there is a significant trend of increasing species richness with increasing invader cell concentration, from 1.8 for the uninvaded treatment to 3.2 for propagule size 100 ($\chi^2 = 13.499$, $df = 1$, $P < 0.01$) (Figure 5-4A).

Effect on the Species Evenness of the Whole Community. The concentration of the invader also positively influences species evenness. On week 5, there is a significant relationship between species evenness and invader cell concentration ($\chi^2 = 12.424$, $df = 1$, $P < 0.01$). Increasing invader cell concentration is associated with increasing values of species evenness, from 0.30 for the uninvaded treatment to 0.67 at propagule size 50. Increasing invader concentration above 63 cells/ml does not provide further benefit (Figure 5-4B).

Effect on the Proportion of Maximum Species Richness Attained. The relationship between *Paramecium bursaria* cell concentration and the proportion of maximum species richness attained by the community is significant ($\chi^2 = 4.228$, $df = 1$, $P = 0.040$) (Figure 5-5A). On week 5, there is a slight trend of increasing richness with increasing invader concentration.

Effect on the Concentration of the Established Community. The concentration of *Paramecium bursaria* does not significantly influence the concentration of the established community. On week 5, community concentration increases with increasing invader concentration, from 236 cells/ml in the uninvaded treatment to a maximum of 302 cells/ml when the invader has a cell concentration of 65 cells/ml. Increasing invader concentration above 65 cells/ml does not provide benefit. The concentration of the established community remains stable at approximately 300 cells/ml as the concentration of the invader rises from 65 cells/ml to 113 cells/ml (Figure 5-5B).

Summary. The presence and concentration of the *Paramecium bursaria* invader significantly promotes the diversity, richness, and evenness of the whole community. *Paramecium bursaria* is able to enter this established community without negatively affecting either the presence or concentration of established community members. The invasion of *Paramecium bursaria* simply adds another species to the community, which increases total species richness. Since *Paramecium bursaria* grows well in this community, forming one of the larger populations in the assemblage, the entrance of this invader also increases species evenness. Both of these effects increase the diversity of the whole community.

Effect of *Tillina magna* Invader

Effect on the Diversity of the Whole Community. There is a positive relationship between the concentration of *Tillina magna* and the diversity of the whole community ($\chi^2 = 90.631$, $df = 1$, $P < 0.01$) (Figure 5-6). On week 2, the presence of the *Tillina magna* invader significantly increases the diversity of the whole community, from 0.26 in the uninvaded treatment to greater than 0.8 for all invaded treatments (Tukey $P < 0.001$). There is a slight increase in community diversity as the concentration of *Tillina magna* rises from 73 cells/ml to 96 cells/ml. However, further increase in *Tillina magna* concentration above 96 cells/ml does not confer benefit, suggesting that there is saturation of invader effects on diversity.

Effect on the Species Richness of the Whole Community. There is a significant relationship between *Tillina magna* cell concentration and species richness ($\chi^2 = 7.241$, $df = 1$, $P < 0.007$). The presence of the *Tillina magna* invader has a strong positive effect on species richness. On week 2, there is a significant increase in the species richness of the whole community from 2.25 for the uninvaded treatment to greater than 3 for the invaded treatments. However, increasing *Tillina magna* concentration above 73 cells/ml provides no benefit. There is little variation in the species richness of the invaded treatments (3.2-4.0), suggesting that there is saturation of invader effects on richness (Figure 5-7A).

Effect on the Species Evenness of the Whole Community. Both the presence and the concentration of the invader appear to influence species evenness. On week 2, there is a significant trend of increasing species evenness with increasing invader cell concentration ($\chi^2 = 57.549$, $df = 1$, $P < 0.01$) (Figure 5-7B). Species evenness rises from 0.34 for the uninvaded treatment to 0.60 when the invader has a concentration of 73 cells/ml to 0.85 when the invader has a concentration of 99 cells/ml. Increasing invader concentration above 99 cells/ml does not provide further benefit, suggesting saturation of invader effect on evenness (Figure 5-7B).

Effect on Dominant Established Species. *Tillina magna* strongly suppresses the growth of the dominant established species in this community. The relationship between *Paramecium bursaria* concentration and *Tillina magna* concentration is significant ($\chi^2 = 23.520$, $df = 1$, $P < 0.01$) (Figure 5-8). As the concentration of *Tillina magna* increases in the community, the concentration of *Paramecium bursaria* decreases, from 240 cells/ml in the uninvaded treatment on week 2 to 125 cell/ml when invader cell concentration is 128 cells/ml. Increasing *Tillina magna* cell concentration up to 96 cells/ml is associated with a rapid reduction of *Paramecium bursaria* growth. However, increasing *Tillina magna* concentration above 96 cells provides little benefit, suggesting saturation of *Tillina magna* effect.

Effect on Weaker Established Species. *Tillina magna* invasion has a negligible effect on the growth of the two slower-growing community members (Figure 5-9). There appears to be a slight increase in *Spirostomum ambiguum* cell concentration with increasing *Tillina magna* concentration, but the trend is not significant. *Paramecium sp.* concentration shows no pattern related to invader concentration.

Summary. The presence of the *Tillina magna* invader promotes diversity, species richness, and species evenness in this community. Increases in *Tillina magna* concentration up to 99 cells/ml significantly enhance community diversity, species richness, and evenness. However, increasing *Tillina* abundance over 99 cells/ml does not confer benefit, suggesting saturation of invader effects at relatively low concentration. *Tillina magna* invasion increases community diversity, species richness, and species evenness because it decreases the cell concentration of the dominant established species, *Paramecium bursaria*, while not influencing the concentration of the two weaker established species. In fact, the pattern of saturation of *Tillina magna* concentration on community diversity can be attributed to a similar saturation pattern on the suppression of *Paramecium bursaria*. It appears that *Tillina magna* is able to enter this established community, negatively affecting the growth only of the dominant established species, and this increases species evenness. The *Tillina magna* invader grows well in the community adding a second substantial population, and

this increases species richness and evenness. Increasing species richness and evenness increases community diversity.

Effect of *Paramecium sp.* Invader

Paramecium sp. is not a strong invader in this system. When inoculated only once, the species does not successfully invade the established community at any tested value of propagule size. The species is able to successfully invade only when inoculated multiple times, probably the result of rescue effect. Since the *Paramecium sp.* invader reaches highest concentration in propagule frequency treatments, these treatments are examined here.

Effect on the Diversity of the Whole Community. The relationship between the propagule frequency of *Paramecium sp.* and the diversity of the whole community is significant ($\chi^2 = 7.847$, $df = 1$, $P = 0.005$) (Figure 5-10). Whole community diversity increases with increasing *Paramecium sp.* propagule frequency. On week 5, the mean diversity of the 25-cell addition series increases from 0 for the 1x(25) treatment to 0.66 for the 3x(25) treatment. The mean diversity of the 50-cell addition series increases from 0.36 for the 2x(50) treatment to 0.67 for the 5x(50) treatment. The effect is caused solely by propagule frequency. The size of additions has a negligible influence on diversity.

Effect on the Species Richness of the Whole Community. Increasing the propagule frequency of the *Paramecium sp.* invader has a significant positive effect on species richness ($\chi^2 = 25.484$, $df = 1$, $P < 0.01$) (Figure 5-11A). In both the 25-cell and 50-cell addition series, there is a clear trend of increasing whole-community species richness with increasing propagule frequency on week 5. The species richness of the 25-cell series increases from 0.5 for the one-addition treatment to 2.8 for the five-addition treatment. The 50-cell addition series increases from 0.8 for the one-addition treatment to 3.0 for the five-addition treatment. This pattern is caused solely by propagule frequency. The size of additions has a negligible effect on species richness.

Effect on the Species Evenness of the Whole Community. Neither the propagule frequency nor the addition size of the *Paramecium sp.* invader influences species evenness. On week 5, the evenness of the whole community varies little across propagule frequency treatments. The evenness of the 25-cell addition series varies from 0.53 to 0.69. The evenness of the 50-cell addition series varies from 0.60 to 0.85 (Figure 5-11B).

Effect on the Dominant Established Species. *Paramecium bursaria* is a dominant established species in this system. Increasing the propagule frequency of the *Paramecium sp.* invader has a significant positive effect on the presence of *Paramecium bursaria* ($\chi^2 = 16.891$, $df = 1$, $P < 0.01$) (Figure 5-12A). On week 5, *Paramecium bursaria* is found in a greater proportion of flasks in high propagule

frequency treatments. The proportion of *Paramecium bursaria* increases from 0.25 in the 1x(25) treatment and 0.4 in the 1x(50) treatment up to 1.0 in the 5x(25) treatment and 0.8 in the 5x(50) treatment (Figure 5-12A). This pattern is caused solely by propagule frequency. Addition size does not significantly affect *Paramecium bursaria* presence.

Tillina magna is also a dominant established species in this system, with cell concentrations nearly identical to that of *Paramecium bursaria*. Increasing propagule frequency has a significant positive effect on *Tillina magna* presence ($\chi^2 = 11.227$, df = 1, P = 0.001) (Figure 5-12B). On week 5, *Tillina magna* is found in a greater proportion of flasks as propagule frequency increases, from 0 for the 1x(25) and 1x(50) treatments to 1.0 for the 3x(25) treatment and 0.8 for the 3x(50) treatment. Propagule frequency causes this effect. Addition size has no effect on *Tillina magna* presence.

Effect on the Weaker Established Species. *Spirostomum ambiguum* grows much more slowly in this community than do the other two established species. Increasing the propagule frequency of the *Paramecium sp.* invader has a significant positive effect on the presence of *Spirostomum ambiguum* when addition size is large (Figure 5-13). In the 50-cell addition series, *Spirostomum ambiguum* is found in a significantly higher proportion of flasks in the two-addition and five-addition treatments (0.8) than in the one-addition treatment (0.4) ($\chi^2 = 8.800$; df = 3; P = 0.032). In the 25-cell addition series,

Spirostomum ambiguum is found in a greater proportion of flasks in higher frequency treatments than in lower frequency treatments. However, this pattern is not significant.

Summary. Propagule frequency influences the abundance of the *Paramecium* sp. invader and in so doing, indirectly influences the diversity and richness of the established community five weeks after invasion. In contrast, the effect of invader propagule frequency on whole-community evenness is negligible. *Paramecium* sp. invasion promotes community diversity and richness because high *Paramecium* sp. propagule frequency strongly increases the presence of the two dominant established species. High *Paramecium* sp. frequency also facilitates the growth of the weaker established species, but only when addition size is large. This pattern suggests that *Paramecium* sp. itself, or something closely associated with *Paramecium* sp., significantly promotes the growth of all established species. The introduction of the invader adds an additional species to the assemblage. Together, these effects increase the richness and diversity of the community.

Invader that Decreases Community Diversity

Effect of *Spirostomum ambiguum* Invader

Effect of Invader Presence on the Growth of the Established Community. The presence of the *Spirostomum ambiguum* invader suppresses the growth of the

established community throughout the experiment. In both the propagule size and propagule frequency experiments, the cell concentrations of all invaded treatments are substantially less than the concentration of the uninvaded treatments (Figure 5-14A).

Effect of Propagule Size on the Concentration of the Established Community.

The concentration of the established community decreases as *Spirostomum ambiguum* propagule size increases. On week 4, there is a significant trend of decreasing community concentration with increasing invader propagule size, from 500 cells/ml for the uninvaded treatment to less than 20 cells/ml for the propagule size 300 treatment ($\chi^2 = 18.765$; $df = 3$; $P < 0.01$) (Figure 5-14B).

Effect on the Diversity of the Whole Community. The relationship between the propagule size of *Spirostomum ambiguum* and whole-community diversity is significant ($\chi^2 = 4.463$; $df = 3$; $P = 0.035$). On week 4, the mean diversity of the community decreases from 0.78 for the uninvaded treatment to 0.30 for the propagule size 300 treatment (Figure 5-15).

Effect on the Species Richness of the Whole Community. The proportion of maximum species richness attained by the whole community decreases as *Spirostomum ambiguum* invader propagule size increases. On week 4, there is a significant trend of decreasing proportion of maximum species richness attained with increasing invader propagule size ($\chi^2 = 9.554$; $df = 3$; $P = 0.002$).

Species richness declines from 0.93 for the uninvaded treatment to 0.40 for the propagule size 300 treatment (Figure 5-16A).

Effect on the Species Evenness of the Whole Community. The species evenness of the whole community is not affected by either the presence or propagule size of the *Spirostomum ambiguum* invader. On week 4, evenness values vary minimally across propagule size treatments, from a minimum of 0.61 to a maximum of 0.87. There is no consistent pattern to the variation (Figure 5-16B).

Effect on Dominant Established Species. *Paramecium bursaria* is the dominant established species in this system. *Paramecium bursaria* growth is strongly suppressed by increasing *Spirostomum ambiguum* propagule size. *Paramecium bursaria* is found in all uninvaded flasks at a mean cell concentration of 314 cells/ml. In contrast, the species is found in only 20% of the propagule size 300 treatment flasks, and at a mean concentration of only 1.7 cells/ml (Figure 5-17). The relationships between invader propagule size and *Paramecium bursaria* presence ($\chi^2 = 10.178$; df = 1; P = 0.001) and concentration ($\chi^2 = 10.101$; df = 1; P = 0.001) are significant (Figure 5-17).

Tillina magna can also be considered a dominant established species in this system, since it is found at cell concentrations just slightly lower than that of *Paramecium bursaria*. Like *Paramecium bursaria*, *Tillina magna* shows strong

patterns of decreasing presence and cell concentration with increasing invader propagule size. On week 5, there are significant trends of decreasing *Tillina magna* presence ($\chi^2 = 15.002$; $df = 1$; $P < 0.01$) and cell concentration ($\chi^2 = 12.172$; $df = 1$; $P < 0.01$), with increasing invader propagule size (Figure 5-18). In the propagule size experiment, *Tillina magna* is found in 100% of the uninvaded treatments, at a mean cell concentration of 300 cells/ml. These values decrease as invader propagule size increases. *Tillina magna* is not found in any flask in the highest propagule size treatment (300) (Figure 5-18).

Effect on the Weaker Established Species. *Paramecium sp.* grows much more slowly in this community than the other two established species. *Spirostomum ambiguum* invasion does not influence *Paramecium sp.* presence or concentration. There is variation in *Paramecium sp.* presence and concentration, but the variation is minimal, error bars are large, and no pattern can be found (Figure 5-19).

Summary. The presence of the *Spirostomum ambiguum* invader, at all tested values of propagule size, substantially suppresses the growth of the established community. Increasing *Spirostomum ambiguum* propagule size produces significant trends of decreased established community concentration, whole-community diversity, and species richness. In contrast, *Spirostomum ambiguum* propagule pressure does not influence species evenness. These community effects occur because increasing invader propagule pressure significantly

decreases the presence and cell concentration of the two dominant established species, but does not affect the growth of the weaker species.

Effect of Invasion on the Growth of Individual Established Species

The percent change in the cell concentration of each established species as a result of invasion by three different invaders is shown across the rows of Table 5-1. In this study, there is wide variation in the effect of invasion on the growth of established species. Most invasion events cause a 20- to 50-percent change in the cell concentration of an established species. However, one invasion is associated with no change in the established species' concentration, while another results in a change of over 200 percent. Established species are affected in different ways by different invaders. The growth of *Paramecium bursaria* and *Spirostomum ambiguum* are positively effected by one invader, but negatively affected by the remaining two invaders. The growth of *Paramecium sp.* is negatively affected by one invader, positively affected by another invader, and the third invader has no effect on the cell concentration of *Paramecium sp.* There is no trend or pattern when these positive and negative responses are compared to the established species' realized growth. The mean change in each established species' concentration, calculated from the absolute value of the species' individual reactions to the three invaders, is shown in the far right column of Table 5-1. The mean effect of invasion varies from 25.7% for *Tillina magna* to 86.7% for *Paramecium sp.* For the established species used in

this study, there is a significant trend of decreased mean change in concentration with increasing realized growth in the system ($\chi^2 = 11.493$; $df = 1$; $P = 0.001$) (Figure 5-20).

Effect of Individual Invaders on Established Species

The effect of an invader on the cell concentration of all established species in the invaded community is shown in the columns of Table 5-1. The mean effect of each invader, calculated from the change in the cell concentration of all established species influenced by that invader, is shown in the bottom row of Table 5-1. The mean effect of invaders ranges from 20.6% for the *Spirostomum ambiguum* invader to 94.8% for the *Paramecium bursaria* invader. The two invaders with the highest realized growth, *Tillina magna* and *Paramecium bursaria*, also cause the greatest mean percent change in the concentration of established species. In this study, there is a significant relationship between the realized growth of invaders and their influence on established species, as measured by change in cell concentration ($\chi^2 = 4.234$; $df = 1$; $P = 0.040$) (Figure 5-21).

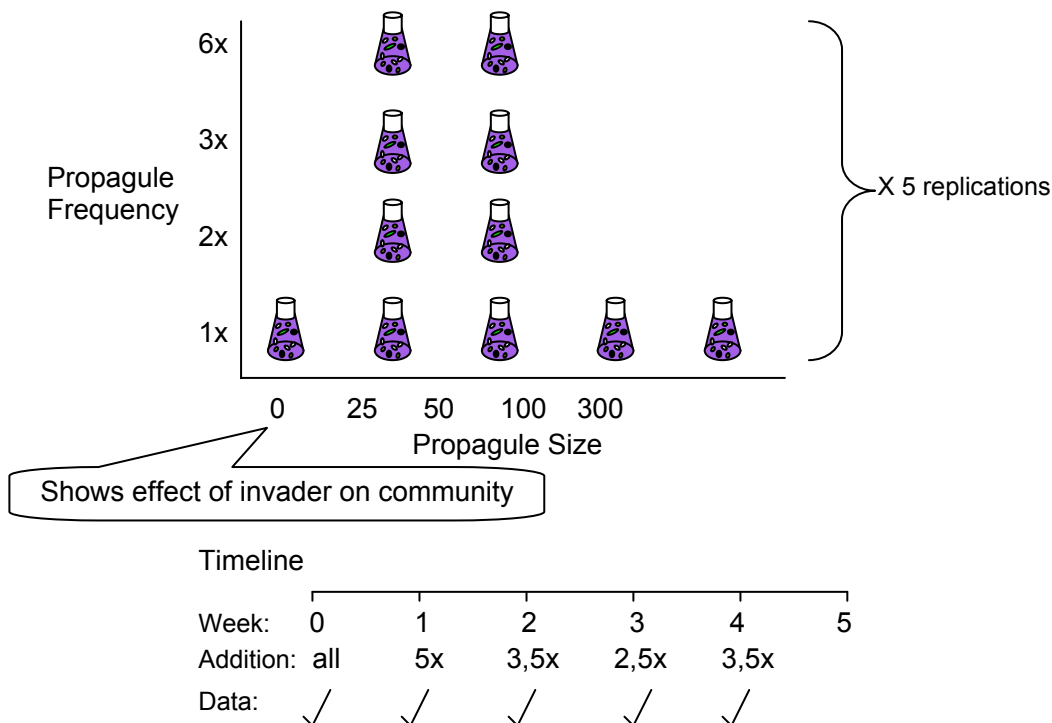


Figure 5-1. Experimental design for Specific Aim #4: To examine how the effects invaders have on the established community and on individual community members vary with the invader's propagule size and frequency. Replicate established communities are inoculated with 0, 25, 50, 100, or 300 cells of an invader species or maintained with 1, 2, 3, or 6 25- or 50-cell additions of the invading species during the first five weeks of culture. All treatments are maintained for six weeks. The cell concentration of all species in every experimental flask is determined every seven days for six weeks.

Effect of Invaders on Community Diversity

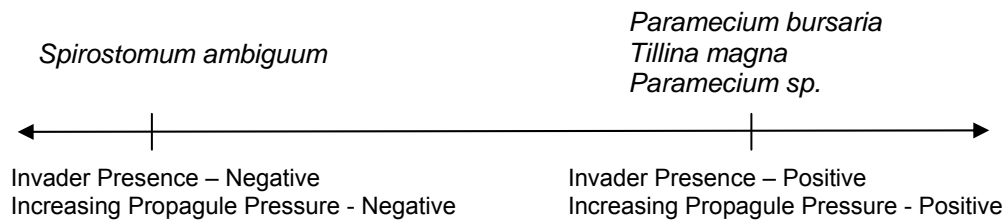


Figure 5-2. The effect of protist invaders on the diversity of experimental communities. The presence and propagule pressure of the four protist invaders have very different effects on the diversity of the established communities.

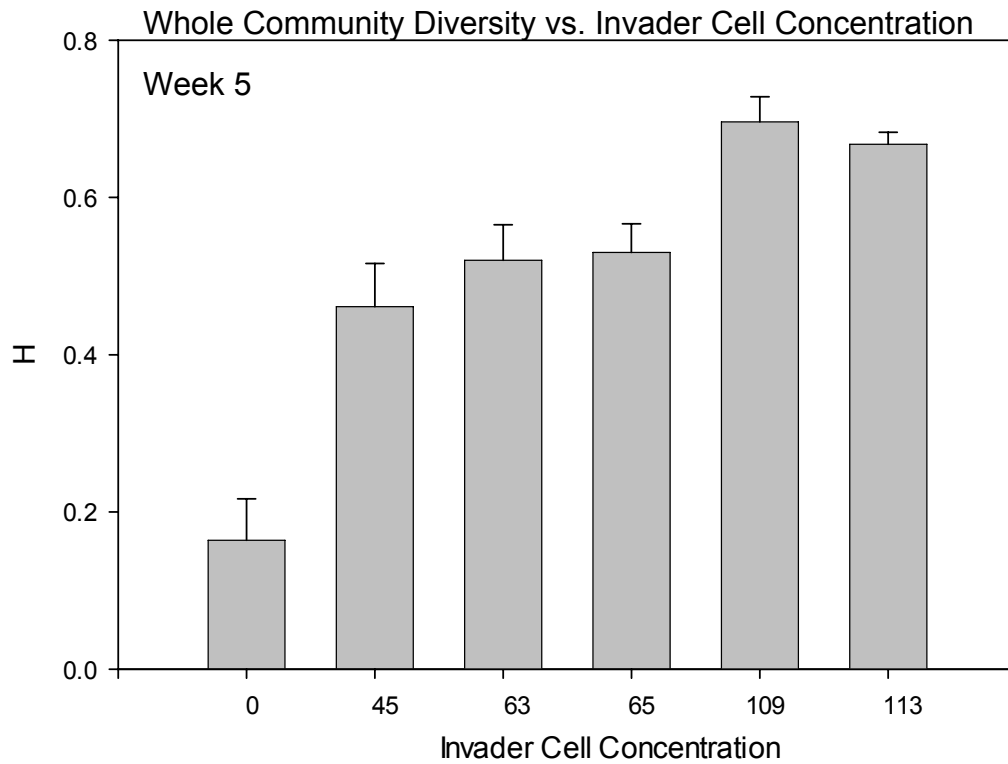
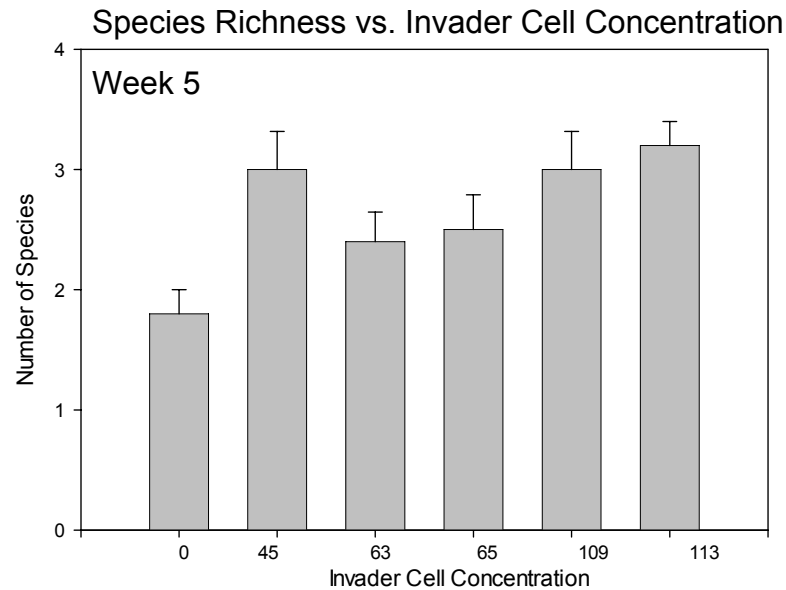


Figure 5-3. The effect of *Paramecium bursaria* cell concentration on the diversity of the whole community five weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, and *Paramecium sp.* and cultured three weeks before the invader was added. Diversity was determined by the Shannon Index. The effect of invader cell concentration on community diversity is significant ($\chi^2 = 105.242$, $df = 1$, $P < 0.01$).

(A)



(B)

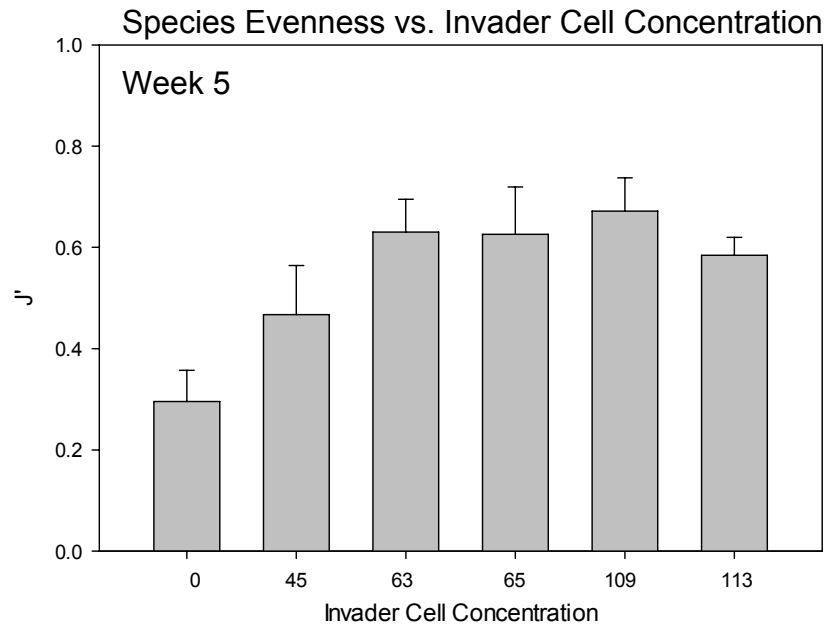
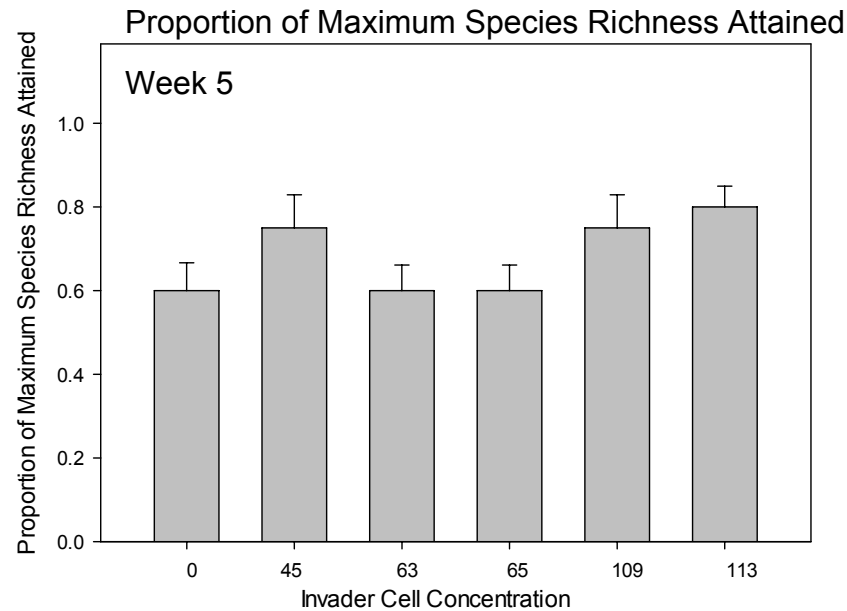


Figure 5-4. The effect of *Paramecium bursaria* cell concentration on the species richness (A) and species evenness (B) of the whole community five weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, and *Paramecium sp.* and cultured three weeks before the invader was added. Evenness values were determined by Pielou's Evenness Index. Plotted values are the means (± 1 SE) of five replications of each treatment, four for propagule size 300. The effect of invader cell concentration on species richness (A) ($\chi^2 = 13.499$, $df = 1$, $P < 0.01$) and species evenness (B) ($\chi^2 = 12.424$, $df = 1$, $P < 0.01$) is significant.

(A)



(B)

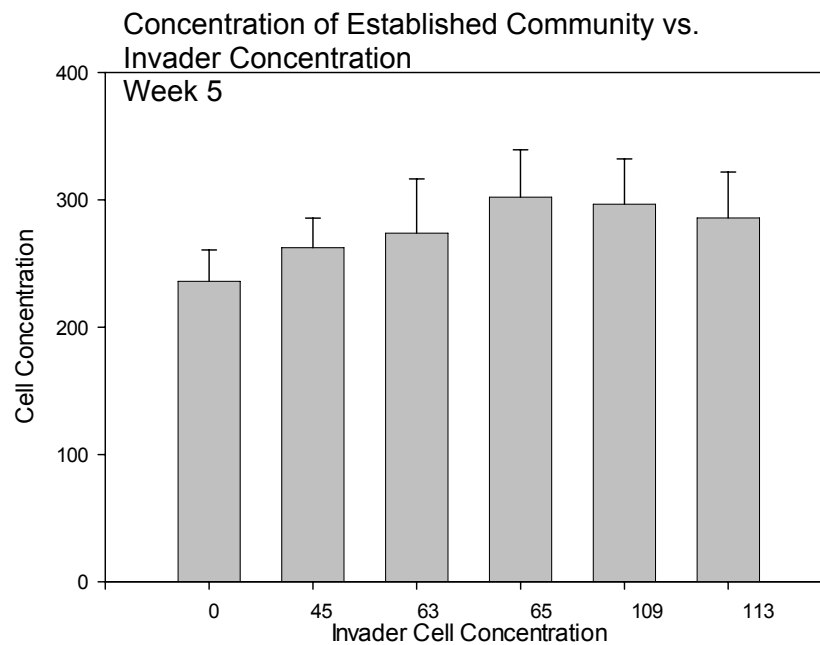


Figure 5-5. The effect of *Paramecium bursaria* cell concentration on the proportion of maximum species richness achieved by the whole community (A) and the concentration of established community (B) five weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Spirostomum ambiguum*, *Tillina magna*, and *Paramecium sp.* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for propagule size 300. The effect of invader cell concentration on maximum species richness is significant (A) ($\chi^2 = 4.228$, $df = 1$, $P = 0.040$).

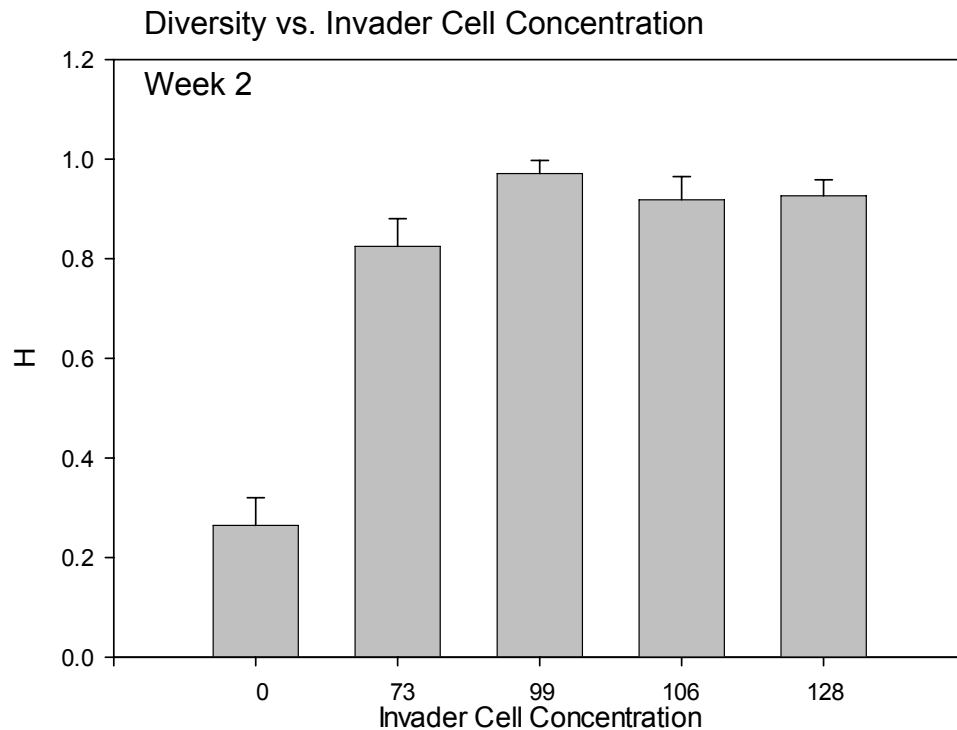
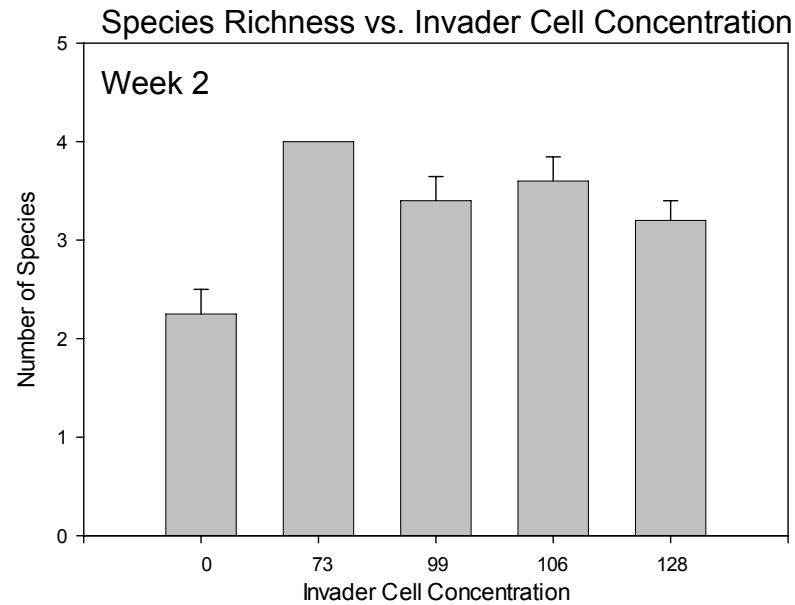


Figure 5-6. The effect of *Tillina magna* cell concentration on the diversity of the whole community two weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. and cultured for three weeks before the invader was added. Diversity was determined by the Shannon Index. Plotted values are the means (± 1 SE) of five replications of each treatment, four for propagule size 0. The effect of invader cell concentration on diversity is significant ($\chi^2 = 90.631$, $df = 1$, $P < 0.01$).

(A)



(B)

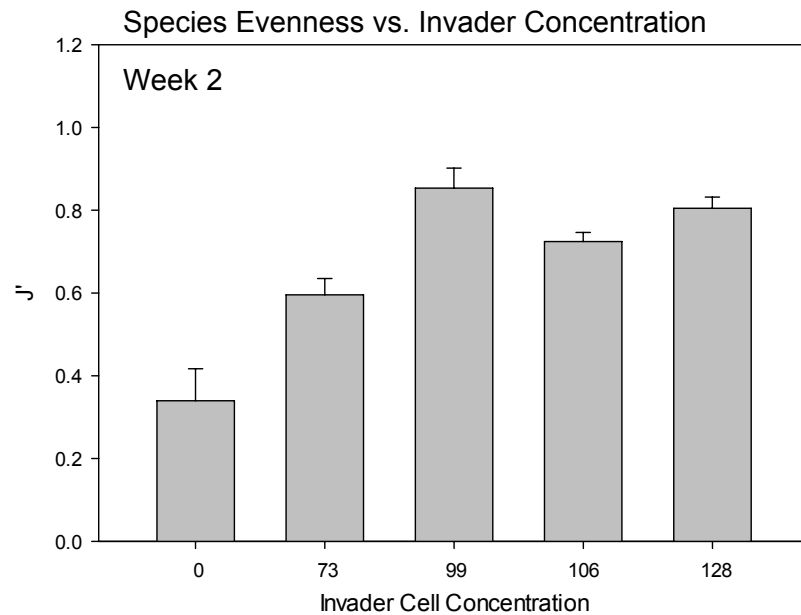


Figure 5-7. The effect of *Tillina magna* cell concentration on the species richness (A) and species evenness (B) of the whole community two weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. and cultured three weeks before the invader was added. Evenness values were determined by Pielou's Evenness Index. Plotted values are the means (± 1 SE) of five replications of each treatment, four for propagule size 0. The effect of invader cell concentration on species richness (A) ($\chi^2 = 7.241$, $df = 1$, $P < 0.007$) and species evenness (B) ($\chi^2 = 57.549$, $df = 1$, $P < 0.01$) is significant.

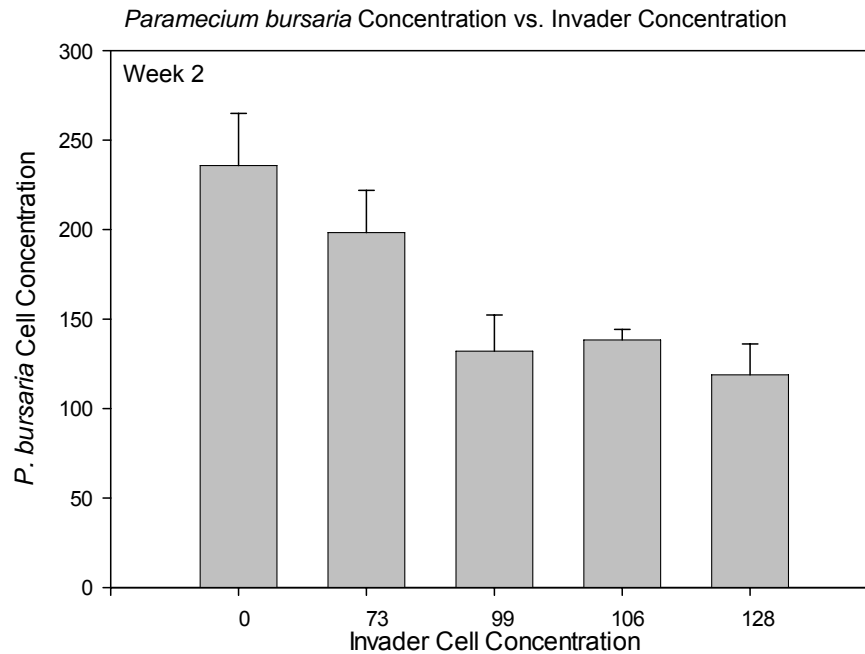
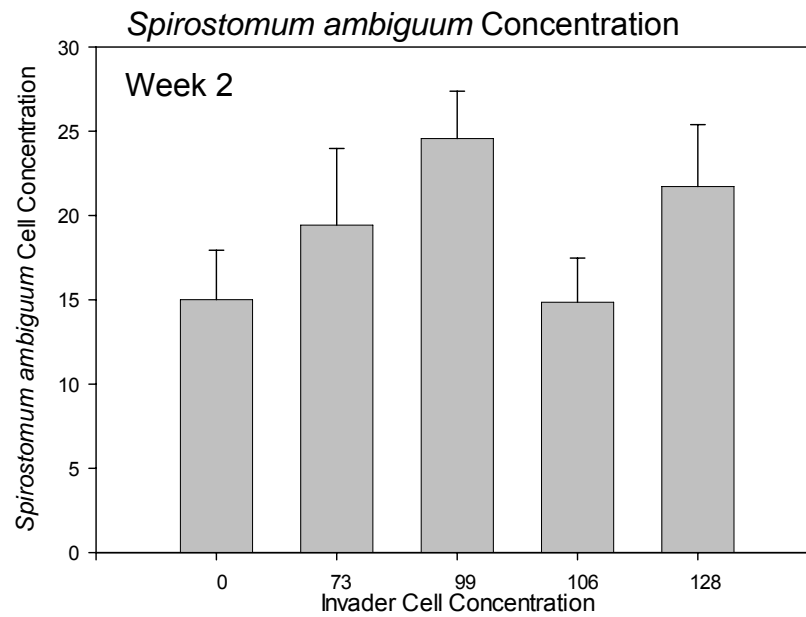


Figure 5-8. The effect of *Tillina magna* cell concentration on the cell concentration of the established species, *Paramecium bursaria*, two weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for propagule size 0. The effect of invader cell concentration on *Paramecium bursaria* cell concentration is significant ($\chi^2 = 23.520$, $df = 1$, $P < 0.01$).

(A)



(B)

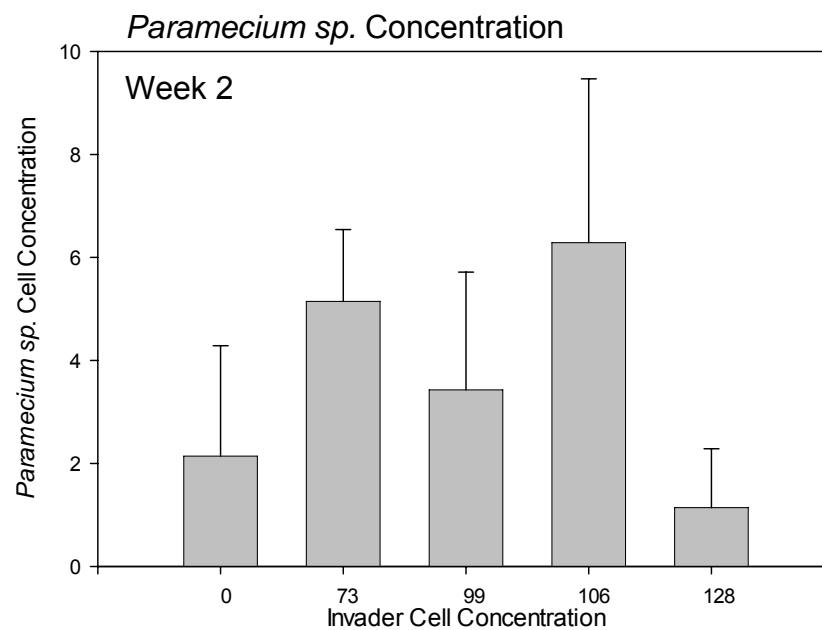


Figure 5-9. The effect of *Tillina magna* cell concentration on the cell concentration of the established species *Spirostomum ambiguum* (A) and *Paramecium* sp. (B) two weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. and cultured three weeks the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for propagule size 0.

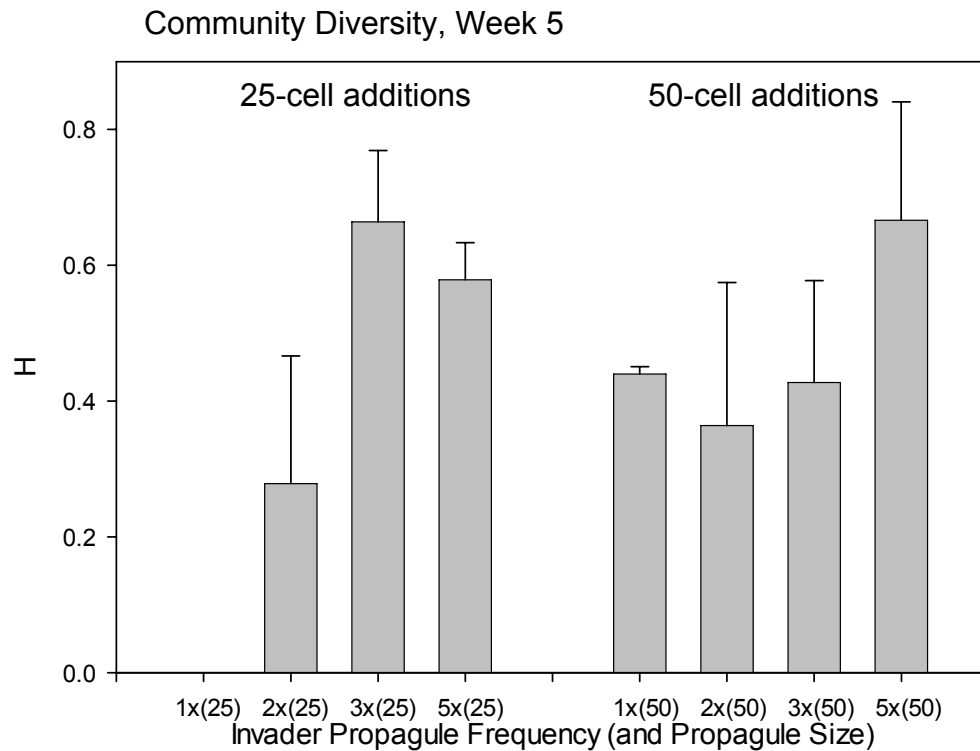
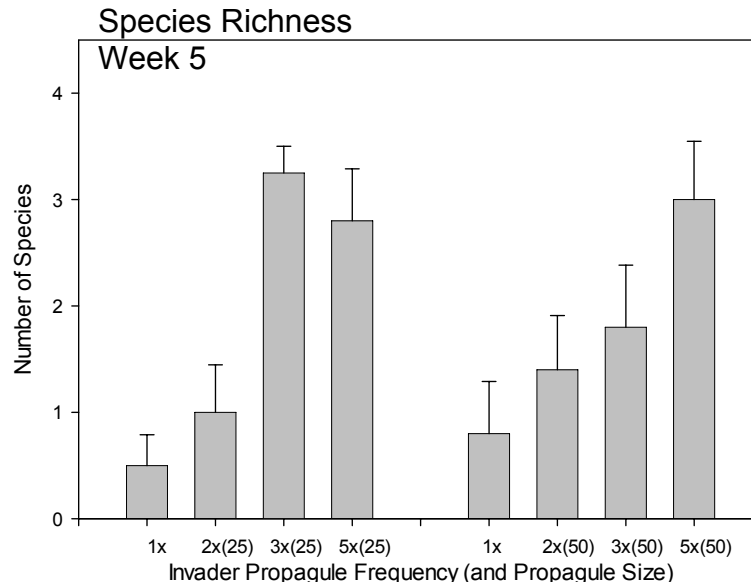


Figure 5-10. The effect of *Paramecium sp.* propagule frequency on the diversity of whole communities five weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Tillina magna* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for 1x(25) and 3x(25). The effect of *Paramecium sp.* propagule frequency on community diversity is significant ($\chi^2 = 7.847$, $df = 1$, $P = 0.005$).

(A)



(B)

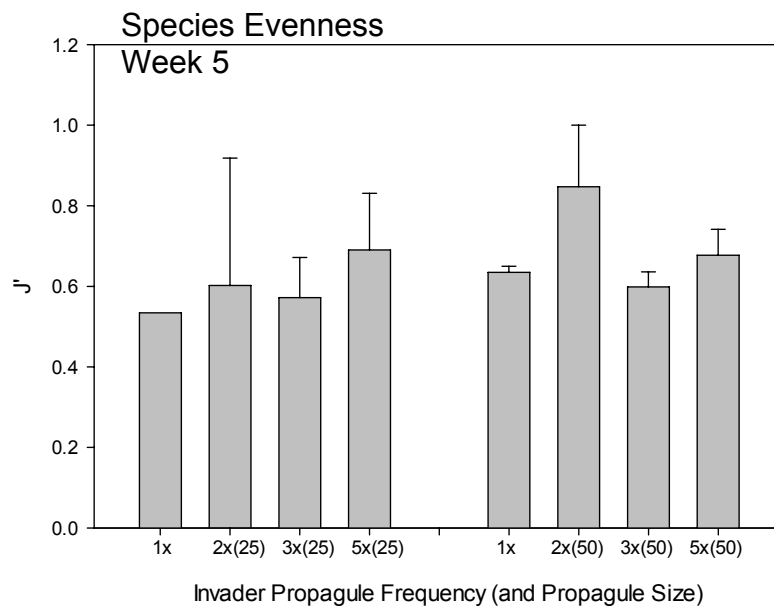
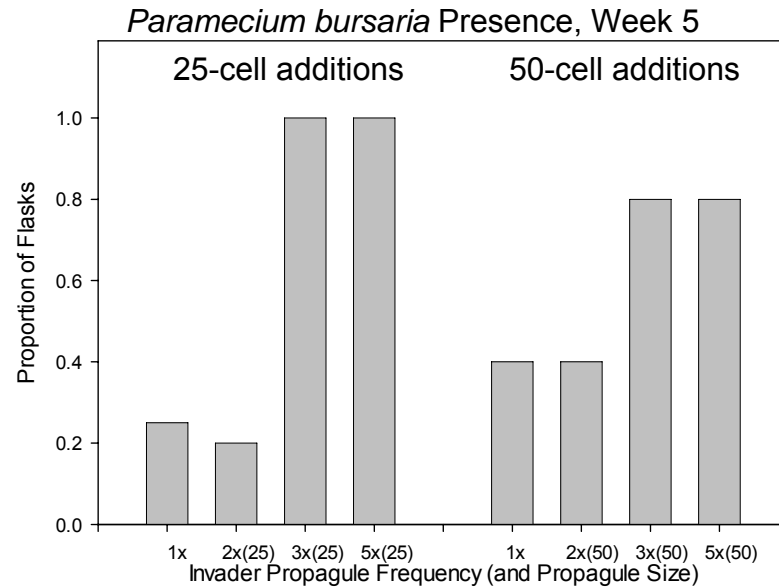


Figure 5-11. The effect of *Paramecium sp.* propagule frequency on the species richness (A) and species evenness (B) of the whole community five weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Tillina magna*, and *Spirostomum ambiguum* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for 1x(25) and 3x(25). The effect of *Paramecium sp.* propagule frequency on species richness (A) is significant ($\chi^2 = 25.484$, $df = 1$, $P < 0.01$).

(A)



(B)

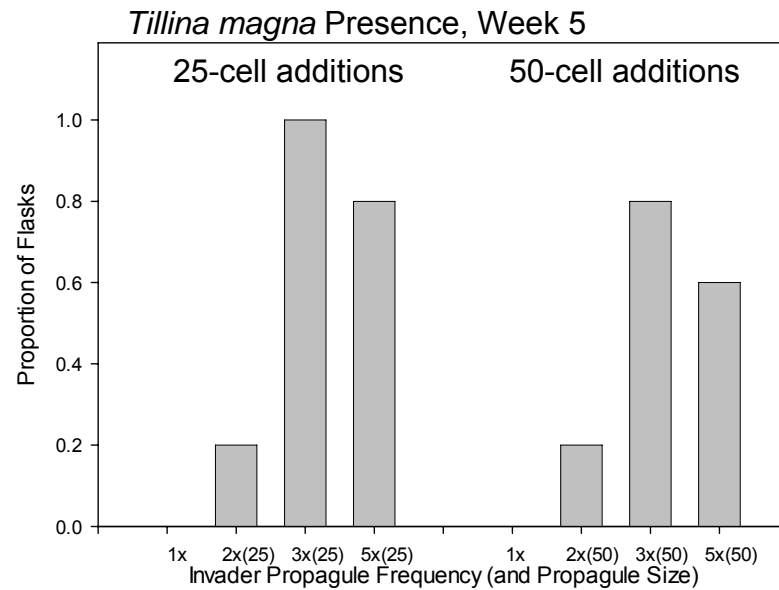


Figure 5-12. The effect of *Paramecium* sp. propagule frequency on the presence of the established species *Paramecium bursaria* (A) and *Tillina magna* (B) five weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Tillina magna* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for 1x(25) and 3x(25). The effect of *Paramecium* sp. propagule frequency on the presence of *Paramecium bursaria* (A) ($\chi^2 = 16.891$, $df = 1$, $P < 0.01$) and *Tillina magna* (B) ($\chi^2 = 11.227$, $df = 1$, $P = 0.001$) is significant.

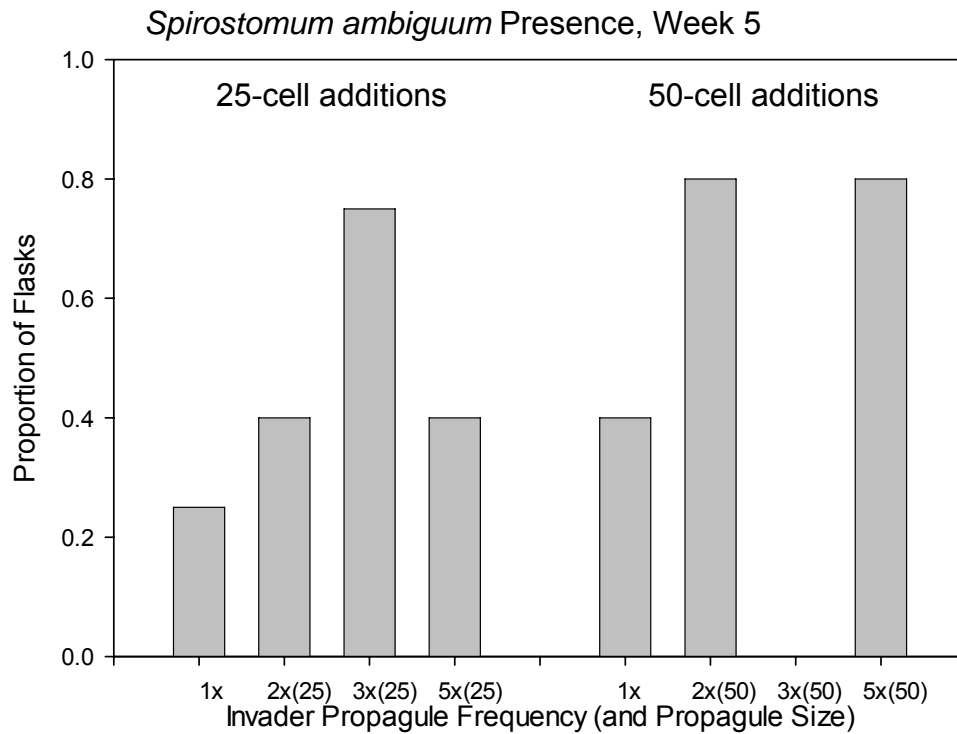
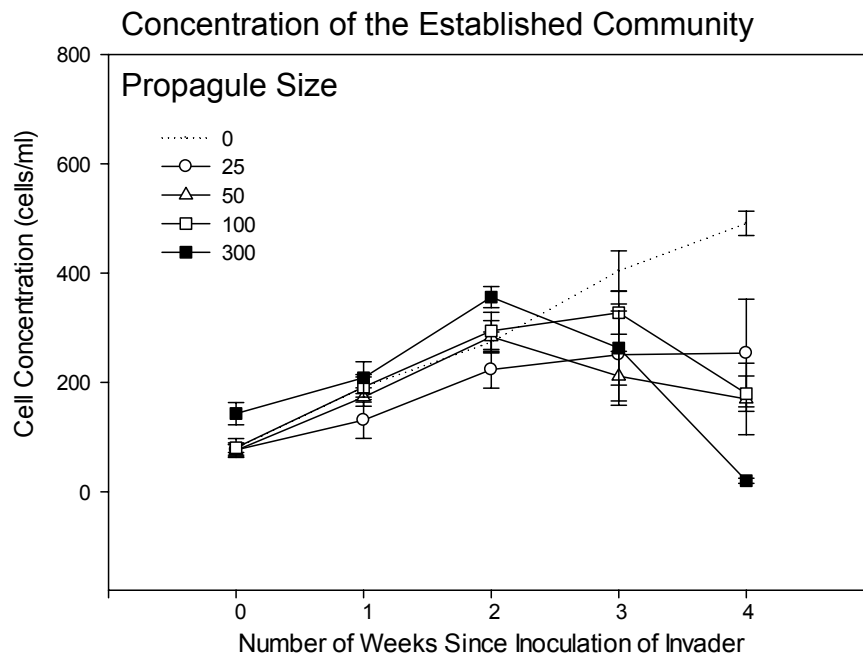


Figure 5-13. The effect of *Paramecium* sp. propagule frequency on the presence of the established species *Spirostomum ambiguum* five weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Paramecium bursaria*, *Spirostomum ambiguum*, and *Tillina magna* and cultured three weeks before the introduction of the invader. Plotted values are the means (± 1 SE) of five replications of each treatment, four for 1x(25) and 3x(25). Presence data were analyzed with Chi-Square tests on 25-cell addition treatments ($\chi^2 = 2.205$; df = 3; P = 0.531) and 50-cell addition treatments ($\chi^2 = 8.800$; df = 3; P = 0.032).

(A)



(B)

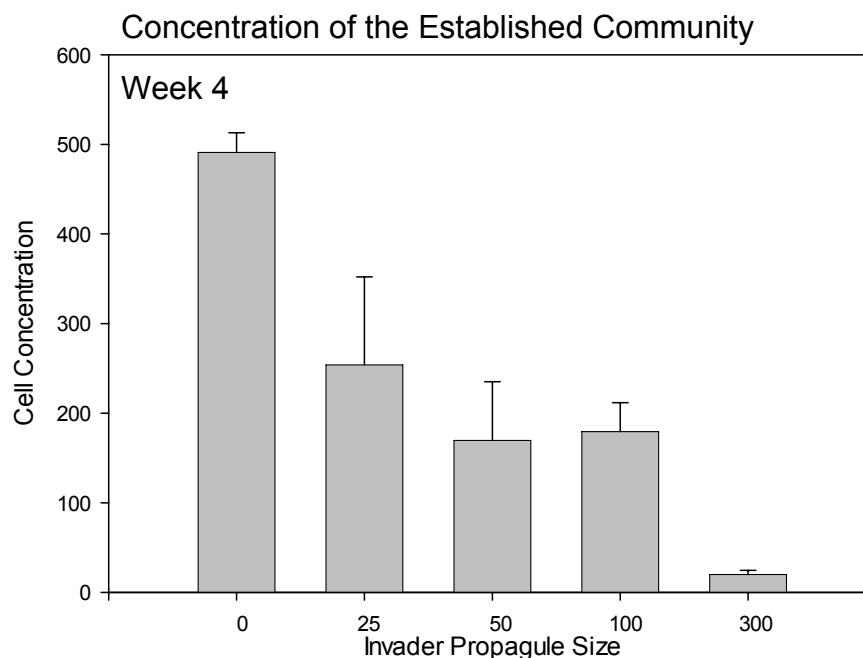


Figure 5-14. The effect of *Spirostomum ambiguum* propagule size on the cell concentration of the established community over the course of the experiment (A) and on week 4 (B). The established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium sp.* and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment, four for propagule size 25 (week 3). The effect of invader cell concentration on the concentration of the established community on week 4 (B) is significant ($\chi^2 = 18.765$; $df = 3$; $P < 0.01$).

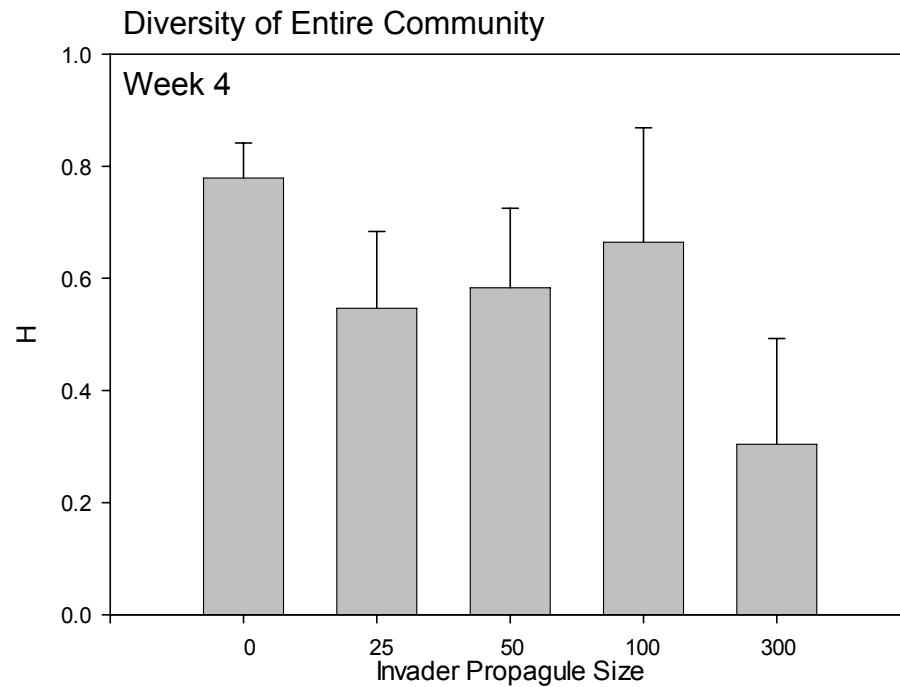
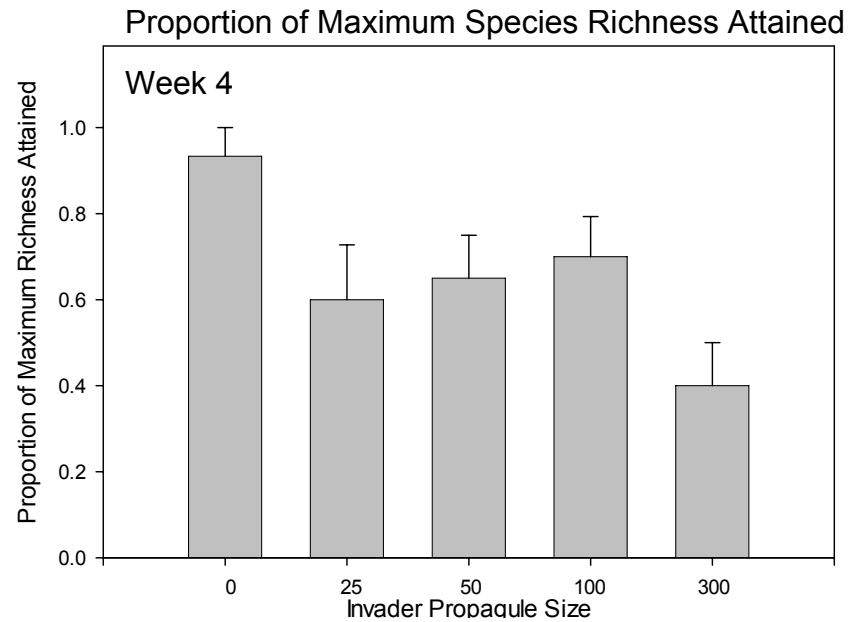


Figure 5-15. The effect of *Spirostomum ambiguum* propagule size on the diversity of the whole community four weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium* sp. and cultured three weeks before the invader was added. Diversity was determined by the Shannon Index. Plotted values are the means (± 1 SE) of five replications of each treatment. The effect of invader propagule size on community diversity is significant ($\chi^2 = 4.463$; $df = 3$; $P = 0.035$).

(A)



(B)

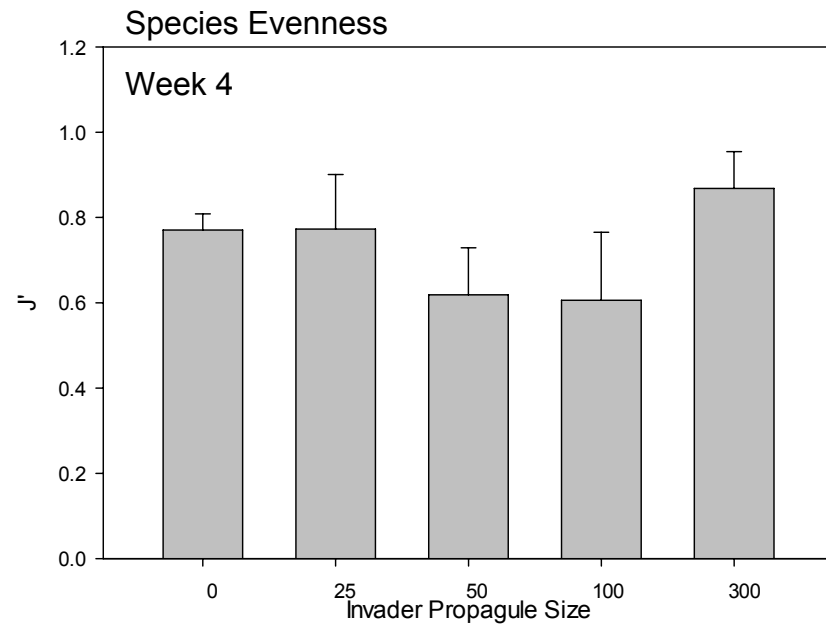
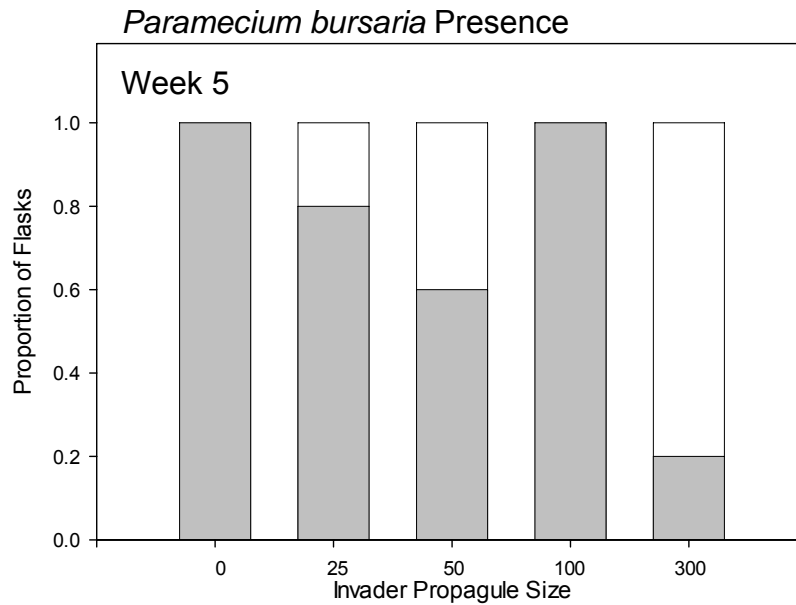


Figure 5-16. The effect of *Spirostomum ambiguum* propagule size on the species richness (A) and species evenness (B) of the whole community four weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium* sp. and cultured three weeks before the invader was added. Evenness was determined by Pielou's Index of Evenness. Plotted values are the means (± 1 SE) of five replications of each treatment. The effect of invader propagule size on species richness (A) is significant ($\chi^2 = 9.554$; $df = 3$; $P = 0.002$).

(A)



(B)

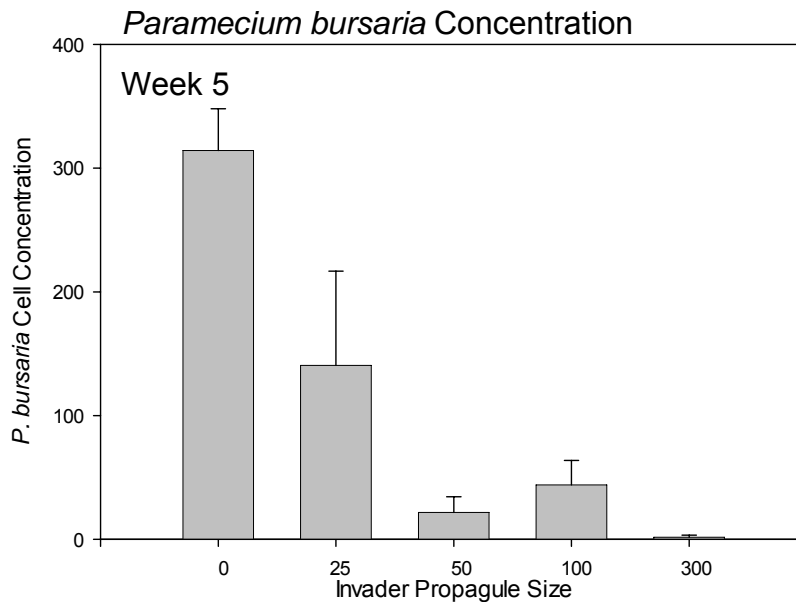
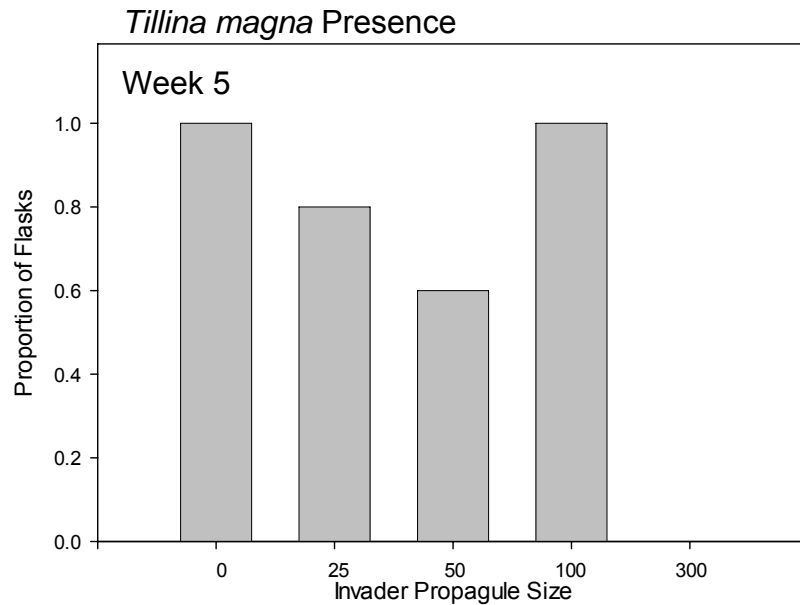


Figure 5-17. The effect of *Spirostomum ambiguum* propagule size on the presence (A) and cell concentration (B) of the established species, *Paramecium bursaria*, five weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium sp.* and cultured for three weeks before the invader was added. The effect of invader propagule size on *Paramecium bursaria* presence (A) ($\chi^2 = 10.178$; $df = 1$; $P = 0.001$) and concentration (B) ($\chi^2 = 10.101$; $df = 1$; $P = 0.001$) is significant.

(A)



(B)

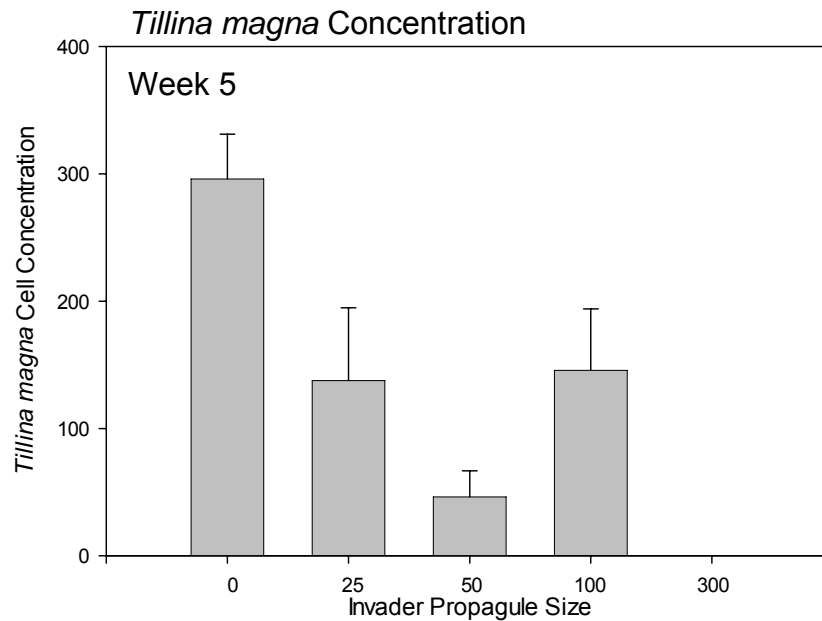
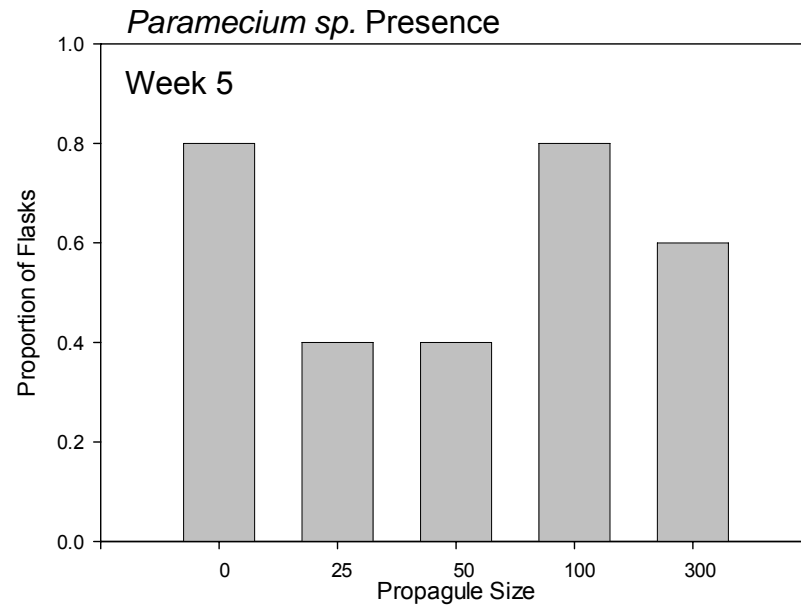


Figure 5-18. The effect of *Spirostomum ambiguum* propagule size on the presence (A) and cell concentration (B) of the established species, *Tillina magna*, five weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium* sp. and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment. The effect of invader propagule size on *Tillina magna* presence (A) ($\chi^2 = 15.002$; df = 1; $P < 0.01$) and concentration (B) ($\chi^2 = 12.172$; df = 1; $P < 0.01$) is significant.

(A)



(B)

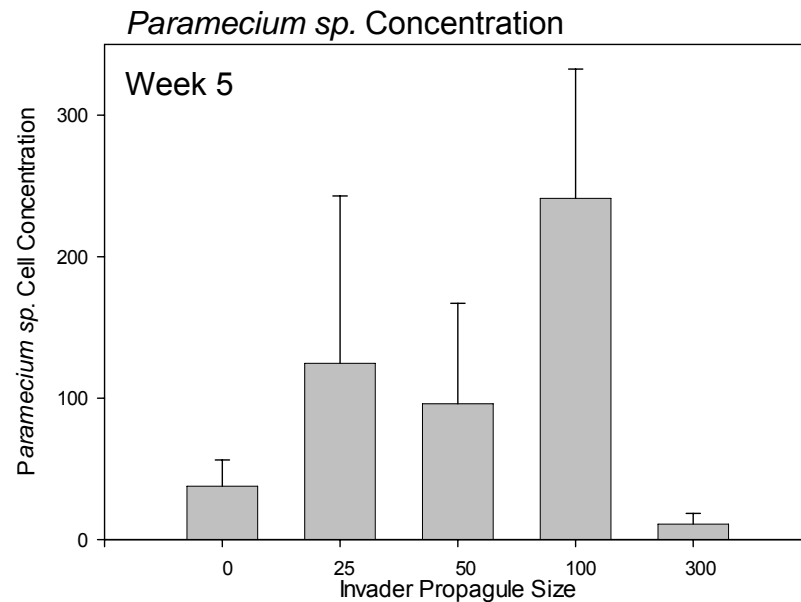


Figure 5-19. The effect of *Spirostomum ambiguum* propagule size on the presence (A) and cell concentration (B) of the established species, *Paramecium* sp., five weeks after the introduction of the invader. The established communities were initiated with 128 cells each of *Tillina magna*, *Paramecium bursaria*, and *Paramecium* sp. and cultured three weeks before the invader was added. Plotted values are the means (± 1 SE) of five replications of each treatment.

		Invaders				Mean Effect of Invaders On Each Est. Species
		<i>Paramecium sp.</i>	<i>Spirostomum ambiguum</i>	<i>Paramecium bursaria</i>	<i>Tillina magna</i>	
Established Species	<i>Paramecium sp</i>		0	+212.5	-47.6	86.7
	<i>Spirostomum ambiguum</i>	-63.3		-47.7	+44.7	51.9
	<i>Paramecium bursaria</i>	+19.1	-37.2		-49.6	35.3
	<i>Tillina magna</i>	+28.3	+24.6	+24.2		25.7
Mean Effect of Each Invader on Established Species		36.9	20.6	94.8	47.3	

Table 5-1. Percent change in the cell concentration of established species as a result of invasion. The proportion of change in cell concentration is determined by dividing the difference between the mean concentration of the established species in the uninvaded treatment and the mean concentration of the established species in the treatment with greatest invader cell concentration by the mean concentration of the established species in the uninvaded treatment, and the quotient multiplied by 100. Values for each species come from the week of maximum cell concentration, week 5 for *Paramecium bursaria* and week 2 for all other species. The symbol proceeding each "proportion of change" value shows whether the cell concentration of the established species increases (+) or decreases (-) as a result of invasion.

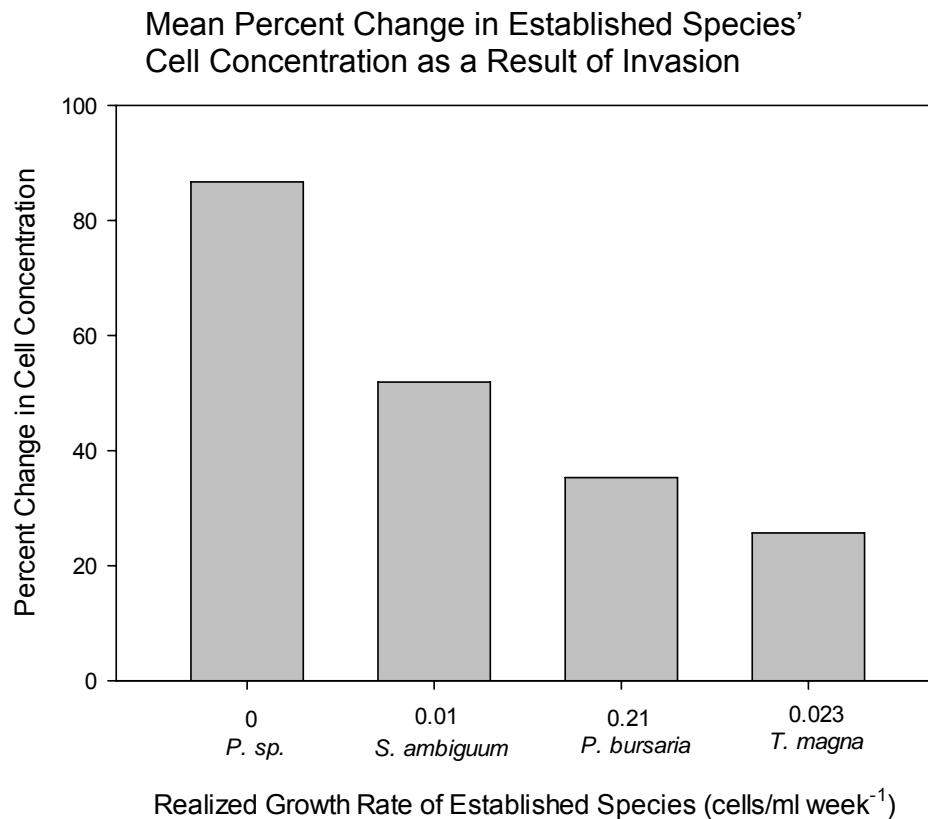


Figure 5-20. The effect of realized growth rate on the mean percent change in the cell concentration each established species as a result of invasion. The percent change in cell concentration is determined by dividing the difference between the mean concentration of each established species in the uninvaded treatment and the mean concentration of the established species in the treatment with greatest invader cell concentration by the mean concentration of the established species in the uninvaded treatment, and the quotient multiplied by 100. Values for each species come from the week of maximum cell concentration, week 5 for *Paramecium bursaria* and week 2 for all other species. The realized growth rate of established species significantly affects the change in its concentration as a result of invasion ($\chi^2 = 11.493$; $df = 1$; $P = 0.001$).

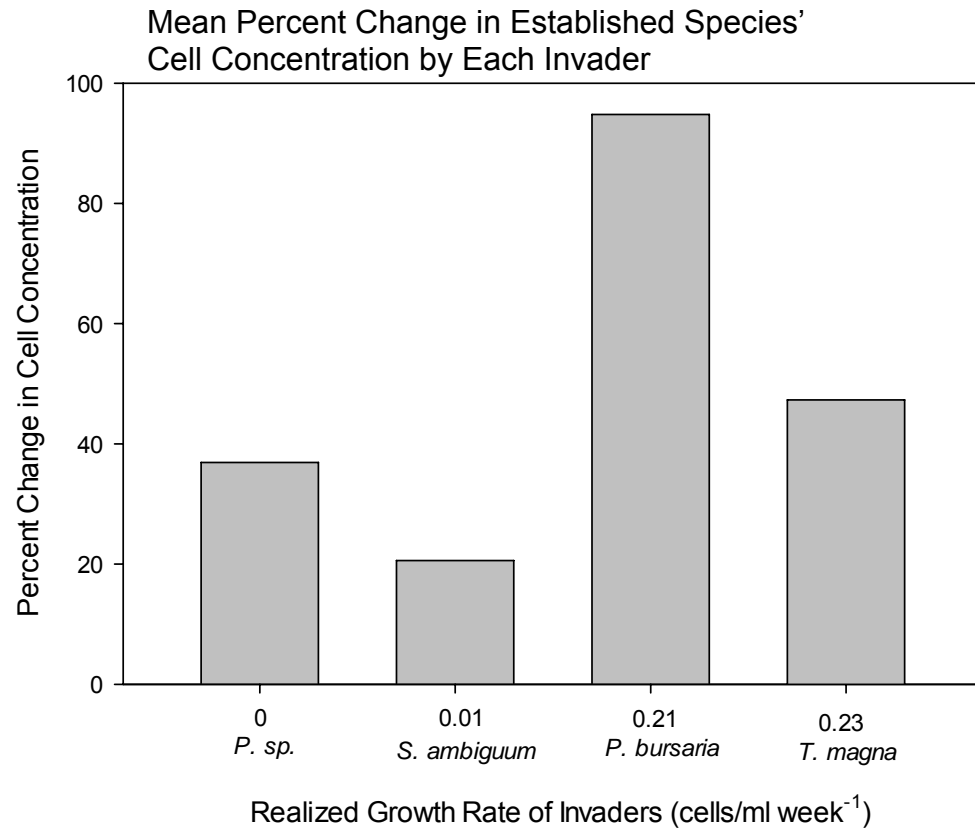


Figure 5-21. The effect of invaders' realized growth rate on the mean percent change in the cell concentration of established species as a result of invasion. The percent change in cell concentration is determined by dividing the difference between the mean concentration of each established species in the uninvaded treatment and the mean concentration of the established species in the treatment with greatest invader cell concentration by the mean concentration of the established species in the uninvaded treatment, and the quotient multiplied by 100. Values for each species come from the week of maximum cell concentration, week 5 for *Paramecium bursaria* and week 2 for all other species. The growth rate of an invader significantly affects the change the concentration of established species ($\chi^2 = 4.234$; df = 1; $P = 0.040$). The plotted values do not look significant because the x-axis is not drawn to scale.

Discussion

By shaping patterns of invader presence and abundance, propagule pressure may influence the effect of invasion on the community. The experiments of Specific Aim #4 examine the influence of four protist invaders on established species and their community. These experiments reveal how propagule size and frequency influence the effect of the invader on the presence and abundance of individual established species and on the concentration, diversity, species richness, and species evenness of invaded communities.

I predicted that invaders would have a negative effect on the growth of individual established species and on community concentration, diversity, richness, and evenness. I expected propagule pressure to influence the strength of the invader's negative effect by determining the abundance of the invader in the community. I assumed that values of propagule pressure that promote invader growth would result in strong negative effects on the community, while treatments that allow only limited invader growth would be associated with minimal negative impact.

The majority of my hypotheses were far too simplistic or downright incorrect. It is true that by influencing invader abundance, invader propagule pressure indirectly influences the effect of the invader on the community. An invader can have very different effects on the native community and its members depending on its propagule pressure. The effect of all four protist invaders varied considerably at

different propagule size and frequency values. At low values of propagule pressure, the invader may have no effect on a particular established species. At higher values of propagule pressure, the same invader may have a strong effect.

What I did not realize earlier was the important role of community composition in influencing the final effect of the invader on community characteristics such as diversity. It is the interaction between the way the invader affects the growth of various established species, the characteristics of the established species, and the specific assemblage of species in the community that actually determines the effect of invasion on community concentration, diversity, richness, and evenness. This principle is clearly illustrated in the way three protist invaders in this study increase community diversity.

I had predicted that all four invaders would reduce the growth of established species and the diversity, richness and evenness of the established community. Actually, the invasion of three of the four protist species in this study increases the diversity of the resulting community. This outcome arises in three very different ways depending on the characteristics of the established species present in the community and the way the invader influences each established species.

Paramecium bursaria is able to enter the established community without negatively affecting the growth or presence of established community

members. The invasion of *Paramecium bursaria* simply adds another species to the community, which increases species richness. Since *Paramecium bursaria* grows well in this community, forming one of the larger populations in the assemblage, the entrance of this invader also increases evenness. Both of these effects increase diversity. The propagule pressure of *Paramecium bursaria* directly affects the cell concentration of the invader, and in doing this, indirectly influences the actual diversity of the established community. Values of propagule pressure that increase *Paramecium bursaria* concentration also indirectly increase the diversity of the established community. In this assemblage, diversity increases because the invader has no effect on the growth of any established species. If the set of established species were different, and included one or more species that were sensitive to *Paramecium bursaria* presence, the outcome of invasion would be different.

Tillina magna enters the established community and negatively affects the growth of the dominant established species, *Paramecium bursaria*. *Tillina magna* invasion does not influence the presence or concentration of the weaker established species in the community. Reducing the concentration of the dominant species, without affecting the concentrations of the other established species in the community, increases evenness. Since *Tillina magna* grows well in this community, forming one of the larger populations in the assemblage, the entrance of

this invader increases both richness and evenness. Increasing richness and evenness enhances community diversity. The specific characteristics of the established species in this community are a critical factor determining the outcome of invasion. Diversity increases as a result of invasion because the community is composed of one dominant established species that is strongly suppressed by the abundance of the invader and two weaker established species that are not influenced by invasion. In this arrangement, invasion reduces dominance, and in doing this, increases diversity. If the set of established species were different, the outcome of invasion would also be different.

Paramecium sp. grows poorly in this system. The species grows only minimally and only at high values of propagule frequency. Nevertheless *Paramecium sp.*, or something closely associated with the invader, strongly promotes the growth of all three established species in the community, increasing their presence and concentration at the end of the experiment. The invasion of *Paramecium sp.* adds an additional species to the assemblage, which also increases the number of established species present the end of the experiment. By increasing richness in these ways, *Paramecium sp.* invasion enhances community diversity. The characteristics of the established species in this assemblage are a critical factor determining the effect of invasion. If the growth of the two dominant

species were not positively influenced by *Paramecium* sp. invasion, richness would not be enhanced and diversity would not increase.

These three examples illustrate the way the particular assemblage of species in a community, the characteristics of each established species, and the influence of the invader at specific values of propagule pressure, interact to promote community diversity. These same factors also interact to influence the way the invasion of the fourth protist species in this study reduces community growth, concentration, diversity, and richness.

The presence of the *Spirostomum ambiguum* invader strongly suppresses the growth of the two dominant established species in the community. Increasing invader propagule pressure creates patterns of decreased presence and concentration for the two dominant established species, but does not influence the growth of the third weaker species. This in turn decreases community diversity and species richness. The characteristics of the established species in the assemblage are critical in determining the negative effect of invasion. Since invasion negatively affects the growth and presence of the two dominant species, and these species comprise the majority of the established community, invasion decreases species richness and community diversity.

When the mean percent change in the established species' cell concentration as a result of invasion are compared across invaders, there is a significant trend of decreasing change in concentration as a result of invasion with increasing realized invader growth (Figure 5-20). In this study, invaders have a strong effect on established species with low realized growth. The effect of invasion on species with higher realized growth is much less. It may be that low realized growth is the result of weak competitive ability, against both established and invasive species. If this is the case, then the effect of invasion being stronger on established species that have low cell concentration may be a general effect seen in other systems.

When the realized growth rate of invaders is compared to the mean percent change in established species' cell concentration as a result of invasion, there is a significant trend of increasing change in concentration with increasing realized invader growth (Figure 5-21). In this study, invaders with strong realized growth have a stronger effect on established species than invaders that grow poorly in the system. Invaders that have high realized growth are found at much greater abundance in the community, which may result in much greater affect on the resource base and media composition. Invaders that are very abundant may deplete the resource base and release waste products at a greater rate than invaders that are found at lower concentration. If this is the case, then the effect of invasion being stronger for invaders that achieve strong realized growth may tend to be a general effect seen in other systems.

This purpose of Specific Aim #4 was to examine whether propagule pressure influences the effect of invasion on the established community and its members. In this experimental system, propagule pressure has a strong indirect influence on the degree to which invasion promotes or diminishes community concentration, diversity, richness, and evenness. Propagule pressure influences invader concentration, which affects the growth of established species. The specific assemblage of established species present in the community, the characteristics of these species, and the effect the invader has on the growth of each species, interact to determine species richness and evenness, and ultimately, community diversity. In this way, propagule pressure plays an important role in the complex interaction of factors that influence the effect invasion has on the established community.

CHAPTER 6. SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS

Background: What is Known and What is Left to Learn About the Way Propagule Pressure Influences Invasion Success

Biological invasion has increased dramatically during the last two-hundred years and is now a critical factor altering global biodiversity and ecosystem stability (Gordon; 1998; Mack *et al.*, 2000; Cassey *et al.*, 2005; Wilson *et al.*, 2009; Eschtruth and Battles, 2009). Many aspects of invasion are strongly influenced by propagule pressure, which is the size of an invading population and the number of times groups of invaders arrive in a location (Richardson *et al.*, 2000b; von Holle and Simberloff, 2005; Kalwij *et al.*, 2008). Propagule pressure can affect the success of invaders (D'Antonio *et al.*, 2000; Lockwood *et al.*, 2005; and Colautti *et al.*, 2006), the diversity and invasibility of communities (Lonsdale, 1999; Williamson and Fitter, 1996a; Green, 1997; Brown and Peet, 2003; Knight and Reich, 2005), and perhaps even the regional diversity-invasibility relationship and the effect invasion has on the native community. Although propagule pressure is a critical factor in invasion, much is left to be learned about how propagule size and frequency determine invasion success and influence the effect of invasion on the established community and its members.

Propagule pressure is critically important to the establishment success of most species, and a large body of work highlights its importance in influencing

invasion. However, the details of the relationship between propagule pressure and invasion are not well known, including the fine-scale relationship between propagule pressure and establishment success (Ruiz and Carlton, 2003; Lockwood *et al.*, 2005). Few studies have quantified the role of propagule pressure to the degree needed to create a dose-response curve that depicts the relationship between propagule pressure and establishment success for an invading species (Williamson, 1996; Travis *et al.*, 2005).

High propagule size and high propagule frequency both influence invasion. However, it is not yet known how the individual effects of propagule size and frequency, functioning together, affect the success of an invader (Lockwood *et al.*, 2005) or how these effects vary from one invader to another (Lockwood *et al.*, 2005). Whether the most important role in invasion is that of propagule size or propagule frequency may depend on the degree of environmental stochasticity and Allee effects (Cassey *et al.*, 2008), or perhaps on the specific assemblage of native species.

A community's invasibility appears to be closely linked its diversity (Davis *et al.*, 2000; Eschtruth and Battles, 2009). Highly diverse communities are classically understood to be inherently stable structures, relatively resistant to invasion (Elton, 1958; Fox and Fox, 1986; Holdgate, 1986; Levine and D'Antonio, 1999; Tilman, 1999; Jiang and Morin, 2004). As species richness increases, community invasibility decreases, creating a negative diversity-invasibility

relationship at the local level that is strongly supported by theoretical and experimental work. A seemingly contradictory diversity-invasibility pattern has been uncovered in large-scale surveys that compare the number of native and invasive species (Lonsdale, 1999; Stohlgren, 1999). In these surveys, areas with high native diversity are associated with a large number of invasive species, creating a positive diversity-invasibility relationship that has been found in a wide variety of community types. The way in which environmental factors shape the positive diversity-invasibility relationship at the regional level is not well understood, and quantifying the different mechanisms by which species coexist is considered to be an important area of future research (Davies *et al.*, 2007). Propagule pressure exhibits the two characteristics thought to be important in promoting a positive regional-level diversity-invasibility relationship: the ability to promote diversity and spatial heterogeneity (Levine, 2000; Brown and Peet, 2003; Knight and Reich, 2005). However, the role of propagule pressure in influencing regional diversity-invasibility relationships in experimental communities is not well tested.

Invaders can affect the native community in multiple ways and at several levels of organization (Melbourne *et al.*, 2007). Invasion can directly influence the abundance and distribution of native species. It can also affect the species richness and diversity of communities (Reaser *et al.*, 2007). By shaping patterns of invader presence and abundance, propagule pressure may influence the effect of invasion on the community. Few experiments have examined the way

propagule pressure influences the effect of an invader on the presence and abundance of individual native species or on the concentration, species richness, and diversity of invaded communities.

Goals of this Project

This project used a bacterivorous protist system to study the role of propagule pressure in shaping many aspects of invasion. The effect of propagule pressure on the diversity of three bacterivorous protist communities was examined to learn whether propagule size or propagule frequency promotes co-existence among established species. The effect of propagule pressure on the establishment and invasion success of four protist invaders was also observed to learn whether propagule size or frequency allows co-existence among invasive and established species in the protist community. Treatment levels were detailed enough to facilitate examination of the fine-scale relationship between propagule pressure and establishment, and to allow dose-response curves to be constructed for each species so that the effect of variation in propagule pressure could be compared both within and among species. The four species' dose-response curves were analyzed to determine whether propagule pressure influences stochastic extinction. To more completely describe invasion success, the effect of propagule pressure on invasion was quantified as the probability of establishment success, as has been commonly done, and also in terms of the concentration and proportion of the invader in the community. The effect of

propagule pressure on the diversity of established communities and on the success of invaders was compared to determine whether propagule pressure could potentially promote a positive regional diversity-invasibility relationship in aquatic protists. The independent and relative effects of propagule size and frequency were compared to learn how these factors interact to influence invasion success. Finally, the effect of invasion on the presence and abundance of established species and on the concentration, species richness, and diversity of established communities was studied across a range of propagule pressure values to determine how propagule pressure influences the effect of invasion on the established community.

What Was Learned From this Project

Propagule Pressure Influences the Richness and Diversity of Protist Communities

At the regional level, community diversity and invasibility are shaped primarily by environmental factors that promote species co-existence and vary in intensity across the landscape (Fridley *et al.*, 2007). Community diversity and invasibility, covarying across a heterogeneous environment, produce a positive diversity-invasibility relationship (Levine and D'Antonio, 1999; Lonsdale, 1999; Stohlgren *et al.*, 1999; Stohlgren *et al.*, 2003; Eschtruth and Battles, 2009). To date, most work has examined the role of heterogeneity of resource availability in allowing

native and exotic species to coexist, and in so doing influencing community diversity and invasibility. Propagule pressure may also be a diversity-promoting factor. The role of propagule pressure in influencing regional diversity-invasibility relationships in experimental communities is not well tested. This study examined the influence of propagule size and propagule frequency on the diversity of three different bacterivorous aquatic protist communities, each with a unique dominant species.

Propagule Pressure Affects the Diversity of Three Experimental Communities.

Increasing propagule size substantially increases community diversity and species richness in all three experimental communities studied. However, high propagule size reduces dominance in only one of the communities. Increasing propagule frequency raises community diversity and species richness values, and lowers dominance, but only in one community. Propagule frequency significantly affects diversity, but not richness or dominance, in another community. The last of the three communities shows no effect of propagule frequency on community diversity, richness, or dominance.

In the experimental community dominated by *Tillina magna*, increasing propagule size and frequency significantly enhances community diversity and species richness and diminishes dominance. These patterns are found when treatments of the same maturity are compared, as well as when treatments of the same cell concentration are compared, regardless of maturity. This suggests that these

patterns are the direct result of increased propagule pressure, and not simply related to increased community concentration in high propagule pressure treatments. Interestingly, the negative relationship between propagule pressure and dominance is found even though the growth of the dominant species, *Tillina magna*, is significantly increased by high propagule size and not affected by high propagule frequency. This suggests that propagule pressure has an exceptionally strong positive influence on the growth of the non-dominant species in this community. In fact, propagule size and propagule frequency do have strong positive effects on the growth of *Paramecium bursaria* and *Spirostomum ambiguum* in this community. The third species in the community, *Paramecium sp.*, grows very weakly and is rarely even found in most experimental flasks during data collection. Increasing propagule size does not benefit *Paramecium sp.* growth. The species is never found in samples from any propagule size flask. In contrast, propagule frequency has significant positive effect on *Paramecium sp.* growth, perhaps the consequence of rescue effect. In all propagule frequency experiments, frequency had a significant effect but addition size did not. This suggests that additional inoculations provide the benefit seen in high propagule frequency treatments, independent of the associated increase in invader cells.

In the experimental community dominated by *Uronema sp.*, increasing propagule size significantly increases community diversity and species richness, but does not influence the dominance of the dominant species. Propagule frequency

significantly increases community diversity, but not species richness, or dominance. The effects of propagule pressure may be reduced in this assemblage because the community is so strongly dominated by *Uronema sp.*, and *Uronema sp.* growth is not significantly affected by propagule frequency. The positive effect propagule frequency might have on the 20% of the community composed of non-dominant species is weakened at the community level by the 80% of the community composed of *Uronema sp.* that is not affected.

In the experimental community dominated by *Colpidium striatum*, high propagule size significantly increases community diversity and species richness, but does not influence dominance. Propagule frequency has no apparent effect on diversity, species richness, or dominance. The effects of propagule pressure are limited in this community perhaps because the dominant species, *Colpidium striatum*, so completely dominates the assemblage, accounting for approximately 97% of the total cell concentration. *Colpidium striatum* is not significantly affected by either propagule size or frequency. The species has nearly identical cell concentration in all propagule size and frequency treatments. Although propagule pressure positively influences the growth of the four non-dominant species in the community, this effect is hidden by the sheer abundance of *Colpidium striatum*.

The Dominant Species May Determine the Effect of Propagule Pressure on the Community. In this system, communities show a clear pattern of increased

diversity and richness, and a strong decrease in dominance, when all species in an assemblage have fairly similar growth rate and the growth of the dominant species is influenced by propagule pressure. As the proportion of the community composed of the dominant species increases, the range of community effects attributed to propagule pressure diminishes. This occurs even if propagule pressure has a positive effect on the non-dominant species in the community. A general trend found throughout this project is that propagule size tends to influence all communities, while propagule frequency primarily influences communities with dominant species that exhibit relative growth rate not much greater than that of other community members. Propagule frequency significantly influences the diversity, richness, and dominance of the community dominated by the slowest-growing dominant species, *Tillina magna*. Propagule frequency significantly influences only the diversity of the community dominated by the dominant species with intermediate realized growth rate, *Uronema sp.* In contrast, propagule frequency does not influence any characteristic of the community dominated by the species with the greatest realized growth, *Colpidium striatum*.

This decline in the range of effects may occur for two reasons that are related and function together. First, the dominant species in a community is dominant because its growth rate in the community is much greater than that of the non-dominant species. When a species grows rapidly, even a small inoculum may be enough to allow maximum population growth. If this is the case, increasing

propagule pressure gives little benefit. Therefore, the greater a dominant species' realized growth rate, the less likely it is that experimental values of propagule pressure will influence its growth. Second, the greater an invader's realized growth rate, the greater its proportion in the community, and therefore, the greater its influence on the effect of propagule pressure at the community level. When the majority of a community is unaffected by propagule pressure, the positive effects exhibited by non-dominant species are less apparent when viewed at the community level. As a dominant species' relative growth rate and its associated proportion within the community increase, the more likely it is that the community will show weakened or even no positive effect from increased propagule pressure.

A Species' Growth Rate May Affect the Influence Propagule Size and Frequency have on the Species' Growth. Propagule pressure positively affects the combined growth of the non-dominant species in all three experimental communities used in this project. It may be valid to assume that propagule pressure generally has a positive influence on non-dominant species. In this experimental system, the effect propagule size and frequency have on a species appears to vary with the species' realized growth rate. The two species in this project with realized growth rates considerably greater than those of all other species are unaffected by propagule pressure. The cell concentration of *Uronema sp.* and *Colpidium striatum* are consistent across all propagule size and propagule frequency treatments. In contrast, the species in this experiment with

a rapid growth rate, *Tillina magna*, is positively affected by propagule size, but not propagule frequency. The species with moderate growth rate, *Paramecium bursaria* and *Spirostomum ambiguum*, exhibit a strong positive relationship with both propagule size and frequency. Finally, the species that grows most poorly in these experiments, *Paramecium sp.*, is positively influenced only by the highest values of propagule frequency. It appears that the benefit conferred by high propagule size and frequency varies with a species' growth rate in the community. Species with strong growth rate are not influenced by propagule pressure or influenced only by propagule size. Since the population grows quickly, inoculating relatively small additions of cells later has no effect. In contrast, species that grow poorly in the protist community are influenced strongly, but only at the highest level of propagule frequency, perhaps the consequence of rescue effect.

The Degree to which Propagule Pressure Promotes Co-existence among Established Species Depends on the Type of Propagule Pressure and the Growth Rate of the Dominant Established Species. Propagule pressure is a diversity-promoting factor in the experimental bacterivorous protist communities, facilitating the co-existence of established species. However, the degree to which propagule pressure increases community diversity and richness, and decreases dominance, depends on the form of the propagule pressure, size or frequency. It also depends on the growth characteristics of the dominant species in the assemblage, its growth rate relative to other species and how strongly it is

influenced by propagule pressure. High propagule size increases the richness and diversity of all experimental communities and may have a similar effect in natural protist communities. In contrast, the influence of high propagule frequency is limited to two experimental communities. Propagule frequency may affect a limited number of natural communities as well. The critical factor determining the effect of propagule frequency on community diversity, richness, and dominance may be the growth characteristics of the dominant species in the assemblage. The positive effect of propagule frequency in influencing community diversity is best seen in communities with members that grow at approximately the same rate and a dominant species whose abundance is influenced by propagule pressure. Communities dominated by a rapidly-growing species that are themselves not influenced by propagule pressure, show only a weak influence.

*Propagule Pressure Influences the
Success of Protist Invaders*

The details of the relationship between propagule pressure and invasion are not well known. We do not yet fully understand the fine-scale relationship between propagule pressure and establishment success (Ruiz and Carlton, 2003; Lockwood *et al.*, 2005). Few experimental studies have quantified the role of propagule pressure in enough detail to create a dose-response curve that depicts the fine-scale relationship between the propagule pressure of an invading

species and the probability it will establish a persistent population (Williamson, 1996; Travis *et al.*, 2005). It is also not well understood how propagule pressure interacts with factors that block invasion, such as demographic stochasticity. Finally, many studies quantify the influence of propagule pressure as the probability of establishment, the likelihood that an invader will establish a reproducing population. This measure may not completely capture all the dimensions of invasion. Perhaps the influence of propagule pressure should also be quantified as invasion success, the proportion of the invasive species in the community.

This project examined the role of propagule size and frequency on the establishment and invasion success of four protist invaders. Treatment levels were scaled finely enough so that the effect of variation in propagule pressure could be compared both within and among species. The curves were examined to determine whether propagule pressure influences the prevalence of stochastic extinction.

General Patterns of Growth Influence the Response of Protist Invaders to Propagule Pressure. Several related, general growth patterns are found in the results of these propagule size and frequency experiments. The patterns are seen repeatedly in multiple experiments throughout this study, so bear mention here. First, the cell concentration of all four of the invaders used in this study is closely associated with the species' proportion in the community. Since

propagule pressure can influence invader cell concentration, it can also indirectly influence the invader's proportion in the community. Second, in general, the higher an invader's growth rate in the community, the more rapidly it reaches maximum cell concentration and the greater the population's cell concentration at maximum value. Finally, the higher an invader's maximum cell concentration, the earlier the population begins to decline. Invader populations tend to decline immediately after reaching maximum cell concentration. Fast growing protists quickly reach a high maximum cell concentration, and then decline through the remainder of the experiment. Slower-growing species take longer to reach maximum cell concentration which delays the beginning of decline.

Propagule Size Influences Establishment and Invasion Success, but the Invader's Growth Rate Affects the Type of Influence Conferred. Propagule size can influence invasion in this protist system in several ways. High propagule size can increase an invader's probability of establishment and increase its cell concentration and proportion during the period of maximum community concentration. Propagule size can also influence an invader's population dynamics, affecting the length of time required to reach maximum invader concentration, the length of time the population remains viable, and whether a second period of invader growth occurs late in the experiment. Although all of these effects can be promoted by high propagule size, and are seen in the experiments, not all effects occur in all species. Characteristics of the invader appear to determine how propagule size influences its establishment and

invasion success. The four invaders used in these experiments differ in their growth rate within the established community. The specific influence of propagule size appears to be associated with this growth rate. Two of the invaders, *Tillina magna* and *Paramecium bursaria*, have relatively high realized growth rate in the experimental community. There are many similarities in the way increasing propagule size affects their invasion. Both species show saturation in their response to propagule size. This saturation is evident in the species' probability of establishment and invasion success. Both strong invaders are able to establish viable populations in all experimental flasks, regardless of propagule size. These species are capable of establishing a persistent population from even an extremely small founding propagule. For both species, increasing propagule size significantly enhances invader cell concentration and proportion, but only up to a point. Both species reach maximum cell concentration and proportion at intermediate propagule size (50, 100). Larger propagule size either has no effect or actually leads to slightly decreased cell concentration and proportion. Increasing propagule size has very different effects on the remaining two invaders used in these experiments.

Spirostomum ambiguum and *Paramecium sp.* are much weaker invaders, grow at substantially lower rate within the experimental community, and form much smaller invader populations. Neither of these species shows saturation in its response to increasing propagule size. The establishment success of these species increases significantly with propagule size. One species shows a trend of increasing establishment with increasing propagule frequency through

propagule size 100. The other species is capable of establishing a viable population only at the highest propagule size values. Unlike the strong invaders which have maximum invasion success at intermediate propagule size, the two weaker invaders exhibit continual increased invasion with increasing propagule size. One weak invader shows a significant trend of increasing cell concentration as propagule size increases. The other invader forms a recoverable population and constitutes a sizable proportion of the community only when inoculated at the highest propagule size. This species grows slowly, reaching an early maximum concentration on week 2. At this time, there are significant patterns of increasing cell concentration and proportion with increasing propagule size. Immediately after reaching maximum, populations decline. High propagule size minimizes this decline and promotes a second period of much greater growth at the end the experiment.

Propagule Frequency and the Size of Additions Influence Establishment and Invasion Success, but the Invader's Growth Rate Affects the Type of Influence Conferred. Propagule frequency can influence invasion in several ways. High propagule frequency can affect an invader's probability of establishment, cell concentration, and proportion one week after the final invader addition is made. Propagule frequency can also influence an invader's population dynamics, affecting whether a second period of invader growth occurs late in the experiment. The size of additions in propagule frequency experiments can influence invader cell concentration and proportion. Although all of these effects

can be promoted by high propagule frequency, and are seen in these experiments, only a subset of effects occurs in any one species. As with propagule size, the growth rate of the invader in the established community appears to determine how propagule frequency influences its establishment and invasion success. The establishment of the two invaders with high growth rate, *Tillina magna* and *Paramecium bursaria*, is not influenced by propagule frequency. Both of these invaders are able to establish persistent populations in all experimental flasks, regardless of propagule frequency or the size of additions. In contrast, propagule frequency does significantly influence the cell concentration and proportion of these invaders, and the size of additions plays a role in determining the type of effect conferred. When the size of additions is small, increasing propagule frequency either has no effect on or significantly increases invader concentration and proportion. When the size of additions is larger, increasing propagule frequency significantly decreases invader cell concentration and proportion. This suggests that saturation plays a role in determining the effect of increasing propagule frequency. Increasing propagule frequency and the size of additions affects invaders with low realized growth rate very differently. Neither of the two weak invaders in this system, *Spirostomum ambiguum* and *Paramecium sp.*, shows saturation in its response to increasing propagule frequency or addition size. Increasing propagule frequency is associated with a significant increase in the establishment, concentration, and proportion of both invaders and a larger size of additions tends to amplify this effect.

Invader Growth Rate Influences the Effect of Propagule Pressure. The growth rate of an invader in the established community appears to determine the influence that propagule pressure has on its subsequent growth. In this study, invaders that have higher realized growth rate are influenced similarly by propagule size and frequency, and these effects are quite different than those experienced by slower-growing invaders. Strong, fast-growing invaders show saturation in their response to both propagule size and propagule frequency. These species are capable of establishing viable populations in all flasks at all treatments, regardless of propagule size. Increasing propagule pressure does not confer benefit. Increasing both propagule size and propagule frequency increases the invasion success of the strong invaders, but only up to a point and the effect is always very weak. Increasing propagule pressure beyond the point of maximum invasion success confers no benefit and, in some cases, results in decreased success. Increasing propagule frequency, when the size of additions is small, increases invasion success or has no effect. However, increasing propagule frequency when the size of additions is large has a negative effect on invader cell concentration and proportion. In contrast, weak invaders never show saturation in their response to propagule pressure. Increasing propagule pressure always has a strong, positive effect on weak invaders. Both weak invaders in this study show clear trends of increasing establishment, cell concentration, and proportion with increasing propagule size and frequency. Increasing addition size tends to amplify the positive effect of propagule frequency. In one species, high propagule pressure minimizes the population

decline that follows maximum cell concentration and promotes a second period of growth at the end of the experiment.

There May be a General Shape to Dose-Response Curves. The fine-scale relationship between propagule pressure and establishment success is not yet well understood (Ruiz and Carlton, 2003; Lockwood *et al.*, 2005). One goal of this study was to determine the dose-response characteristics of four protist invaders. The effect of increasing propagule size and frequency on the probability of invader establishment was examined, as suggested by Ruiz and Carlton (2003). The effect of increasing propagule pressure on invasion success, defined as the cell concentration and proportion of the invader in the community, was also studied. The results suggest that dose-response curves for invader establishment and invasion success may have a general Gaussian distribution, the particular characteristics of which vary across systems. Increasing propagule size or frequency increases establishment and invasion success up to a point after which increasing propagule pressure confers no benefit or even results in decreased growth. An invader's realized growth may determine the way specific values of propagule size and frequency fall along the x-axis. For strong fast-growing invaders, saturation is reached at lower propagule size and frequency values. For weak slow-growing invaders, the curve levels at much greater values of propagule size and frequency. This suggests that the responses seen in this study depend on the experimental values of propagule size and frequency used. If this study had utilized lower

values, even the fast growing invaders might have shown continuous benefit from increasing propagule size and frequency. If the study had used larger values, even the slow-growing invaders might have shown saturation in their response to propagule size and frequency.

Propagule Pressure Influences the Presence of Demographic Stochasticity in Invasion. To establish a persistent population, an invader must avoid extinction and achieve positive population growth at low density (Chesson, 2000; Sakai *et al.*, 2001, Theoharides and Dukes, 2007). Demographic stochasticity may strongly impede the establishment of some invaders (Grevstad, 1999). Few studies have examined how this barrier might be weakened by high propagule pressure. Propagule size and propagule frequency influence the size of the founding invader population and the degree of rescue effect available. Therefore, propagule pressure could theoretically influence the effect of demographic stochasticity on establishment.

Stochastic extinction is an important factor for the weak invaders in some treatments in this study. It can be seen on the probability of establishment graphs, in the treatments that have probability of establishment values between 0 and 1.0. Stochastic extinction can be quantified as:

$$\text{Stochastic extinction} = 1 - \text{probability of establishment}$$

Both *Spirostomum ambiguum* and *Paramecium* sp. show significant trends of decreased stochastic extinction with increasing propagule size and propagule frequency. By increasing the size of the founding population or contributing rescue effect, high values of propagule pressure decrease the effect of demographic stochasticity on the establishment of the slow-growing invaders examined in this study. High propagule pressure may generally decrease the stochastic extinction of invading populations in many different taxa.

*Propagule Pressure May Influence the
Diversity-Invasibility Relationship of the Protist System*

Propagule pressure exhibits the two characteristics necessary to promote a positive diversity-invasibility relationship at the regional level. High levels of propagule pressure are known to increase community diversity and there is heterogeneity in the intensity of propagule pressure across the landscape. High propagule pressure has been linked to a positive diversity-invasibility relationship in a riparian plant community (Brown and Peet, 2003). Nevertheless, the role of propagule pressure in influencing regional diversity-invasibility relationships in experimental communities is not well tested.

Natural Aquatic Protist Communities May Have a Positive Diversity-Invasibility Relationship at the Regional Level. This study examined the influence of propagule size and propagule frequency on species coexistence. The

experiments of Specific Aim #1 examined the influence of propagule pressure on the coexistence of established species. The experiments of Specific Aim #2 focused on the effect of propagule pressure on the coexistence of established and invasive species. High propagule size and high propagule frequency were found to promote coexistence of established community members, resulting in greater species richness and community diversity, and lower dominance. The positive effect of propagule pressure in influencing community diversity is best seen in communities with members that grow at approximately the same rate and a dominant species whose abundance is positively influenced by propagule pressure. The experimental community composed of *Tillina magna*, *Paramecium bursaria*, *Spirostomum ambiguum*, and *Paramecium* sp. displays particularly strong positive effects of both propagule size and propagule frequency. These four species were used to examine the role of propagule pressure on invasion success, a characteristic that reflects the coexistence of established and invasive species. High propagule pressure, in the form of large propagule size, high propagule frequency, and large addition size, promotes invasion success in all four protist invaders. It facilitates the coexistence of all experimental invader and established species.

In this system, propagule size and propagule frequency promote coexistence among all species, established and invasive, and in so doing, increase both community diversity and invasibility. Therefore, this study suggests that propagule pressure could create a positive regional diversity-invasibility

relationship in naturally-occurring bacterivorous communities. Of course, natural communities are influenced by biotic and environmental factors not included in this study, including predation and environmental fluctuation. Since the regional diversity-invasibility relationship of natural protist communities is influenced by so many factors not considered here, the actual relationship found in many natural communities may not be positive. However, propagule pressure, functioning in a community shaped by competition and experiencing fairly constant environmental conditions, could potentially create a positive regional diversity-invasibility relationship.

*The Interaction Between Propagule Size and Propagule Frequency
Influences Invasion Success*

It is not yet known how the effects of propagule size and frequency, functioning together, affect the success of an invader or how these effects vary from one invader to another (Lockwood *et al.*, 2005).

Growth Rate Influences the Relative Effects of Propagule Size and Frequency on Establishment and Invasion Success. In this protist system, an invader's growth rate affects the strength of propagule size and frequency in influencing the probability of its establishment. Strong fast-growing invaders establish populations in all experimental flasks in all treatments, regardless of propagule size or frequency. In contrast, propagule size and frequency influence the

establishment of the two slower-growing invaders. Independently increasing propagule size and propagule frequency increases the establishment of the two slow-growing invaders. In the relative effects experiments, there is variation the way the establishment of these two species varies across treatments. The establishment of *Spirostomum ambiguum* is influenced only by propagule frequency. Increasing propagule frequency significantly increases establishment,. In contrast, both propagule size and propagule frequency influence *Paramecium sp.* establishment. The negative effects of low propagule size and low propagule frequency both seem to suppress *Paramecium sp.* establishment in treatments with low values of either propagule size or frequency. Maximum establishment is achieved in treatments that have intermediate values of both propagule size and frequency.

The way propagule size and frequency interact to influence invasion success varies from species to species, and is loosely associated with the invader's growth rate. For three protist species, the effects of both propagule size and propagule frequency influence invasion when the two factors interact. The invasion of the other species is shaped primarily by propagule frequency. The effect of propagule size, clearly seen when this factor functions independently in influencing invasion, is lost in the relative effects treatments. In the experiments that examine the independent effects of propagule size and frequency, the two fast-growing species show saturation in their response to increasing propagule size and propagule frequency. These invaders show saturation in the relative

effects treatments as well. Increasing propagule size or propagule frequency above a particular value does not increase invasion, and can in fact suppress growth.

Although the four protist invaders can be grouped based on the number of factors that influence invasion and whether saturation is shown, the way propagule size and frequency interact to influence invasion is unique for each species.

Tillina magna. Propagule size and frequency interact to determine the success of *Tillina magna* invasion. Propagule size is like a switch, regulating whether the negative effect of increasing propagule frequency will influence invasion. When the size of additions is small, the effect of propagule frequency is not significant. However, when the size of additions is larger, the negative effect of propagule frequency significantly influences the concentration and proportion of the invader.

Paramecium sp. Propagule size and frequency both influence *Paramecium sp.* invasion, but in a way that is very different from the way the two factors influence *Tillina magna*. Low propagule size and low propagule frequency both suppress *Paramecium sp.* growth. Therefore, maximum *Paramecium sp.* invasion occurs when moderate propagule size and frequency values co-occur.

Paramecium bursaria. The invasion of *Paramecium bursaria* appears to be shaped only by propagule frequency. Propagule size does have an effect, but the effect of saturation often masks its influence. Increasing propagule frequency suppresses *Paramecium bursaria* growth, regardless of the total number of cells inoculated or the size of additions. The independent effect of propagule size is not prevalent because almost all treatments have propagule sizes of 50-100, values shown in the invasion experiments to produce nearly identical *Paramecium bursaria* growth. The influence of propagule size can be seen in two treatments. Treatment 1x(50) has significantly greater cell concentration and proportion than treatment 2x(25). Invasion experiments have shown that increasing propagule frequency when addition size is small has no effect on *Paramecium bursaria* growth, so these two treatments are expected to have similar values. The depression of growth must be the consequence of low propagule size, since a propagule size of 25 reduces *Paramecium bursaria* growth in invasion experiments. The value of the 1x(300) treatment is also substantially lower than that of the 2x(150) treatment. Since the 2x(150) treatment has multiple inoculations of high propagule size, it is expected to have concentration and proportion values much lower than those of a single addition treatment. The unexpectedly low *Paramecium bursaria* concentration and proportion values in the 1x(300) treatment must be caused by the treatment's large propagule size, a size shown in invasion experiments to depress the species' growth.

Spirostomum ambiguum. For *Spirostomum ambiguum*, the influence of propagule size is hidden by the vastly stronger influence of propagule frequency six weeks after introduction. Individually, increasing propagule size and increasing propagule frequency both lead to increasing *Spirostomum ambiguum* cell concentration and proportion. However, populations initiated with one large addition of cells reach a high maximum cell concentration on week 2, and then the population declines and is lost from the community by week 6. The relative effects treatments show a strong trend of increasing invasion with increasing propagule frequency because the positive effect of propagule frequency at week 6 is much stronger than that of propagule size.

The combined results from the four relative effects experiments suggest that the individual effects of propagule size and propagule frequency simultaneously influence establishment and invasion success. When the two effects vary, the negative effect appears to more powerfully shape the resulting pattern of invasion. When a species shows saturation in its response to propagule size or frequency, its effect may be equivalent across treatments and invasion success is therefore shaped only by the remaining factor. The time at which relative effects are compared may also influence whether propagule size, propagule frequency or both factors most strongly influence invasion success. Propagule size effects occur early in culture, and then populations with high concentration decline. In contrast, high propagule frequency maintains a population's

concentration for many weeks. If relative effects are compared very early in culture, they may show a stronger effect from propagule size. When compared many weeks after invasion, they may show an effect primarily from propagule frequency. Cultures compared three to four weeks after invasion may show strong effects of both propagule size and frequency.

*Propagule Pressure Influences the Effect of Invaders on the
Established Community and on Individual Community Members*

Invaders can affect the native community in multiple ways and at several levels of organization (Melbourne *et al.*, 2007). Invasion can directly influence the abundance and distribution of established species. It can also affect the species richness and diversity of communities (Reaser *et al.*, 2007). Few studies have examined whether the way invaders are introduced influences their effect on the presence and abundance of individual established species or on the richness and diversity of the established community.

This project examines the influence of four protist invaders on established species and their community. Treatments are designed to show the effect of the invaders, at various values of propagule pressure, on the presence and concentration of individual established species and on the richness, diversity, and evenness of invaded communities.

By shaping patterns of invader presence and abundance, propagule pressure indirectly influences the effect invasion has on the protist community. The effect of all four protist invaders on the concentration of established species and on the diversity, richness, and evenness of the established community varies considerably at different propagule pressure values. At low propagule size the invader may have no effect on a particular established species. At higher values of propagule pressure, the same invader may have a strong effect.

The Propagule Pressure of an Invader Interacts with Community Composition and the Characteristics of Established Species to Influence the Effect of Invasion on Community Diversity. Community composition strongly influences the final effect an invader has on community characteristics such as species richness, species evenness, and diversity. It is the interaction between the way the invader affects the growth of various established species, the characteristics of the established species, and the specific assemblage of species in the community that actually determines the effect of invasion on community diversity, richness, and evenness. This principle is clearly illustrated in the way three protist invaders in this study increase community diversity.

Of the four protist invaders in this study, three increase the diversity of the community they invade. This outcome is itself surprising. Invaders are generally believed to have negative effects on the native community. However, the majority of protist invaders in this study actually increase community diversity.

This outcome arises in three very different ways depending on the characteristics of the established species present in the community and the way the invader influences each of the established species.

Paramecium bursaria is able to enter the established community without negatively affecting the growth or presence of established community members. The invasion of *Paramecium bursaria* simply adds another species to the community, which increases total species richness. Since *Paramecium bursaria* grows well in this community, forming one of the larger populations in the assemblage, the entrance of this invader also increases evenness. Both of these effects increase diversity. If the set of established species were different, and included one or more species that were sensitive to *Paramecium bursaria* presence, the outcome of invasion would be different. The propagule pressure of *Paramecium bursaria* directly affects the cell concentration of the invader, and in doing this, influences the actual diversity of the established community. Propagule pressure values that increase *Paramecium bursaria* concentration indirectly increase the diversity of the established community.

Tillina magna enters the established community and negatively affects the growth of the dominant established species, *Paramecium bursaria*, without influencing the presence or concentration of the weaker established species in the community. By reducing the concentration only of the

dominant species, *Tillina magna* invasion increases evenness. Since *Tillina magna* grows well in this community, forming one of the larger populations in the assemblage, the entrance of this invader also increases evenness and richness. Increasing richness and evenness enhances community diversity. Values of propagule pressure that promote strong *Tillina magna* growth indirectly enhance community diversity, species richness, and evenness. The specific characteristics of the established species in this community are a critical factor determining the outcome of invasion. Diversity increases as a result of invasion because the community is composed of one dominant established species that is strongly suppressed by the abundance of the invader and two weaker established species that are not influenced by invasion. If the set of established species were affected differently by *Tillina magna*, the outcome of invasion would be different.

Paramecium sp. itself, or something closely associated with *Paramecium sp.*, strongly promotes the growth and presence of all three established species in the community. The invasion of *Paramecium sp.* also adds an additional species to the assemblage. By increasing richness in these ways, *Paramecium sp.* invasion enhances community diversity. As the propagule frequency of *Paramecium sp.* increases, the benefit to the community also increases. The characteristics of the established species in this assemblage are critical in determining the

effect of invasion. If the growth of the established species were not positively influenced by *Paramecium* sp. invasion, richness would not be enhanced and diversity would not increase.

The results of these experiments illustrate the way the particular assemblage of species in a community, the characteristics of each established species, and the influence of the invader at specific values of propagule pressure, interact to promote community diversity. These same factors also interact to influence the way the invasion of the fourth protist species in this study reduces community diversity.

Spirostomum ambiguum invasion significantly depresses the growth, concentration, diversity, and richness of the established community. Increasing invader propagule pressure significantly enhances *Spirostomum ambiguum* growth and this indirectly decreases the presence and concentration of dominant community members without influencing the growth of the weaker established species. This causes significant decreases in species richness and community diversity.

*Protist Invaders May Have a Stronger Effect on
Community Members that have Low Realized Growth Rate*

The results of these experiments suggest a general pattern relating the effect of invasion to the realized growth of established species. In the communities used in this study, invaders alter the cell concentration of established species that have low realized growth rate and abundance more strongly than community members with greater realized growth and abundance. Perhaps species with low realized growth are weaker competitors and are, therefore, strongly depressed by the addition of an invader. If it is the case that species with low realized growth rate are weaker competitors, then this may be a general pattern in many systems, natural and experimental, and might be worth studying in more detail.

*Invaders with High Realized Growth Rate Have a Stronger Effect
on Established Community Members*

In this study, invaders with strong realized growth have a greater effect on established species than do invaders that grow poorly in the system. Invaders that have high realized growth are found at much greater abundance in the community, which may result in much greater affect on the resource base and media composition. Invaders that are very abundant may deplete the resource base and release waste products at a greater rate than do invaders that are found at lower concentration. If this is the case, then the effect of invasion being

stronger for invaders that achieve strong realized growth may tend to be a general effect seen in other systems.

Propagule Pressure, Interacting with Many Other Factors, Influences the Effect of Invasion on the Established Community and its Members

In this experimental system, propagule pressure has a strong, but indirect, influence on the degree to which invasion promotes or diminishes community concentration, richness, and diversity. Propagule size and frequency influence invader abundance, which affects the growth of established species. The specific assemblage of established species present in the community, the characteristics of these species, and the effect the invader has on the growth of each species, interact to determine species richness and evenness, and community diversity. In this way, propagule pressure plays an important role in the complex interaction of factors that influence the effect invasion has on the established community.

Conclusions

This study suggests that high values of propagule size and frequency promote the co-existence of bacterivorous protist species. Propagule pressure increases community diversity, having the greatest effect on communities that are composed of species with similar realized growth rates and that have a dominant

species whose growth is itself influenced by propagule pressure. High propagule pressure also promotes establishment and invasion success in this system. The invader's realized growth rate in the community influences the specific effect of propagule pressure on invasion. Increasing propagule pressure increases establishment and invasion success for slow-growing invaders. In contrast, faster-growing invaders show saturation in their response to propagule pressure. Increasing propagule pressure increases the concentration of these species, but only up to a point. Increasing propagule pressure beyond this point does not give additional benefit, and in some instances, even leads to decreased growth. This suggests that there might be a general dose-response curve with Gaussian distribution. A species' growth rate determines how specific propagule size and frequency values fall along the curve. Slow-growing invaders reach saturation only at high values of propagule size and frequency, while fast-growing invaders show saturation at much lower values. These results suggest that propagule pressure could create a positive diversity-invasibility relationship in protist communities at the regional level. Of course, natural communities are influenced by biotic and environmental factors not included in this study, so the actual regional diversity-invasibility relationship found in nature may not be positive. However, this study shows that the way propagule pressure, functioning independently in a community shaped by competition and experiencing fairly constant environmental conditions, could potentially create a positive regional diversity-invasibility relationship.

This study suggests that propagule size and frequency interact to influence the probability of establishment and invasion success. The particular outcome of this interaction varies from one species of invader to the next, and appears to be loosely related to the invader's realized growth rate. Fast-growing protist invaders in this system achieve the greatest invasion success when cells are added in one large addition. In contrast, the establishment and invasion success of the slow-growing invaders in this study are enhanced by high propagule frequency.

Stochastic extinction influences the establishment of slow-growing protist invaders. This study demonstrates that high propagule pressure significantly decreases the effect of stochastic extinction for these species. As propagule size and frequency increase, the probability of stochastic extinction decreases and establishment increases.

In this system, propagule pressure influences the effect invaders have on the established community, including the diversity that occurs as a result of invasion. The entrance of three protist invaders increases community diversity, but the way this occurs varies from species to species. One invader enters the established community without effecting the growth of established species. Another invader depresses the growth only of the dominant species in the community. The third invader promotes the growth of all three established species in the community. The last protist invader decreases community diversity by suppressing the

growth of the two dominant established species in the community. In all cases, the invader's propagule pressure influences the specific effects that occur as a result of invasion.

This project demonstrates that propagule pressure has a strong influence on many aspects of invasion. It also suggests that the specific effect produced by propagule pressure is influenced by characteristics of the invader, the community, and the environment.

Future Directions

While this project suggests that propagule pressure has a strong influence on community diversity and invasion success in the experimental bacterivorous protist system, there is much left to learn. It is not well understood how propagule pressure influences Allee effects. Very little is known about how environmental factors influence the effect of propagule pressure on invasion success. The model system used in this project would work well for a study of these topics.

Invading populations tend to be quite small. To establish a persistent population, the invader must avoid extinction and achieve positive population growth at low density (Chesson, 2000; Sakai *et al.*, 2001, Theoharides and Dukes, 2007). Some small invading populations exhibit reduced growth rate at low density, the

defining characteristic of the Allee effect. The fitness of these species is positively related to their population density. When the species is at low density, individual fitness is also low, and this reduces per capita growth rate (Taylor and Hastings, 2005). Allee effects reduce the establishment, increase the lag time, and slow the growth of small invading populations (Taylor and Hastings, 2005). Because it has a direct influence on the size of founding invader populations, propagule pressure may determine whether Allee effects influence a founding population's invasion success (Taylor and Hastings, 2005). Allee effects are pervasive in many animal and plant groups, and have been found in several invasive plants, birds, insects, mollusks, and pathogens (Taylor and Hastings, 2005). If Allee effects are prevalent among invasive species, propagule pressure may determine their influence on invasion processes. It would be interesting to examine the influence of Allee effects by comparing the slope of invader growth curves across propagule size and number treatments. With minor modification, the design of the invasion experiments utilized in this project could be used to examine Allee effects. Data would need to be collected more frequently than done in this project so that curves reflect a finer scale and slope can be determined more accurately. The invasion experiments would also need to be conducted at cool temperature,, not in high heat as was done in this project. This would extend the period of invader growth, and delay the timing of maximum cell concentration and subsequent population decline.

This project focused on the effect of propagule pressure on the establishment and success of an invader in only in a single established community. It would be interesting to repeat this experimental design in multiple communities that differ in species richness and species composition. The experimental community in this study had three established species. Experimental communities could be constructed that represent all combinations of two species. Single established species cultures could also be invaded. This collection of treatments would allow identification of the independent effects of species richness and species composition on invasion success at various values of propagule size and frequency. Comparing the independent effects of species richness and community composition addresses an area of research not yet well studied. Most previous studies have examined the influence of species richness on invasive success, and have not directly examined other community factors such as composition (Levine *et al.*, 2004). Several workers have suggested that the specific species in a community might be a stronger determinant of invasibility than simple species richness (Crawley *et al.*, 1999; Richardson *et al.*, 2000a), but to date, few studies have addressed this hypothesis.

Community density and resource availability may strongly influence the effect of propagule pressure. Since invasion is promoted by the availability of resources, but depressed by strong competition among native community members, I expect that higher values of propagule pressure are required to enhance establishment and invasion success as the density of the established community

increases or the availability of resources decreases. It might be interesting to conduct a study with treatments that independently manipulate community density and resource availability. Standard culture can be filtered to remove protist cells, but leave prokaryotes and nutrients at near-original concentration. Mixing the filtered media with an equal volume of standard media produces a treatment that has standard media concentration, but reduced community concentration. The success of the invader in this treatment, at different values of propagule pressure, can be compared to the species' success in standard culture to identify the effect of reduced community density. The success of the invader in media prepared with different quantities of protist pellet can also be compared to identify the effect of reduced resource abundance. A combined set of treatments that vary community density and resource abundance will allow the independent effects of reduced community concentration and reduced resource base to be identified cleanly and clearly. Repeating the experimental design with multiple invaders, and with a good number of replicates, would demonstrate the way resource availability and community concentration affect invasion.

In addition, it would be interesting to examine how the effect of propagule pressure varies in environments subjected to disturbance. I expect that propagule pressure may be less important to invasion success when there is intermediate environmental disturbance since disturbance may reduce community concentration, increasing resource availability. Extremely high levels of environmental disturbance may instead increase the importance of propagule

pressure, especially propagule frequency, since rescue effect may be more important in a harsh environment. The standard experimental design used in this project to investigate the role of propagule pressure on invasion success could be repeated in a series of environments that differ only in the degree of environmental disturbance.

Since this project includes only bacterivorous protist species that could all consume the prokaryotic resource base, experimental communities were structured by competition. Natural communities also include the influence of predation. Therefore, it is important to study the effect of propagule pressure on the success of invaders subjected to predation. I expect that high propagule pressure will be even more important in ensuring establishment and invasion success when the community includes a predator that targets the invader. Several predatory protists are known to consume the four bacterivorous species studied in this project. The standard experimental design used in this project to study the effect of propagule pressure on invasion could be repeated in multiple communities, each with a different established predator species. This would allow any general effect of predation to be separated from effects related to individual predators.

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APPENDIX: RESULTS OF STATISTICAL ANALYSIS

Figure	Predictor	χ^2 Value	Degrees of Freedom	P Value
2-2B	Log propagule size	39.706	1	< 0.01
2-3B	Log propagule size	19.104	1	< 0.01
2-4	Log propagule size	35.007	1	< 0.01
2-5	Log propagule size	18.804	1	< 0.01
2-6A	Log propagule size	41.704	1	< 0.01
2-6B	Log propagule size	41.366	1	< 0.01
2-7A	Log propagule size	13.158	1	< 0.01
2-7B	Log propagule size	7.009	1	< 0.01
2-8A	Log propagule size	35.237	1	< 0.01
2-8B	Log propagule size	41.362	1	< 0.01
2-9	Log propagule size	6.642	1	0.010
2-10	Log propagule size	76.905	1	< 0.01
2-11	Log propagule size	15.105	1	< 0.01
2-12	Log propagule size	11.677	1	0.001
2-13	Log propagule size	67.431	1	< 0.01
2-14	Log propagule size	7.732	1	0.005
2-15	Log propagule size	18.621	1	< 0.01
2-17	Propagule frequency	31.360	1	< 0.01
	Addition size	0.003	1	0.954
	Interaction	0.172	1	0.678
2-18	Propagule frequency	36.414	1	< 0.01
	Addition size	0.279	1	0.597
	Interaction	0.293	1	0.588
2-19	Propagule frequency	20.391	1	< 0.01
	Addition size	0.939	1	0.332
	Interaction	2.356	1	0.125
2-20	Propagule frequency	9.745	1	0.002
	Addition size	1.214	1	0.270
	Interaction	2.197	1	0.138
2-21A	Propagule frequency	27.826	1	< 0.01
	Addition size	0.101	1	0.750
	Interaction	0.172	1	0.678
2-21B	Propagule frequency	15.022	1	< 0.01
	Addition size	0.641	1	0.423
	Interaction	0.657	1	0.418

Figure	Predictor	χ^2 Value	Degrees of Freedom	P Value
2.22	Propagule frequency	10.276	1	0.001
	Addition size	0.829	1	0.362
	Interaction	1.553	1	0.213
2-23	Propagule frequency	8.544	1	0.003
	Addition size	0.910	1	0.340
	Interaction	8.544	1	0.003
2-25	Propagule frequency	11.790	1	0.001
	Addition size	2.621	1	0.105
	Interaction	4.273	1	0.039
2-26	Propagule frequency	4.009	1	0.045
	Addition size	0.098	1	0.745
	Interaction	0.017	1	0.897
2-27●	Propagule frequency	19.426	1	< 0.01
	Addition size	0.14	1	0.907
2-28	Propagule frequency	11.236	1	0.001
3-5A▼	Propagule size	0.929	1	0.345
	1-way ANOVA (df 3, 16; F = 2.563; P = 0.091)			
3-5B▼	Propagule size	1.207	1	0.272
	1-way ANOVA (df3, 16; F = 6.942, P = 0.003) Tukey post-hoc comparison (propagule size 25<100, P=0.002)			
3-6A	Propagule size	13.175	1	< 0.01
3-6B	Propagule size	0.188	1	0.664
	1-way ANOVA (df 3, 16; F = 2.705; P = 0.080)			
3-8A▼	Propagule size	0.597	1	0.440
	1-way ANOVA (df 4, 19; F = 4.860; P = 0.007) Tukey post-hoc comparison (propagule size 25 < 50 and 100; P≤0.027)			
3-8B▼	Prop. size	0.747	1	0.387
	1-way ANOVA (df 4, 19; F = 4.156, P = 0.014) Tukey post-hoc comparison (propagule size 25<100; P=0.033)			
3-9	Propagule size	20.898	1	< 0.01
3-10	Propagule size	1.273	1	0.259
	■ Independent regression analysis of propagule sizes 25, 50, and 100 using an exponential function ($\chi^2 = 279.101$, df = 1, P < 0.01)			
3-11A	Propagule size	7.004	1	0.008
3-11B	Propagule size	7.744	1	0.005

Figure	Predictor	χ^2 Value	Degrees of Freedom	P Value
3-13	Propagule size	12.277	1	< 0.01
3-14	Propagule size	8.181	1	0.004
3-15	Propagule size	5.767	1	0.016
3-16	Propagule size	5.934	1	0.015
3-18A	Propagule frequency	0.773	1	0.379
	Addition size	5.544	1	0.019
	Interaction	6.154	1	0.013
	Independent regression analysis using propagule frequency as the predictor for 25-cell additions ($\chi^2 = 4.134$, df = 1, P = 0.042) and 50-cell additions ($\chi^2 = 2.020$, df = 1, P = 0.155)			
3-18B	Propagule frequency	0.140	1	0.708
	Addition size	8.367	1	0.004
	Interaction	5.242	1	0.022
	Independent regression analysis using propagule frequency as the predictor for 25-cell additions ($\chi^2 = 1.518$, df = 1, P = 0.218) and 50-cell additions ($\chi^2 = 4.481$, df = 1, P = 0.034)			
3-20A	Propagule frequency	2.455	1	0.117
	Addition size	9.741	1	0.002
	Interaction	4.471	1	0.034
	Independent regression analysis using propagule frequency as the predictor for 25-cell additions ($\chi^2 = 0.192$, df = 1, P = 0.661) and 50-cell additions ($\chi^2 = 5.422$, df = 1, P = 0.020)			
3-20B	Propagule frequency	0.046	1	0.830
	Addition size	5.580	1	0.018
	Interaction	3.973	1	0.046
3-21	Propagule frequency	6.145	1	0.013
	Addition size	0.734	1	0.392
	Interaction	1.108	1	0.293
3-22	Propagule frequency	24.011	1	< 0.01
	Addition size	4.013	1	0.045
	Interaction	6.793	1	0.009
3-23A	Propagule frequency	2.411	1	0.121
	Addition size	0.679	1	0.410
	Interaction	0.368	1	0.544
	Independent regression analysis using propagule frequency as the predictor for 25-cell additions ($\chi^2 = 4.358$, df = 1, P = 0.037) and 50-cell additions ($\chi^2 = 0.306$, df = 1, P = 0.580)			

Figure	Predictor	χ^2 Value	Degrees of Freedom	P Value
3-23B	Propagule frequency	7.796	1	0.005
	Addition size	1.194	1	0.274
	Interaction	6.055	1	0.014
3-24	Propagule frequency	87.584	1	< 0.01
	Addition size	2.798	1	0.094
	Interaction	2.784	1	0.095
3-26A	Propagule frequency	8.286	1	0.004
	Addition size	1.367	1	0.242
	Interaction	3.734	1	0.053
3-26B	Propagule frequency	3.478	1	0.062
	Addition size	0.696	1	0.404
	Interaction	1.256	1	0.262
	Independent regression analysis using propagule frequency as the predictor for 25-cell additions ($\chi^2 = 0.465$, df = 1, P = 0.495) and 50-cell additions ($\chi^2 = 3.281$, df = 1, P = 0.070)			
3-27A■	Propagule size	5.694	1	0.017
3-27B	Propagule frequency	24.011	1	< 0.01
	Addition size	4.013	1	0.045
	Interaction	6.793	1	0.009
3-28A	Propagule size	8.181	1	0.004
3-28B	Propagule frequency	87.584	1	< 0.01
	Addition size	2.798	1	0.094
	Interaction	2.784	1	0.095
4-3A	Frequency/size	2.408	1	0.121
	Number of cells	6.635	1	0.010
	Interaction	7.639	1	0.006
	Independent regression analysis using frequency/size as the predictor for 50-cell comparison ($\chi^2 = 0.785$, df = 1, P = 0.376) and 100-cell comparison ($\chi^2 = 8.743$, df = 1, P = 0.003)			
4-3B	Frequency/size	4.934	1	0.026
	Number of cells	6.626	1	0.010
	Interaction	7.707	1	0.006
	Independent regression analysis using frequency/size as the predictor for 50-cell comparison ($\chi^2 = 0.151$, df = 1, P = 0.698) and 100-cell comparison ($\chi^2 = 13.051$, df = 1, P < 0.01)			
4-5A	Frequency/size	0.507	1	0.477
	1-way ANOVA (df 3, 15; F = 1.168, P = 0.355)			
4-5B	Frequency/size	1.589	1	0.208

Figure	Predictor	χ^2 Value	Degrees of Freedom	P Value
4-6A	Frequency/size	13.353	1	< 0.01
	Number of cells	0.005	1	0.941
	Interaction	0.074	1	0.786
4-6B	Frequency/size	8.355	1	0.004
	Number of cells	0.008	1	0.928
	Interaction	0.000	1	0.997
4-7	Frequency/size	11.200	1	0.001
4-8A	Frequency/size	0.326	1	0.568
	Kruskal-Wallis 1-way ANOVA on ranks (H = 3.278, df = 3, P = 0.351)			
4-8B	Frequency/size	2.845	1	0.92
4-9	Frequency/size	1.267	1	0.260
	Chi-square test of data ($\chi^2 = 4.560$, df = 3, P = 0.207)			
4-10A	Frequency/size	0.463	1	0.496
	1-way ANOVA (df 3, 15; F = 2.533; P = 0.096)			
4-10B ▼	Frequency/size	0.067	1	0.796
	1-way ANOVA (df 3, 14; G = 7.055; P = 0.004)			
	Tukey post-hoc comparisons (2x(150) > 1x(300) and 6x(50), P ≤ 0.078; 3x(100) > 1x(300), P = 0.026).			
5-3	Invader concentration	105.24	1	<0.01
5-4A	Invader concentration	13.499	1	<0.01
5-4B	Invader concentration	12.424	1	<0.01
5-5A	Invader concentration	4.228	1	0.040
5-5B	Invader concentration	2.249	1	0.134
5-6 ▼	Propagule size	90.631	1	<0.01
	1-way ANOVA (df 4, 18; F = 37.555; P < 0.001) Tukey post-hoc comparisons (concentration 0 < 73, 99, 106, and 128, P < 0.001).			
5-7A	Propagule size	7.241	1	0.007
5-7B	Invader concentration	57.549	1	<0.01
5-8	Invader concentration	23.520	1	<0.01
5-9A	Invader concentration	1.498	1	0.221
5-9B	Propagule size	0.024	1	0.878
5-10	Propagule frequency	7.847	1	0.005
	Addition size	0.495	1	0.482
	Interaction	0.333	1	0.564
5-11A	Propagule frequency	25.484	1	<0.01
	Addition size	0.017	1	0.897
	Interaction	0.084	1	0.772

Figure	Predictor	χ^2 Value	Degrees of Freedom	P Value
5-11B	Propagule frequency	0.314	1	0.575
	Addition size	1.047	1	0.306
	Interaction	0.641	1	0.423
5-12A	Propagule frequency	16.891	1	<0.01
	Addition size	1.176	1	0.278
	Interaction	1.658	1	0.198
5-12B	Propagule frequency	11.227	1	0.001
	Addition size	0.026	1	0.871
	Interaction	0.258	1	0.612
5-13	Propagule frequency	0.604	1	0.437
	Addition size	0.000	1	0.988
	Interaction	0.015	1	0.901
	Independent Chi-Square analysis of the 25-cell addition treatments ($\chi^2 = 2.205$; df = 3; P = 0.531) and 50-cell addition treatments ($\chi^2 = 8.800$; df = 3; P = 0.032).			
5-14B	Propagule size	18.765	1	<0.01
5-15	Propagule size	4.463	1	0.035
5-16A	Propagule size	9.554	1	0.002
5-16B	Propagule size	0.141	1	0.708
5-17A	Propagule size	10.178	1	0.001
5-17B	Propagule size	10.101	1	0.001
5-18A	Propagule size	15.002	1	< 0.01
5-18B	Propagule size	12.172	1	< 0.01
5-19A	Propagule size	0.014	1	0.907
5-19B	Propagule size	0.534	1	0.465
5-20	Realized growth rate	11.493	1	0.001
5-21	Realized growth rate	4.234	1	0.040

Statistical Analysis.

Propagule Size. To determine whether propagule size influenced cell concentration, diversity, species richness, or species evenness, values of the dependent variable were compared across propagule size treatments with regression analysis using SPSS Statistics 17.0 software (SPSS, Inc., 2008). Propagule size values, transformed as log base 2, and measured dependent variable data were both identified as numeric scale measures. A generalized linear model used propagule size as the predictor and the different variables as the response to fit a linear curve to the data set. A Wald's Chi-Square test determined the significance of the relationship.

Propagule Frequency. To determine whether propagule frequency influenced values of the dependent variable, cell concentration, diversity, species richness, and species evenness data were compared across propagule frequency treatments with ANCOVA using SPSS Statistics 17.0 software (SPSS, Inc., 2008). Values of propagule number and measured dependent variable data were both identified as numeric scale measures. The categorical data of addition size was identified as a factor. A generalized linear model used propagule frequency data as the covariate predictor, addition size as the factor, and the dependent variable data as the response to fit a linear curve. The model considered the main effects of propagule frequency and addition size, as well as the interaction of propagule frequency and addition size, on the values of the dependent variable. A Wald's Chi-Square test determined the significance of the factor and covariate.

Exceptions

- No interaction term used in ANCOVA

In one case, Figure 2-27, an insignificant interaction term hindered the ability of the model to identify the significance of the main effects. The model was run without the interaction term for this data set.

- Exponential function used in regression analysis

In two cases, Figures 3-10 and 3-27, the y-intercept of the linear curve was negative. A generalized linear model using propagule size as the predictor and probability of stochastic extinction as the response fit an exponential curve with Normal errors to the data set.

- ▼ ANOVA used instead of ANCOVA

For two experimental species, *Tillina magna* and *Paramecium bursaria*, the relationship between increasing invader propagule size and cell concentration, and between propagule size and proportion, was unexpectedly Gaussian. Regression analysis could not be used on these data sets. Treatments were instead compared with one-way ANOVA followed by Tukey post-hoc comparisons using SigmaPlot for Windows 11.00 (Systat Software, 2008).

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