

UNIVERSITY OF CALGARY

Comparing Methods of Quantifying Coexistence Using Protist Microcosms

by

Katherine Flora Badwey

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF MASTER OF SCIENCE

GRADUATE PROGRAM IN BIOLOGICAL SCIENCES

CALGARY, ALBERTA

FEBRUARY, 2026

© Katherine Flora Badwey 2026

## **Abstract**

Species in a community must be able to increase from rarity for coexistence to be possible. It is possible to quantify this ability, and thus the strength of coexistence, by estimating invasion growth rates. This is typically done by fitting a mathematical model to the community. However, modeling is far from a simple task, and resulting estimates can depend upon model choice. A more direct method, one that bypasses the need to fit a model, could be a better option. Using protist microcosms, I conducted numerous small-scale invasion assays to estimate invasion growth rates directly. I compared these direct estimates with those I obtained by fitting a model. I obtained quantitatively different results depending on the method used. This may be due to differences in spatial environment as the culture vessels used for invasions were much smaller than those used for the long-term competition experiment. Modifications to this method of direct invasion could be made to obtain more comparable estimates and to further investigate the importance of spatial variables in protist microcosms.

## **Preface**

This thesis is original, unpublished, independent work by the author, Katherine Flora Badwey.

## Table of Contents

Abstract	ii
Preface	iii
Table of Contents	iv
List of Figures	vi
CHAPTER 1: INTRODUCTION	1
1.1 Defining and Measuring Coexistence	1
1.2 Coexistence Mechanisms	5
1.3 Challenges of Empirical Analysis	7
1.4 Invasive Species and Invasibility	9
1.5 Protists as Model Organisms	12
1.6 Study Overview and Objectives	13
CHAPTER 2: ASSESSING THE EFFECTS OF COMMUNITY COMPOSITION AND DIFFERENT RESOURCE LEVELS ON COEXISTENCE	14
2.1 Introduction	14
2.2 Methods	16
2.2.1 Study Species	16
2.2.2 Microcosm Methods	17
2.2.3 Microcosm Sampling	20
2.2.4 Analyses	20
2.3 Results	23
2.3.1 Time Series and Population Dynamics	23
2.3.2 Three-Species Treatments	26
2.3.3 Modeling Results	34
2.4 Discussion	40
2.4.1 Interpreting the Estimates	40

2.4.2 Data Features Affecting Model Fit	42
2.4.3 Future Directions and Modeling Approaches	45
2.5 Conclusions	46
CHAPTER 3: COMPARING METHODS OF ESTIMATING INVASION GROWTH RATE	47
3.1 Introduction	47
3.2 Methods	49
3.2.1 Study Species and Microcosm Methods	49
3.2.2 Microcosm Sampling	49
3.2.3 Invasion and Census	50
3.2.4 Analyses	52
3.3 Results	55
3.3.1 Colpidium Invasion Results	55
3.3.2 Tetrahymena Invasion Results	58
3.4 Discussion	60
3.5 Conclusions	64
CHAPTER 4: CONCLUSIONS	65
4.1 Summary	65
4.2 Value of Direct Invasion	66
4.3 Future Directions	67
REFERENCES	72

## List of Figures

Plate 2.1: Representative images of <i>Euplotes patella</i> , <i>Colpidium striatum</i> , and <i>Tetrahymena pyriformis</i> taken via fluid imaging-----	17
Figure 2.1: A graphical representation of the experimental design-----	19
Figure 2.2: <i>Tetrahymena</i> grown alone in 0.4 g/L -----	24
Figure 2.3: <i>Tetrahymena</i> and <i>Colpidium</i> grown together in 0.4 g/L -----	24
Figure 2.4: <i>Tetrahymena</i> and <i>Euplotes</i> grown together in 0.4 g/L -----	25
Figure 2.5: <i>Colpidium</i> grown alone in 0.4 g/L -----	26
Figure 2.6: Box plot of time averaged <i>Colpidium</i> density for each resource level-----	29
Figure 2.7: <i>Tetrahymena</i> , <i>Colpidium</i> , and <i>Euplotes</i> grown in 1.0 g/L -----	29
Figure 2.8: Box plot of time averaged <i>Colpidium</i> density for each species combination-----	30
Figure 2.9: <i>Tetrahymena</i> , <i>Colpidium</i> , and <i>Euplotes</i> grown in 0.7 g/L -----	30
Figure 2.10: <i>Tetrahymena</i> , <i>Colpidium</i> , and <i>Euplotes</i> grown in 0.4 g/L -----	31
Figure 2.11: Box plot of time averaged <i>Tetrahymena</i> density for each species combination -----	31
Figure 2.12: Box plot of maximum <i>Tetrahymena</i> density for each resource level-----	32
Figure 2.13: Box plot of time averaged <i>Tetrahymena</i> density for each resource level-----	32
Figure 2.14: <i>Tetrahymena</i> , <i>Colpidium</i> , and <i>Euplotes</i> grown in 0.1 g/L -----	33
Figure 2.15: Linear regression between maximum <i>Euplotes</i> density and resource level -----	33
Figure 2.16: Linear regression between <i>Euplotes</i> cycle period and resource level -----	34
Figure 2.17: Lotka-Volterra competition model fit to time series of <i>Colpidium</i> and <i>Tetrahymena</i> grown in 0.4 g/L -----	35
Figure 2.18: Modified Lotka-Volterra competition model with decaying carrying capacity fit to time series of <i>Colpidium</i> and <i>Tetrahymena</i> grown in 0.4 g/L-----	36
Figure 2.19: Model fit for three-species microcosms at 0.4 g/L-----	38
Figure 2.20: Model fit for three-species microcosms at 0.1 g/L-----	39
Figure 2.21: Model fit for three-species microcosms at 0.7 g/L-----	39
Figure 2.22: Model fit for three-species microcosms at 1.0 g/L-----	40
Figure 3.1: A graphical representation of the species combinations used-----	50
Figure 3.2: Graphic showing experimental design of direct invasions -----	51

Figure 3.3: Per-capita growth rates of <i>Colpidium</i> invading either <i>Tetrahymena</i> alone or <i>Tetrahymena</i> and <i>Euplotes</i> -----	57
Figure 3.4: : Comparison of the invasion growth rate estimates of <i>Colpidium</i> invading <i>Tetrahymena</i> -----	58
Figure 3.5: Per-capita growth rates of <i>Tetrahymena</i> invading <i>Colpidium</i> -----	59
Figure 3.6: Comparison of the invasion growth rate estimates of <i>Tetrahymena</i> invading <i>Colpidium</i> -----	60

## CHAPTER 1: INTRODUCTION

Think of an open, grassland habitat. Even though we might not be able to easily tell them all apart, there are vast numbers of plant species living together in this one location. Each of these species requires similar resources to survive like sunlight, water, and soil nutrients. Despite all competing for the same thing, they can persist for long periods of time. Something similar is happening in all types of habitats and nearly everywhere you look. Attempting to understand how such a wide diversity of species can persist in the same environment is one of the primary goals in community ecology. Over the years and as our understanding of communities has improved, extensive theory has been developed to better answer this question of how species are able to coexist.

### 1.1 Defining and Measuring Coexistence

In ecology, “coexistence” means more than species existing at the same time and in the same place as each other. Species can co-occur for long periods of time, but over a long enough timespan, one species might eventually become extinct. Thus, a stricter definition is needed to differentiate coexistence from co-occurrence. To be considered as coexistence, each species in a community must be able to increase from low density (Chesson 2000a). Without the ability to increase from being rare, a species will die out when perturbed to low densities and will not be able to increase in density when entering a new community.

In order to increase from rare, species typically require negatively density-dependent per-capita growth rates (Nicholson 1954). This means their growth rate is faster when at low density and slower when at high density. Without this, species would either grow infinitely, which is not possible, or decline to extinction. In nature, species are most often found in communities alongside competitors. Under the effects of competition, negative *frequency*-dependence is also

necessary to increase from rare. Density-dependence is only influenced by the absolute abundance of a species, such as in competition coefficients which measure the effect each individual of one species has on the per-capita growth rate of itself or another species. However, frequency-dependence involves the *relative* abundance of one species compared to the total number of individuals in the community. In this case, both the effects of competition and the relative abundance of conspecifics versus heterospecifics are important. It is in this way that negative frequency-dependence arises indirectly from negative density-dependence (Yenni et al. 2017; Rovere and Fox 2019). For example, if species A has an unknown abundance, and every conspecific decreases the per-capita growth rate of species A by 0.1, whether species A has 2 or 2000 individuals makes a large difference in its growth rate (frequency-dependence). However, the growth rate of species A remains dependent on its own abundance regardless of how many individuals there are (density-dependence).

Negatively frequency-dependent per-capita growth rates go hand in hand with intraspecific competition exceeding interspecific competition (Rovere and Fox 2019), which is a requirement for stable coexistence (Chesson 2000b). When a species limits itself more than it limits other species, frequencies are regulated at both extremes. In this situation, a species at too high of a frequency will be heavily competing with itself, resulting in a decrease in frequency while also allowing other species to increase from low frequencies. At the same time, those species at low frequencies will be able to increase due to the decreased amount of both inter- and intraspecific competition. If the opposite is true, and interspecific competition is greater, then a species will be competitively excluded and unable to coexist in the community.

Coexistence is commonly investigated using the mutual invasibility criterion. The mutual invasibility criterion says that for coexistence to occur, each species in a community must be able

to increase from very low density with all other species at their equilibrium densities (Turelli 1978; Chesson 2000b). This criterion is applied to communities by determining each species' invasion growth rate. The invasion growth rate is a value that describes how rapidly a species can recover from low density and also provides information on the strength of coexistence (Chesson 2008). If this value is positive, coexistence can occur. If this value is negative, the species will instead be competitively excluded. Invasion growth rates are mathematically defined as the average growth rate over time when a species is rare. An average is necessary to capture the variation in environmental conditions and species densities across time and space. A species may have negative invasion growth rates under certain conditions, but so long as this value is positive on average, they are expected to be able to increase from rare. This value is most commonly obtained by fitting a mathematical model to a time series of community data. It is also possible to calculate invasion growth rates by conducting empirical experiments on mutual invasion, where a pair of species is grown alone and while invading the other species, but this is done much less often.

When discussing coexistence and the invasibility criterion, "invader" is the term used for the species that is entering the community at a low density and does not necessarily indicate an invasive species. Conversely, "resident" is the term used for any species already present in the community. Invasibility is measured when the invader is at a very low density, basically zero, while all resident species are at their equilibrium densities. The invader must be at as low a density as possible when invasion growth rates are determined so that the invader's density is not large enough to affect the resident community, and intraspecific competition is minimized. If the residents do not remain at a stable density, such as with predator-prey cycling, then invasibility is instead measured when the community is following its usual dynamics in the absence of the

invading species. As invasion growth rate is calculated as an average, this value is capable of capturing information from multiple points in time, such as in a resident community with fluctuating densities.

Meeting the invasibility criterion or having a positive invasion growth rate is a strong indication of coexistence, but coexistence is possible without mutual invasibility (Chesson and Ellner 1989; Chesson 2018). Invasion growth rates are a good predictor of coexistence for pairs of species, but issues arise in larger communities or those with alternative stable states. One assumption of the mutual invasibility criterion is that each  $N - 1$  sub-community exists, where  $N$  is the number of species in the final community of interest (Saavedra et al. 2017). However, there are situations when a community of species appears to coexist, but some species are unable to survive in or invade one or more  $N - 1$  sub-communities (Spaak and Schreiber 2023). For example, if a prey species is removed from the final community, that species' predator will not be able to persist due to its missing food source. Similarly, if a trio of species exhibit intransitive (“rock-paper-scissors”) competition where there is no single species that is always competitively dominant, removing one species will change the dynamics of the remaining two such that one will persist, and the other will be excluded (May and Leonard 1975; Hofbauer and Schreiber 2022). Species with Allee effects are also unable to be investigated with the invasibility criterion as their positive density-dependence does not allow them to increase from low densities (Barabás et al. 2018; but see Walker and Gilbert 2025).

Although invasion growth rates have been subject to criticism (Pande et al. 2020), they are still able provide useful information. Meeting the mutual invasibility criterion implies coexistence in the two-species case and in simpler multispecies cases (Armstrong and McGehee 1980; Chesson and Ellner 1989; Chesson 2018), but in more complicated communities, invasion

growth rates can still be useful in deriving a more appropriate multispecies measure (e.g., Chesson 2018 community average approach). Other criterion, such as permanence theory which requires the density of each species to exceed and remain above some positive constant, are much stricter, more complicated, and do not provide as useful of results (Chesson 2018). Additionally, positive invasion growth rates mean there is *some* coexistence mechanism at play, even without knowing what that mechanism is. Invasion growth rates can then be partitioned into different coexistence mechanisms using counterfactuals, “what if” scenarios that look at what the community dynamics might look like under different circumstances or without certain mechanisms. There are two different methods of accomplishing this (Chesson 1994; Ellner et al. 2019), but both involve breaking up the invasion growth rate into environmental and competitive features that contribute to it and then comparing differences in each term between the invader and the residents. This allows us to quantify and determine the importance of each mechanism, rather than only being able to show the presence or absence of a mechanism.

## **1.2 Coexistence Mechanisms**

Coexistence can be achieved by a variety of different mechanisms. Any mechanism that either helps the rare species or harms the common ones can promote coexistence. These mechanisms can be divided into two conceptual categories: equalizing and stabilizing. Equalizing mechanisms are those that decrease the fitness inequality between two species which reduces the strength of stabilizing mechanisms necessary for coexistence, while stabilizing mechanisms are those that increase intraspecific competition relative to interspecific competition such that a species limits itself more than it limits its competitors (Chesson 2000b; Adler et al. 2007). On their own, equalizing mechanisms cannot lead to coexistence and will only slow the

rate of competitive exclusion, but together with stabilizing mechanisms they can result in coexistence.

Mechanisms affecting coexistence can be further split into fluctuation-independent and -dependent groupings (Chesson 2000b). A fluctuation can be any reoccurring change in the environment over time or space such as cycling predator abundances, changes in weather, or fluctuating resource availability. Fluctuation-*independent* mechanisms, such as resource partitioning, can still occur in the presence of fluctuations but are not reliant on fluctuations to operate. Using resource partitioning as an example, a species that preferentially consumes one resource will still prefer to utilize that resource regardless of changes in its availability. Fluctuation-*dependent* mechanisms rely on fluctuations in the community or the environment and will not function otherwise.

Fluctuation-dependent mechanisms show how trade-offs or other species-specific responses can enable coexistence when competing species experience beneficial and poor conditions at different times by creating opportunities for niche differentiation (Chesson and Huntly 1997; Chesson 2000a). For temporal fluctuations, fluctuation-dependent mechanisms can be summarized by relative nonlinearity of competition and the storage effect. These broad categories also have spatial analogues. Relative nonlinearity describes how species have different responses in their per-capita growth rate to limiting factors, like resources or predators, which can be quantified by comparing how nonlinear one species' growth function is compared to another (Chesson 1994). The main idea is that species-specific responses can result in the species with higher fitness without fluctuations being put at a disadvantage in the presence of fluctuations. The storage effect can occur when species who have differential responses to the environment also exhibit buffered population growth and covariance between the environment

and competition (Chesson 2000b). Environment-competition covariance concentrates intraspecific competition such that it is strongest when the environment is benefiting a species, while buffered population growth (e.g., seed banks or long-lived adults) limits the impact of competition when environmental conditions are unfavorable. With the storage effect, a species will increase to high density under good conditions and become limited by competition, and poor conditions will allow a species to persist due to lower competition. Thus, these three conditions combine to generate negative-frequency dependence and promote coexistence.

### **1.3 Challenges of Empirical Analysis**

Although the tools to study coexistence are available, it is still a difficult task. Current methods are easier to apply to some species than others. A majority of empirical coexistence work has been done on annual plants or aquatic microcosms (Holyoak and Lawler 2005; Siepielski and McPeck 2010). These groups of organisms have relatively fast generation times, known population dynamics, and are easy to count. Thus, obtaining the data necessary to calculate invasion growth rates is more straight-forward. In longer-lived species, the length of time a population would need to be monitored to get enough data makes empirical study of coexistence impractical.

Further challenges may arise when translating theory to application. Many claims of coexistence in the literature are not properly testing for it. In a literature review by Siepielski and McPeck (2010), 78% of the 323 studies identified did not report any evidence to support claims of coexistence, and only 7 studies (0.02%) assessed invasibility in any way. Whether this is due to a more complicated theory, ignorance of potential methods and applications (Grainger, Levine, et al. 2019), or a consequence of the large amount of data necessary to apply the theory

(HilleRisLambers et al. 2012), the lack of empirical application and quantitative analysis signals a difficulty in adopting and implementing coexistence theory.

The most used method of calculating invasion growth rates and quantifying coexistence involves fitting a mathematical model to the community of interest. Predictions from the models used to study coexistence are susceptible to uncertainty from a variety of sources such as the environment data was collected in, biological variation among individuals, or the model itself (Bowler et al. 2022; Cervantes-Loreto et al. 2023). This can result in different functional forms of models that describe the same ecological process producing qualitatively different predictions despite fitting the data equally well (Cordoleani et al. 2011). An example of this in coexistence research comes from Cervantes-Loreto et al. (2023) who tested how using different models and accounting for different sources of uncertainty affected the final predictions of whether two species could coexist. They found that the experimental data gave different predictions depending on which model was used even though both models described the same process with parameters that were interpreted the same way. Additionally, Bowler et al (2022) found that propagating uncertainty affected predictions of coexistence for 3 out of 14 species pairs studied. However, not all studies of coexistence apply the same amount of statistical rigor, and uncertainty is not often propagated through to the final coexistence prediction along with model validation being rare (Terry and Armitage 2024).

As mentioned briefly in Section 1.1, invasion growth rates are more complicated to apply to multispecies cases. There are a variety of situations where all possible subcommunities might be unable to persist, despite the full community coexisting. As a result, a large amount of coexistence research only looks at two species at a time (Siepielski and McPeck 2010). Natural communities have many more than two species at a time, so the inability to quantify coexistence

with greater numbers of species is a definite drawback. However, recent work has been expanding theory and developing methods more applicable to multispecies studies (e.g., Barabás et al. 2016; Saavedra et al. 2017; Spaak and Schreiber 2023).

#### **1.4 Invasive Species and Invasibility**

Coexistence and invasion are important not only to understand how species can persist in the same community, but also in learning how to prevent harmful species from becoming established (Vitousek et al. 1997). Thus, a lot of empirical work has been done to investigate what factors make invasion more likely, although the primary goals did not involve coexistence theory or invasion growth rates. This work has been conducted under the umbrellas of community assembly, invasion ecology, and conservation just to name a few (Hobbs and Huenneke 1992; Vitousek et al. 1997; Law et al. 2000). These data have been collected through invader addition studies like seed addition experiments where a fixed number of propagules are added to a resident community, spatial pattern studies that record the presence and absence of non-native species and correlate them with environmental variables, and experiments with constructed communities where both species composition and the environment can be controlled and manipulated (Levine and D'Antonio 1999).

No single trait, nor a specific set of traits, is associated with invasiveness across all species (Richardson and Pyšek 2006). In fact, the performance of most invaders is context-dependent (Law et al. 2000; Daehler 2003). This means that the inability to invade might be due to the environmental conditions during the study. Thus, invasion, and therefore coexistence could still be possible under different conditions (Clark et al. 2025).

While there is no widespread pattern affecting invasibility at the species level, there are some general factors at the community level that can influence invasion outcomes: competition,

the amount and frequency of invading individuals, disturbance, and resource supply (Levine and D'Antonio 1999; Richardson and Pyšek 2006). The presence of a predator or species that compete with an invader can decrease invasion success (Levine et al. 2004; Thomaz et al. 2015), though successful invasive species are often better competitors than residents (Vilà and Weiner 2004). When a large number of individuals invade a community, the chances of the species becoming established are greater than if only a few individuals were to invade (Levine and D'Antonio 1999; Richardson and Pyšek 2006). A disturbance is any event that affects community structure and changes resource availability or the physical environment (Pickett and White 1985). Disturbances can increase the invasibility of a community by providing a “window of opportunity” for invaders (Hobbs and Huenneke 1992), but invaders may not be able to persist long-term (Flory et al. 2017). Disturbances that result in higher resource availability can increase invasibility even more, especially when looking across larger spatial scales (Richardson and Pyšek 2006; Liu et al. 2012; Thomaz et al. 2015). In fact, short fluctuations in resource availability can have long-term impacts on invasion outcomes (Davis and Pelsor 2001; Richardson and Pyšek 2006).

Among empirical work, findings are mixed as to whether species-rich communities are more or less invulnerable (Levine and D'Antonio 1999; Law et al. 2000; Richardson and Pyšek 2006). In general, a negative correlation between diversity and invasibility is found in experimental manipulations and small spatial scales where competition with residents is more important, while observational studies done across broader scales show a positive relationship (Levine and D'Antonio 1999; Richardson and Pyšek 2006; Thomaz et al. 2015). It is not that competition gets weaker at larger spatial scales, but averaging over more abiotic variation ends up swamping the effects of these local interactions (Levine and D'Antonio 1999). The positive

diversity-invasibility relationship is also in part due to favorable biotic and abiotic factors that covary with increased diversity such as nutrients, competitors, and habitat heterogeneity (Levine and D'Antonio 1999; Sakai et al. 2001). Intuitively, aspects of the environment that are beneficial for residents will be beneficial for invaders too. More variable or heterogeneous habitats are more invasible due to the increased likelihood that an invader can find suitable habitat (Lonsdale 1999; Richardson and Pyšek 2006).

While a lot of the previously cited work on invasibility is referring to terrestrial plants, there is evidence that the general patterns are the same in other systems (e.g., Kneitel and Perrault 2006; Liu et al. 2012; Thomaz et al. 2015). Both observational and experimental studies on tropical macrophytes have found the same factors that influence invasion success as in terrestrial plant studies, although additional abiotic filters unique to aquatic environments are also important to invasibility for these species (Thomaz et al. 2015). Studies using microorganisms typically involve constructed communities and have investigated invasibility by manipulating invasion frequency, disturbance, community composition, and resource availability (McGrady-Steed et al. 1997; Kneitel and Perrault 2006; Liu et al. 2012; Mallon et al. 2015). Invasion studies on birds and mammals are primarily observational, correlating presence of invaders with environmental variables or the frequency of invasion attempts (Smallwood 1994; Kolar and Lodge 2001). Across this wide range of taxa, invasion frequency, disturbance, and resource availability increase invasion success while competition and predation decrease invasion success just as they do for terrestrial plants. Thus, the factors affecting invasibility should still apply whether the species under study are microscopic, macroscopic, terrestrial, or aquatic.

The literature on invasibility provides us with some statistical predictors as to whether invasions will happen. However, these studies often do not estimate metrics like long-term low density growth rates due to their goal of identifying correlates of invasions. Therefore, they are unable to make predictions about coexistence and whether these invasions will persist in the long-term. Some studies may have additionally been too short-term to address questions of coexistence due to the large amount of data that would be necessary (Adler et al. 2007; Siepielski and McPeck 2010). These studies can only tell us whether an invader can increase in density short-term under a specific set of conditions. To know whether the invader slowly declines or is able to persist after this short “window of opportunity”, we need longer-term data that spans across multiple generations (Siepielski and McPeck 2010).

### **1.5 Protists as Model Organisms**

Protists are commonly used as model systems in research across many biological disciplines, including ecology (Corliss 1954; Holyoak and Lawler 2005). Their small size (<200  $\mu\text{m}$  in length for the free-swimming ciliates in my experiments) makes them easy to handle and manipulate, yet they are large enough to reliably count and observe under a microscope. Many species can be easily grown in a laboratory environment (Altermatt et al. 2015), making them even more appealing as study organisms. Protist microcosms allow for a high degree of experimental control and replicability, making them an ideal system for coexistence related experiments (Altermatt et al. 2015). A microcosm is a microbial community contained in a small vessel, such as a jar or flask. Much like scale model airplanes can be used to investigate engineering, microcosms can be used as a model system to answer ecological questions. Just within the discipline of ecology, protists have been used to empirically test foundational theory for nearly a century (Gause 1934; reviewed by Holyoak and Lawler 2005). In addition to their

size, protists' short generation times (on the scale of hours) allow for a large amount of data to be collected in a relatively short period of time (Fenchel 1987). Multi-generational experiments that accurately capture community dynamics can be conducted in a few weeks instead of taking multiple years. With asexually reproducing species, we can assume there is minimal genetic diversity, and that any results are likely due to environmental factors rather than genetic ones – a variable that would otherwise be impossible to control for.

## **1.6 Study Overview and Objectives**

In my study, I used protist microcosms to investigate two aspects of species coexistence with a focus on invasion growth rates. First, I looked at how changes in the environment affected the invasion growth rates of two competing species. The microcosms for these experiments were modified in two ways: the addition of a predator and modifying the amount of resources available. I then fit models to these communities and used model parameters to calculate invasion growth rates for the competitors to compare across each treatment. By using invasion growth rates to quantify coexistence, I was able to compare the strength of coexistence based on how positive or negative the value was for each species. Second, I conducted hundreds of small-scale invasions to estimate invasion growth rates directly. Instead of fitting a model and estimating invasion growth rates in that way, I “cut out the middleman” with the hope that these direct measurements would have fewer and smaller sources of error than model-based estimates. Mutual invasion experiments have been done before (e.g., Narwani et al. 2013; Grainger, Letten, et al. 2019; Terry et al. 2021), but to my knowledge none have tried to emulate the conditions for invasion growth rates by adding only one individual to a community of competitors. This novel method was then compared to the estimates obtained from model fitting to assess whether the two methods provided similar results.

## CHAPTER 2: ASSESSING THE EFFECTS OF COMMUNITY COMPOSITION AND DIFFERENT RESOURCE LEVELS ON COEXISTENCE

### 2.1 Introduction

Predation can modify the effects of interspecific competition and promote either of coexistence or competitive exclusion when competition alone would not (Holt et al. 1994; Chesson and Kuang 2008). Competition and predation tend to undermine each other such that the interaction with the greater ability to regulate density-dependence will have the dominant effect (Chesson and Kuang 2008). If the stronger interaction would normally result in competitive exclusion on its own, then competitive exclusion will still be the outcome with both together. For example, Balciunas and Lawler (1995) found that the addition of a predator to protist microcosms containing *Chilomonas paramecium* and *Colpidium striatum* resulted in the exclusion of *Chilomonas* despite being able to coexist with *Colpidium* in the absence of the predator. However, coexistence can occur if there is partitioning of the stronger interaction whether it is competition or predation (Chesson and Kuang 2008).

If predation has more influence on growth rates of the focal species, then predator partitioning, where predators utilize prey species in different ways or show preferences in prey, can promote coexistence. This can be seen in Lawler's (1993) protist microcosm study where *Chilomonas* was unable to persist alone with a predator *Actinosphaerium* sp., but the addition of a competitor *Tetrahymena* sp. enabled all three species to coexist. If competition is stronger than predation, then resource partitioning needs to occur for coexistence. For example, wolves were found to reduce niche overlap in two competing prey species due to differing anti-predator behaviors, one species avoids encounters entirely and the other species flees from them, thus promoting coexistence by weakening apparent competition via spatial partitioning (Sommers and

Chesson 2019; Dellinger et al. 2022). Additionally, interactive partitioning of both resources and predators can make coexistence more likely (Chesson 2018). If species have different preferred habitats and are additionally limited by species-specific natural enemies within their respective preferred habitats, then coexistence is more likely than if there was only resource partitioning (Stump and Chesson 2015).

Another situation that can result in coexistence is when one species is more limited by predation and its competitor is more limited by resources. The combination of predation and competition creates more opportunities for niche differentiation than with competition alone (Chesson and Huntly 1997). Thus, coexistence is possible if there is a trade-off between sensitivity to predation and sensitivity to resource abundances (Chesson and Kuang 2008). This occurs in grasslands where herbivory can increase plant diversity by increasing light availability (Borer et al. 2014). Coexistence could also occur in a scenario with two competitors and a specialized predator that only consumes one species, such that each competitor is most limited by a different interaction (Chase et al. 2002).

Protists are commonly used as study organisms when investigating aspects of competition and predation (reviewed in Holyoak and Lawler 2005). Communities of protists have been used to test the predictions of competition models (Fox 2002), investigate community assembly rules and apparent competition (Lawler 1993; Weatherby et al. 1998), study predator diet breadth (Jiang and Morin 2005), and assess how community composition influences population dynamics across trophic levels (Lawler and Morin 1993; Balciunas and Lawler 1995). Using a similar system of protist microcosms, I focused on viewing the community through the lens of coexistence theory to provide new and complimentary information to what is already known from using these organisms in ecological research.

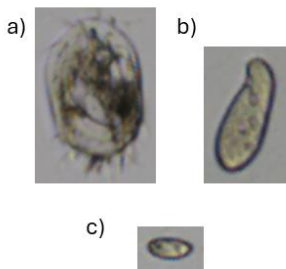
Much of research surrounding coexistence primarily focuses on competition (Grainger, Levine, et al. 2019), and experiments containing both competition and predation are primarily focused on just one of the two interactions. Because of this, I wanted to investigate how the interaction between predation and competition might affect coexistence. Using a model community consisting of two competing and one predator protist species, I quantified coexistence of the two competitors using invasion growth rates. I additionally assessed how population dynamics changed between two-species microcosms and the complete three-species community. The two-species microcosms provided information on either competition or predation on their own while the three-species microcosms included both interactions. Additionally, a number of previous microcosm studies have been conducted across resource gradients (Fox 2002; Jiang and Morin 2005; Laan and Fox 2020), although there is typically a focus on only one of either competition or predation. For this reason, I additionally looked at how the combined effects of these interactions changed across a resource gradient for the three-species protist community.

## **2.2 Methods**

### **2.2.1 Study Species**

The species used in my experiments were the ciliate protists *Tetrahymena pyriformis*, *Colpidium striatum*, and *Euplotes patella* (Plate 2.1). Ciliates are covered in large amounts of hair-like protrusions, cilia, that allow them to swim through water. All three study organisms are unicellular, free-living, freshwater species that reproduce asexually. *Tetrahymena* and *Colpidium* are competitors and feed on various species of bacteria. *Euplotes* is a predator of *Tetrahymena* and cannot survive on bacteria alone. There is evidence of *Euplotes* species consuming *Colpidium* (Balciunas and Lawler 1995; Jiang and Morin 2005), but I was unable to successfully

culture them in two-species microcosms for my study. All three species are filter-feeders, generating water currents to draw in suspended food particles or prey (Fenchel 1987). The smallest, *Tetrahymena*, has an area-based diameter (ABD, calculated as the diameter of a circle with an area equal to that of the organism) of 28  $\mu\text{m}$ , *Colpidium* has an ABD of 50  $\mu\text{m}$ , and *Euplotes* is the largest with an ABD of 100  $\mu\text{m}$  (values are approximate averages from unpublished fluid imaging data). Their approximate generation times, under conditions of optimal temperature and high per-capita food availability, are 3-6 hours (*Tetrahymena*; (Hill 2014; Laan and Fox 2020)), 6-12 hours (*Colpidium*; (Jiang and Morin 2007)), and 24-48 hours (*Euplotes*; (Laan and Fox 2020)).



**Plate 2.1:** Representative images demonstrating the relative scale of (a) *Euplotes patella*, (b) *Colpidium striatum*, and (c) *Tetrahymena pyriformis* taken via fluid imaging.

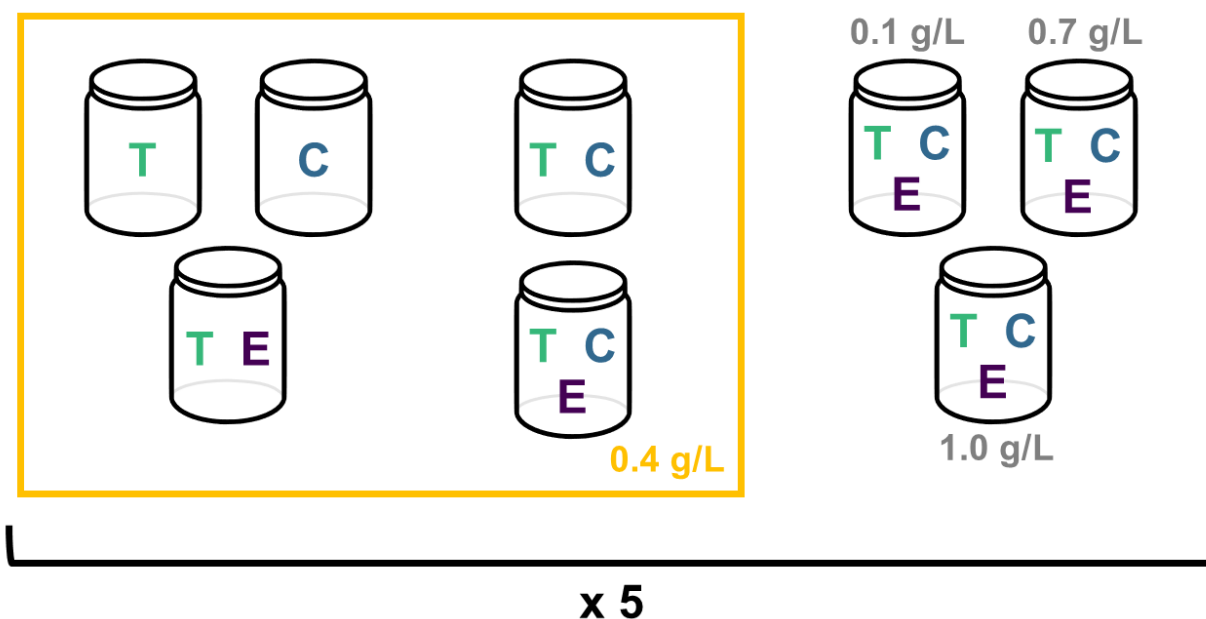
### 2.2.2 Microcosm Methods

Microcosm set-up and maintenance followed previously established protocol (Lawler and Morin 1993) with modifications to better suit available supplies and experimental goals. All dry materials and fresh media were autoclaved before use for sterilization. Each microcosm consisted of 80 mL sterile timothy hay medium in a 250 mL glass jar with a screw-on cap. One sterile wheat seed was added to each jar as an additional, slow-release carbon source (Balciunas

and Lawler 1995). Jar lids were left slightly loose to allow for gas exchange. All microcosms were stored in an incubator maintained at 20°C.

Media was made by autoclaving timothy hay (TH) in 1.0 L of spring water. The hay was contained in a cloth teabag to minimize large particulate matter that could result in among-replicate differences in the medium. The amount of timothy hay per liter used was determined through pilot experiments, with the chosen concentration of 0.4 g/L allowing for similar growth rates and carrying capacities to previous studies using a different medium. There were five replicates of each feasible species combination of *Tetrahymena*, *Colpidium*, and *Euplotes* (Figure 2.1). Additional concentrations (0.1, 0.7, and 1.0 g/L) were used to create a resource gradient among three-species microcosms, and each resource level was also replicated five times.

Bacteria respond strongly to nutrient levels such that an increase in nutrients results in higher bacterial carrying capacity and potential to support higher protist densities (Balciunas and Lawler 1995; Kaunzinger and Morin 1998).



**Figure 2.1:** A graphical representation of the experimental design of my study. Each species is represented by the first letter of its genus name. Jars within the yellow box are all at the same resource level of 0.4 g TH/L. The remaining three jars have their resource levels labelled in grey. Each treatment shown in the figure was replicated five times, as indicated by the bracket at the bottom of the graphic.

Media was inoculated with taxonomically unidentified bacteria 24 hours before being added into experimental jars. This allowed the bacteria to reach a density sufficient for supporting the focal protist species, which use bacteria as a food source. Bacteria were isolated by spreading a small amount of liquid from a microcosm stock culture on an LB agar plate.

All protist species were added to their respective jars at the same time and given 24 hours to grow before sampling began. Initial densities were 24.1 protists/mL for *Tetrahymena*, 14.5 protists/mL for *Colpidium*, and 0.14 protists/mL for *Euplotes*. These densities were calculated using the density of each species' stock culture, the volume of stock culture added (~0.3 mL), and the volume of the new microcosm jars (80 mL).

For any microcosms containing the predator *Euplotes*, all other species were added 24 hours before adding *Euplotes* to allow prey species to reach a high enough density to sustain the

predators. Protists were sourced from maintained stock cultures and added to experimental jars via micropipette. Stock culture density was counted before any protists were removed so experimental starting densities could be estimated.

### **2.2.3 Microcosm Sampling**

Prior to sampling, each jar was gently swirled to homogenize the contents. Then, 0.3 mL was removed from the jar using a micropipette and added to a petri dish. Samples were counted using a binocular light microscope. If any species was too abundant to count easily, species that could be counted were counted first. Then, the sample was diluted with a known volume of bacteria-free medium, and another 0.3 mL subsample was taken. Abundance counts were used to calculate estimated density per mL in each microcosm while accounting for the sample volume and any dilutions. One was added to the result to allow for log transformations in case of any zero counts.

### **2.2.4 Analyses**

All data manipulation and analyses were conducted using R 4.5.0 (R Core Team 2025). To most accurately capture the effects of any species interactions, days past the extinction of any species were trimmed from the dataset before statistical analysis. A species was considered extinct if three consecutive samples recorded the species as absent.

ANOVA was used to assess differences in time averaged densities for each species across all treatments. Significant results were followed up with a Tukey's test to determine which treatments differed. Simple linear regression was used to evaluate the effect of resource level on average densities, maximum densities, and predator-prey cycle period in three-species treatments. To estimate cycle period, I conducted spectral analysis using the R package 'lomb' (Ruf 1999) to compute Lomb-Scargle periodograms from *Euplotes* densities. This method was

used to account for uneven sampling intervals in the time series due to sampling only being conducted on weekdays.

I used the R package ‘FME’ (Soetaert and Petzoldt 2010) to fit a model to time series data obtained from microcosm sampling. First, I defined a system of equations describing the community to use as my model. For either *Tetrahymena* or *Colpidium* alone, a logistic growth equation was used (Equation 2.1). For both *Tetrahymena* and *Colpidium* together, I used the Lotka-Volterra competition equations (Equation 2.2).

$$\frac{dN_i}{dt} = r_i N_i \left(1 - \frac{N_i}{K_i}\right) \quad (2.1)$$

$$\frac{dN_i}{dt} = r_i N_i \left(1 - \frac{N_i + \alpha_{ij} N_j}{K_i}\right), \quad i \neq j \quad (2.2)$$

In these equations,  $r$  is the intrinsic rate of increase or growth rate,  $N$  is species density,  $K$  is carrying capacity, and  $\alpha$  is the competition coefficient for the resident’s competitive effect on the invader. This system of equations is then solved using initial density values measured from microcosm stock cultures, starting parameter values, and time values to output the fitted state variables at. The function ‘modCost’ estimates the weighted residuals of the model compared to the data, and the function ‘modFit’ then fits the model to the data. Together, these functions were used to find the best-fit parameter values that minimized model cost using the Levenberg-Marquardt algorithm, an iterative least-squares method. Residuals were weighted by 1/mean of the absolute value of the sampling data to avoid picking parameter values that fit higher means well at the cost of fitting lower means poorly.

While I initially planned to fit the Lotka-Volterra competition model (Equation 2.2) to *Tetrahymena* and *Colpidium*, the sampling data indicated that carrying capacity did not appear to be constant for these species (see Section 2.3.1). Carrying capacity declined over time, especially

for *Tetrahymena*. For this reason, I also fit an alternative model that includes a decay in carrying capacity:

$$\frac{dN_i}{dt} = r_i N_i \left( 1 - \frac{N_i + \alpha_{ij} N_j}{K_i e^{D_i t}} \right), \quad i \neq j \quad (2.3)$$

where  $e$  is the base of the natural logarithm and  $D$  is the exponential rate of decline in carrying capacity over time.

For fitting a model to the data of *Tetrahymena* and *Euplotes* together, I used a Rosenzweig-MacArthur model (Equations 2.4 and 2.5) which has been used previously to model the population dynamics of these two species of protists (Vasseur and Fox 2009). In this model,  $P$  is the predator's density,  $d$  is the predator's per-capita mortality rate,  $e$  is the predator's conversion efficiency,  $b$  is the attack rate, and  $h$  is the handling time:

$$\frac{dN}{dt} = rN \left( 1 - \frac{N}{K} \right) - \frac{bNP}{1 + bhN} \quad (2.4)$$

$$\frac{dP}{dt} = e \left( \frac{bNP}{1 + bhN} \right) - Pd \quad (2.5)$$

For fitting a model to all three species together, *Colpidium* still uses Equation 2.2 and *Euplotes* still uses Equation 2.5, but *Tetrahymena* uses a modified equation to combine aspects of competition and predation together:

$$\frac{dN_i}{dt} = r_i N_i \left( 1 - \frac{N_i + \alpha_{ij} N_j}{K_i} \right) - \frac{bN_i P}{1 + bhN_i}, \quad i \neq j \quad (2.6)$$

To estimate invasion growth rates from the fitted model, I used 100 sets of parameters randomly pulled from the model's variance-covariance matrix, assuming a multivariate normal distribution. This allowed for possible correlations between estimated parameters such as growth rate correlating with competition coefficients, for example. I calculated invasion growth rate for each parameter set using Equation 2.7 which is a modified per-capita form of the Lotka-Volterra

competition equation (Equation 2.1) where the invader is at a density of zero and the resident is at its equilibrium density. Carrying capacity ( $K$ ) was used as an approximation for equilibrium density of the resident. From these 100 estimates of invasion growth rate, I then calculated an average and 95% confidence interval.

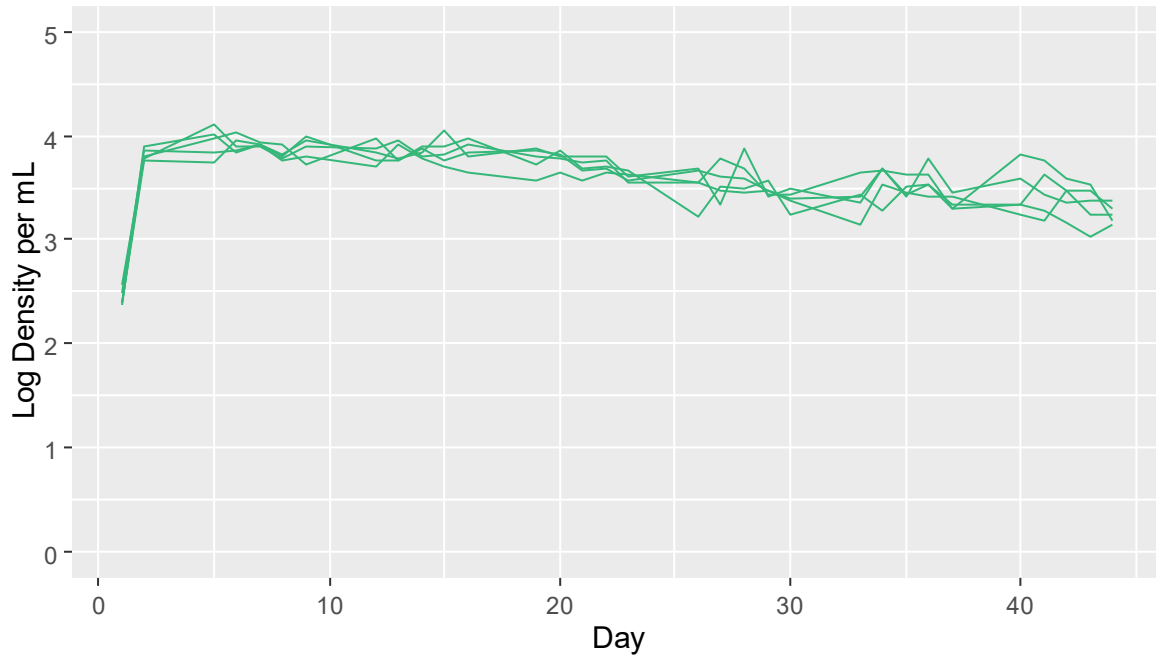
$$\text{invasion growth rate} = r_i \left( 1 - \frac{\alpha_{ij} K_j}{K_i} \right) \quad (2.7)$$

*Colpidium*'s invasion growth rate was then calculated using Equation 2.7 for each parameter set, as the model equation for this species did not change when going from two to three species. *Tetrahymena*'s invasion growth for the three-species models was also calculated using Equation 2.7, as setting invader density ( $N$ ) to zero reduces the predation term of Equation 2.6 to zero. *Euplotes*, being a predator of *Tetrahymena*, is not able to persist alone without its prey, so invasion growth rate was not calculated for this species.

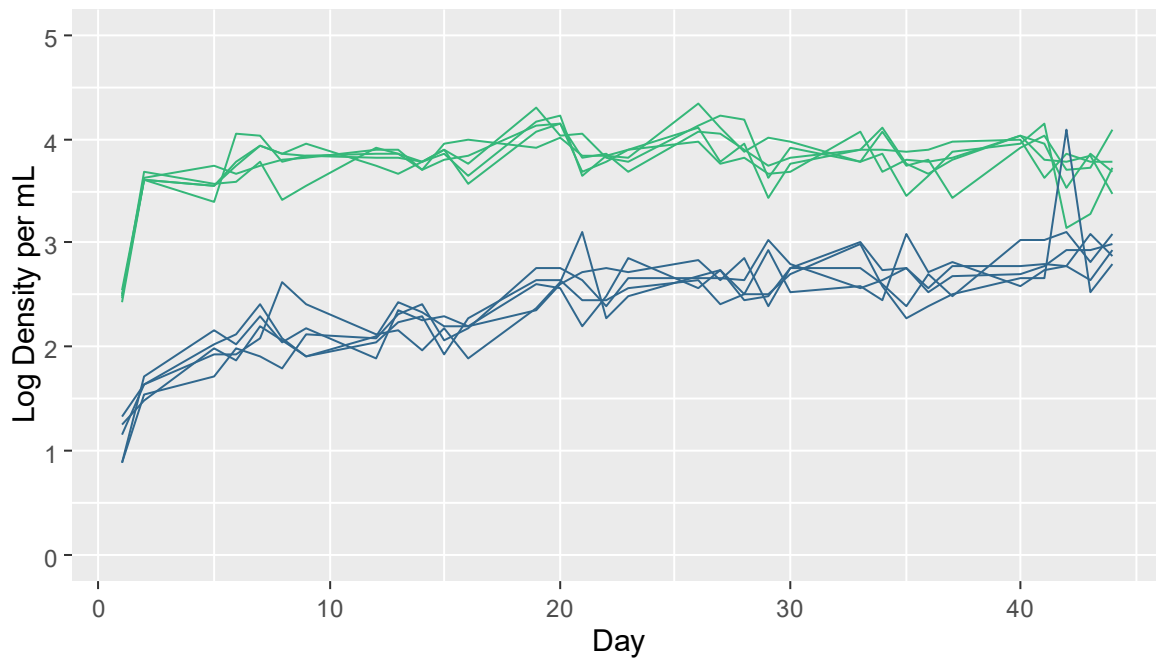
## 2.3 Results

### 2.3.1 Time Series and Population Dynamics

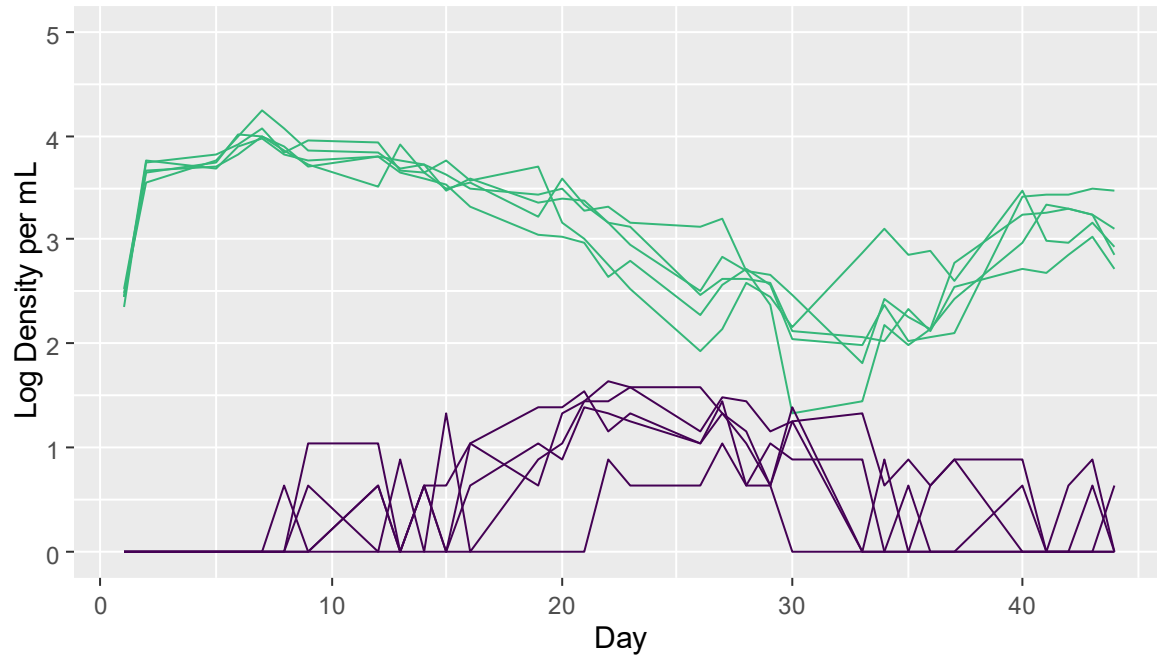
*Tetrahymena* growing alone increased rapidly and reached carrying capacity in only a few days (Figure 2.2). Across the full length of sampling, *Tetrahymena* densities trended downward, with samples in the final days being a few thousand cells per mL lower than at the start of the experiment. When grown in the same microcosm as *Colpidium*, *Tetrahymena* still increased quickly and reached carrying capacity within the first week of the experiment (Figure 2.3).



**Figure 2.2:** *Tetrahymena* (green)  $\log_{10}(\text{density per mL} + 1)$  when grown alone in 0.4 g/L timothy hay medium. Each line gives data from one replicate.



**Figure 2.3:** *Tetrahymena* (green) and *Colpidium* (blue)  $\log_{10}(\text{density per mL} + 1)$  when grown together in 0.4 g/L timothy hay medium. Each line gives data from one replicate.

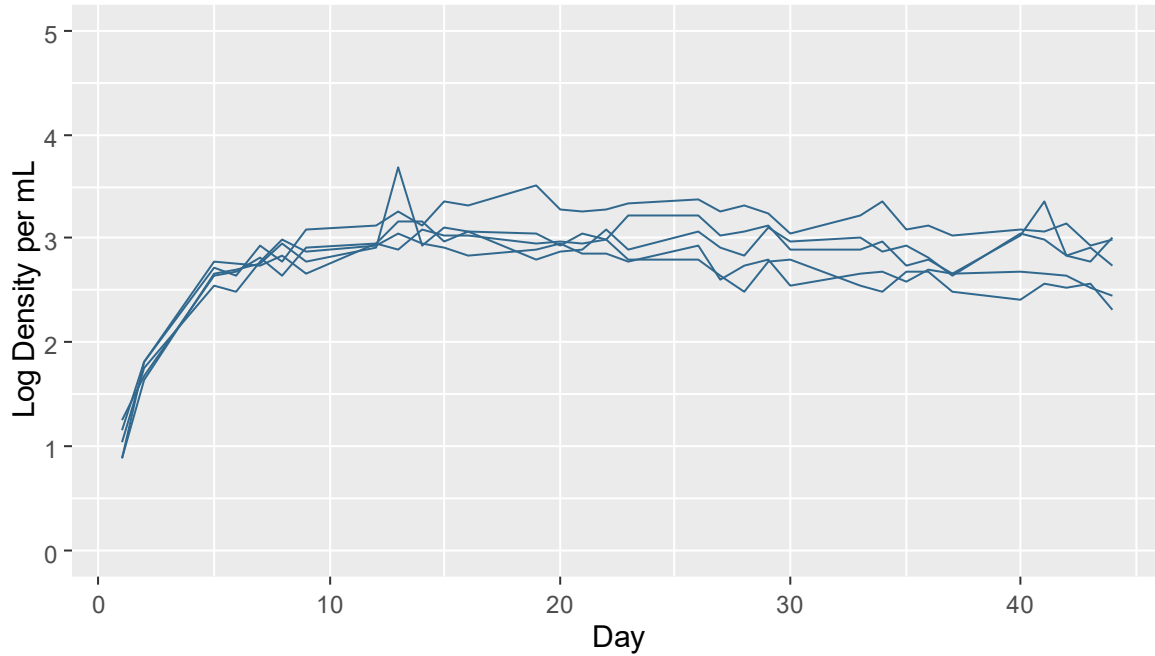


**Figure 2.4:** *Tetrahymena* (green) and *Euplotes* (purple)  $\log_{10}(\text{density per mL} + 1)$  when grown together in 0.4 g/L timothy hay medium. Each line gives data from one replicate.

The addition of the predator *Euplotes* to *Tetrahymena* alone increased variability in *Tetrahymena* densities. Average *Tetrahymena* densities were significantly lower when grown with *Euplotes* compared to densities when grown alone (Tukey's HSD,  $p < 0.001$ ). Density of *Tetrahymena* showed a large, gradual decrease between days 20 and 30 (Figure 2.4). This decrease coincided with an increase in *Euplotes* density. After day 30, *Tetrahymena* began to grow in density again while *Euplotes* decreased.

In the microcosm jars growing *Colpidium* alone, the population grew approximately exponentially until reaching carrying capacity at about day 10 (Figure 2.5). Shortly after reaching carrying capacity, the replicate populations started to diverge with some jars showing consistently higher or lower densities than the others. When grown alongside *Tetrahymena*, *Colpidium* increased gradually over the course of the experiment (Figure 2.3). Average

*Colpidium* densities were significantly lower when growing with *Tetrahymena* than when growing alone (Tukey's HSD,  $p = 0.006$ ). Additionally, the maximum density per mL reached alongside *Tetrahymena* was lower than when *Colpidium* grew on its own.



**Figure 2.5:** *Colpidium* (blue)  $\log_{10}(\text{density per mL} + 1)$  when grown alone in 0.4 g/L timothy hay medium. Each line gives data from one replicate.

### 2.3.2 Three-Species Treatments

Increasing resource level did not have a significant effect on *Colpidium* average density (Figure 2.6; ANOVA,  $p = 0.0802$ ) or max density (ANOVA,  $p = 0.628$ ). Mean *Colpidium* density was highest at the highest enrichment level of 1.0 g/L (Figure 2.7). Mean *Colpidium* density in three-species treatments was higher than when grown with only *Tetrahymena* (Figure 2.8; Tukey's HSD  $p = 0.0078$ ). The addition of *Euplotes* increased *Colpidium*'s mean density by roughly 300 cells per mL compared to *Colpidium* growing with just *Tetrahymena*. In all resource levels, *Colpidium* displayed similar growth patterns and reached carrying capacity around day 20

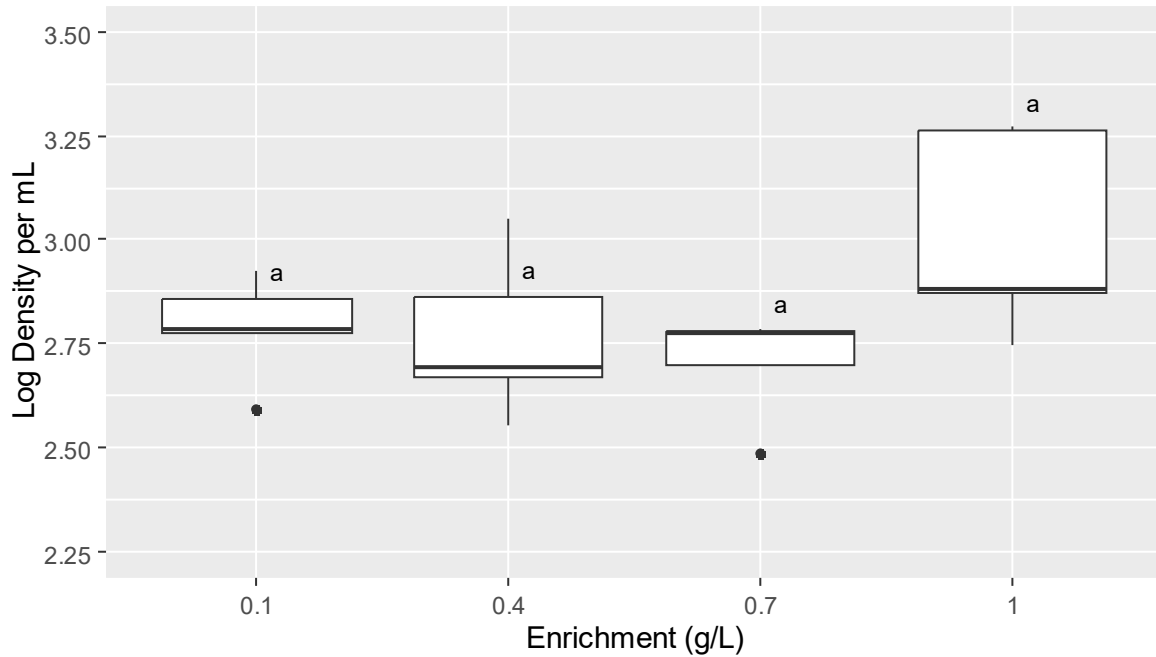
of sampling. *Colpidium* went extinct in one of the 0.7 g/L replicates (Figure 2.9) and persisted in all other microcosms.

Across the same resource level of 0.4 g/L, maximum density for *Tetrahymena* in a three-species community (Figure 2.10) was similar to maximums reached with only *Colpidium* (Figure 2.11; Tukey's HSD,  $p = 0.721$ ). Average *Tetrahymena* densities in treatments without *Euplotes* (T and T+C) were significantly higher than in treatments with *Euplotes* (T+E and T+C+E) (Figure 2.11; Tukey's HSD,  $p < 0.001$  for each comparison). *Tetrahymena* max density (Figure 2.12; Tukey's HSD,  $p < 0.001$ ) and average density (Figure 2.13; Tukey's HSD,  $p = 0.014$ ) significantly increased when going from the lowest resource level to the highest. Maximum densities were reached in less than week for all resource levels. The 0.1 g/L three-species treatment had the lowest average *Tetrahymena* density across all resource levels and species combinations (Figure 2.14). *Tetrahymena* persisted in all three-species microcosms.

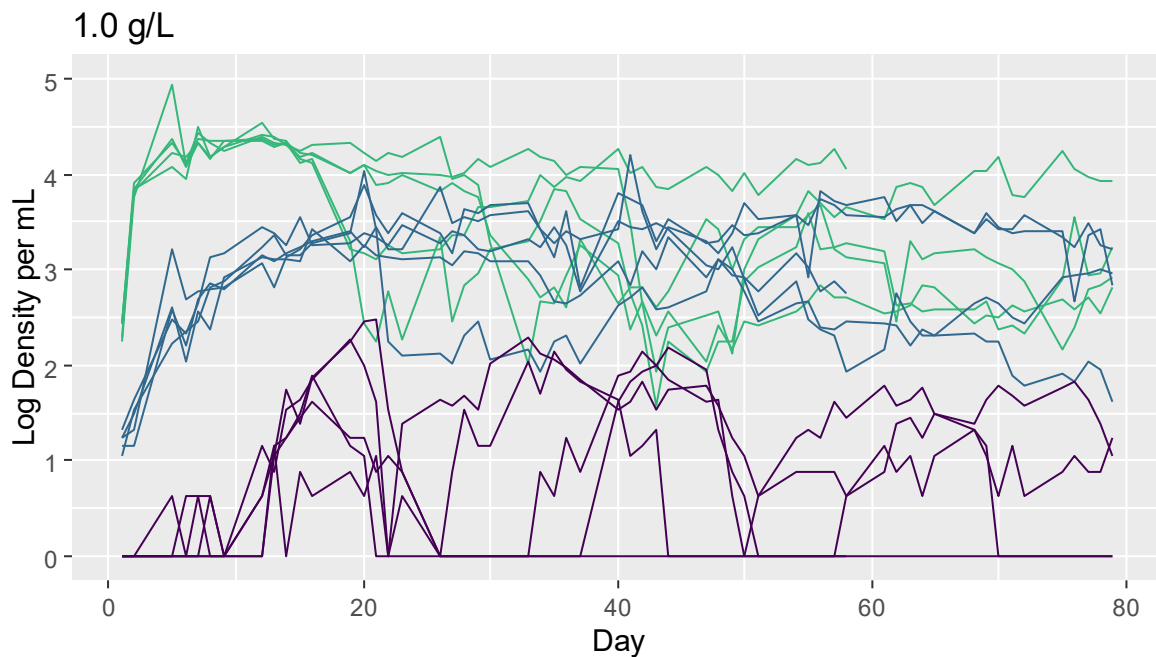
*Euplotes* was able to reach higher average densities in the three-species treatment compared to when grown with only *Tetrahymena* (ANOVA,  $p < 0.001$ ). Predator-prey cycle amplitude increased with resource level (Figure 2.15; linear regression,  $p = 0.006$ ), using maximum *Euplotes* density as an approximation. Peak density was lowest in 0.1 g/L ( $101 \text{ mL}^{-1}$ ), but only slightly increased among the higher resource levels with 0.4, 0.7, and 1.0 g/L showing similar maximums ( $304 - 394 \text{ mL}^{-1}$ ). Cycle period of *Euplotes* was not significantly different across resource levels (Figure 2.16; linear regression,  $p = 0.061$ ).

At 0.4 and 0.7 g/L, *Euplotes* went extinct in all but one replicate per treatment. *Euplotes* also went extinct in one of the 1.0 g/L microcosms. For 0.1 g/L, three microcosms had *Euplotes* densities of zero at the end of the experiment. In another 0.1 g/L replicate, however, *Euplotes*

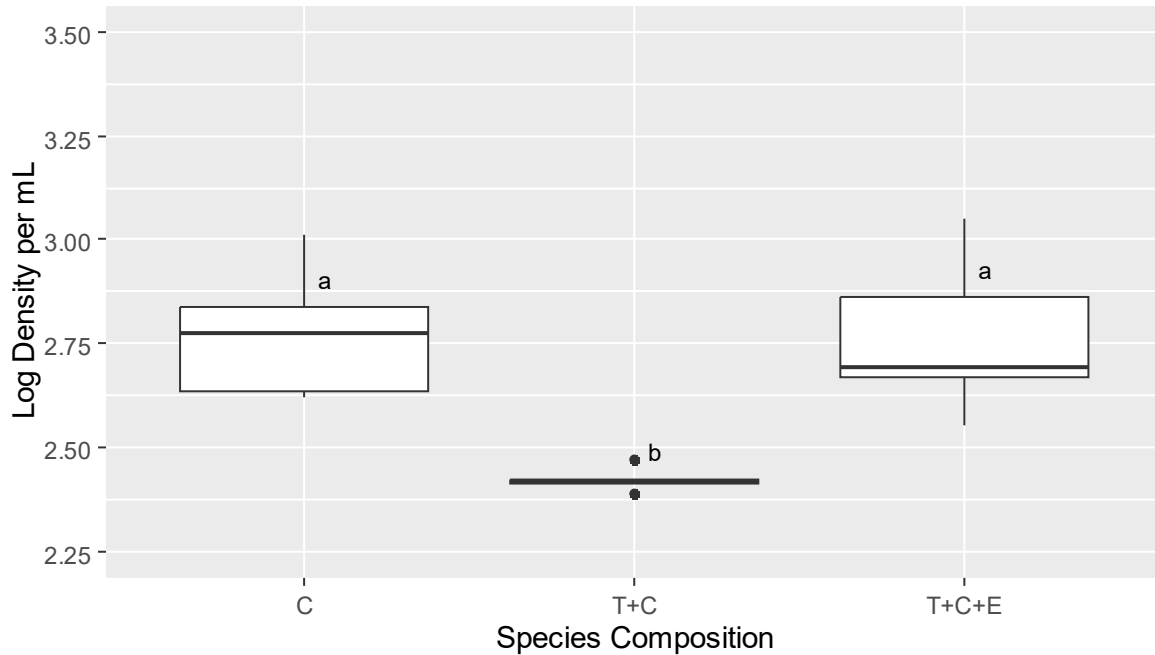
increased again after a 20-day stretch of not being found in sampling. Therefore, I cannot be certain that *Euplotes* truly went extinct in any of the lowest resource level jars.



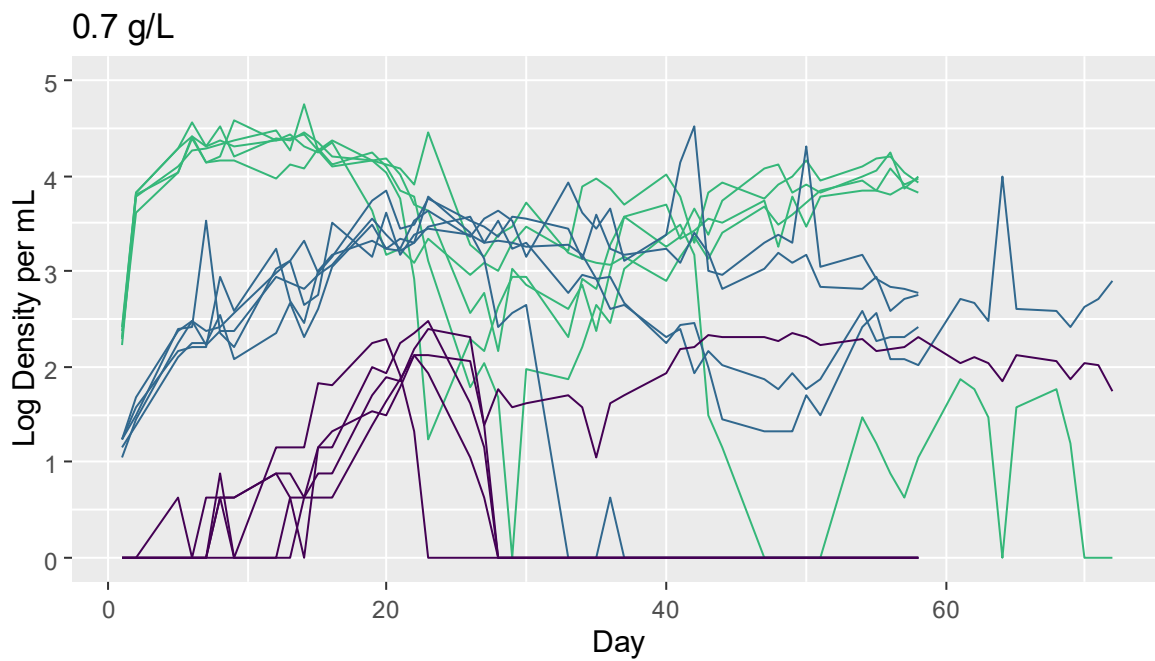
**Figure 2.6:** Box plot of time averaged *Colpidium*  $\log_{10}$ (density per mL) for each resource level in a three-species community. Letters next to each box indicate each mean does not differ significantly.



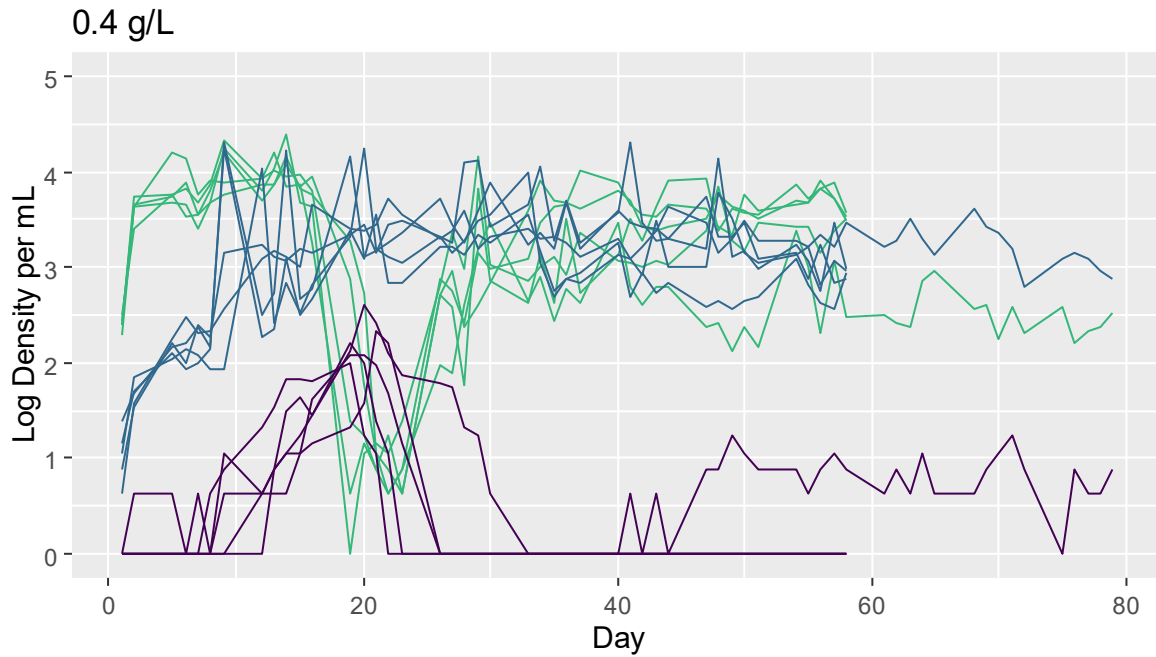
**Figure 2.7:** *Tetrahymena* (green), *Colpidium* (blue), and *Euplotes* (purple)  $\log_{10}$ (density per mL + 1) grown in 1.0 g/L timothy hay medium. Each line gives data from one replicate.



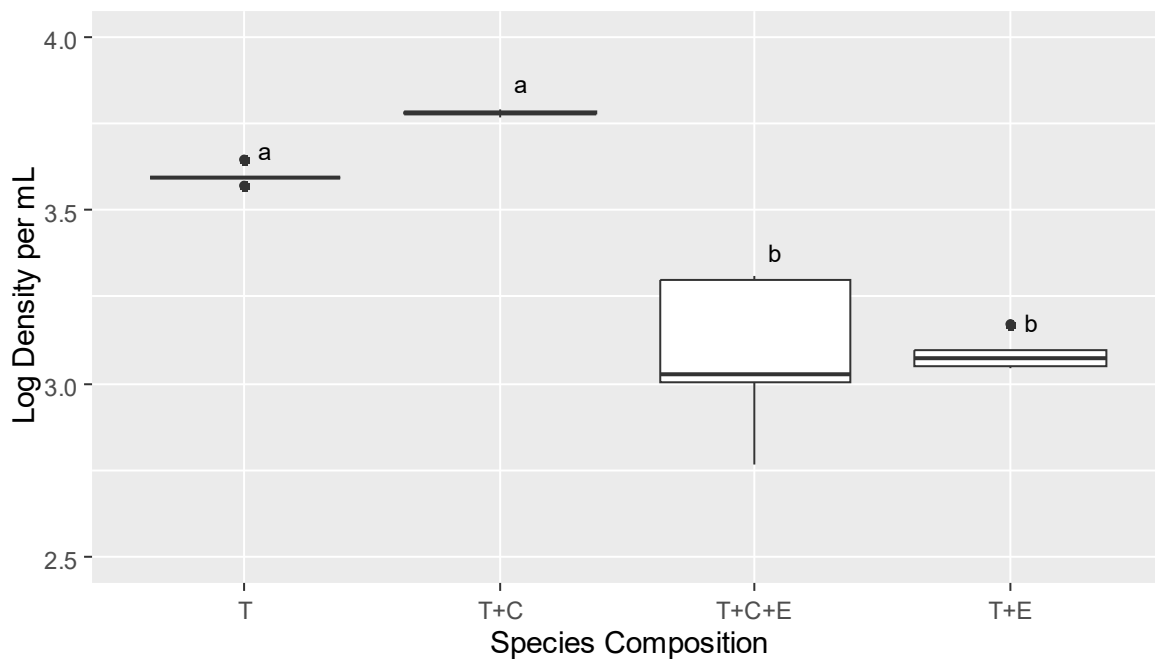
**Figure 2.8:** Box plot of time averaged *Colpidium*  $\log_{10}(\text{density per mL})$  for each combination of species with *Colpidium* present at 0.4 g/L. Letters next to each box indicate significantly different means from a Tukey's test. X-axis labels consist of the first letter of a present species' name.



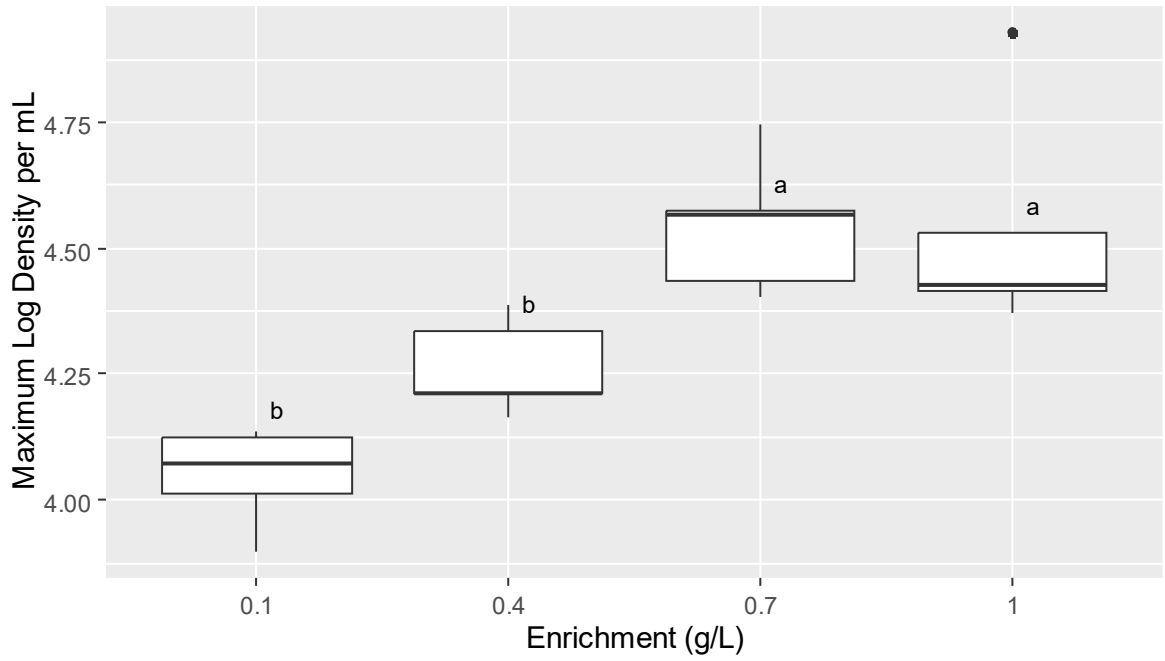
**Figure 2.9:** *Tetrahymena* (green), *Colpidium* (blue), and *Euplotes* (purple)  $\log_{10}(\text{density per mL} + 1)$  grown in 0.7 g/L timothy hay medium. Each line gives data from one replicate.



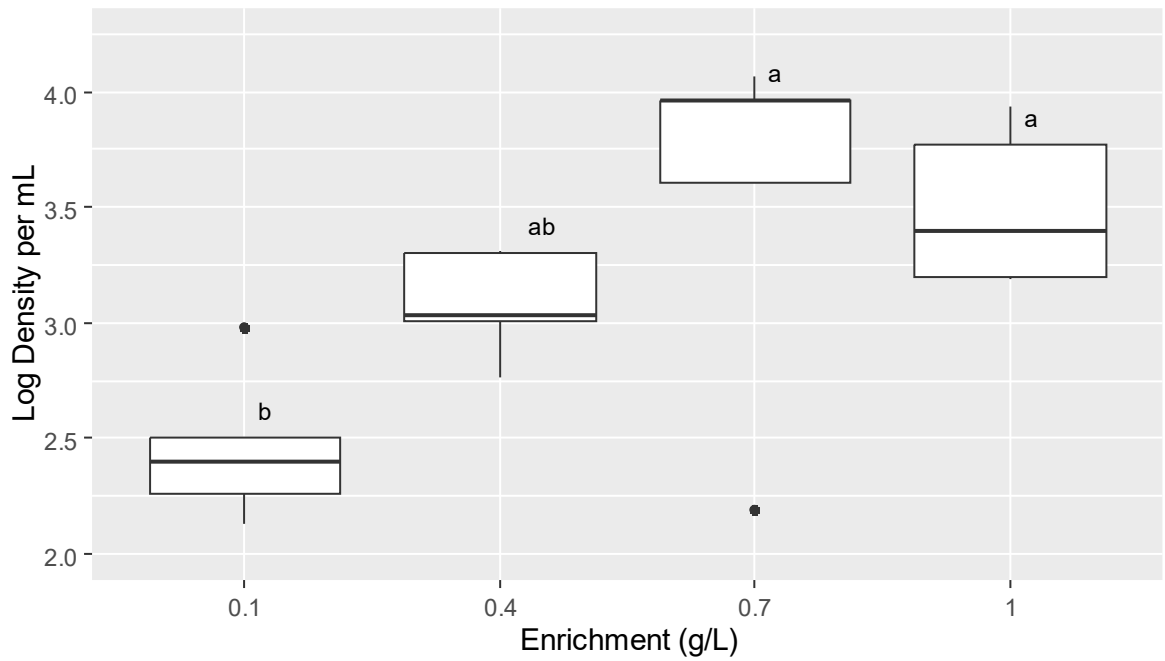
**Figure 2.10:** *Tetrahymena* (green), *Colpidium* (blue), and *Euplotes* (purple)  $\log_{10}(\text{density per mL} + 1)$  grown in 0.4 g/L timothy hay medium. Each line gives data from one replicate.



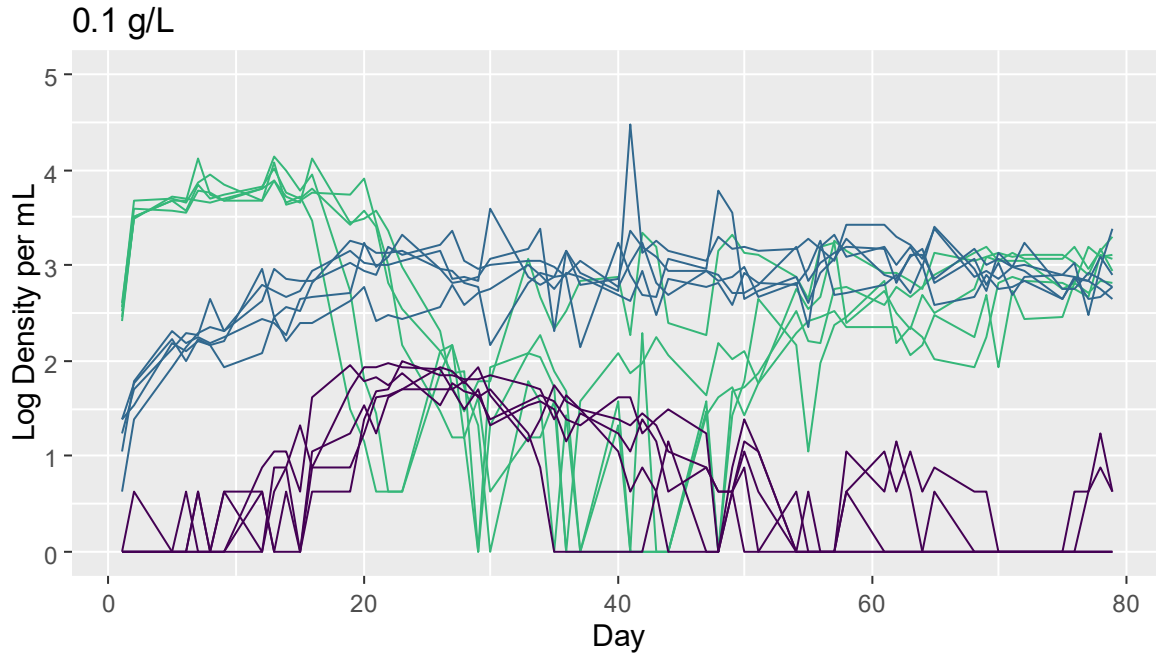
**Figure 2.11:** Box plot of time averaged *Tetrahymena*  $\log_{10}(\text{density per mL})$  for each combination of species with *Tetrahymena* present at 0.4 g/L. Letters next to each box indicate significantly different means from a Tukey's test. X-axis labels consist of the first letter of a present species' name.



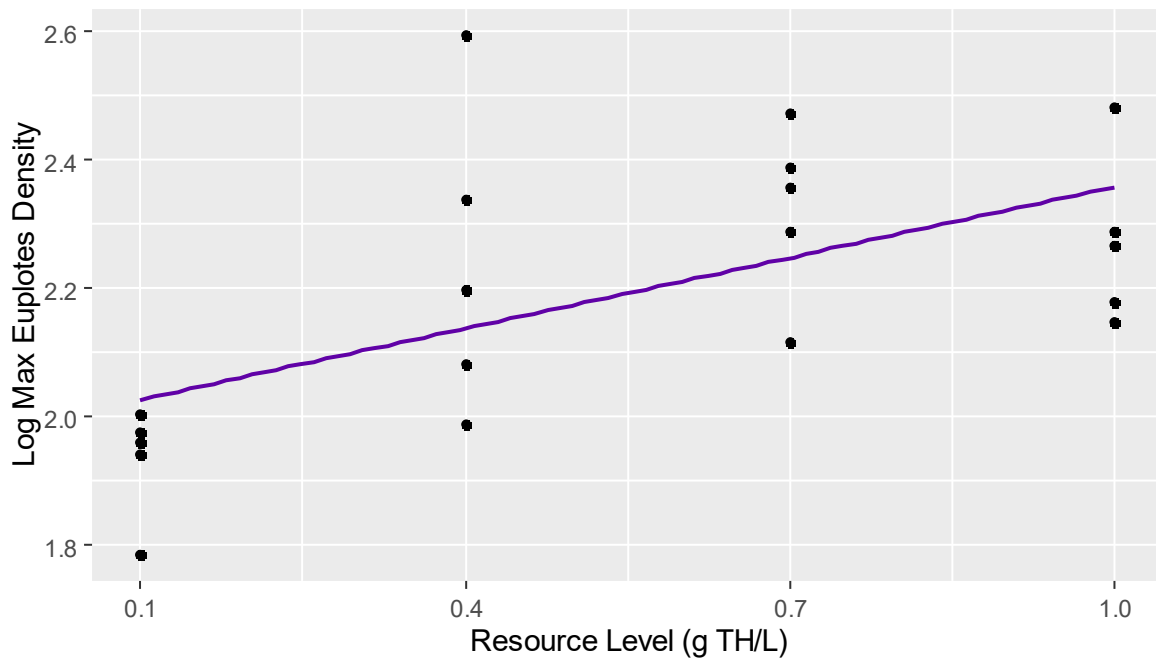
**Figure 2.12:** Box plot of maximum *Tetrahymena*  $\log_{10}$ (density per mL) for each resource level in a three-species community. Letters next to each box indicate significantly different means from a Tukey's test.



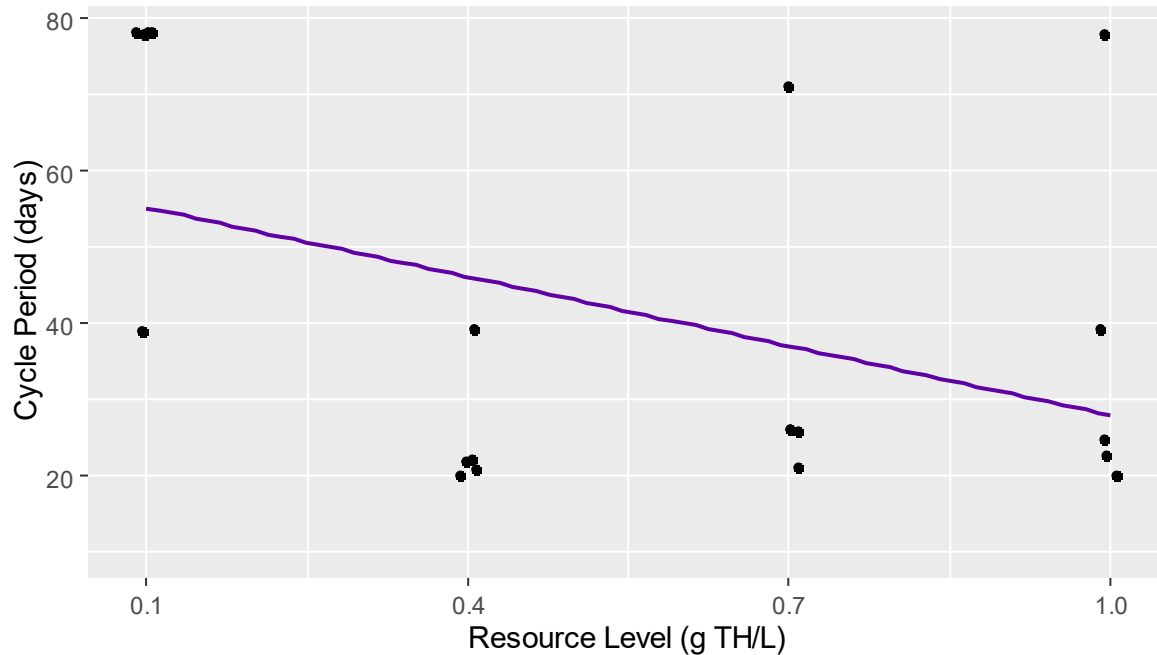
**Figure 2.13:** Box plot of time averaged *Tetrahymena*  $\log_{10}$ (density per mL) for each resource level in a three-species community. Letters next to each box indicate significantly different means from a Tukey's test.



**Figure 2.14:** *Tetrahymena* (green), *Colpidium* (blue), and *Euplotes* (purple)  $\log_{10}(\text{density per mL} + 1)$  grown in 0.1 g/L timothy hay medium. Each line gives data from one replicate.



**Figure 2.15:** Simple linear regression between  $\log_{10}$  maximum *Euplotes* density and resource level. Max *Euplotes* density was used as an approximation of predator-prey cycle amplitude.  $R^2 = 0.3$ ,  $p = 0.006$



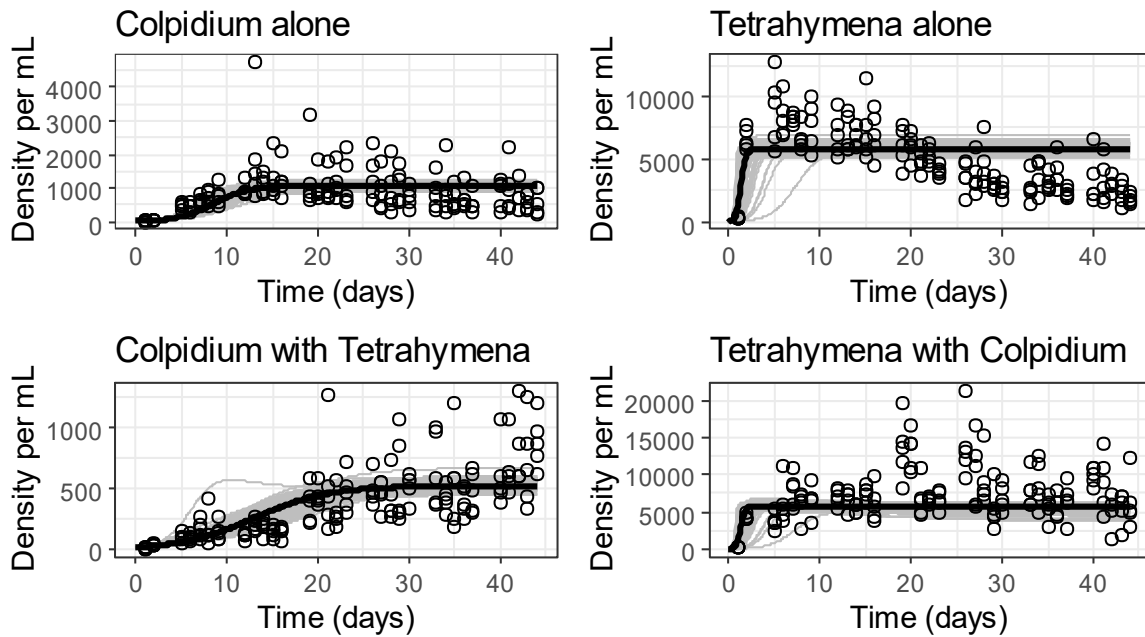
**Figure 2.16:** Simple linear regression between *Euplotes* cycle period and resource level. Cycle period was estimated from *Euplotes* density using Lomb-Scargle periodograms.  $R^2 = 0.135$ ,  $p = 0.061$

### 2.3.3 Modeling Results

The single-species *Colpidium* and *Tetrahymena* models were fitted simultaneously with the two-species model of them grown together (Figure 2.17). This combined model had a sum of squared residuals (SSR) of 202.08 after fitting. No parameters were strongly correlated with one another. One outlier was removed on day 42 where *Colpidium* reached a density per mL over 10,000. This had minimal effect on fitted parameter values. Model fit is initially quite good but diverges slightly from the data points in the latter half of the experiment. As the goal of fitting this model was to estimate invasion growth rates, which rely on average growth rates when rare and therefore making the model fit of initial growth period most important, this was deemed acceptable. Additionally, an alternative model (Figure 2.18) was fit to the data to address this mismatch.

Best-fit model parameters for *Colpidium* were a growth rate ( $r$ ) of 0.50 per day and a carrying capacity ( $K$ ) of 1072. For *Tetrahymena*, the best-fit growth rate was 4.19 per day with a carrying capacity of 5780. Competition coefficients were estimated to be 0.10 for the effect *Tetrahymena* has on *Colpidium*, and 0.003 for the effect of *Colpidium* on *Tetrahymena*.

For *Tetrahymena* invading *Colpidium*, invasion growth rate was calculated as  $3.37 \text{ day}^{-1}$  (95% CI: 3.09 – 3.65). For *Colpidium* invading *Tetrahymena*, the calculated invasion growth rate was  $0.234 \text{ day}^{-1}$  (95% CI: 0.228 – 0.241). I could not calculate invasion growth rates for *Colpidium* invading *Tetrahymena* and *Euplotes* due to poor model fit.

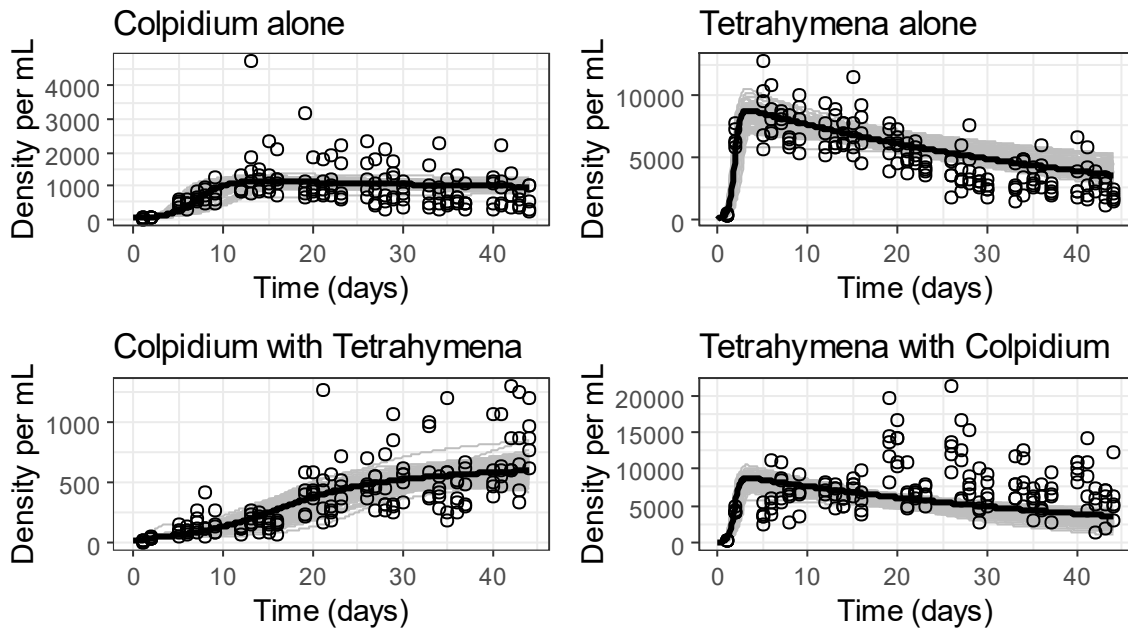


**Figure 2.17:** Lotka-Volterra competition model fit to time series of *Colpidium* and *Tetrahymena* grown in 0.4 g/L timothy hay medium. Empty circles are real data points, dark line is the best-fit parameter set from the model, grey lines are fits from 100 random parameter sets generated from the model’s covariance matrix.

I also fit an alternative Lotka-Volterra model with decaying carrying capacity to the same *Tetrahymena* and *Colpidium* data (Figure 2.18). This resulted in a better fit (SSR = 174.81) compared to the unmodified Lotka-Volterra model. Best-fit model parameters for *Colpidium*

were a growth rate ( $r$ ) of 0.62 per day, a carrying capacity ( $K$ ) of 1216, and a decline rate ( $D$ ) of -0.005. For *Tetrahymena*, the best-fit growth rate was 3.28 per day with a carrying capacity of 9461 and a decline rate of -0.022. Competition coefficients were estimated to be 0.10 for the effect *Tetrahymena* has on *Colpidium*, and 0.003 for the effect of *Colpidium* on *Tetrahymena*.

Estimated invasion growth rates for the alternative model were lower than the estimates from the unmodified model. For *Tetrahymena* invading *Colpidium*, invasion growth rate was calculated as 2.60 day<sup>-1</sup> (95% CI: 2.48 – 2.72). For *Colpidium* invading *Tetrahymena*, the calculated invasion growth rate was 0.126 day<sup>-1</sup> (95% CI: 0.114 – 0.138).



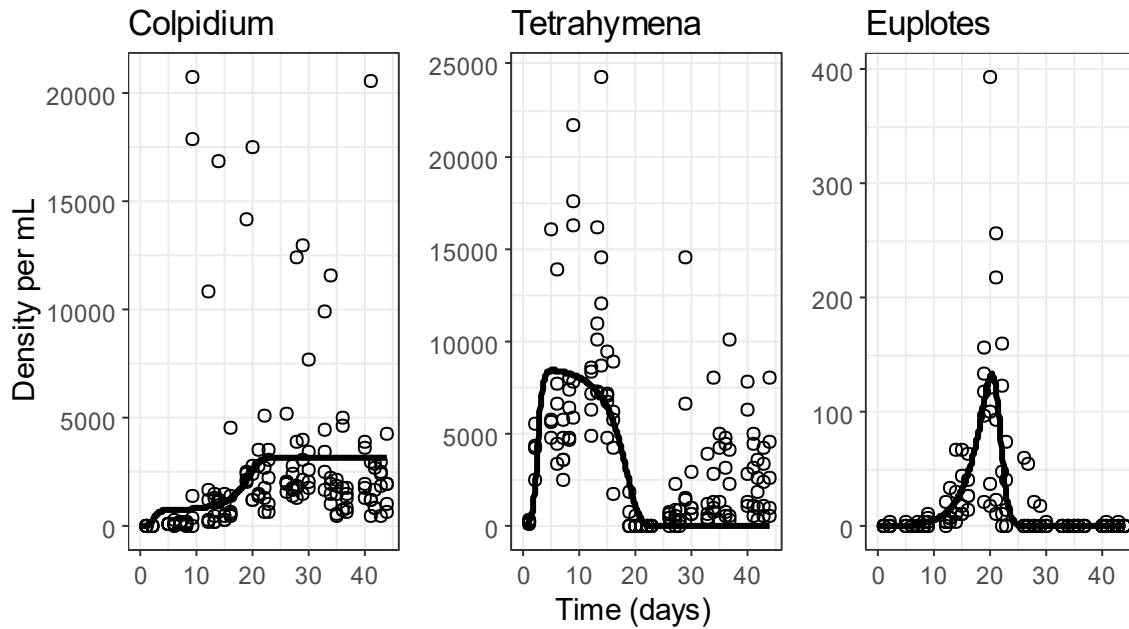
**Figure 2.18:** Modified Lotka-Volterra competition model with decaying carrying capacity fit to time series of *Colpidium* and *Tetrahymena* grown in 0.4 g/L timothy hay medium. Empty circles are real data points, dark line is the best-fit parameter set from the model, grey lines are fits from 100 random parameter sets generated from the model’s covariance matrix.

Each three-species resource level was modeled individually using only the data from its respective treatment due to the different resource levels used. The best-fit parameters from the one- and two-species models were used to inform initial parameter values for the three-species

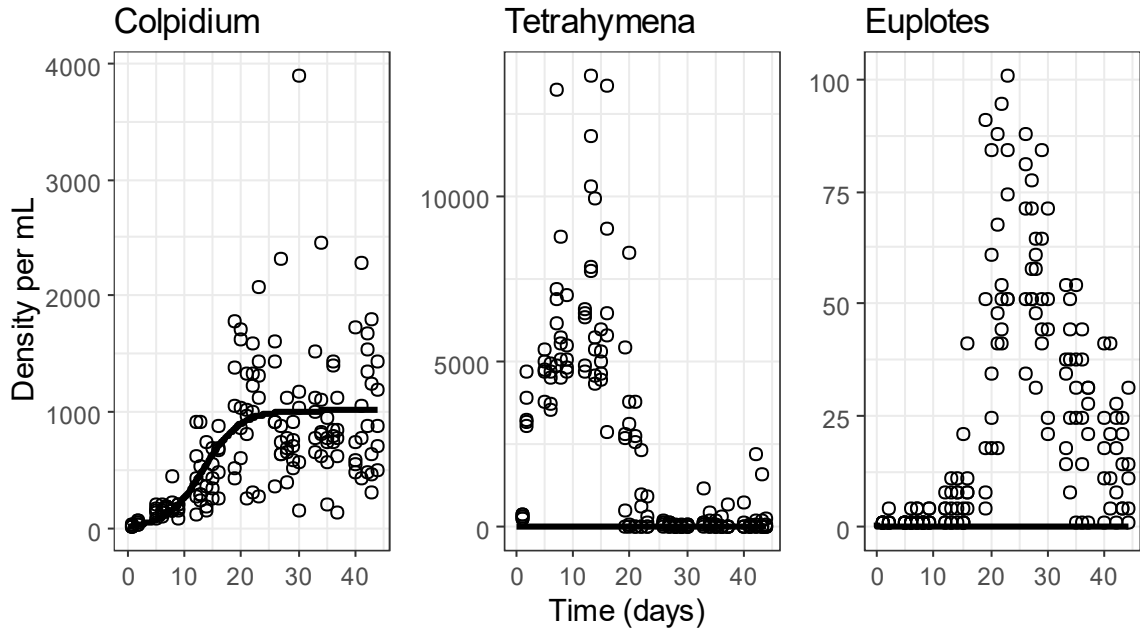
models. When fitting a model to all replicates in the 0.4 g/L treatment (Figure 2.19), all species appear to be accurately estimated (SSR = 1015.838). However, predator conversion efficiency ( $e$ ) and the competition coefficient for *Colpidium*'s effect on *Tetrahymena* were pushed unreasonably high indicating the model cannot properly estimate the dynamics of this community. Parameter estimates were initially constrained between 0 and  $10^6$ , but decreasing the upper bound only resulted in the problematic parameters being estimated at that limit. I then fit the model to each replicate separately, with one replicate unable to estimate the residual weighing for *Colpidium*, two replicates having poor fits for all species with parameters pushing against the upper bound of  $10^6$ , and two replicates having reasonable fits for at least one species while having at least one unrealistic parameter estimate. As confidence in the model's ability to accurately explain the data was low, I did not go on to estimate invasion growth rates for this treatment.

Similar outcomes occurred for the three-species models at other resource levels. For the 0.1 g/L model (Figure 2.20), multiple parameters were estimated at the upper bounds and only *Colpidium* could be accurately modelled (SSR = 827.3347). When fit per-replicate, *Colpidium* had a good fit in all but one replicate where weighing could not be estimated, and *Tetrahymena* and *Euplotes* could not be fit in any replicate. Additionally, some parameters affecting *Tetrahymena* and *Euplotes* were estimated to be unreasonably large values. The 0.7 g/L model (Figure 2.21) gave an unrealistic estimate of  $e$  and predator attack rate ( $a$ ) pushed against the specified upper bound of  $10^6$  (SSR = 1838.175). Similar issues occurred when fitting this resource level per-replicate where  $e$  was often estimated to be greater than 1.0 and predator attack rate ( $a$ ) or handling time ( $h$ ) were pushing upper bounds. The 1.0 g/L model had a decent visual fit (Figure 2.22), but the competition coefficient for *Colpidium*'s effect on *Tetrahymena*

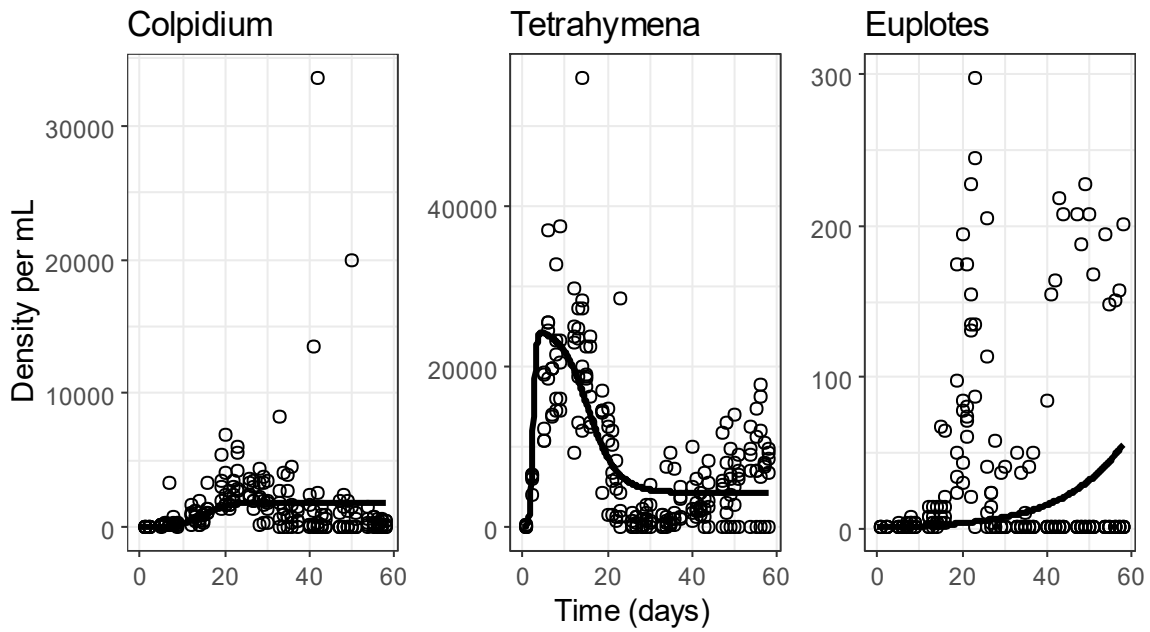
was much larger than expected ( $SSR = 1282.635$ ). Individual replicates had a variety of problems with some replicates unable to estimate the weighing for *Colpidium*, and various parameters being estimated as unreasonably high values depending on the replicate.



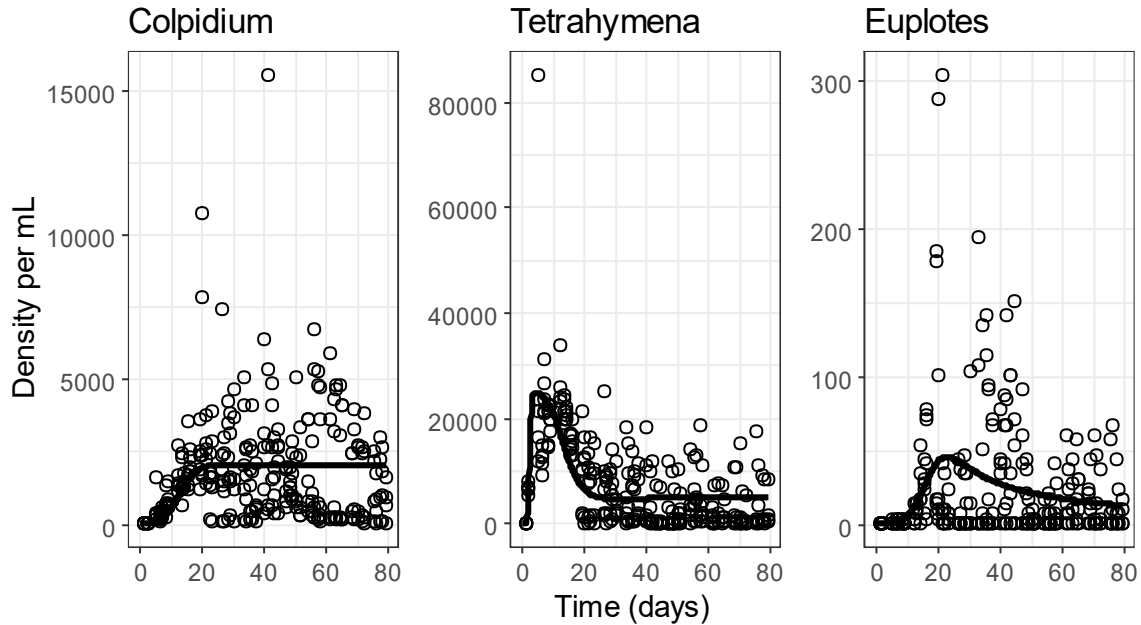
**Figure 2.19:** Model fit for three-species microcosms at 0.4 g/L. Empty circles are real data points, and the line is the best-fit parameter set from the model.



**Figure 2.20:** Model fit for three-species microcosms at 0.1 g/L. Empty circles are real data points, and the line is the best-fit parameter set from the model.



**Figure 2.21:** Model fit for three-species microcosms at 0.7 g/L. Empty circles are real data points, and the line is the best-fit parameter set from the model.



**Figure 2.22:** Model fit for three-species microcosms at 1.0 g/L. Empty circles are real data points, and the line is the best-fit parameter set from the model.

## 2.4 Discussion

### 2.4.1 Interpreting the Estimates

The models fit to any of the treatments including the predator *Euplotes* did not produce a reliable enough estimate to confidently calculate invasion growth rate estimates. This was due to unrealistic estimated parameter values and inaccurate fits to predator-prey dynamics. If *Euplotes* is not modeled correctly, parameter estimates will overstate the importance of *Colpidium*'s effect on *Tetrahymena* and calculated invasion growth rates for *Tetrahymena* will be underestimated.

*Colpidium* tended to have a reasonable fit in three-species models even when the fit for the other two species was poor. The equation used to model *Colpidium*'s population dynamics in three-species treatments is the same as the one used for the model with only *Tetrahymena*. The two-species model with *Tetrahymena* and *Colpidium* had a very good fit, showing that the Lotka-

Volterra competition model is an accurate representation of resource competition in this system. Thus, the model fitting problem lies with the predator-prey equations. Even in a two-species model with *Tetrahymena* and the predator *Euplotes*, the model used could not accurately capture population dynamics.

Both *Tetrahymena* and *Colpidium* had positive invasion growth rates (3.37 and 0.234 day<sup>-1</sup> respectively) when invading each other indicating the two species are coexisting. This also aligns with what was observed experimentally where both species were able to persist for the whole length of the experiment. The lower value of *Colpidium*'s invasion growth rate reflects the differences in estimated competition coefficients (0.10 vs. 0.003). *Colpidium* experiences much more competition from *Tetrahymena* than vice versa. This means that while intraspecific competition is still greater, as is required for coexistence, *Colpidium* experiences proportionally more interspecific competition than *Tetrahymena* which makes it harder to increase from rare.

Supported by model estimates of competition coefficients, *Colpidium* was more affected by *Tetrahymena* ( $\alpha = 0.10$ ) than vice versa ( $\alpha = 0.003$ ). For the microcosms with both *Tetrahymena* and *Colpidium* grown together, *Colpidium* densities would generally react to *Tetrahymena* densities and not vice versa. A decrease in *Tetrahymena* density would often be followed by an increase in *Colpidium* density so long as the *Tetrahymena* decrease was large enough that *Colpidium* briefly experienced less interspecific competition. As *Tetrahymena* was able to increase more quickly due to a greater intrinsic rate of increase combined with low interspecific competition, not every decrease in *Tetrahymena* would lead to an increase in *Colpidium*.

This difference in competitive ability contradicts what was expected based on cell size. If the competitors were eating the same species of bacteria, a single *Colpidium* should be

equivalent to multiple *Tetrahymena* as *Colpidium* is the larger species. Additionally, previous research found that *Colpidium* was a much stronger competitor than *Tetrahymena* (Fox 2002). However, this older study used a different species of *Tetrahymena* (*T. thermophila* instead of *T. pyriformis*), which could explain the difference in outcomes.

Regardless, several other studies provide support for the finding that *Tetrahymena* has a larger effect on *Colpidium* than vice versa. Asymmetric competition has been found in protist microcosms where *Colpidium* had no effect on *Chilomonas paramecium* in the presence of the predator *Euplotes* (Balciunas and Lawler 1995). Fox (2007) also found *Colpidium* is a weaker competitor than *Tetrahymena* with *Tetrahymena* decreasing *Colpidium* density compared to monoculture data. In a series of experiments looking at priority effects, Bright (2018) found similar competitive outcomes such that *Tetrahymena* could invade *Colpidium* across a range of temperatures, but *Colpidium* could never successfully invade *Tetrahymena*.

#### **2.4.2 Data Features Affecting Model Fit**

A few characteristics of the collected data could have contributed to the poor model fits for many treatments. *Tetrahymena* likely overshot carrying capacity at the start of the experiment in some treatments. This is most evident in the monoculture data showing a gradual decrease in density over time (Figure 2.2). The model-estimated carrying capacity of 5780 cells per mL provides further reasoning for the decrease in density that follows after *Tetrahymena* exceeds a density of 10,000 cells per mL. This can also be seen at the start of the three-species 0.7 and 1.0 g/L time series (Figures 2.7 and 2.9), and occasionally throughout the two-species time series of *Tetrahymena* with *Colpidium* (Figure 2.3). The overshooting carrying capacity could be due to the protist stock cultures being maintained at a much higher resource level (2.0 g TH/L) than any of my experimental treatments. Thus, when *Tetrahymena* was added to the experimental jars

from the stock cultures, they were still experiencing the benefits of a higher resource level for a short period of time which resulted in temporary increased growth.

The two competing species, *Tetrahymena pyriformis* and *Colpidium striatum*, have both been shown to persist as prey for *Euplotes* species (Balciunas and Lawler 1995; Vasseur and Fox 2009). However, I was unable to successfully grow *Euplotes* on *Colpidium* alone. This could be due to using different species of *Euplotes*, as another study where *Euplotes* preyed on *Colpidium* used the species *Euplotes aediculatus* (Jiang and Morin 2005) while I used the species *Euplotes patella*. *Euplotes* is scarce when feeding on only *Colpidium* (Balciunas and Lawler 1995) which suggests that *Euplotes* either has some difficulty with eating *Colpidium* or does not prefer it as prey compared to other species like *Tetrahymena* or *Chilomonas paramecium*. Additionally, previous experiments have reported that *Colpidium* size increases with resource level, resulting in a size refuge where cells are too big for *Euplotes* to consume (Balciunas and Lawler 1995; Jiang and Morin 2005). Thus, the reason I was unable to grow *Euplotes* on only *Colpidium* could have been because the cells were too large, especially after growing at a much higher (2.0 g TH/L) resource level in stock cultures.

Despite not being able to grow *Euplotes* and *Colpidium* together, there are some indications that *Euplotes* might have been consuming *Colpidium* in addition to *Tetrahymena* in the three-species replicates. *Euplotes* was able to reach higher maximum and mean densities in all three-species treatments compared to when grown with only *Tetrahymena*. Looking at the 0.1 g/L treatment specifically, both the average and maximum *Tetrahymena* density was lower than when grown with just *Euplotes* at 0.4 g/L. For *Tetrahymena*, this lower density can be attributed to the lower resource level. That *Euplotes* attained higher densities despite the lower density of prey could suggest that *Euplotes* supplemented its diet with *Colpidium*. However, this is not the

strongest piece of evidence as densities of both *Tetrahymena* and *Euplotes* at 0.1 g/L were on the same order of magnitude as their respective densities when grown without *Colpidium*.

In theoretical and experimental systems of shared predation, alternative prey allows predators to reach higher densities than would otherwise be possible with just one prey species (Lawton 1993; Holt and Lawton 1994). This means that the addition of alternative prey (*Colpidium* in my study) can result in reduced density of the focal prey (*Tetrahymena*) like what was observed in some replicates.

As *Colpidium* went through its growth cycle, newly divided cells may have been small enough for *Euplotes* to consume despite older cells being large enough to have a size refuge. Alternatively, *Colpidium* may have reached a small enough size after acclimating to the lower resource levels of the experimental microcosms, thus allowing *Euplotes* to eat them. However, it is not clear as to why this only occurred in a few specific microcosms. Fluid imaging data was taken during my experiment, but it has not yet been filtered and analyzed. These data provide an interesting opportunity for follow-up to determine whether variables like size or transparency changed across the length of the experiment and if this influenced species densities.

*Euplotes* reaches much lower densities than either of the competing species. These lower densities mean that sampling error is much more likely. The sample volume is so small compared to the total microcosm volume that it is not impossible to observe zero *Euplotes* despite their presence in the culture. In fact, this almost certainly occurred given that *Euplotes* would often increase in density following multiple days of being undetectable. This would have contributed to greater among-replicate variability and difficulty in model fitting.

### 2.4.3 Future Directions and Modeling Approaches

The inability to accurately model the population dynamics in any of the treatments including *Euplotes*, and therefore the inability to estimate invasion growth rates, highlights one of the primary difficulties with applying coexistence theory empirically. Modeling is a difficult task, and this is not something that is unique to ecology, but it becomes even more apparent when adding in the inherent complexity that comes with living organisms. However, the fact that one model did not produce an adequate fit does not mean it is impossible to quantify coexistence in this system. There are several modeling approaches and suggestions I was unable to implement due to time constraints and lack of familiarity with modeling which could provide improved fits when utilized.

Many studies, including my own, choose models based on their use in previous research (Terry and Armitage 2024). Evidently, this is not the best course of action despite it being the simplest. Running and comparing many different candidate models adds to the reliability and confidence of the results (Terry and Armitage 2024). Even so, I did not compare multiple candidate models due to time constraints. Fitting a variety of models to my data would be a necessary step toward obtaining a better fit.

The choice between complex and simple models is up to a researcher's preferences and research goals. A complex model is more realistic at the cost of increased uncertainty while simpler models can be applied more generally but result in more bias (Aho et al. 2014). The model I chose is one of the simpler predator-prey models available. Even so, combining it with a competition model for the three-species microcosms made the final model more complex with nearly twice as many parameters to estimate. A three-species model with fewer parameters to fit or a different predator functional response may fit my data better than the model I used.

Model selection is an area of active research in statistics (Tredennick et al. 2021; Terry and Armitage 2024). However, there is no agreement on best practices for model selection, and given the wide variety of data sources and modeling goals, there likely never will be (Tredennick et al. 2021). The “best” model will depend on the purpose of the analysis and what aspects or capabilities of the model (e.g., predictive accuracy, realism, etc.) are most important to the researcher (Aho et al. 2014; Tredennick et al. 2021). It is also possible to combine the estimates from all candidate models or use model averaging techniques which may provide better predictions while accounting for uncertainty (Longford 2012; Dormann et al. 2018). As models used for quantifying coexistence are prone to providing different predictions despite similar fits, the model averaging approach could be worthwhile.

## **2.5 Conclusions**

The main method of estimating invasion growth rates, model fitting, is difficult. This isn't a problem unique to coexistence theory. In my study, the competition model I chose proved to be a good match for *Tetrahymena* and *Colpidium*, and I was able to estimate invasion growth rates for these two species. However, the simplifications inherent in the predator-prey model I chose proved to be too much, and I was unable to use the model estimates to calculate any other invasion growth rates confidently. Regardless, I still gained valuable information about how different resources affect the population dynamics of this model system with both competition and predation present. Additionally, I was able to demonstrate how community composition and the interactions present can modify population dynamics like carrying capacity or predator-prey cycling. With these data already collected, it would be possible to return and apply different model forms or techniques with the goal of eventually being able to estimate invasion growth rates for all treatments.

## CHAPTER 3: COMPARING METHODS OF ESTIMATING INVASION GROWTH RATE

### 3.1 Introduction

Quantifying coexistence almost always involves simulations and model fitting. It is usually not tractable to directly measure invasion growth rates for each species in a community at many different points in time or space and average them. Many systems do not have fast enough generation times to make such an experiment practical (Grainger, Levine, et al. 2019). By building and parameterizing a model of the community of interest, invasion growth rates can be estimated in a larger variety of study systems. However, if invasion growth rates will be estimated by fitted models, we want to be sure that the estimates are accurate, and this is easier said than done.

Modeling on its own is a difficult task, as evidenced by the numerous articles and books providing advice and strategies (e.g., Tarantola 2005; Bolker et al. 2013; Grainger et al. 2022). It is often up to the researcher to determine how realistic their model should be (Stouffer 2019). Additional model parameters can decrease bias at the cost of increasing variance in model estimates and vice versa for simpler models (Adkison 2009). Thus, an already challenging task becomes harder with increasingly complex data, as the researcher must decide on which and how many parameters are necessary to accurately capture a system's dynamics.

Model estimates can strongly differ in their predictions of coexistence depending on the model used and whether uncertainty is propagated (Bowler et al. 2022; Cervantes-Loreto et al. 2023). However, even if one source of uncertainty is accounted for, other sources can end up being ignored (Cervantes-Loreto et al. 2023). The outcome of species interactions like competition can depend on the environment it takes place in which can cause predictions to vary across a heterogeneous environment (Chamberlain et al. 2014; Bimler et al. 2018). Model

outputs can be sensitive to variations in initial parameter values, and parameter uncertainty can affect predictions not propagated properly (Terry et al. 2021; Cervantes-Loreto et al. 2023). Of particular concern is structural sensitivity, which characterizes how phenomenologically similar mathematical functions can produce different model outcomes and can lead to qualitatively different predictions of coexistence in different models with similar fit to data (Cordoleani et al. 2011; Cervantes-Loreto et al. 2023). Biological models, including those used to study coexistence, are especially prone to this due to the nature of the models summarizing complex processes and applying simplifying assumptions (Aldebert and Stouffer 2018). As a result, there have been concerns with the modeling approaches used to assess coexistence and whether model estimates should be trusted.

Given these recent concerns over the reliability of model estimates for quantifying coexistence, I wanted to see if another method would provide comparable results to those obtained from model fitting. If an alternative method can provide reliable estimates of invasion growth rates with less sources of error, then the fitting of a model could be cut out entirely. Using a model system of protists, I conducted hundreds of short-term, small-scale invasion assays to directly estimate invasion growth rates. I also fit a model to long-term competition data from the same protist species and calculated invasion growth rate estimates. Using both the data from invasion assays and the long-term competition data, I then compared the direct estimates with those obtained via the more typical method of fitting a model to data to assess whether these methods are capable of producing similar results.

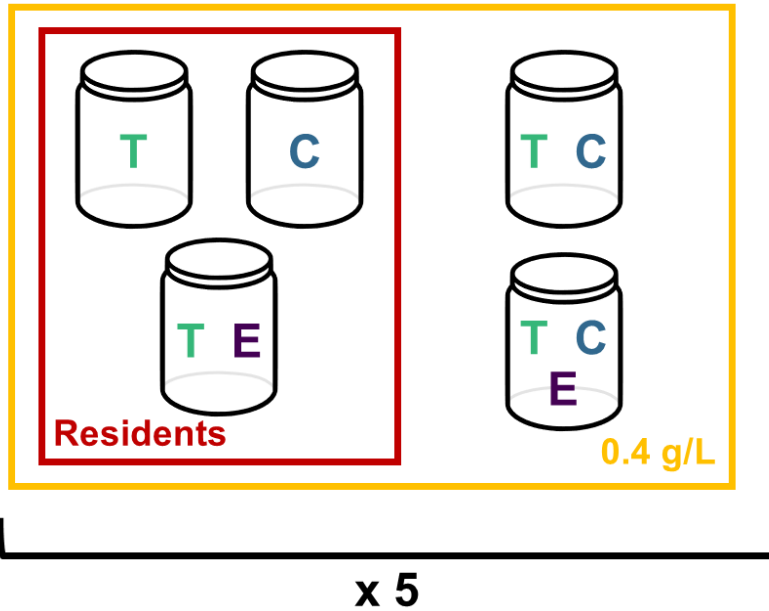
## 3.2 Methods

### 3.2.1 Study Species and Microcosm Methods

For this set of experiments, I used the ciliate protists *Tetrahymena pyriformis*, *Colpidium striatum*, and *Euplotes patella*. *Tetrahymena* and *Colpidium* are competitors that feed on various species of bacteria, and *Euplotes* is a predator of *Tetrahymena*. These species are described in further detail in Section 2.2.1. Microcosms were established the same as described in Section 2.2.2, but with only one resource level (0.4 grams of timothy hay (TH) per liter of spring water) and periodic media replacement differing based on experimental treatment (more details in Section 3.2.3).

### 3.2.2 Microcosm Sampling

To build a model for comparison to direct invasion, a set of microcosms were sampled every weekday to create a time series of protist species densities. There were five replicates of each feasible species combination of *Tetrahymena*, *Colpidium*, and *Euplotes* for a total of 25 jars (Figure 3.1). *Euplotes*, being a predator of *Tetrahymena*, could not survive on its own. While *Euplotes* species have been reported to consume *Colpidium* (Balciunas and Lawler 1995; Jiang and Morin 2005), I was not able to successfully grow together in the absence of *Tetrahymena*. Jars containing *Tetrahymena* alone, *Colpidium* alone, or *Tetrahymena* with *Euplotes* were additionally used for invasions as sources of “residents” and “invaders”. All daily sampling was conducted before any volume was removed for invasions. Sampling protocol was identical to that described in Section 2.2.3.

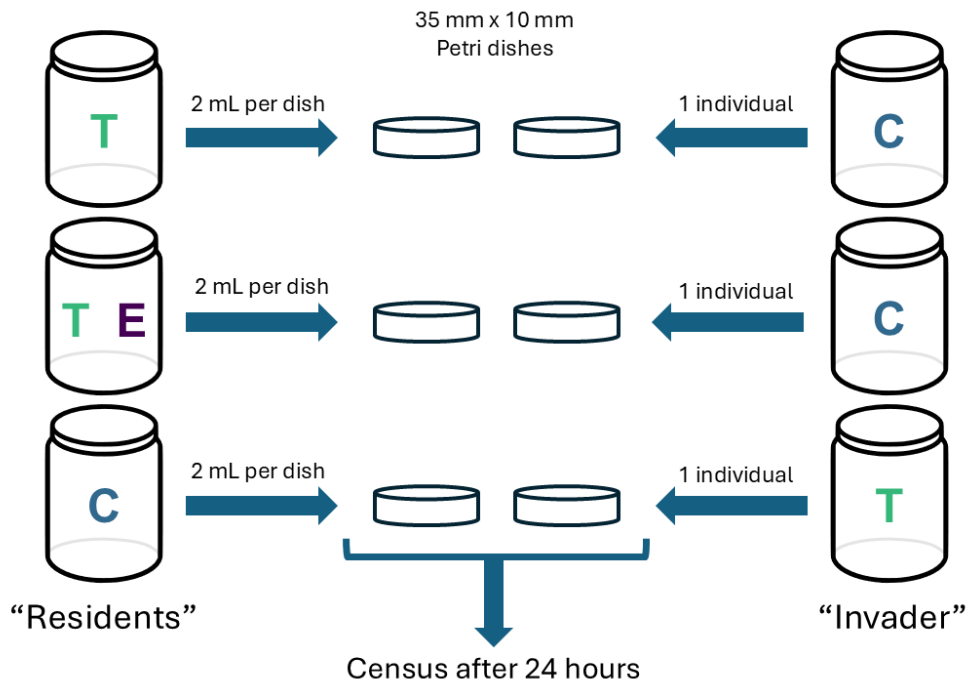


**Figure 3.1:** A graphical representation of the species combinations used in long-term microcosms. Each species is represented by the first letter of its genus name. All jars used the same resource level of 0.4 g TH/L. Species combinations in the red box were used as residents for invasion assays in addition to being sampled. Each treatment shown in the figure was replicated five times, as indicated by the bracket at the bottom of the graphic.

### 3.2.3 Invasion and Census

I used hundreds of small (35x15 mm) plastic, disposable petri dishes for conducting invasions. Each petri dish could hold a maximum volume of approximately 5 mL. All petri dishes were labeled for identification with the label including a unique dish ID number, the resident species with their original jar ID, and the invading species with their original jar ID.

Each day of invasions had two replicates per resident jar (Figure 3.2). For example, five different resident jars being invaded with two replicates per jar equals 10 petri dishes invaded per day. Over the course of all invasions, invaders were sourced from each possible jar replicate. However, only one source jar was used per day of invasions, such that all invaders on a given day originated from the same replicate.



**Figure 3.2:** A graphic showing the experimental design of the direct invasions. Letters on the jars represent the first letter of the species present. Any given petri dish contained 2 mL taken from a jar of “residents” and 1 individual cell as the “invader”. This was repeated for each replicate resident jar.

Petri dishes were set up for invasion by first adding 2 mL taken from the respective resident jar and then adding one individual cell from the invader jar. Petri dishes consisted of 2 mL from the respective “resident” jar and one individual cell from the “invader” jar. To obtain single cells for invasion, 0.3 mL was removed from the “invader” jar and added to a separate, larger petri dish. A Pasteur pipette was modified to have a smaller opening and used to pick up individual cells one at a time. New pipettes were used each day to prevent contamination from different source jars. As a cell was added to the experimental petri dishes, the invasion time was noted down to ensure accurate calculations for time between invasion and census. After invasion, all petri dishes were stored in an incubator maintained at 20°C until censusing.

As conducting invasions resulted in a larger volume of media being removed per microcosm, media was replaced more often than in jars where only sampling occurred. Every weekday, 10% of a microcosm's volume was removed and replaced with fresh, sterile medium of the same nutrient concentration. This ensures each jar maintains a volume of approximately 80 mL while also providing additional resources to increase the longevity of the microcosms.

Censusing occurred approximately 24 hours after invasion. Invaders were censused, rather than sampled, to ensure that all individuals were identified and obtain the most accurate count. With the invaders being at such a low abundance, samples taken from the petri dishes would have had large amounts of sampling error and among-sample variation. The start time of censusing for each dish was recorded and would be used to calculate the actual duration of the invasion. Every petri dish was visually examined under a light microscope for individuals of the invading species. Resident species were not counted as this would not be viable given the large number of replicates. Each petri dish was scanned for approximately 3 minutes.

### 3.2.4 Analyses

All data manipulation and analyses were conducted using R 4.5.0 (R Core Team 2025).

After invaded petri dishes have been censused, the final and initial counts of the invading species are used to calculate a per capita growth rate. This is done by taking the natural logarithm of both counts, subtracting the initial count from the final count, and dividing the difference by the hours since invasion (Equation 3.1).

$$\frac{\ln(\textit{final}) - \ln(\textit{initial})}{\textit{hours}} \quad (3.1)$$

In some replicates, the invader died off between invasion and censusing, resulting in a final count of zero. This means that growth rates cannot be calculated the same way for these replicates, as the logarithm of zero is undefined.

To estimate the negative growth rate of these petri dishes, I utilized the R package ‘msm’ (Jackson 2011). This package fits a continuous-time Markov multi-state model to data. The model’s main assumptions are that the system moves through a series of states in continuous time, and that future states depend only on the current state of the system. A matrix,  $Q$ , which describes the allowed transitions is provided as input to the model. Within this matrix, a “0” indicates a disallowed transition, and any other value is a placeholder for a valid transition. Diagonal entries are left as zeroes, as their value is calculated from the off-diagonal entries in the same row, such that each row sums to zero.

For my purposes, the “states” of the model are different invader counts, such that each possible integer value of invaders is a state (i.e., 0, 1, 2). Any dish that reached an invader count greater than 2 was combined into state 2 as a general “successful invasion” state. As state 0 represents no invading cells, and it is impossible to produce new individuals from none, state 0 is an absorbing state. Once the system reaches this state of no invaders, it is not able to transition to a different state again. Transitions between states 1 and 2 can occur freely, but a system in state 2 must first pass through state 1 before it can reach state 0.

The model fitting function ‘msm’ takes an input of a data frame that contains, at minimum: the time of observation, the observed state, and a subject identification number. The data frame is grouped by subject, with observations ordered by time for each subject. For my dataset, each petri dish is an individual “subject” and is associated with a unique ID number. For each petri dish, there are two observation times, with the invasion time being at 0 hours, and the censusing time being time passed (in hours) relative to the invasion. The modeling function takes the dataset, and a provided  $Q$  matrix to calculate initial values for the model to use.

The function works by finding the unknown (non-zero) values of the matrix such that likelihood is maximized. Specifically, the base-R function ‘optim’ is used to minimize the minus log-likelihood of the model, using the BFGS algorithm as a default. The main output is a matrix of transition intensities: instantaneous rates of moving between states per unit time. Each possible transition has its own transition intensity, such that the transition intensity from 1 to 2 is different from that of 1 to 0, and so on. As diagonal entries in the matrix are calculated based on the other entries in each row, these values have a different meaning. Diagonal entries are used to calculate the mean sojourn time, the average time spent in a state before transitioning by taking -1 divided by the diagonal entry.

An additional function, ‘prevalence.msm’, from the same package uses the model output to provide a list of observed and expected numbers and percentages for each state at each timestep. To calculate an estimated growth rate for petri dishes that decreased to zero, I used these percentages alongside the transition intensities. Since I am interested in the transition from state 1 to state 0, I only needed to look at one transition intensity per invader-resident combination.

First, I averaged the proportion of dishes in state 1 at a time of  $t=0$  with the proportion at censusing time, which ranged from  $t=21$  to  $t=26$ . This is to account for part of the fact that the single invader cell could have died at any time between invading and censusing. Then, I multiplied this average proportion by the transition intensity to obtain an estimation of growth rate. As transition intensities are already instantaneous rates, I did not need to further manipulate the value to give a per hour growth rate. Additionally, this final value must be multiplied by -1 to properly indicate the negative growth, as all transition intensities are given as positive values.

These estimates of negative growth rates were then added back into the data frame, replacing the NA values from the undefined logarithm calculations. Estimates were substituted in on a per-replicate basis depending on the length of time between invasion and census. For example, a dish that was censused 22 hours after invasion would use the growth rate calculated from the expected proportion at  $t=22$ . Finally, the average growth rate and 95% confidence interval was calculated per day and across all replicates for each resident-invader combination to arrive at final estimates of invasion growth rates.

To compare my direct estimates to a more common method of estimating invasion growth rates, I used the R package 'FME' (Soetaert and Petzoldt 2010) to fit a model to time series data obtained from microcosm sampling. Microcosms sampled for model fitting used the same resource level as petri dishes used for invasion (0.4 g/L). A model of *Tetrahymena* and *Colpidium* together was used to calculate the invasion growth rates for either species invading the other, and a three species model was used to calculate invasion growth rates for *Colpidium* invading both *Tetrahymena* and *Euplotes* together. These are the same models described in Chapter 2 (Section 2.2.4). As the unit of time for my model estimates of invasion growth rate differs from the unit of time in my direct invasion estimates (days vs. hours), estimates calculated from direct invasion were multiplied by 24. This value could then be compared to the invasion growth rates I calculated from my repeated direct invasions.

### **3.3 Results**

#### **3.3.1 Colpidium Invasion Results**

*Colpidium* was unable to invade either of the resident species combinations on average (Figure 3.3). Most replicates resulted in either zero growth, where only one individual invader was censused, or negative growth, where no individuals were censused. When invading

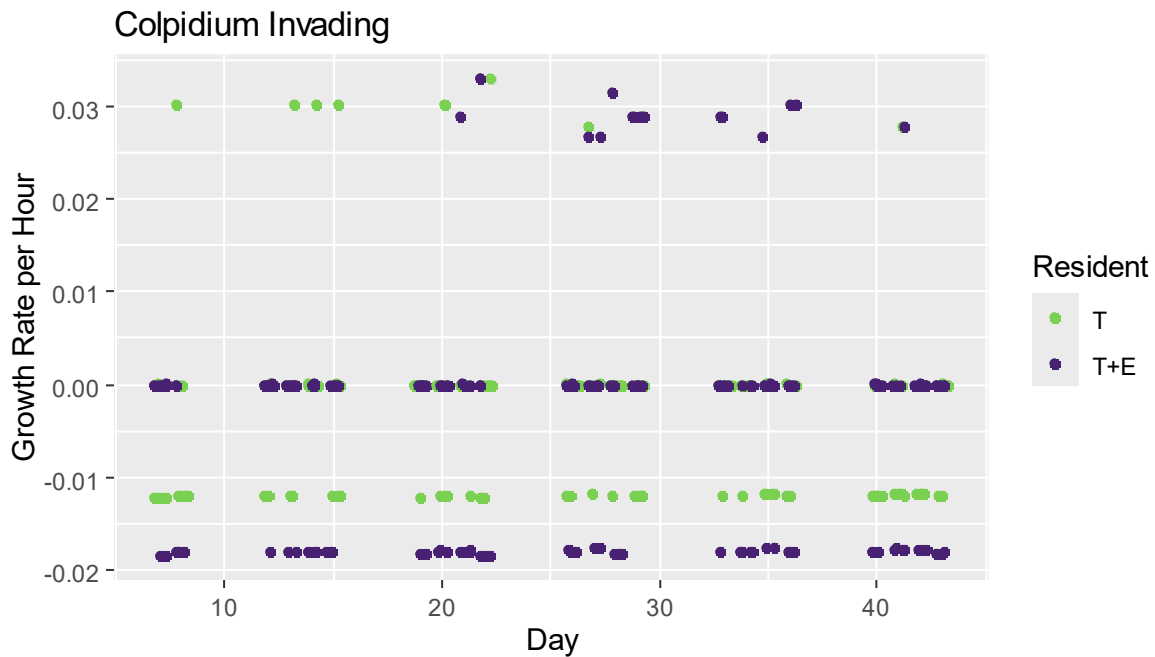
*Tetrahymena* as the only resident species, *Colpidium* had zero growth in 150 replicates and negative growth in 61 replicates. When both *Tetrahymena* and *Euplotes* were residents, *Colpidium* had zero growth in 112 replicates and negative growth in 91 replicates. *Colpidium* successfully invaded slightly more often when the resident species was both *Tetrahymena* and *Euplotes* compared to *Tetrahymena* alone. In later days of the experiment, with resident cultures having been established for over 20 days, a greater proportion of successes occurred when invading *Tetrahymena* and *Euplotes* compared to invasions into *Tetrahymena* alone. With both *Tetrahymena* and *Euplotes* as residents, *Colpidium* successfully invaded 17 times, and with only *Tetrahymena* as the resident, *Colpidium* successfully invaded 8 times.

For calculating estimates of negative growth rates, transition intensities for *Colpidium* decreasing from 1 to 0 individuals were 0.014 (95% CI: 0.011 – 0.018) for *Tetrahymena* as the resident and 0.024 (95% CI: 0.019 – 0.029) for *Tetrahymena* and *Euplotes* as the residents. This means *Colpidium* had a roughly 1.4% probability per hour of dying out when invading *Tetrahymena* and a roughly 2.4% probability per hour when invading *Tetrahymena* and *Euplotes*. These transition intensities were multiplied by the expected frequency of replicates with one individual invader, as estimated by the multi-state models, for each of the six possible census time values. For *Colpidium* invading *Tetrahymena*, negative growth rate estimates ranged from -0.0121 to -0.0118 at 21- and 26-hours post invasion, respectively. For invasions with *Tetrahymena* and *Euplotes* as the residents, estimates ranged from -0.0185 to -0.0176 at 21- and 26-hours post invasion, respectively.

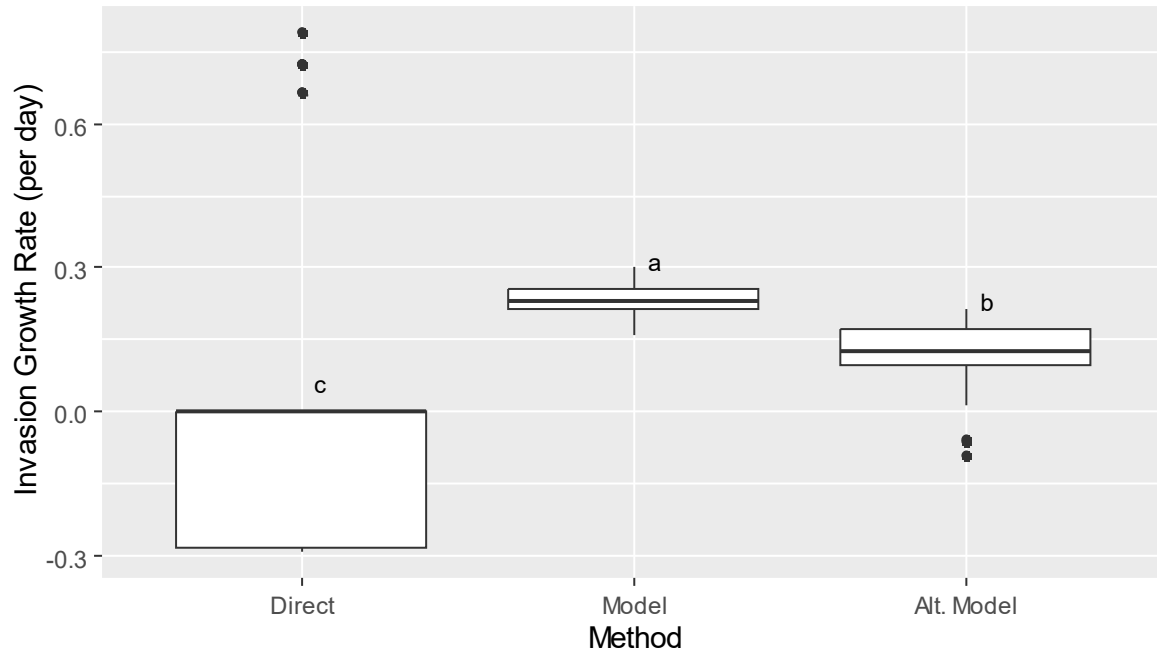
After substituting in the estimated negative growth rate values, the final estimated invasion growth rate across all replicates was -0.054 (95% CI: -0.08 – -0.02) when invading *Tetrahymena* and -0.125 (95% CI: -0.167 – -0.083) when invading both *Tetrahymena* and

*Euplotes*. The value obtained from model fitting for *Colpidium* invading *Tetrahymena* was 0.234 (95% CI: 0.228 – 0.241). This is larger than the estimate from direct invasions and is positive rather than negative. The value obtained from the alternative model was 0.126 (95% CI: 0.114 – 0.138). This is closer to the direct estimate, but still the opposite sign. All estimates are significantly different from one another (Figure 3.4).

I was unable to fit models containing *Euplotes* well enough to give informative parameter values, so I do not have a comparison for *Colpidium* invading *Tetrahymena* and *Euplotes*.



**Figure 3.3:** Calculated per-capita growth rates of *Colpidium* invading either *Tetrahymena* alone (T, in green) or *Tetrahymena* and *Euplotes* (T+E, in purple). Each point represents the result of one invasion.



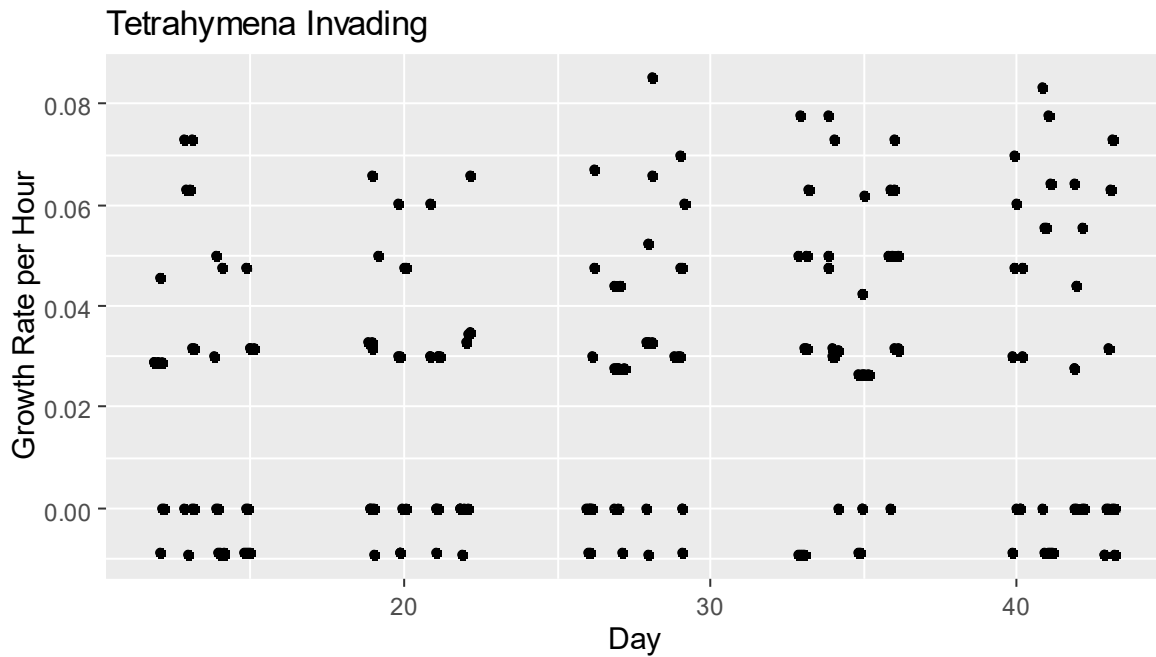
**Figure 3.4:** Comparison of the invasion growth rate estimates of *Colpidium* invading *Tetrahymena*. Letters next to each box indicate significant differences from a Tukey’s test.

### 3.3.2 *Tetrahymena* Invasion Results

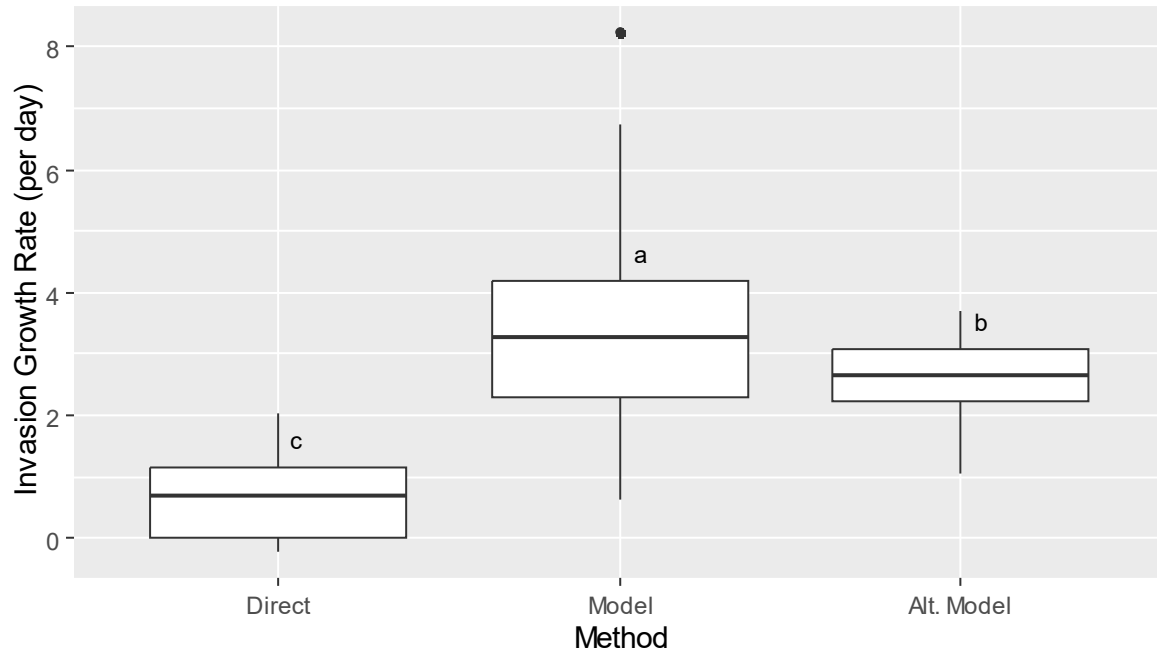
*Tetrahymena* was consistently able to invade a resident community of *Colpidium* (Figure 3.5). 114 replicates were successful invasions. There were still cases where *Tetrahymena* could not invade with 49 replicates exhibiting no growth and 36 replicates with negative growth.

The transition intensity for *Tetrahymena* decreasing from 1 to 0 cells when invading *Colpidium* was 0.014 (95% CI: 0.010 – 0.020). This translates to a roughly 1.4% probability per hour of the invading cell dying. This transition intensity was multiplied by the expected frequency of replicates with one individual invader, as estimated by the multi-state model, for each of the seven possible census time values. The resulting negative growth rate estimates ranged from -0.0094 to -0.0087 at 20- and 26-hours post invasion, respectively.

The final estimated invasion growth rate across all replicates of *Tetrahymena* invading *Colpidium* was 0.575 (95% CI: 0.484 – 0.666). The invasion growth rate obtained from model fitting was 3.37 (95% CI: 3.09 – 3.65). While these estimates are both positive, the value obtained from model fitting is approximately one order of magnitude larger. The value from the alternative model was 2.60 (95% CI: 2.48 – 2.72). This estimate is of a similar magnitude to the original model estimate and larger than the direct estimate. All invasion growth rate estimates are significantly different from one another (Figure 3.6).



**Figure 3.5:** Calculated per-capita growth rates of *Tetrahymena* invading *Colpidium*. Each point represents the result of one invasion.



**Figure 3.6:** Comparison of the invasion growth rate estimates of *Tetrahymena* invading *Colpidium*. Letters near each box indicate significant differences from a Tukey's test.

### 3.4 Discussion

Estimates of invasion growth rate obtained from direct invasion were not comparable with those obtained from model fitting. *Colpidium* was unable to invade resident communities of *Tetrahymena* alone or with *Euplotes* despite being able to coexist with these species in the microcosm jars. While *Tetrahymena* was able to both invade and coexist with *Colpidium*, invasion growth rate estimates differed by roughly two orders of magnitude between methods.

Estimates of negative growth rate for direct invasions, regardless of the method used to calculate them, will likely have a large amount of error due to the lack of observation time points available to calculate them from. Regardless, *Colpidium* failed to invade in most replicates, which contradicts what was expected based on the sampling data where the two species coexisted over many generations. A previous study found strong priority effects between

*Colpidium* and *Tetrahymena* such that *Colpidium* was always excluded when invading *Tetrahymena*, but *Tetrahymena* was always able to invade *Colpidium* (Bright 2018). This could potentially explain the difference in estimates for *Colpidium*, but *Tetrahymena*, having a smaller proportion of negative invasions, should be minimally impacted by inaccurate negative growth rate estimates. Despite this, *Tetrahymena*'s calculated invasion growth rate is still quite different from the model derived value. This poses the question: Why is there such a large difference in invasion growth rates, and even in invasion outcomes, between methods?

Protists, being very small, are useful for experiments as they can be manipulated more easily. However, their size also makes them more susceptible to changes or inconsistencies that we, being much larger organisms, might not perceive to be a meaningful difference. My results suggest that these species of protists are sensitive to relatively small changes in their environment. This "change" could have been the difference in material between the jars and petri dishes, bacterial composition, or volume of the container.

The difference in competitive outcomes between methods could be due to two coexistence mechanisms having to do with habitat partitioning: the spatial storage effect and fitness-density covariance. The spatial storage effect is a coexistence mechanism where the environment modifies how competition affects growth rates (Chesson 2000a). When there is spatial variation in the environment, a species might perform better in some areas than others. This better performance "buffers" the population against poor performance in other locations and allows the species to persist in the environment. The spatial storage effect is mathematically the same as the temporal version (Barabás et al. 2018). However, while the temporal storage effect is a fairly weak mechanism (Stump and Vasseur 2023), the spatial version is much stronger (Snyder 2008).

Fitness-density covariance, a coexistence mechanism with no temporal analog, occurs when population densities vary across space such that species are more concentrated in areas that are more favorable to them (Stump and Chesson 2015; Barabás et al. 2018). Fitness-density covariance is thought to be even stronger than the spatial storage effect (Shoemaker and Melbourne 2016), though these distinct mechanisms are often found together (Stump and Chesson 2015). A species that is a worse competitor but a better disperser is benefited by fitness-density covariance (Shoemaker and Melbourne 2016), so this mechanism would be especially advantageous to *Colpidium* as the faster disperser (Fox 2007).

It is possible that the differences in the spatial environment between the jar microcosms and smaller petri dishes caused these spatial coexistence mechanisms to weaken. The petri dishes I used for invasion, being much smaller than the glass jars used for the long-term microcosms, likely resulted in a more homogenous environment. This would mean that the protists were not able to utilize the spatial niches they perform best in. The smaller volume would also mean individuals could not distribute themselves as widely as was possible in a microcosm jar. It has been found previously that culture volume can affect whether a predator-prey pair can coexist by limiting the frequency that predators encounter prey (Luckinbill 1974). Similarly, Balciunas and Lawler (1995) saw unexpected extinctions of *Chilomonas paramecium* with a predator despite the species being able to coexist in similar, albeit 70 mL larger, microcosms. This same thing may have been happening between competitors in my study where the smaller 2 mL petri dishes made interactions more likely than in the 80 mL microcosm jars. On the other hand, experiments conducted by Fox and Barreto (2006) found that continuous mixing of microcosms containing an unidentified mixture of bacteria did not affect competitive outcomes between *Colpidium striatum* and *Tetrahymena thermophila*.

Additionally, the petri dishes did not have sterile wheat seeds added to them which were present in the jar microcosms. This choice was made as a wheat seed of the same size might have had a much larger impact on the resource level of the petri dish due to the smaller volume. However, the lack of wheat seed could have affected the bacterial distribution of the petri dishes. Bacteria typically cluster around the wheat seed and form biofilms (Cremin et al. 2023; personal observations). Anecdotally, I have seen *Tetrahymena* cluster around these wheat seeds more so than *Colpidium*. The absence of the wheat seed in the petri dishes, combined with the smaller culture volume, could have led to *Tetrahymena* being more dispersed and encroaching upon what would typically be *Colpidium*'s spatial niche.

The decrease in volume might have affected the distribution of bacterial species as well. While we do not definitively know whether *Tetrahymena* and *Colpidium* eat similar species to one another, they have been shown to display species-specific preferences when provided with multiple types of prey (Balciunas and Lawler 1995; Fox and Barreto 2006; Thurman et al. 2010). Additionally, bacteria can prefer different depths of water, for example, due to differences in available oxygen (Salmaso 2019). Two bacterial species confirmed to be edible to both *Tetrahymena* and *Colpidium*, *Escherichia coli* and *Bacillus subtilis* (Fox and Barreto 2006; Fox 2007; Thurman et al. 2010), tend to be found in different parts of an aquatic environment. *E. coli* is a facultative anaerobe and is most common in the sediments at the bottom of a body of water (Solo-Gabriele et al. 2000; Von Wulffen et al. 2016), while *B. subtilis* is an aerobic species and found at the water-air interface (Krajnc et al. 2022).

While I do not know exactly what species of bacteria were present in my experimental cultures, these two species are plausible candidates given their ubiquity and use in prior microcosm studies. It is possible that the change from jar to petri dish removed some of the

bacteria's preferred spatial niches, or at the very least reduced the distance between them by decreasing the size of the water column. Any impact on the bacterial community has the potential to affect the protists that consume them, which could contribute to decreased growth rates.

### **3.5 Conclusions**

I used a direct method of estimating invasion growth rates and compared that to the more common method of model fitting. Although it was a reasonable hope that they would be comparable, each method ended up providing different results. The sources of error when fitting a model were thus traded for a different set of problems when estimating coexistence more directly. However, that does not necessarily mean that the results are inaccurate. Modifying the spatial environment, specifically by decreasing the culture volume, was necessary to make accurate censusing of invaders tractable. These changes proved to have a greater impact than was expected. Thus, the difference in invasion growth rates may simply reflect the difference in culture vessel. My experiments additionally illustrate the importance of studying and understanding multiple environmental conditions as seemingly insignificant variables may affect results when not properly accounted for.

## CHAPTER 4: CONCLUSIONS

### 4.1 Summary

In order to coexist, species need to be able to increase when rare. This ability to increase from low densities can be quantified using invasion growth rates. The difficulty of obtaining estimates of invasion growth rates through modeling suggests a need for a more direct measurement. However, I found that direct estimates using invasion assays have their own difficulties.

In my study, I conducted two main experiments focusing on coexistence in protist microcosms. For the first experiment, detailed in chapter 2, I grew three species of protists in all feasible combinations. Then I calculated invasion growth rates where possible using fitted models. I was only able to calculate invasion growth rates for *Tetrahymena* and *Colpidium* growing together, as there were difficulties with the predator-prey model used fitting the data for the other treatments. Both estimated invasion growth rates were positive, suggesting these two species were coexisting in the experimental microcosms. *Tetrahymena*'s invasion growth rate was larger than *Colpidium*'s which indicates that *Colpidium* was experiencing more interspecific competition than *Tetrahymena* and therefore would have a harder time invading.

The second set of experiments, detailed in chapter 3, compared two methods of estimating invasion growth rates. I used the model fitting estimates obtained in chapter 2 and compared these with invasion growth rates obtained directly via repeated small-scale invasions. In these direct invasions, *Tetrahymena* was able to invade *Colpidium* on average, and *Colpidium* was unable to invade either *Tetrahymena* alone or *Tetrahymena* with *Euplotes* on average. However, *Colpidium* was successful more often when invading *Tetrahymena* with *Euplotes*. This alternate method of obtaining invasion growth rates, unfortunately, did not result in fewer

sources of error as I had hoped, and the different methods did not provide comparable results. The model fitting estimates differed in magnitude from the direct estimates for both species. Additionally, the estimates for *Colpidium* were of different signs with the model estimate being positive and the direct estimate being negative. The inconsistency in estimates across methods most likely has to do with the difference in volume between the experimental microcosms used for sampling and the petri dishes used for invasions.

#### **4.2 Value of Direct Invasion**

I sought out to test a new method of estimating invasion growth rates so that coexistence theory could be more easily applied to some systems. While it didn't work out perfectly, there is still potential in this alternative method and the results I obtained. Much like there is still value in modeling even though I encountered difficulties, I think there is still value in direct invasions as a potential method of estimating invasion growth rates.

Estimating invasion growth rate by direct invasions could still work for other microscopic species. It would be worth trying this approach again with different species to see if the problems I faced are specific to bacterivorous ciliates. Additionally, this method could benefit from more replication in the same system to ensure that the same results are able to be obtained. Applying this same method again in the future could tell us whether my mismatch between direct and model estimates is due to human error, the species used, or something else entirely.

The long-term competitive outcomes from the microcosm jars did not match what I would expect given the results from the direct invasion assays. This indicates that the direct estimates were not an accurate representation of the competitive interactions in the long-term cultures, but they could still be accurate for the smaller environment the invasions took place in. I expect that my direct method would work well if the long-term cultures and invasion growth

rate assays occurred in culture vessels of the same volume. For example, a larger culture volume per invasion could be used depending on available time and equipment. The culture volume of the long-term microcosms used for comparison could also be varied, as incidental findings have shown this can impact results of competition and predation (Luckinbill 1974; Balciunas and Lawler 1995). Either or both modifications would make the spatial environment of the jars and petri dishes more similar in addition to providing further information on how available space affects the community.

While direct invasion might only be possible using small organisms, it could still be a valuable method once the issues are worked out. The nature of microcosms and the faster generation time of species used make it a good system to troubleshoot how to best apply coexistence theory to different and more complicated scenarios. With the right equipment, the censusing process could be streamlined such that petri dishes are photographed and then analyzed by software. If this method is shown to provide accurate estimates of invasion growth rate, then the application of coexistence theory can be more accessible to those unfamiliar or uncomfortable with model fitting. Regardless, I believe that useful information and interesting data will come from further testing of direct invasions, and it is too soon to discredit it fully.

### **4.3 Future Directions**

Given what I now know, there are many ways my study could be improved and expanded upon. Although I had microcosm jars at multiple resource levels, I only conducted invasions at one. A future study could vary the resource level used in these direct invasions and then compare the changes along this gradient to those observed using full-size microcosms. This would give more insight into what effect the smaller culture volume has on the protist species used. The invasions could also be conducted at higher volumes, as previously mentioned, to see how

estimated invasion growth rates change. However, as the volume of media used for the invasion increases, so does the difficulty of censusing. Thus, this would only be feasible up to a certain volume and might require more specialized equipment.

From my experiments, it is impossible to tell whether *Colpidium* or *Tetrahymena* were partitioning space in the larger jars. By swirling and homogenizing microcosm jars prior to sampling, any spatial variation in species density was temporarily removed at the point of sampling. Thus, while species might be partitioning space in the microcosm, this information would not be translated to the sampling data obtained. To properly investigate the extent of spatial partitioning in these protist species, and in the bacteria they consume, further experiments need to be conducted where different areas of the jar are sampled without disturbing or mixing the jar's contents.

There are still many unanswered questions regarding the three-species microcosm results such as whether there is a pattern to *Colpidium* becoming small enough to be consumed by *Euplotes*. To look further into this, more data is needed using the same three species with tighter controls and more replication. With more replication at the same time, batch differences across experiments can be mitigated while obtaining more information on what might be causing the variation across replicates that I saw in my results. More data will make any potential patterns more obvious, and if no patterns can be found then this might indicate the system has chaotic behavior. In terms of tighter controls, identifying and manipulating the composition of bacterial species used in microcosms such that only a few known species are present could address some of the sources of large among-replicate variation. Protists could also be grown on one type of bacteria at a time by thoroughly washing individual protists in sterile medium, and then transferring the protists to high density cultures containing the known bacteria (Fox and Barreto

2006; Bright 2018). Identification of bacteria used in experiments will also allow for more accurate replication of entire studies and aid in understanding competition between *Tetrahymena* and *Colpidium*.

Additionally, I think it would be beneficial to create and maintain stock cultures at experimental resource levels, such that each protist species spends at least one month at this resource level before the start of the experiment. This would allow the protists time to acclimate to the new, lower resource level. If all individuals are acclimated to the resource level of the experiment, I would expect there to be less variability in cell size due to the sudden drop in resources. As *Colpidium*'s susceptibility to predation depends on its size (Balciunas and Lawler 1995; Jiang and Morin 2005), this change could give more consistent results across the same resource level. In general, I think microcosm experiments could benefit from more standardized methodology as past studies exhibit a high degree of variation in protocol (Altermatt et al. 2015). If more studies use the same microcosm volume, type of culture medium, resource level, and bacterial species, then results can be more easily compared across experiments.

While direct invasion did not go as anticipated, modeling didn't work very well either. I was unable to fit a model to any treatment involving *Euplotes*, so a logical next step would be to try different predator-prey models as mentioned in the chapter 2 discussion. Using different functional responses for *Euplotes* or allowing *Euplotes* to eat both *Tetrahymena* and *Colpidium* could provide a better fit. A different model could additionally place constraints on how often *Colpidium* would be consumed, although this would be more difficult to implement. If simply changing the model equations is not enough to obtain a good fit, a different approach using Bayesian methods could be used. This could include model aggregation or better error propagation (Bowler et al. 2022; Cervantes-Loreto et al. 2023). If invasion growth rates can be

obtained, then future work would be able to look at the specific coexistence mechanisms at play between these species by partitioning the invasion growth rates. This would provide more concrete information on how the species in this system interact and coexist.

Despite the issues I ran into with modeling, other researchers have instead found success when applying models to protist microcosms. Gause (1934) provided some of the first empirical evidence for the Lotka-Volterra equations using species of *Paramecium* (Halperin 2025). Lotka-Volterra competition models have also been fit to communities including *Tetrahymena* and *Colpidium* (Fox 2023). Even predator-prey models have been successfully fit to time series of protists. Using data of *Paramecium aurelia* and the predator *Didinium nasutum* (Luckinbill 1973), Harrison (1995) made many modifications to a predator-prey model in search of the best fit. Simply changing the predator functional response from a saturating type II, present in the model I used, to a sigmoid type III substantially improved the model fit (Harrison 1995). Thus, my own troubles with modeling do not indicate that it is impossible to reliably estimate invasion growth rates in this system.

Coexistence theory is an extensive framework that I have barely scratched the surface of in this study. The theory, techniques, and research surrounding it are constantly evolving, especially given coexistence theory has only really become cemented in the literature over the past 30-40 years. (Johnson and Hastings 2022; Simha et al. 2022; Halperin 2025). Despite the challenges continuing to emerge, solutions continue to be found. Difficulty understanding models central to coexistence has been met with guides on using theory (Grainger et al. 2022) and suggestions to incorporate verbal formulations reminiscent of Gause (Halperin 2025). While the original theory is not applicable to all situations, this is being addressed with approaches that can be applied to multispecies communities (Saavedra et al. 2017; Spaak and Schreiber 2023).

Even our understanding of certain coexistence mechanisms is still changing (Stump and Vasseur 2023). If it turns out that invasion growth rates just can't be reliably estimated, this could be an indication that predictions of coexistence might be better as a range of probabilities rather than a distinct "yes" or "no". (Bowler et al. 2022; Cervantes-Loreto et al. 2023). Understanding the diversity of species and what allows them to coexist has been a goal of community ecology for decades. Thus, I am confident that coexistence theory will move forward and continue to innovate despite methodological complications.

## REFERENCES

- Adkison MD. 2009. Drawbacks of complex models in frequentist and Bayesian approaches to natural-resource management. *Ecol Appl.* 19(1):198–205. doi:10.1890/07-1641.1.
- Adler PB, HilleRisLambers J, Levine JM. 2007. A niche for neutrality. *Ecol Lett.* 10(2):95–104. doi:10.1111/j.1461-0248.2006.00996.x.
- Aho K, Derryberry D, Peterson T. 2014. Model selection for ecologists: the worldviews of AIC and BIC. *Ecology.* 95(3):631–636. doi:10.1890/13-1452.1.
- Aldebert C, Stouffer DB. 2018. Community dynamics and sensitivity to model structure: towards a probabilistic view of process-based model predictions. *J R Soc Interface.* 15(149):20180741. doi:10.1098/rsif.2018.0741.
- Altermatt F, Fronhofer EA, Garnier A, Giometto A, Hammes F, Klecka J, Legrand D, Mächler E, Massie TM, Pennekamp F, et al. 2015. Big answers from small worlds: a user’s guide for protist microcosms as a model system in ecology and evolution. Spencer M, editor. *Methods Ecol Evol.* 6(2):218–231. doi:10.1111/2041-210X.12312.
- Armstrong RA, McGehee R. 1980. Competitive Exclusion. *Am Nat.* 115(2):151–170. doi:10.1086/283553.
- Balciunas D, Lawler SP. 1995. Effects of Basal Resources, Predation, and Alternative Prey in Microcosm Food Chains. *Ecology.* 76(4):1327–1336. doi:10.2307/1940939.
- Barabás G, D’Andrea R, Stump SM. 2018. Chesson’s coexistence theory. *Ecol Monogr.* 88(3):277–303. doi:10.1002/ecm.1302.
- Barabás G, J. Michalska-Smith M, Allesina S. 2016. The Effect of Intra- and Interspecific Competition on Coexistence in Multispecies Communities. *Am Nat.* 188(1):E1–E12. doi:10.1086/686901.
- Bimler MD, Stouffer DB, Lai HR, Mayfield MM. 2018. Accurate predictions of coexistence in natural systems require the inclusion of facilitative interactions and environmental dependency. Godoy O, editor. *J Ecol.* 106(5):1839–1852. doi:10.1111/1365-2745.13030.

- Bolker BM, Gardner B, Maunder M, Berg CW, Brooks M, Comita L, Crone E, Cubaynes S, Davies T, De Valpine P, et al. 2013. Strategies for fitting nonlinear ecological models in R, ADM model Builder, and BUGS. Ramula S, editor. *Methods Ecol Evol.* 4(6):501–512. doi:10.1111/2041-210X.12044.
- Borer ET, Seabloom EW, Gruner DS, Harpole WS, Hillebrand H, Lind EM, Adler PB, Alberti J, Anderson TM, Bakker JD, et al. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. *Nature.* 508(7497):517–520. doi:10.1038/nature13144.
- Bowler CH, Weiss-Lehman C, Towers IR, Mayfield MM, Shoemaker LG. 2022. Accounting for demographic uncertainty increases predictions for species coexistence: A case study with annual plants. Enquist B, editor. *Ecol Lett.* 25(7):1618–1628. doi:10.1111/ele.14011.
- Bright E. 2018. The resilience of alternative community states driven by priority effects: a microcosm investigation [MScRes Thesis]. University of Bedfordshire. [accessed 2025 July 17]. <https://uobrep.openrepository.com/handle/10547/623587>.
- Cervantes-Loreto A, Pastore AI, Brown CRP, Marraffini ML, Aldebert C, Mayfield MM, Stouffer DB. 2023. Environmental context, parameter sensitivity, and structural sensitivity impact predictions of annual-plant coexistence. *Ecol Monogr.* 93(4):e1592. doi:10.1002/ecm.1592.
- Chamberlain SA, Bronstein JL, Rudgers JA. 2014. How context dependent are species interactions? Etienne R, editor. *Ecol Lett.* 17(7):881–890. doi:10.1111/ele.12279.
- Chase JM, Abrams PA, Grover JP, Diehl S, Chesson P, Holt RD, Richards SA, Nisbet RM, Case TJ. 2002. The interaction between predation and competition: a review and synthesis. *Ecol Lett.* 5(2):302–315. doi:10.1046/j.1461-0248.2002.00315.x.
- Chesson P. 1994. Multispecies Competition in Variable Environments. *Theor Popul Biol.* 45(3):227–276. doi:10.1006/tpbi.1994.1013.
- Chesson P. 2000a. General Theory of Competitive Coexistence in Spatially-Varying Environments. *Theor Popul Biol.* 58(3):211–237. doi:10.1006/tpbi.2000.1486.

- Chesson P. 2000b. Mechanisms of Maintenance of Species Diversity. *Annu Rev Ecol Syst.* 31(1):343–366. doi:10.1146/annurev.ecolsys.31.1.343.
- Chesson P. 2008. Quantifying and testing species coexistence mechanisms. In: Valladares F, Camacho A, Elosegi A, Gracia C, Estrada M, Senar JC, Gili JM, editors. *Unity in diversity: reflections on ecology after the legacy of Ramon Margalef*. Bilbao: Fundación BBVA. p. 119–164.  
[https://www.zoology.ubc.ca/bdg/pdfs\\_bdg/2013/fall/chesson/Chesson\\_2008.pdf](https://www.zoology.ubc.ca/bdg/pdfs_bdg/2013/fall/chesson/Chesson_2008.pdf).
- Chesson P. 2018. Updates on mechanisms of maintenance of species diversity. Godoy O, editor. *J Ecol.* 106(5):1773–1794. doi:10.1111/1365-2745.13035.
- Chesson P, Huntly N. 1997. The Roles of Harsh and Fluctuating Conditions in the Dynamics of Ecological Communities. *Am Nat.* 150(5):519–553. doi:10.1086/286080.
- Chesson P, Kuang JJ. 2008. The interaction between predation and competition. *Nature.* 456(7219):235–238. doi:10.1038/nature07248.
- Chesson PL, Ellner S. 1989. Invasibility and stochastic boundedness in monotonic competition models. *J Math Biol.* 27(2):117–138. doi:10.1007/BF00276099.
- Clark AT, Shoemaker LG, Arnoldi J, Barabás G, Germain R, Godoy O, Hallett L, Karakoç C, Saavedra S, Schreiber SJ. 2025. A practical guide to characterising ecological coexistence. *Biol Rev.:brv.70079*. doi:10.1111/brv.70079.
- Cordoleani F, Nerini D, Gauduchon M, Morozov A, Poggiale J-C. 2011. Structural sensitivity of biological models revisited. *J Theor Biol.* 283(1):82–91. doi:10.1016/j.jtbi.2011.05.021.
- Corliss JO. 1954. The Literature on Tetrahymena: Its History, Growth, and Recent Trends. *J Protozool.* 1(3):156–169. doi:10.1111/j.1550-7408.1954.tb00809.x.
- Cremin K, Duxbury SJN, Rosko J, Soyer OS. 2023. Formation and emergent dynamics of spatially organized microbial systems. *Interface Focus.* 13(2):20220062. doi:10.1098/rsfs.2022.0062.

- Daehler CC. 2003. Performance Comparisons of Co-Occurring Native and Alien Invasive Plants: Implications for Conservation and Restoration. *Annu Rev Ecol Evol Syst.* 34(1):183–211. doi:10.1146/annurev.ecolsys.34.011802.132403.
- Davis MA, Pelsor M. 2001. Experimental support for a resource-based mechanistic model of invasibility. *Ecol Lett.* 4(5):421–428. doi:10.1046/j.1461-0248.2001.00246.x.
- Dellinger JA, Shores CR, Craig AD, Kachel SM, Heithaus MR, Ripple WJ, Wirsing AJ. 2022. Predators reduce niche overlap between sympatric prey. *Oikos.* 2022(1):oik.08628. doi:10.1111/oik.08628.
- Dormann CF, Calabrese JM, Guillera-Arroita G, Matechou E, Bahn V, Bartoń K, Beale CM, Ciuti S, Elith J, Gerstner K, et al. 2018. Model averaging in ecology: a review of Bayesian, information-theoretic, and tactical approaches for predictive inference. *Ecol Monogr.* 88(4):485–504. doi:10.1002/ecm.1309.
- Ellner SP, Snyder RE, Adler PB, Hooker G. 2019. An expanded modern coexistence theory for empirical applications. Metcalf J, editor. *Ecol Lett.* 22(1):3–18. doi:10.1111/ele.13159.
- Fenchel T. 1987. *Ecology of protozoa: the biology of free-living phagotrophic protists.* Madison, Wis.: Science Tech Publishers.
- Flory SL, Bauer J, Phillips RP, Clay K. 2017. Effects of a non-native grass invasion decline over time. D'Antonio C, editor. *J Ecol.* 105(6):1475–1484. doi:10.1111/1365-2745.12850.
- Fox J, Barreto C. 2006. Surprising competitive coexistence in a classic model system. *Community Ecol.* 7(2):143–154. doi:10.1556/ComEc.7.2006.2.2.
- Fox JW. 2002. Testing a Simple Rule for Dominance in Resource Competition. *Am Nat.* 159(3):305–319. doi:10.1086/338543.
- Fox JW. 2007. Testing the Mechanisms by Which Source-Sink Dynamics Alter Competitive Outcomes in a Model System. *Am Nat.* 170(3):396–408. doi:10.1086/519855.

- Fox JW. 2023. The existence and strength of higher order interactions is sensitive to environmental context. *Ecology*. 104(10):e4156. doi:10.1002/ecy.4156.
- Gause GF. 1934. *The struggle for existence*. Baltimore: The Williams & Wilkins Company. [accessed 2025 June 16]. <http://archive.org/details/struggleforexist00gauz>.
- Grainger TN, Letten AD, Gilbert B, Fukami T. 2019. Applying modern coexistence theory to priority effects. *Proc Natl Acad Sci*. 116(13):6205–6210. doi:10.1073/pnas.1803122116.
- Grainger TN, Levine JM, Gilbert B. 2019. The Invasion Criterion: A Common Currency for Ecological Research. *Trends Ecol Evol*. 34(10):925–935. doi:10.1016/j.tree.2019.05.007.
- Grainger TN, Senthilnathan A, Ke P-J, Barbour MA, Jones NT, DeLong JP, Otto SP, O'Connor MI, Coblenz KE, Goel N, et al. 2022. An Empiricist's Guide to Using Ecological Theory. *Am Nat*. 199(1):1–20. doi:10.1086/717206.
- Halperin T. 2025. Georgii F. Gause's *The Struggle for Existence* and the Integration of Natural History and Mathematical Models. *Am Nat*. 205(3):251–264. doi:10.1086/734003.
- Harrison GW. 1995. Comparing Predator-Prey Models to Luckinbill's Experiment with *Didinium* and *Paramecium*. *Ecology*. 76(2):357–374. doi:10.2307/1941195.
- Hill DG. 2014. *The Biochemistry and Physiology of Tetrahymena*. Saint Louis: Elsevier Science.
- HilleRisLambers J, Adler PB, Harpole WS, Levine JM, Mayfield MM. 2012. Rethinking Community Assembly through the Lens of Coexistence Theory. *Annu Rev Ecol Evol Syst*. 43(1):227–248. doi:10.1146/annurev-ecolsys-110411-160411.
- Hobbs RJ, Huenneke LF. 1992. Disturbance, Diversity, and Invasion: Implications for Conservation. *Conserv Biol*. 6(3):324–337. doi:10.1046/j.1523-1739.1992.06030324.x.
- Hofbauer J, Schreiber SJ. 2022. Permanence via invasion graphs: incorporating community assembly into modern coexistence theory. *J Math Biol*. 85(5):54. doi:10.1007/s00285-022-01815-2.

- Holt RD, Grover J, Tilman D. 1994. Simple Rules for Interspecific Dominance in Systems with Exploitative and Apparent Competition. *Am Nat.* 144(5):741–771. doi:10.1086/285705.
- Holyoak M, Lawler SP. 2005. The Contribution of Laboratory Experiments on Protists to Understanding Population and Metapopulation Dynamics. In: *Advances in Ecological Research*. Vol. 37. Elsevier. p. 245–271. [accessed 2025 June 17]. <https://linkinghub.elsevier.com/retrieve/pii/S006525040437008X>.
- Jackson CH. 2011. Multi-State Models for Panel Data: The msm Package for R. *J Stat Softw.* 38(8):1–29. doi:10.18637/jss.v038.i08.
- Jiang L, Morin PJ. 2005. Predator Diet Breadth Influences the Relative Importance of Bottom-Up and Top-Down Control of Prey Biomass and Diversity. *Am Nat.* 165(3):350–363. doi:10.1086/428300.
- Jiang L, Morin PJ. 2007. Temperature fluctuation facilitates coexistence of competing species in experimental microbial communities. *J Anim Ecol.* 76(4):660–668. doi:10.1111/j.1365-2656.2007.01252.x.
- Johnson E, Hastings A. 2022. Resolving conceptual issues in Modern Coexistence Theory. doi:10.48550/arXiv.2201.07926. [accessed 2025 Sept 24]. <http://arxiv.org/abs/2201.07926>.
- Kaunzinger CMK, Morin PJ. 1998. Productivity controls food-chain properties in microbial communities. *Nature.* 395(6701):495–497. doi:10.1038/26741.
- Kneitel J, Perrault D. 2006. Disturbance-induced changes in community composition increase species invasion success. *Community Ecol.* 7(2):245–252. doi:10.1556/ComEc.7.2006.2.11.
- Kolar CS, Lodge DM. 2001. Progress in invasion biology: predicting invaders. *Trends Ecol Evol.* 16(4):199–204. doi:10.1016/S0169-5347(01)02101-2.

- Krajnc M, Stefanic P, Kostanjšek R, Mandic-Mulec I, Dogsa I, Stopar D. 2022. Systems view of *Bacillus subtilis* pellicle development. *Npj Biofilms Microbiomes*. 8(1):25. doi:10.1038/s41522-022-00293-0.
- Laan E, Fox JW. 2020. An experimental test of the effects of dispersal and the paradox of enrichment on metapopulation persistence. *Oikos*. 129(1):49–58. doi:10.1111/oik.06552.
- Law R, Weatherby AJ, Warren PH. 2000. On the invasibility of persistent protist communities. *Oikos*. 88(2):319–326. doi:10.1034/j.1600-0706.2000.880210.x.
- Lawler SP. 1993. Direct and Indirect Effects in Microcosm Communities of Protists. *Oecologia*. 93(2):184–190.
- Lawler SP, Morin PJ. 1993. Food Web Architecture and Population Dynamics in Laboratory Microcosms of Protists. *Am Nat*. 141(5):675–686. doi:10.1086/285499.
- Levine JM, Adler PB, Yelenik SG. 2004. A meta-analysis of biotic resistance to exotic plant invasions. *Ecol Lett*. 7(10):975–989. doi:10.1111/j.1461-0248.2004.00657.x.
- Levine JM, D’Antonio CM. 1999. Elton Revisited: A Review of Evidence Linking Diversity and Invasibility. *Oikos*. 87(1):15. doi:10.2307/3546992.
- Liu M, Bjørnlund L, Rønn R, Christensen S, Ekelund F. 2012. Disturbance Promotes Non-Indigenous Bacterial Invasion in Soil Microcosms: Analysis of the Roles of Resource Availability and Community Structure. *Ibekwe AM, editor. PLoS ONE*. 7(10):e45306. doi:10.1371/journal.pone.0045306.
- Longford NT. 2012. ‘Which model?’ is the wrong question. *Stat Neerlandica*. 66(3):237–252. doi:10.1111/j.1467-9574.2011.00517.x.
- Lonsdale WM. 1999. GLOBAL PATTERNS OF PLANT INVASIONS AND THE CONCEPT OF INVASIBILITY. *Ecology*. 80(5):1522–1536. doi:10.1890/0012-9658(1999)080%5B1522:GPOPIA%5D2.0.CO;2.

- Luckinbill LS. 1973. Coexistence in Laboratory Populations of *Paramecium Aurelia* and Its Predator *Didinium Nasutum*. *Ecology*. 54(6):1320–1327. doi:10.2307/1934194.
- Luckinbill LS. 1974. The Effects of Space and Enrichment on a Predator-Prey System. *Ecology*. 55(5):1142–1147. doi:10.2307/1940365.
- Mallon CA, Poly F, Le Roux X, Marring I, Van Elsas JD, Salles JF. 2015. Resource pulses can alleviate the biodiversity–invasion relationship in soil microbial communities. *Ecology*. 96(4):915–926. doi:10.1890/14-1001.1.
- May RM, Leonard WJ. 1975. Nonlinear Aspects of Competition Between Three Species. *SIAM J Appl Math*. 29(2):243–253. doi:10.1137/0129022.
- McGrady-Steed J, Harris PM, Morin PJ. 1997. Biodiversity regulates ecosystem predictability. *Nature*. 390(6656):162–165. doi:10.1038/36561.
- Narwani A, Alexandrou MA, Oakley TH, Carroll IT, Cardinale BJ. 2013. Experimental evidence that evolutionary relatedness does not affect the ecological mechanisms of coexistence in freshwater green algae. De Meester L, editor. *Ecol Lett*. 16(11):1373–1381. doi:10.1111/ele.12182.
- Nicholson A. 1954. An outline of the dynamics of animal populations. *Aust J Zool*. 2(1):9. doi:10.1071/ZO9540009.
- Pande J, Fung T, Chisholm R, Shnerb NM. 2020. Mean growth rate when rare is not a reliable metric for persistence of species. Coulson T, editor. *Ecol Lett*. 23(2):274–282. doi:10.1111/ele.13430.
- Pickett STA, White PS. 1985. *The ecology of natural disturbance and patch dynamics*. San Diego New York Berkeley: Academic press.  
[https://api.pageplace.de/preview/DT0400.9780323138932\\_A23647343/preview-9780323138932\\_A23647343.pdf](https://api.pageplace.de/preview/DT0400.9780323138932_A23647343/preview-9780323138932_A23647343.pdf).
- R Core Team. 2025. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>.

- Richardson DM, Pyšek P. 2006. Plant invasions: merging the concepts of species invasiveness and community invasibility. *Prog Phys Geogr Earth Environ.* 30(3):409–431. doi:10.1191/0309133306pp490pr.
- Rovere J, Fox JW. 2019. Persistently rare species experience stronger negative frequency dependence than common species: A statistical attractor that is hard to avoid. Tomasovych A, editor. *Glob Ecol Biogeogr.* 28(4):508–520. doi:10.1111/geb.12871.
- Ruf T. 1999. The Lomb-Scargle Periodogram in Biological Rhythm Research: Analysis of Incomplete and Unequally Spaced Time-Series. *Biol Rhythm Res.* 30:178–201.
- Saavedra S, Rohr RP, Bascompte J, Godoy O, Kraft NJB, Levine JM. 2017. A structural approach for understanding multispecies coexistence. *Ecol Monogr.* 87(3):470–486. doi:10.1002/ecm.1263.
- Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, et al. 2001. The Population Biology of Invasive Species. *Annu Rev Ecol Syst.* 32(1):305–332. doi:10.1146/annurev.ecolsys.32.081501.114037.
- Salmaso N. 2019. Effects of Habitat Partitioning on the Distribution of Bacterioplankton in Deep Lakes. *Front Microbiol.* 10:2257. doi:10.3389/fmicb.2019.02257.
- Shoemaker LG, Melbourne BA. 2016. Linking metacommunity paradigms to spatial coexistence mechanisms. *Ecology.* 97(9):2436–2446. doi:10.1002/ecy.1454.
- Siepielski AM, McPeck MA. 2010. On the evidence for species coexistence: a critique of the coexistence program. *Ecology.* 91(11):3153–3164. doi:10.1890/10-0154.1.
- Simha A, Pardo-De La Hoz CJ, Carley LN. 2022. Moving beyond the “Diversity Paradox”: The Limitations of Competition-Based Frameworks in Understanding Species Diversity. *Am Nat.* 200(1):89–100. doi:10.1086/720002.
- Smallwood SK. 1994. Site invasibility by exotic birds and mammals. *Biol Conserv.* 69(3):251–259. doi:10.1016/0006-3207(94)90424-3.

- Snyder RE. 2008. When does environmental variation most influence species coexistence? *Theor Ecol.* 1(3):129–139. doi:10.1007/s12080-008-0015-3.
- Soetaert K, Petzoldt T. 2010. Inverse Modelling, Sensitivity and Monte Carlo Analysis in *R* Using Package **FME**. *J Stat Softw.* 33(3). doi:10.18637/jss.v033.i03. [accessed 2024 Mar 20]. <http://www.jstatsoft.org/v33/i03/>.
- Solo-Gabriele HM, Wolfert MA, Desmarais TR, Palmer CJ. 2000. Sources of *Escherichia coli* in a Coastal Subtropical Environment. *Appl Environ Microbiol.* 66(1):230–237. doi:10.1128/AEM.66.1.230-237.2000.
- Sommers P, Chesson P. 2019. Effects of Predator Avoidance Behavior on the Coexistence of Competing Prey. *Am Nat.* 193(5):E132–E148. doi:10.1086/701780.
- Spaak JW, Schreiber SJ. 2023. Building modern coexistence theory from the ground up: The role of community assembly. *Ecol Lett.* 26(11):1840–1861. doi:10.1111/ele.14302.
- Stouffer DB. 2019. All ecological models are wrong, but some are useful. Gill J, editor. *J Anim Ecol.* 88(2):192–195. doi:10.1111/1365-2656.12949.
- Stump SM, Chesson P. 2015. Distance-responsive predation is not necessary for the Janzen–Connell hypothesis. *Theor Popul Biol.* 106:60–70. doi:10.1016/j.tpb.2015.10.006.
- Stump SM, Vasseur DA. 2023. Reexamining the storage effect: Why temporal variation in abiotic factors seems unlikely to cause coexistence. *Ecol Monogr.* 93(4):e1585. doi:10.1002/ecm.1585.
- Tarantola A. 2005. Inverse problem theory: and methods for model parameter estimation. Philadelphia: SIAM, Society for Industrial and Applied Mathematics.
- Terry JCD, Armitage DW. 2024. Widespread analytical pitfalls in empirical coexistence studies and a checklist for improving their statistical robustness. *Methods Ecol Evol.* 15(4):594–611. doi:10.1111/2041-210X.14227.

- Terry JCD, Chen J, Lewis OT. 2021. Natural enemies have inconsistent impacts on the coexistence of competing species. *J Anim Ecol.* 90(10):2277–2288. doi:10.1111/1365-2656.13534.
- Thomaz SM, Mormul RP, Michelan TS. 2015. Propagule pressure, invasibility of freshwater ecosystems by macrophytes and their ecological impacts: a review of tropical freshwater ecosystems. *Hydrobiologia.* 746(1):39–59. doi:10.1007/s10750-014-2044-9.
- Thurman J, Parry JD, Hill PJ, Laybourn-Parry J. 2010. The Filter-Feeding Ciliates *Colpidium striatum* and *Tetrahymena pyriformis* Display Selective Feeding Behaviours in the Presence of Mixed, Equally-Sized, Bacterial Prey. *Protist.* 161(4):577–588. doi:10.1016/j.protis.2010.04.001.
- Tredennick AT, Hooker G, Ellner SP, Adler PB. 2021. A practical guide to selecting models for exploration, inference, and prediction in ecology. *Ecology.* 102(6):e03336. doi:10.1002/ecy.3336.
- Turelli M. 1978. Does environmental variability limit niche overlap? *Proc Natl Acad Sci.* 75(10):5085–5089. doi:10.1073/pnas.75.10.5085.
- Vasseur DA, Fox JW. 2009. Phase-locking and environmental fluctuations generate synchrony in a predator–prey community. *Nature.* 460(7258):1007–1010. doi:10.1038/nature08208.
- Vilà M, Weiner J. 2004. Are invasive plant species better competitors than native plant species? – evidence from pair-wise experiments. *Oikos.* 105(2):229–238. doi:10.1111/j.0030-1299.2004.12682.x.
- Vitousek PM, D’antonio CM, Loope LL, Rejmanek M, Westbrooks R. 1997. Introduced species: a significant component of human-caused global change. *N Z J Ecol.*:1–16.
- Von Wulffen J, RecognNice-Team, Sawodny O, Feuer R. 2016. Transition of an Anaerobic *Escherichia coli* Culture to Aerobiosis: Balancing mRNA and Protein Levels in a Demand-Directed Dynamic Flux Balance Analysis. Parkinson J, editor. *PLOS ONE.* 11(7):e0158711. doi:10.1371/journal.pone.0158711.

Walker EJ, Gilbert B. 2025. Allee Effects and Coexistence. *Am Nat.* 205(6):559–571.  
doi:10.1086/735419.

Weatherby AJ, Warren PH, Law R. 1998. Coexistence and collapse: an experimental investigation of the persistent communities of a protist species pool. *J Anim Ecol.* 67(4):554–566. doi:10.1046/j.1365-2656.1998.00212.x.

Yenni G, Adler PB, Ernest SKM. 2017. Do persistent rare species experience stronger negative frequency dependence than common species? *Glob Ecol Biogeogr.* 26(5):513–523.  
doi:10.1111/geb.12566.