
# Title: The Resilience of Alternative Community States Driven by Priority Effects: A Microcosm Investigation

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# The Resilience of Alternative Community States Driven by Priority Effects: A Microcosm Investigation

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I, Emma Bright declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

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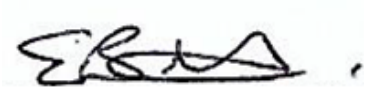

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**Abstract**

Within an ecosystem, there are a variety of interactions between species which affect the overall community. One of the strongest influences of community structure within a habitat is the order in which species arrive and establish; resulting in populations either coexisting or excluding one another to extinction. This can either be invading species excluding residents (competitive exclusion) or residents excluding invaders (priority effects), often due to freely depleting any shared resources before invaders arrive. Priority effects are predicted to be weaker when the invasion occurs simultaneously with warming towards and above the thermal tolerance of one species as the pressure put on the species can be too much to allow a population to grow or establish to survive. This experiment investigated whether an 8°C temperature range altered protist ability to invade or be invaded in simple aquatic microcosms, where the order of invasion of *Colpidium* and *Tetrahymena* was varied. I measured the changes to population density of both species over time, to identify changes in maximum population density and time to extinction. Results showed very strong priority effects between the two species, but this was never affected by temperature. In all treatments, resident *Tetrahymena* could never be invaded by *Colpidium*. However, *Tetrahymena* can invade resident *Colpidium* and populations can coexist for weeks, but *Colpidium* always eventually exclude *Tetrahymena* to extinction. The only factors temperature affected were maximum population density and time to extinction in single species microcosms, with earlier extinction and lower maximum population densities at warmer temperatures. This study suggests that arrival of species into an environment is vital in determining the final habitat composition. Although temperature does not affect priority effects, it does alter the duration species may be able to survive and coexist, which could be fundamental in conservation work in a world with changing habitats and climates.

**Contents**

# 1. Introduction

## 1.1. Community Assembly

Within a community, all individuals interact in a variety of complex, intertwining ways to affect one another (Fukami, 2015). This can occur in relationships between individuals of different species, interspecifically, or the same species, intraspecifically. These interactions can affect individuals of each species and the community as a whole, consequently affecting species richness, productivity and energy flow within the ecosystem (Fukami, 2015).

Community structure and assembly has been a focus within ecology since the early nineteenth century, initially deriving from the disagreements between Clements and Gleason on plant succession (Egerton, 2009). Clements created a theory that diverse successions would result in one single climax for a variety of regions, being naive about the complexity between individuals and their relationships (Egerton, 2009; Eliot, 2007). Gleason became a regular, strong critic of Clements with his proposal that there is no single climax possibility, but multiple possibilities, after taking into consideration the variety of different flora and their complex interactions with local environments (Egerton, 2009; Eliot, 2007). Although Clements' single climax theory has since proved unpopular, it has sparked interest and investigations into more complex areas of ecology such as succession, assembly history and the development of environments over time whilst considering the intricacy of species relationships and interactions (Egerton, 2009; Eliot, 2007).

Since then, one of the most popular topics to surface and gain attention within community ecology research is priority effects. Priority effects can be defined as the influence species

can have on one another dependant on the order of arrival of species into a habitat (Fukami, *et al*. 2016; Leopold, *et al*. 2017). The order and timing of species colonisation within community assembly is arguably one of the most significant determinants of species composition within a community, as well as how individuals interact together and with the environment (Fukami, *et al.* 2010; Ejrnaes, *et al.* 2006).

However, priority effects can be altered by a variety of different mechanisms and species specific traits (Sarneel, *et al*. 2016). Mechanisms affecting this can be equalising, stabilising or destabilising resulting in: (1) priority effects – the new species cannot establish and the resident either excludes, or is overwhelmingly more abundant than the invading species; (2) stable coexistence – the new species can establish and it coexists with the resident species long term with little change to population density; or (3) competitive exclusion – the late arriving species establishes and excludes the resident species (Little and Altermatt, 2018). The mechanisms behind these outcomes all work at varying strengths to create the different community structures.

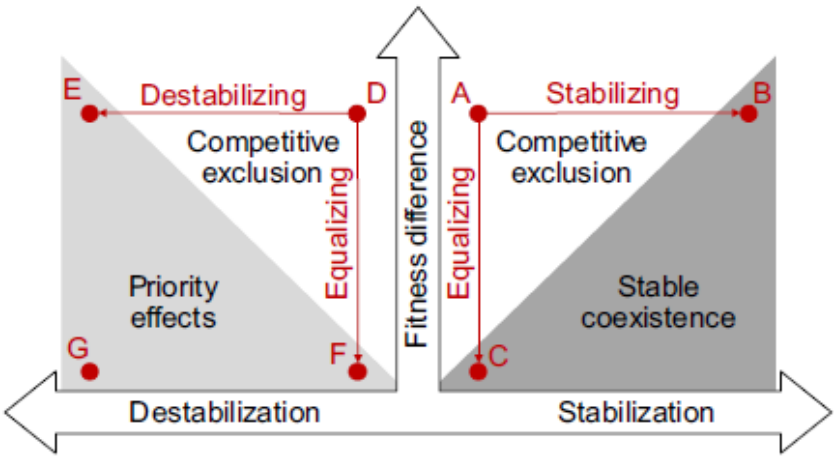


**Figure 1.1 - Fukami, *et al*. (2016) Conceptual Framework for Priority Effects showing mechanisms behind coexistence, exclusion and priority effects.**

Fukami, *et al.* (2016) developed a framework for priority effects based on Chesson (2000) and Mordecai's (2011) original conceptual outlines (refer to figure 2.1). This framework illustrates the strength of equalising (e.g. fitness difference), destabilising and stabilising mechanisms in order for priority effects, competitive exclusion or stable coexistence to occur (Fukami, 2016). This was created in order to allow possible predictions of how and when priority effects can occur depending on the strength and presence of certain mechanisms. The individual points plotted on the model (A, B, C, D, E, F and G) are all examples of possible results dependant on the mechanisms present within the community.

Stabilising mechanisms are processes that cause species to limit themselves but not others; the best example of this is fitness difference used in Fukami's framework (Fukami, 2016; Alder, *et al*. 2017). This is dependent on each species individual growth rate (Alder, *et al*. 2017). Equalising mechanisms are those that reduce relative fitness difference between species to allow coexistence rather than competitive exclusion of a species (Chesson, 2000).

Stable coexistence can be defined as there being no long-term trends, with populations remaining at steady quantities and always being able to recover from any pressures (Chesson, 2000). However, Chesson (2000) argues that there can also be unstable coexistence, where either population are never able to recover if densities begin to decrease. This could include population counts being dramatically different at a single point in time, with one species being far more abundant than the other. The strength of these mechanisms is influenced by other factors including competition and predation, productivity and external environmental conditions (Fukami, *et al*. 2010; Sarneel, *et al*. 2016).

Simpler ecosystems tend to be more significantly affected by community assembly, showing stronger priority effects (Fukami, 2004). Moreover, species that are more successful in lone populations normally are less affected by priority effects when in a community with other species (Sarneel, *et al*. 2016). This allows some basic predictions to be made on the outcomes and consequences of invasions and priority effects; but in reality, predicting the results can be difficult due to the wide range of variable interactions between species and species-specific changes such as temporary phenotypic plasticity or long-term evolution (Buckley, 2017). The impact of an invasion can vary, from mild short-term effects such as small population density changes to stronger effects which develop over a longer period of time, where generations can pass before any changes arise leading to environmental and evolutionary changes and possible species extinction (Buckley, 2017).

Early arriving species can gain several benefits, including depleting shared resourced and changing the environment for late arriving species, possibly amplifying the competitive exclusion of the inferior competitors (Grainger, *et al.* 2018). This niche modification or niche pre-emption (depending on what the species actually does to the environment) can limit late arriving species ability to invade due to limited niche availability (Little and Altermatt, 2018). This means that species that share similar requirements can be unable to coexist if environmental conditions do not allow it (Little and Altermatt, 2018). However, even with knowledge of species, environment and resources, it can be difficult to predict the availability of niche spaces, especially with interspecific competition too (Little and Altermatt, 2018).

### 1.2. Environmental conditions

Environmental conditions can influence many key processes in ecosystems. Temperature in particular has been known to affect physical processes including metabolic rate, behaviour, activity levels, population growth, feeding rates, competitive ability and phenology (Beveridge, *et al*. 2010a; Griffiths, *et al.* 2014; Grainger, *et al.* 2018). Due to this multitude of interlinked effects, temperature in turn alters the strength of interactions between species and the stability of communities and populations (Beveridge, *et al.* 2010a). Different species have distinct thermal tolerances and are therefore able to survive at different temperatures more successfully than others (Frankel and Nelson, 2001). For example, some *Tetrahymena* species are able to survive in temperatures that exceed 32°C, whereas other protists cannot tolerate temperatures that are this high (Frankel and Nelson, 2001).

How a species reacts to varying temperatures in their environment is largely dependent on the species initial intrinsic rate of increase (Fox and Morin, 2001; Letten, *et al.* 2018). The intrinsic rate of increase can be defined as the rate of population growth whilst assuming growth is exponential (Weisse, *et al.* 2016). Machler and Alermatt (2012) argue that species with a faster intrinsic rate of increase are more successful at establishing and persevering, particularly in microcosm experiments (Mata, *et al*. 2012). It is worth noting that those species with faster growth rates and greater carrying capacities tend to tolerate warmer temperatures better, with higher optimal temperatures or can at least respond quicker to rapid warming (Grainger, *et al.* 2018). Not only are these species able to dominate the environment spatially and in abundance, but also consume and utilise more resources available to hinder the success of future establishments (Young, *et al.* 2017). Therefore,

conditions increasing population growth and impact on the environment and resources should strengthen priority effects (Grainger, *et al.* 2018).

Growth rate and maximum population density of species can be species dependant making it essential to understand each species specific qualities (Fjerdingstad, *et al.* 2007). Gray, *et al*. (2015) identified that as well as larger invader propagules, high resource availability also allowed more successful establishment of invading species. However, successful invaders are often those species that are flexible in their ability to adjust their own growth and densities to meet resource fluctuations (Mata, *et al*. 2012). This allows them to overcome any environmental pressures they may encounter easier than those species that may be less adaptable to any environmental changes and stresses.

Beveridge, *et al.* (2010a) showed that colder temperatures allow greater community and population stability, due to slower and steadier growth. Warmer conditions decreased community stability due to the faster and more unpredictable population growth, rather than faster growth rates allowing greater success. Therefore, species within a community can greatly differ in their responses to environmental changes such as climate change and warming, but it can be difficult to predict which species will be more or less affected than the other (Rudolph and Singh, 2013).

Warmer temperatures would not only increase growth rate, but also all other rates. Faster growth and greater population densities would cause a non-linear increase in resource use, possibly resulting in lower maximum population sizes that would peak and reach maximum population density much earlier than in cooler temperatures (Grainger, *et al.* 2018). This in

turn would cause the community to run out of energy and resources faster, resulting in earlier extinction of all species within the community (Grainger, *et al.* 2018). This makes weaker competitors, and those with cooler optimum temperatures, much more dependent on early arrival to be able to establish a population and persist successfully (Grainger, *et al.*, 2018). Due to higher vital rates of species at warmer temperatures, impacts on the community tend to be more rapid and more extreme, often resulting in stronger priority effects (Grainger, *et al.* 2018).

### 1.3. Species specific traits

Species vary so drastically in their species specific behaviours, morphology, phenology and physiology that even without invasion pressures, they can struggle with competition. Competition between species for resources is a key determinant of invasion success and whether a species is able to colonise within the new environment (Mata, *et al.* 2012). Nonetheless, species that compete for the same food/nutrients can often coexist. For example, in microcosm communities, protists compete for bacteria but can still coexist despite their differences in factors such as their grazing ability (Fox and Barreto, 2006; Holdridge, *et al*. 2017).

Fox and Barreto (2006) state that *Colpidium* are much stronger grazers than *Tetrahymena* as they reduce bacteria density much more, as a result *Tetrahymena* are rapidly excluded by *Colpidium* in nutrient-rich media (Fox and Barreto, 2006). Resident species often hamper invasion success due to the initial freedom and lack of restriction they have within the environment. Their population density is not controlled by competition or predation from other species, therefore they readily consume and utilise any resources available in order to

reach higher maximum population densities and are consequently able to gain an advantage over late arriving species (Gray, *et al*. 2015; Sarneel, *et al.* 2016). However, some species can cope better with resource limits and fluctuations than others, also shifting invasion success (Mata, *et al*. 2012).

By altering the resource availability, resident species can pre-empt niches, possibly taking advantage of more of their fundamental niche (the niche they should theoretically be able to fill) (Weisse, *et al*. 2016). This reduces the availability of niches for later arriving species, limiting them to a much smaller realised niche (the niche they fill in reality) (Weisse, *et al*. 2016). This could be a method of niche modification within *Colpidium* and *Tetrahymena* microcosm experiments. Resident *Colpidium* could consume resources with no limitations as very successful grazers to modify the environment enough to hamper *Tetrahymena* establishment and invasion through lack of resources. On the other hand, as an invader, *Colpidium* may have a slower growth rate and lower maximum population density than *Tetrahymena* and therefore will not be able to reach the maximum population densities that *Tetrahymena* can in the same period of time. Nevertheless, *Colpidium* may be able to deplete the resources enough for *Tetrahymena* to reduce their population growth enough to allow them to be invaded successfully. Alternatively the empty niche hypothesis, states that the invader may be able to more effectively utilise any resources that are unexploited to fill the niches available more successfully than resident species (Young, *et al*. 2017).

Therefore, not only can order of arrival influence the abundance of species and community structure, but also the competitive ability of the individuals (Clements, *et al*. 2013; Dickie, *et al*. 2012). Interactions and individual traits can be strengthened or weakened, making

species more competitively superior or competitively inferior. Therefore, species that would normally dominate under ordinary circumstances can end up being inferior and become outcompeted by those species that would normally be weaker. The stronger competition there is between species, the more unstable the community becomes (Carrera, *et al*. 2015). The similarity in resource demand and use between species can also affect the strength of priority effects, with greater similarities leading to more competition for resources (Jiang, *et al*. 2017). However, it is possible that competitively inferior species can still be successful in invasions so long as there are enough individuals to overwhelm competitively superior resident species (Jones, *et al*. 2017).

### 1.4. Phenotypic plasticity

Individual species can react to pressures such as invasion or environmental change which can affect their chances of survival, their feeding rates and their growth rates. Therefore, dynamics like these should be taken into consideration when predicting invasion outcomes. An example of this is when species can optimise their fitness by altering their traits by phenotypic plasticity, (most commonly altering cell size or shape) to match environmental pressures to enhance their chances of survival (Luhring and DeLong, 2016; Young, *et al*. 2017; Faillace and Morin, 2016).

For example, *Colpidium* strains have the ability to alter their cell size and shape, from large cylindrical bodies, to smaller spherical shapes to alter their feeding ability. Glucksman, *et al.* (2010) identified that feeding differences can be highly influenced by individual microorganism cell size (Fyda, 1998). *Tetrahymena thermophila* possess the ability to transform into morphs that differ in swim-speed, with weaker performing strains producing

vast amounts of morphs under normal conditions compared to those who were naturally competitively superior, to ensure a greater likelihood for survival (Fjerdingstad, *et al*. 2007).

### 1.5. Microcosm and protist studies

Protists are beneficial for use in microcosm communities due to their short generation time and fast reproductive rate, allowing long-term population dynamics to instead develop rapidly over many generations (Beveridge, *et al*. 2010a; Jiang, *et al*. 2011). In addition, assembly order effects are easy to demonstrate in either small scale experimental or field based models and protist microcosms are a classic method for modelling competition (Clements, *et al*. 2013; Fox and Barreto, 2006). Laboratory microcosm experiments are commonly used for community ecology experiments due to the ability to easily manipulate multiple environmental variables to study mechanisms and processes as well as physical traits of individual microorganisms (Altermatt, *et al*. 2015). However, they rarely capture realistic complexity in natural ecological situations (Buckley, 2017).

Protists such as *Tetrahymena* and *Colpidium* are useful to use in microcosm models as they are both bacterivorous ciliate protists that are able to survive on just *Pseudomonas fluorescens* SBW25 alone (Fox and Barreto, 2001). Both species are quite morphologically similar, yet distinct enough to be able to distinguish between them when in a community together. Additionally, there is knowledge that they can coexist together under certain environmental conditions despite their differences, but also that they can exclude one another (Fox and Barreto, 2001). Furthermore, Jiang, *et al*. (2011) identified that communities with *Colpidium* or *Tetrahymena* as the primary colonisers caused the strongest

priority effects, so creating an experiment with the two together would be an interesting way to test Fukami's model (Fukami, *et al.* 2016).

### 1.6. Rationale

With climate change altering temperatures (terrestrial and aquatic) all around the world, ecological processes will be varying in speed. Species are also extending their territories and shifting their ranges to remain within their thermal tolerances, often entering new habitats and encountering species that they have not been in contact with previously. Therefore, predicting which species will eventually enter the same habitat, and attempting to forecast the arrival order and possible outcomes is vital in order to avoid species deaths and extinctions. This should allow better management for conservation of species who are threatened by invasive species and climate change.

Frameworks and concepts must be examined, tested and refined to better understand the mechanisms behind the different community climaxes. In this case, it is vital to understand mechanisms behind priority effects in order to determine how and when they will occur, and to what strength (Fukami, *et al*. 2016). Therefore, this study aims to test Fukami's framework to investigate how priority effects interact with temperature to determine community assembly trajectories.

Since environmental conditions can influence community assembly, it is vital to investigate the mechanisms behind priority effects in different ecological and environmental conditions due to the extensive habitat changes occurring in reality (Gray, *et al*. 2015). Furthermore, it is important to understand these mechanisms to begin to predict the species specific

responses species can have in response to either temperature change or invasion (Clements, *et al*. 2013).

### 1.7. Aim and hypotheses

I aim to investigate the effect of temperature on the establishment of priority effects by constructing controlled aquatic microcosms using the protists *Tetrahymena* and *Colpidium*. This could provide evidence that with waters warming due to global climate change, individual species processes and characteristics could change and alter the possibility of successful invasions and the possible outcomes.

The experimental design ensured the resident population was always at the species maximum population density at the point of invasion in order to alter fitness difference between the two species and push the boundaries of Fukami's (2016) conceptual model of priority effects. This model's purpose was to examine whether fitness difference can alter when priority effects or stable coexistence can occur and the strength of these effects. Therefore, I was less interested in the effect temperature has on growth rates in the early stages on community development with the resident (as I allowed residents to reach maximum population density) and more interested in how a rapid change of temperature over a single generation affects the potential for immigration into a competitor at maximum population density.

The experimental design ensured that all 'resident' populations were treated identically up to the point of invasion, upon which they experienced rapid warming. This design removes

the possibility that invasibility is dependent upon differences that have occurred before the arrival of a new species due to temperature dependent growth rates and changes to the bacteria. I predicted that priority effects would be less intense at temperatures where the invader was closer to its optimal temperature for growth.

In order to fully understand the mechanisms behind priority effects and how and when they occur, it is vital to understand how temperature and invasions can affect other factors that could in turn have consequences on the strength of priority effects. This includes individual species time to extinction in different temperatures as well as the maximum population density of both species in each temperature.

Therefore, I hypothesised that:

*Hypothesis 1 – testing the effect of temperature on maximum population density of both species*

I hypothesised that warmer temperatures would decrease maximum population density when single species are invaded immediately into each temperature. Although this may not directly link to priority effects, it is important to identify whether differences in maximum population density in different temperatures could encourage a species to be more competitively superior and greater abundances may allow a species to overwhelm the other species. Where a species is in an optimum temperature, they will reach a larger population density than those in non optimum temperatures. If temperatures are too hot or cold, species may be unable to grow sufficiently to establish or be at lower abundances compared to those in optimum conditions that will easily replicate and reach high population densities.

*Hypothesis 2 – testing the effect of temperature on time to extinction for both species*

I hypothesised that warmer temperatures would make extinction occur earlier when single species are invaded immediately into each temperature. Whether temperature affects a species time to extinction is important in understanding priority effects; if a species is more likely to go extinct earlier in a particular temperature then it would be likely that they would be easier to invade and outnumber the resident species and consequently alter the strength of priority effects.

This is due to temperature speeding up all vital processes within the microcosms. Increased temperatures result in faster growth rates, metabolic rates and activity (such as swimming speeds and grazing rates). This will in turn result in resources depleting faster, causing the microcosm system to run out of energy much quicker, making species extinction occur much earlier than it probably would in cooler temperatures. Cooler temperatures will have the opposite effect on populations, with slower growth and activity, longer lasting resources and prolonged life with later extinction to create a more stable community.

*Hypothesis 3 – testing the effect of rapid warming with/without simultaneous invasion on time to extinction for both species*

a) I hypothesised that warmer temperatures would make extinction faster when single species are moved from 20°C to warmer temperatures. Understanding the effect of different temperatures on time to extinction is important, but to make the theory more relatable to real life climate change, it is important to try to underpin the effect of rapid

warming on a species and their extinction rate. This is mainly due to the possibility of it altering the species invasibility and consequently the strength of priority effects between the species.

Rapid warming could have dramatic effects on populations due to the shock of such a drastic environmental change in such a short period of time. Although this time frame of less than two hours is not realistic, it can highlight which mechanisms and interactions rapid environmental change could affect within an ecosystem.

b) I hypothesised that extinction will be even faster still when species are moved from 20°C to warmer temperatures and invaded by a competitor. The effect of simultaneous invasion along with rapid warming should be taken into consideration for priority effects due to the huge competition pressure it can put on a species. The greater stress a species is under can dramatically change the invasibility of the species and consequently alter the strength of priority effects.

*Hypothesis 4 – testing the effect of rapid warming and simultaneous invasion on priority effects for both species.*

Priority effects will be weakened when the invasion occurs simultaneously with warming towards and above the thermal tolerance of one species in both directions (*Colpidium* as an invader to resident *Tetrahymena* and *Colpidium* as a resident to invading *Tetrahymena*). It would be expected that in a temperature which is not optimum, one species will be competitively inferior to the other, allowing the strength of priority effects to vary with each

temperature and invasion arrival order. You would expect the more competitively superior species in the optimum temperature to be more successful than the other species, to result in greater mechanism strength.

*Simultaneous Arrival Experiment – testing the effect of temperature and simultaneous arrival on priority effects for both species.*

Once the experiment had been carried out, it was logical to explore the effect of simultaneous arrival of the competitors, to see if coexistence could possibly be promoted and made stable for a prolonged period of time. These results were not analysed or used as comparisons to the first experiment; despite it being as similar to the first experiment as possible, it was still completed in a different time block and the protists would have evolved over the time period between experiments.

# 2. Methods

## 2.1. Establishing microcosm communities

For all experiments, microcosm communities were assembled in 50ml centrifuge tubes containing 25ml of liquid growth media, the ciliates *Tetrahymena pyriformis* and *Colpidium striatum* and the bacteria *Pseudomonas fluorescens* SBW25. Both ciliate species were acquired from Sciento Scientific Supplies, Manchester, UK. *P. fluorescens* was supplied by Dr Andrew Spiers, Abertay University, Scotland, UK. Growth medium, on which the bacteria fed, was made by adding 1L of Ashbeck mineral water (available from Tesco) with 0.5g freeze-dried *Chlorella* powder (Naturya Organic). Medium was autoclaved after the *Chlorella* powder was added. *P.fluorescens* was stored on nutrient agar plates until introduced into the liquid growth medium. Once inoculated with the *P. fluorescens*, the medium was incubated at 26°C for 3 days to allow sufficient bacterial growth for the later introduced ciliates (ciliate stock cultures were already created and surviving on *P. fluorescens* in *Chlorella* medium – see section 2.1.1.).

Microcosm experiments use different media, such as Clements, *et al*. (2013) and Warren, *et al*. (2003) who used Chalkey's media and some even used LB media or King's B media with wheat grains and protozoan pellets. Alternatively, Machler and Alermatt (2012) used the natural pool water from which the resident community species were collected from in their natural habitat. However, pond water could contain lots of unidentified bacteria and is not as safe as sterile chlorella media containing one known bacteria and would not have been suitable for my experiment. An alternative to this is to make artificial pond water using

chlorella medium. Chlorella is unicellular green algae which the bacteria can feed on (Krienitz, *et al.* 2015). There are no additional benefits to using the other media types apart from chlorella media can appear very 'bitty' and can aggregate into clumps which could lead to different niches within a single microcosm. However, this shows no issues for this experiment where the microcosm is agitated and the aggregated clusters are broken up before sampling for population counts.

### 2.1.1. Isolating ciliates to grow on only *P. fluorescens*

From high density stock cultures growing on a mixed unknown bacterial flora, one individual ciliate cell was isolated under a dissecting microscope. It was then washed multiple times in sterile medium before being transferred to high density culture of *P. fluorescens*. This process involved selecting the larger, active individuals of each species and pipetting them into 1ml of sterile chlorella medium. Once the single *Colpidium* or *Tetrahymena* cell had made its way through enough sterile medium to sufficiently clean it, the individual was then isolated again into a new 1ml of sterile medium. This process was carried out until no bacteria remained (~10 times).

The individual was then inserted into a new centrifuge tube containing fresh medium inoculated with *P. fluorescens* at 20°C. After a few days, if ciliate growth had been successful, the stock was checked using nutrient agar plates to identify if it was still contaminated with other bacteria or if protists had successfully grown on *P.fluorescens* only. The known clean stocks were then used for all future work. Both protist species were sub-cultured approximately monthly onto fresh medium inoculated with *P.fluorescens* and were checked

regularly to ensure they were still growing on the single bacterium and no contamination had occurred.

## 2.2. Preliminary pilot experiment testing the thermal tolerances of *Tetrahymena* and *Colpidium*

A preliminary pilot experiment was performed in order to identify which temperatures (10°C, 16°C, 20°C, 22°C and 26°C) *Tetrahymena* and *Colpidium*: a) were able to grow and establish in and, b) reached a greater maximum population density in. This would allow a suitable temperature range to be selected for the experiment that included temperatures that could push the thermal tolerance limits of each species. This in turn could push the boundaries of the probability of each species successfully or unsuccessfully establishing, whilst including the optimum temperature for each species to reach their highest maximum population count.

On experimental day 0, 50 microcosms were set up with 10 microcosms used in the 5 different temperatures; 10°C, 16°C, 20°C, 22°C and 26°C. Five of the 10 microcosms contained *Colpidium* only and the remaining five contained *Tetrahymena* only. Five replicates of each species in each temperature were considered adequate for this experiment as it provided enough replicates to gain an accurate average and it did not require excessive amounts of time sampling populations. On day 3 the microcosms were inoculated with just one individual cell to gain knowledge of the growth and maximum population density of just a single individual over a 14 day period (data collected on experimental days 0, 1, 2, 3, 4, 5, 8, 9, 10, 11, 12 and 15).

Data were collected by means of population counts using a Nikon SMZ800N microscope on varying days throughout the experiment. A small volume of the microcosm was removed (this depended on the density of the population; more dense populations required a smaller volume) to count no more than 30 individuals within the sample. The population counts were then used to calculate the total population density of each species for each microcosm on each day of data collection.

### 2.3. Staggered Arrival Experiment

This experiment manipulated temperature (20°C, 22°C, 24°C, 26°C and 28°C based on the preliminary pilot experiment results) and the assembly order of species into a community to explore whether temperature affected priority effects. Less than 20°C was disregarded as *Tetrahymena* still reached higher maximum population densities than *Colpidium* (it was assumed that even then Tetrahymena would be competitively superior) and 20°C is *Colpidium's* optimum. After this, 2 intervals were used; specifically including 28°C as Fox and Morin (2001) identified that *Tetrahymena* can thrive well in this warmer range. Additionally, other invasion studies used similar ranges (Clements, *et al*. 2013; Beveridge, *et al*. 2010a; Beveridge, *et al*. 2010b; Fox and Morin, 2001).

On day 0, all liquid growth medium was inoculated with *P. fluorescens* and on day 3, a single individual of the 'resident' species was added to each microcosm. All residents were from a single stock culture of each species and were 7 days old.

There were: (300 microcosms in total)

*Colpidium* resident – no invader x 5 temperatures x 10 replicates

*Colpidium* resident – *Tetrahymena* invader x 5 temperatures x 10 replicates

*Tetrahymena* resident – no invader x 5 temperatures x 10 replicates

*Tetrahymena* resident – *Colpidium* invader x 5 temperatures x 10 replicates

No resident – *Colpidium* invader x 5 temperatures x 10 replicates

No resident – *Tetrahymena* invader x 5 temperatures x 10 replicates

Each single species was established as a resident species and later invaded with 10 cells of the other species on experimental day 14 – at which point both populations had stopped exponential growth and were at, or very near, maximum population density as population increase was starting to slow (Griffiths, *et al*. 2015; Jiang, *et al*. 2017; Chase, 2000). It is worth noting that invading protists in the experiment were used from the same age stocks (7 days old) to ensure all individuals of both species were at a standardised state (Jiang, *et al*. 2008). Since I was interested in the effects of simultaneous warming and invasion, and not the effect of temperature on pre-arrival niche pre-emption or modification, all 'resident' populations were kept at 20°C for the first 11 days. They were then rapidly warmed (by moving them immediately to a different incubator) and invaded with other species simultaneously.

It is worth noting that initially, one single individual was used for the resident propagule, despite other experiments always using more than one (Jiang, *et al*. 2008; Weatherby, *et al*. 1998; Machler and Alermatt, 2012). This was decided due to already having detailed

maximum population density data on each species and the knowledge from previous attempts that a propagule of one has always been successful and survived an invasion, apart from when priority effects have occurred. If one individual is able to invade then a propagule of 10 or 100 definitely would too. Additionally, in reality, when invasion occurs it is very unlikely that more than one individual of a species would all invade the same place simultaneously as species invasions are primarily stochastic; it's more realistic that one individual would – especially for a single celled asexual microorganism (Fukami, 2004).

From day 0 until day 14, data was collected daily in order to monitor the population density and population growth of each species in all the temperatures (the first day after being added to the microcosm was excluded due to the lack of growth from one individual in 24 hours; and the difficulty in finding the protists). After invasion, data was collected every two to three days until day 35 when data started being collected weekly as populations began to decline up until the end of the experiment on day 84. These counts were then used to calculate the total population of each species in each microcosm throughout time.

### 2.4. Simultaneous Arrival Experiment

This experiment was carried out as an exploratory experiment to investigate the maximum population densities and time to extinction for both species invading simultaneously in equal quantities into each temperature. This was to emphasise whether arrival order was an important factor for priority effects. This experiment would identify if having a period of time for a resident species to occupy a habitat before another species invaded resulted in a different community structure compared to both species arriving together and having to

compete from the start. 50 microcosms were set up with 10 cells of *Tetrahymena* and 10 cells of *Colpidium* added to each microcosm simultaneously. 10 microcosms were then moved to each temperature immediately (20°C, 22°C, 24°C, 26°C and 28°C). Populations were then sampled over 84 days.

It was decided that 10 cells of each species would be more suitable for this experimental set up than one single individual as was carried out in the staggered arrival experiment. This was to ensure that both species could have enough of an opportunity to establish a population simultaneously; it was more reliable than using one single cell that could be lost immediately for common reasons such as the fact it could have been a weak or already dying cell. This ensured that if a species became extinct, I could be certain that it was due to competitive exclusion or priority effects.

### 2.5. Photographing cell size

Separate *Colpidium* and *Tetrahymena* microcosms were set up with 1 individual per microcosm and left to grow at 20°C. The *Colpidium* were photographed on day 17 and *Tetrahymena* on day 16 in order to measure cell size of the protists once exponential population growth had finished and populations were at carrying capacity.

For *Colpidium*, from each microcosm, a volume of approximately 20 microlitres containing at least 20 individuals was removed from the microcosm onto a petri dish. 20 microlitres of 20% polyethylene glycol solution was added to the cells and mixed to slow down the cells

movement to avoid blurry photographs. Once mixed, individual cells were isolated into a smaller volume of solution to create a denser population to make it easier to capture more cells per photograph. This volume (no more than 10 microlitres) was pipetted onto a $1mm^2$ haemacytometer and covered with a glass cover slip. The haemocytometer was placed onto an Olympus CX41 microscope at 10 times magnification and photographed with a Nikon D5300 digital camera. A minimum of 10 cells were photographed per microcosm.

For *Tetrahymena*, the same process was carried out except no polyethylene glycol solution was used as even small volumes of low concentration solution rapidly killed the cells. Therefore, a small dense volume straight from the microcosm was placed on the haemocytometer and covered with a cover slip and photographed. This was carried out on the Olympus CX41 microscope at 4 times magnification.

All photographs were then analysed using the programme ImageJ to measure the length and width of each cell. Where there were more than 10 cells per photograph, the photograph was treated as a grid, with cells counted firstly in the top left corner along to the top right, then the next row down from left to right again, with the last possible quadrant being the bottom right corner. Once all measurements had been taken, the volume was then calculated using the formula $(4/3) \times \pi b^2 a$ (Marie, *et al.* 2010) where $a$ is the cell length and $b$ is the cell width to calculate the volume for a prolate spheroid.

However, this data was not useful to use as there was there large variance in cell size (some were half the size of larger cells) within each population. This meant that even when more than 10 cells were measured, when a mean cell size was calculated for each population, variance was still too vast between all replicates.

### 2.6. Experimental assumptions and validity

For all experiments, to ensure the microcosms were all incubated at the correct temperature, the temperature of the top and bottom of each incubator was measured using a Testo 174T mini temperature datalogger. This identified if there was any slight variance in temperature within each incubator. There was some slight variance within some incubators (maximum of 0.3°C between top and bottom and a maximum of 0.3°C from actual target temperature), thus all microcosms in all experiments were kept on the same shelf in the middle of each incubator throughout the duration of both experiments.

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To test the time taken for the media to warm up to the new temperature after being moved from 20°C, a centrifuge tube filled with 25ml of water was left in a 20°C incubator overnight. The next day, the tube was moved to 28°C and the water temperature was measured every 15 minutes until it had reached the new target temperature. Only 28°C was measured as this was the maximum target temperature, all other temperatures (20°C, 22°C, 24°C, 26°C) would be reached sooner. The microcosm reached 28°C after 1 hour and 45 minutes, indicating that all microcosms changed temperature well within a single generation of both ciliate species.

## 2.7. Statistical Analysis

It is worth noting that some microcosm data was not included in any of the graph analysis (means and error bars) or statistical analysis (5 microcosms in the entire experiment were disregarded) due to these populations never establishing at the beginning of the experiment and consequently, these microcosms were not counted for population density throughout the rest of data collection. These microcosms were *Colpidium* invaded by *Tetrahymena* (replicate 5 at 20°C), *Tetrahymena* invaded by *Colpidium* (replicate 1 at 22°C), *Colpidium* invaded by *Tetrahymena* (replicate 3 at 26°C) and *Colpidium* invaded by *Tetrahymena* (replicate 4 at 28°C and replicate 7 at 28°C).

Error bars were created by calculating the standard deviation of all microcosm replicates for each treatment in each temperature. This identified the variance between all sets of data for each treatment. A linear regression was carried out on the data for *Colpidium* alone and *Tetrahymena* alone immediately invaded into each temperature on day 14. This was performed to identify if there is a significant relationship between temperature and maximum population density measured.

## 2.8. Ethics

Ethical approval has been approved by the iBEST Research Ethics Committee. The use of protists in research raises no ethical issues under the Home Office and they are suitable for research use. Additionally, no pathogenic microorganisms are used meaning no risks to public health are caused. To avoid any further health issues, all biological waste was

appropriately disposed of (autoclaved to destroy the organisms) in line with all procedures and policies of the School of Life Sciences. All research was professionally carried out with acknowledgement given to the authorship of any data and ideas. All data and information was stored in a safe and confidential manner and presented honestly.

# 3. Results

## 3.1. Testing the thermal tolerances of *Tetrahymena* and *Colpidium*

The preliminary pilot study was carried out to investigate the thermal tolerance range and maximum population density of each species to find the optimum temperature for each species to reach their largest maximum population density. It is clear to identify that *Tetrahymena* and *Colpidium* never reached similar densities; *Tetrahymena* always reached over 250,000 cells in all temperatures, whereas *Colpidium* could never reach over 30,000 cells in any temperature (see figure 4.1).

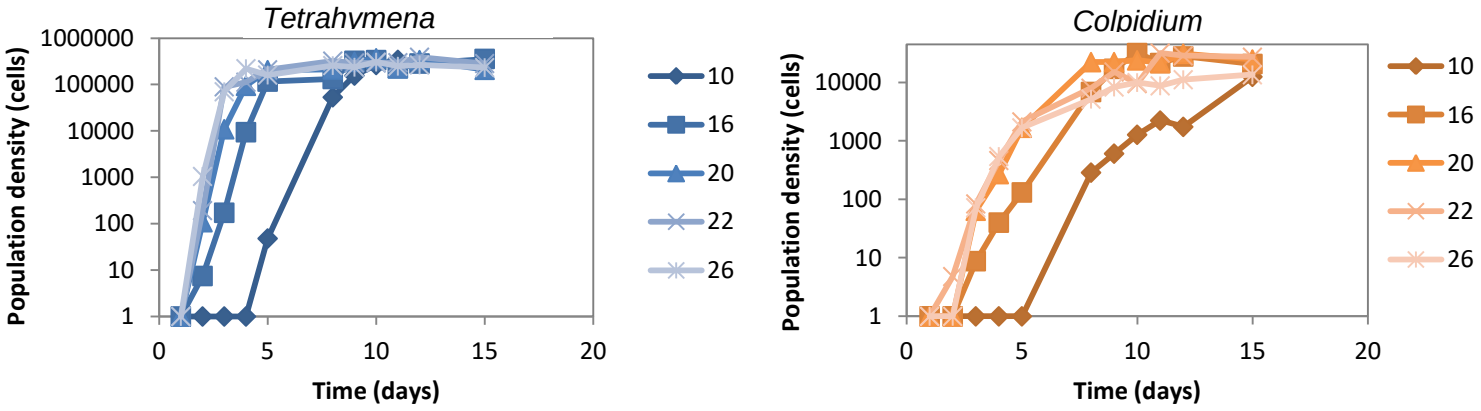


**Figure 4.1. Population density of *Tetrahymena* (blue) and *Colpidium* (orange) over time in different temperatures. The Y axis is on a Log10 scale.**

*Tetrahymena* reached their maximum population density faster at 26°C compared to *Colpidium* having an optimum temperature of 20°C. Both species grew slowest at 10°C, but despite a long period of very little growth, both species had the ability to establish and grow up to larger abundances (*Tetrahymena* at around 300,000 cells on day 15 and *Colpidium* reached over 25,000 cells). *Tetrahymena* showed very little temperature dependence on growth over the 16°C range, always establishing and reaching very high densities after 2 weeks. However, *Colpidium* showed much stronger temperature dependence, with extreme cold and warm conditions reducing the population density by at least 10,000 cells on day 15.

### 3.2. Testing the effect of temperature on maximum population density for both species

*Hypothesis 1 - Warmer temperatures would decrease maximum population density when single species are invaded immediately into each temperature.*

Warmer temperatures did decrease maximum population density when invaded immediately into each different temperature for *Colpidium*, but had a less profound effect on *Tetrahymena*. *Colpidium* reached a much larger mean maximum population density in cooler temperatures, decreasing as temperatures rise (see figure 4.2). However, *Tetrahymena* populations varied by 25,000 cells between temperatures (in the scale of how large *Tetrahymena* populations were, this change is quite small). *Tetrahymena* always reached higher maximum densities than *Colpidium* when both species were alone. *Tetrahymena* populations were around 10 times larger than *Colpidium*'s maximum population density, making them far more abundant (see figure 4.2).

*Colpidium* at 20°C reached a mean 30,000 cells, decreasing by 10,000 cells with a 2°C difference to 22°C. This happened again when populations reached up to 20,000 cells at 24°C and decreased to around 11,000 cells at 26°C. In contrast, at 28°C populations began to grow, or cells were already committed to a division which resulted in a mean maximum population density of around 30 cells (see figure 4.2). However, despite the initial growth, populations could not establish and went extinct shortly after. On the other hand, *Tetrahymena* were successful in all temperatures, reaching similar densities regardless of temperature, and were always able to grow and establish a population. Over all temperatures in all replicates, there was a variation of 25,000 cells between microcosms, the lowest being 232,500 cells at 28°C and the highest reached 257,500 cells at 24°C.

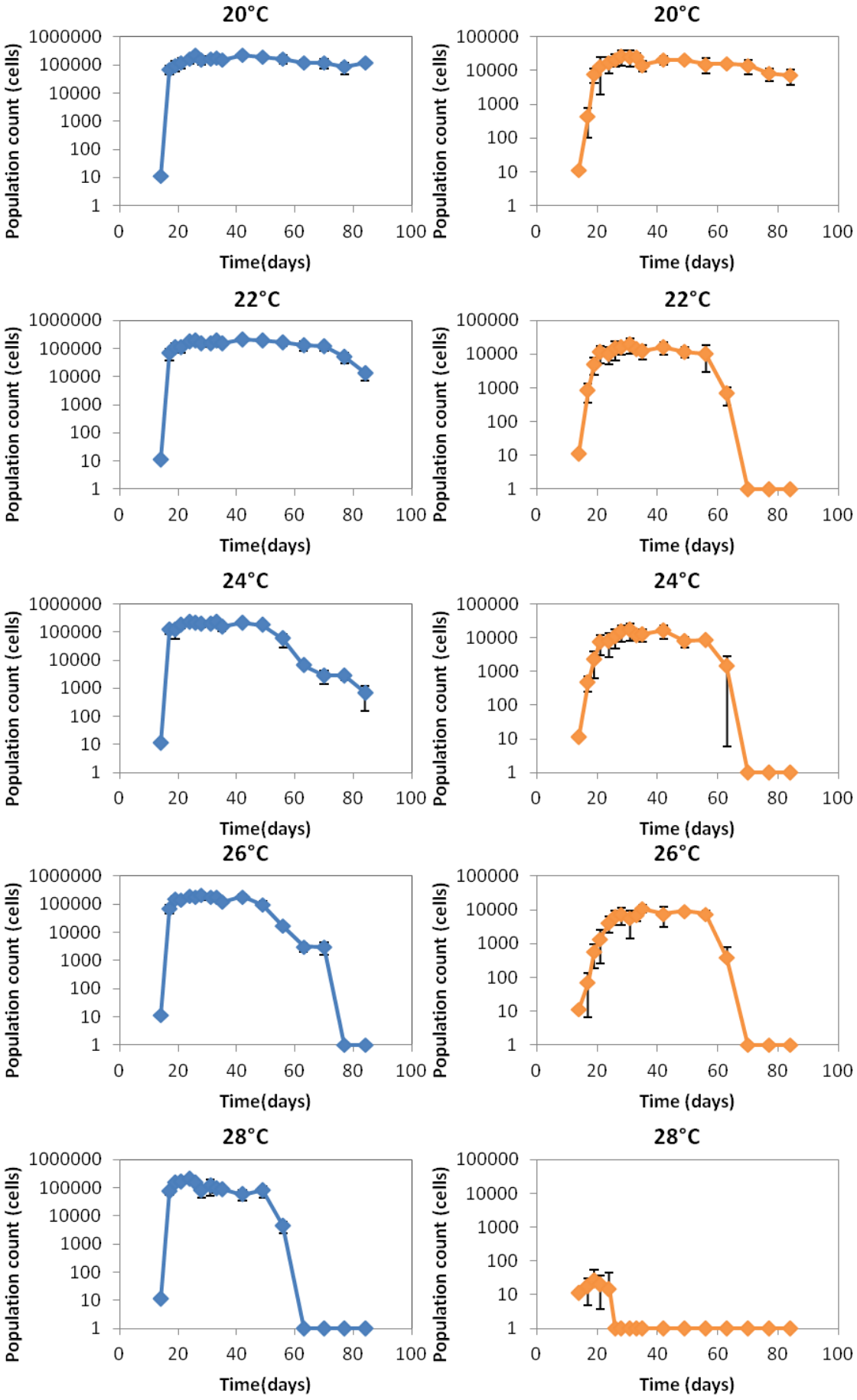


**Figure4.2. Mean population density over time of *Tetrahymena* alone (blue) and *Colpidium* alone (orange) invaded immediately into each temperature on day 14. The Y axis is on a Log10 scale. Black bars represent one standard error.**

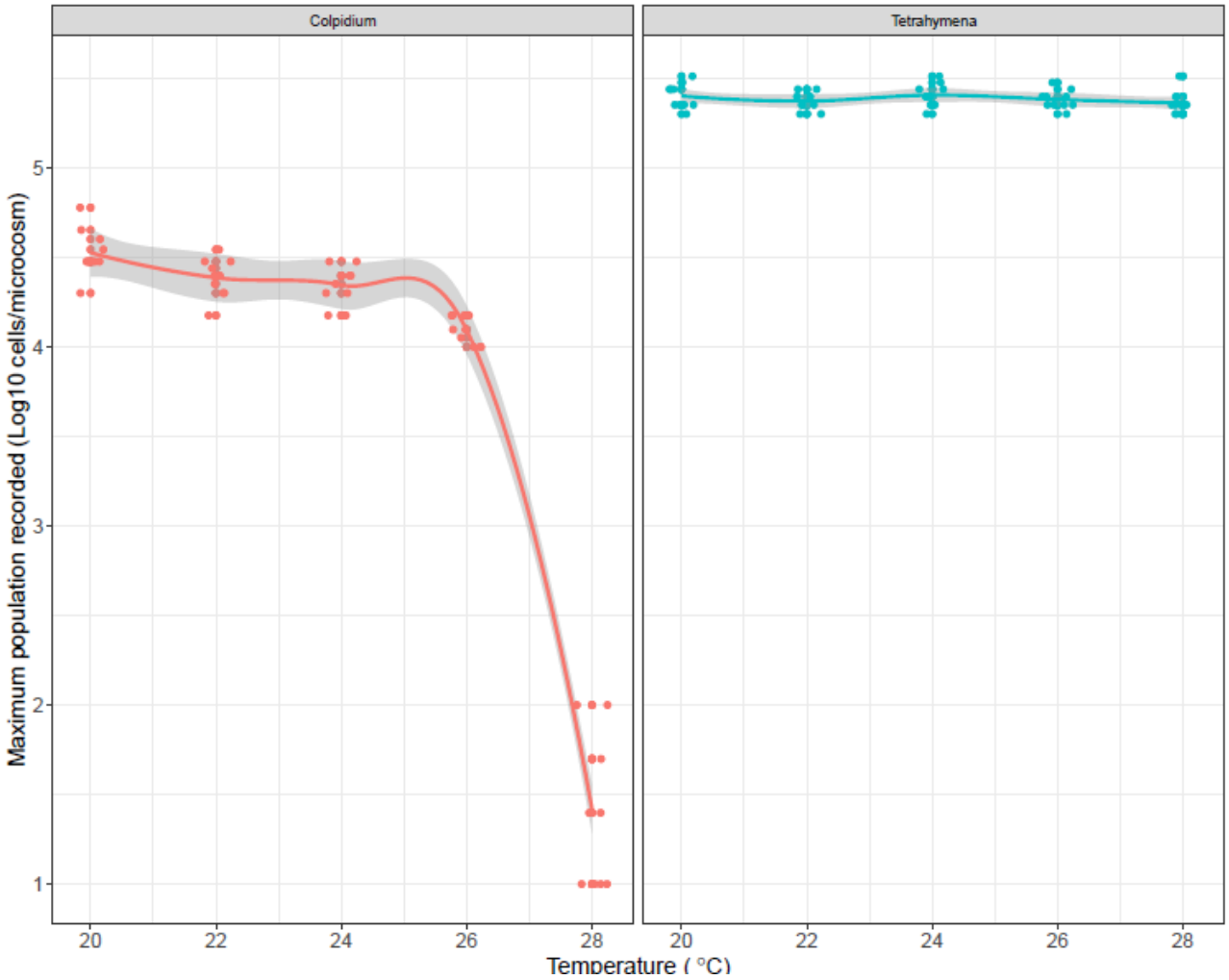


**Figure 4.3. Maximum population reached in each microcosm as a function of temperature for *Tetrahymena* (blue) and *Colpidium* (orange) invading into empty (bacteria only) microcosms on day 14. Coloured lines represent a loess model fitted to the data and grey shading represents 95% confidence intervals.**

A linear regression revealed no significant effect on the maximum population of *Tetrahymena* across all five temperatures (data transformed to the log base 10 before analysis; d.f. 1, 48; F=1.4137; P=0.2403). Due to the extreme non-linear effects of temperature on *Colpidium* between 26°C and 28°C, a linear regression was performed on the temperature range 20°C -26°C only. This showed that there was a significant negative effect of increasing temperature on the maximum population (data transformed to the log base 10 before analysis; d.f. 1.38; F= 69.067; P<0.001).

It is clear to see that *Tetrahymena* maintained very consistent maximum population densities, with temperature showing very little effect (refer to figure 4.3) where *Tetrahymena* showed little variability (25,000 cells over all temperatures). In contrast, *Colpidium* were greatly affected by temperature, where a drastic decrease in mean maximum population density occurred from 26°C to 28°C (refer to figure 4.3). *Colpidium* were several orders of magnitude less abundant at 28°C than at 20°C, 22°C, 24°C and 26°C.

### 3.3. Testing the effect of temperature on time to extinction for both species

*Hypothesis 2 - Warmer temperatures would make extinction occur earlier when single species are invaded immediately into each temperature.*

Warmer temperatures did make extinction occur at an earlier date when invaded immediately into each different temperature. Both species went extinct first at 28°C (*Colpidium* always went extinct earlier than *Tetrahymena* in all temperatures) and had individuals still present at 20°C by the end of the experiment on day 84.

*Tetrahymena* populations were still present in all microcosms at 20°C, 22°C and 24°C (see figure 4.2). However, densities declined earlier in warmer temperatures. At 20°C there was a mean of around 117,500 cells on day 84 compared to 13,000 cells at 22°C and 700 cells in 24°C, with populations not far from extinction. It was 2 weeks later (day 70) when they went extinct at 26°C and a week after that (day 63) at 28°C.

On the other hand, *Colpidium* at 20°C were all still alive at the end of the experiment apart from one microcosm where all individuals were gone by day 77 (see figure 4.2). All microcosms that still had populations present were declining. Temperatures from 22°C to 26°C had very little effect, where all microcosms had gone extinct by day 70 (apart from two microcosms at 22°C that had gone extinct a week before this). 28°C had the largest effect, where all microcosms were extinct by day 26, only 12 days after the initial propagule was added on day 14.

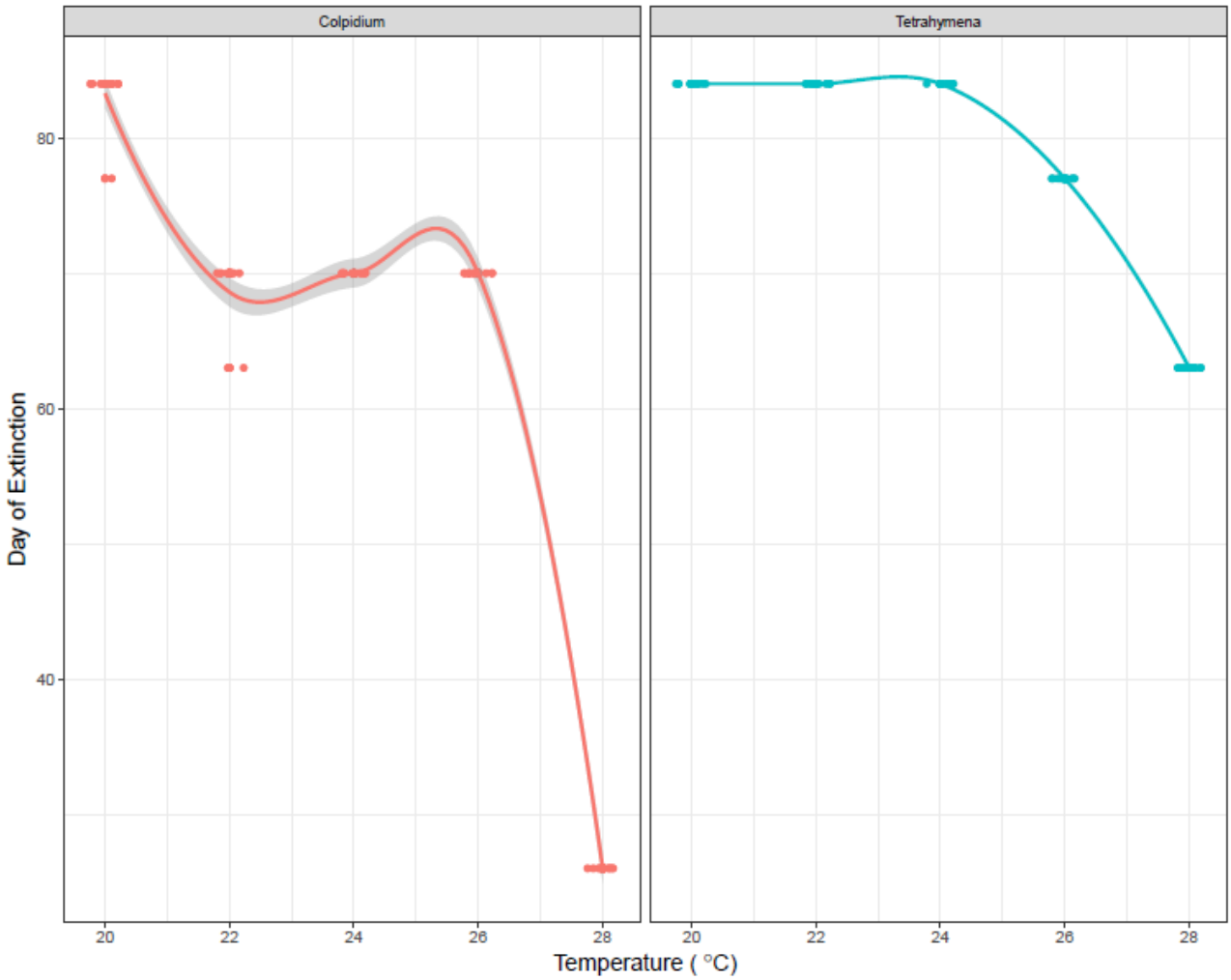


**Figure 4.4. Day of population extinction as a function of temperature for *Tetrahymena* (blue) and *Colpidium* (orange) invading into empty (bacteria only) microcosms on day 14. Coloured lines represent a loess model fitted to the data and grey shading represents 95% confidence intervals.**

*Tetrahymena* and *Colpidium* were strongly affected by temperature, but especially 28°C. *Colpidium*'s time to extinction clearly decreased from 20°C to 22°C but then remained fairly stable, nearly 3 times later than populations at 28°C. *Tetrahymena* populations showed a similar trend, with populations very stable with similar extinction times from 20°C to 24°C, but then gradually started to lessen at 26°C and 28°C at very predictable intervals (refer to figure 4.4).

For *Colpidium* at 22°C, there were large differences in day of extinction (some on day 63 and some on day 70). However, this is due to the length of time between sampling populations; with a week between samples, the exact date of extinction cannot be identified. For example, it may have been one day after sampling (day 64) or it may have been on day 70.

### 3.4. Testing the effect of rapid warming without invasion on time to extinction for both species

*Hypothesis 3a - Warmer temperatures would make extinction faster when single species are moved from 20°C to warmer temperatures.*

Temperature had the same effect on time to extinction for different temperatures as for when populations were moved from 20°C to warmer temperatures; warmer temperatures caused earlier time to extinction. Again, *Colpidium* populations always went extinct earlier than *Tetrahymena*. When the time to extinction was counted after warming on day 14 rather than from day 3 where all microcosms were at 20°C, extinction occurred much faster than in microcosms invaded immediately into each temperature (see figure 4.2).

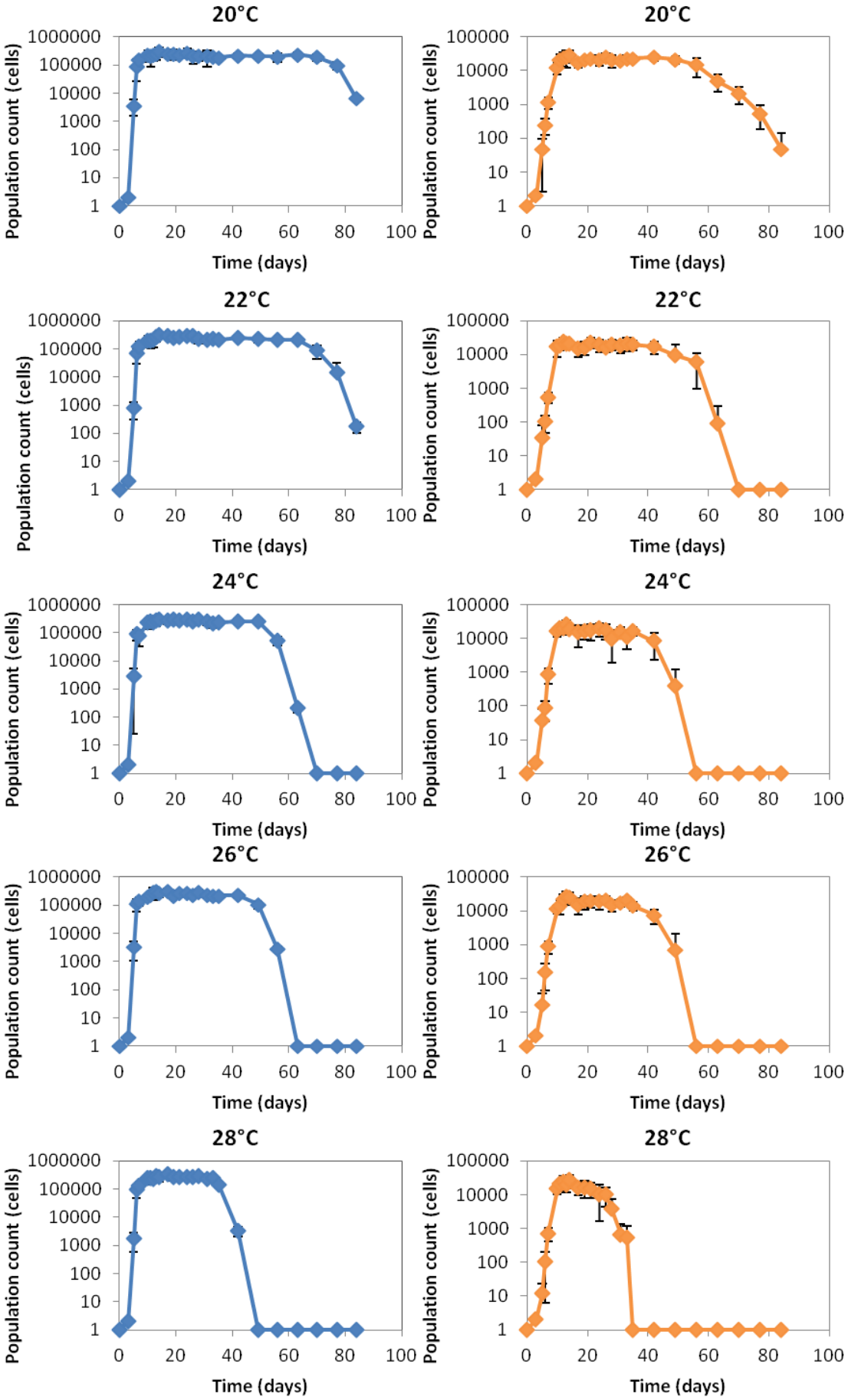


**Figure 4.5. Mean population density over time of *Tetrahymena* (blue) and *Colpidium* (orange) kept in 20°C until day 14 and then rapidly warmed to each temperature. Black bars represent one standard error.**

*Tetrahymena* populations were still alive in all microcosms at 20°C and 22°C at the end of the experiment on day 84 (see figure 4.5). These populations were a mean 6,500 cells and at 22°C they were a mean 170 cells, clearly on the way to extinction. Those that were moved from 20°C went extinct on day 70 (56 days after warming) at 24°C while microcosms at 26°C all went extinct earlier still on day 63 (49 days after warming) and at 28°C on day 49 (35 days after warming).

In most of the treatments for warming, all *Tetrahymena* microcosms experienced the same time of extinction, but *Colpidium* that were warmed varied much more, where some populations survived a week longer than others in the same temperature (see figure 4.5). *Colpidium* populations were still surviving at 20°C but only in four microcosms, where five went extinct on day 84 and one microcosm a week before on day 77 (70 or 63 days after warming). Those still alive were in very low densities, with a mean of 50 cells still surviving. At 22°C, microcosms went extinct around two weeks later on either day 70 or 63 (56 or 49 days after warming) with those at 24°C and 26°C reaching extinction two weeks after that on day 56 or 49 (42 or 35 days after warming). At 28°C all microcosms were extinct by day 35 (21 days after warming), with one microcosm extinct on day 31.

### 3.5. Testing the effect of rapid warming with simultaneous invasion on time to extinction for both species

*Hypothesis 3b - Extinction will be even faster still when a species is moved from 20°C to warmer temperatures and invaded by a competitor.*

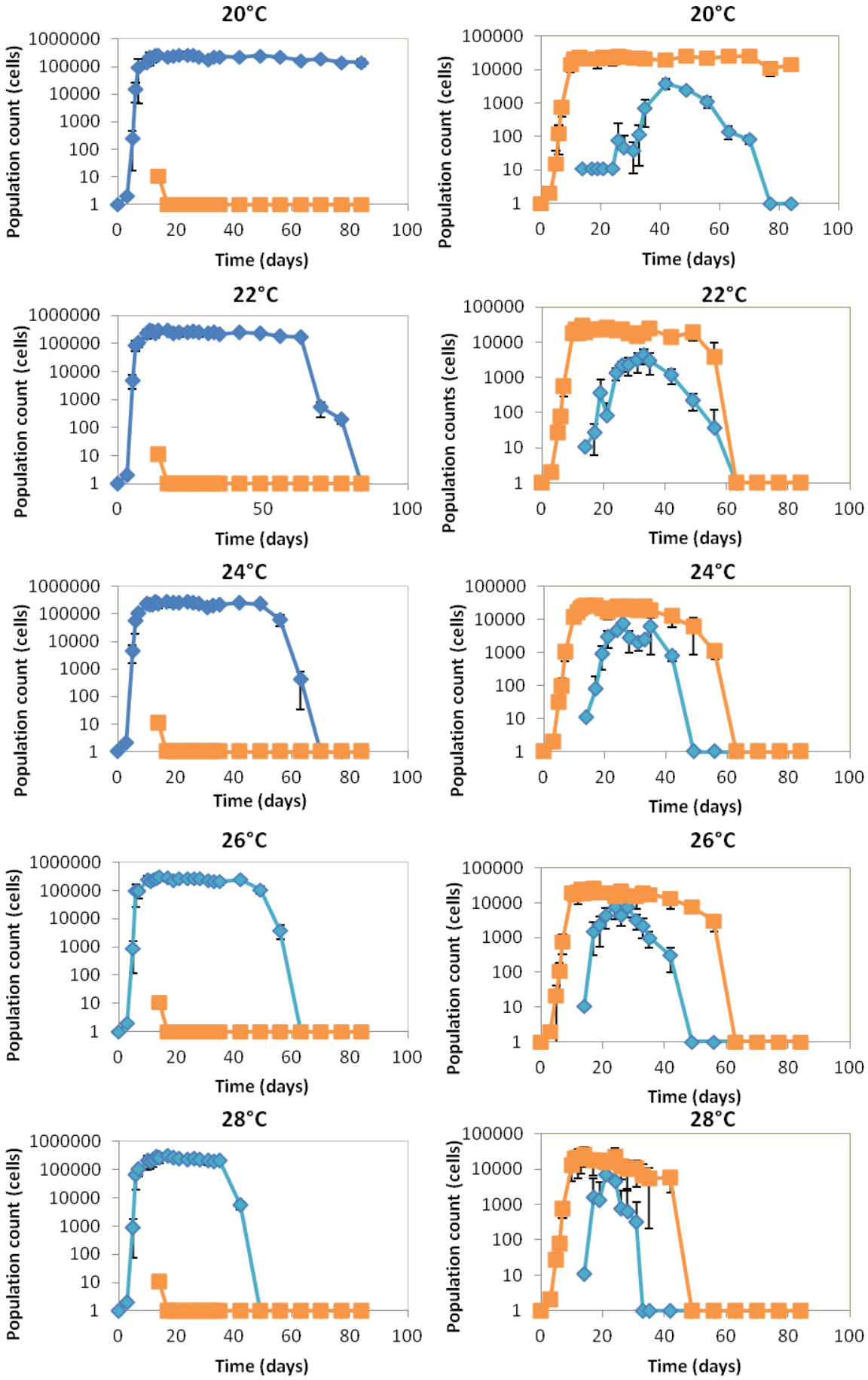


**Figure 4.6. Population density over time of resident *Tetrahymena* (blue) invaded by *Colpidium (*orange) in all temperatures. Also population density over time of resident *Colpidium* (orange) invaded by *Tetrahymena* (blue) in all temperatures The Y axis is on a Log10 scale. Black bars represent one standard error.**

Extinction was later when species experienced rapid warming and invasion by a competitor; extinction was earlier when alone species experienced rapid warming and remained uninvaded. Invaded *Tetrahymena* populations were still alive at 20°C on day 84 in all populations, but in much larger densities than uninvaded populations, with a mean population of 145,000 cells compared to 7,000 (see figure 4.5 and 4.6). At 22°C, all microcosms were extinct by day 84 whereas uninvaded populations were still alive, despite declining towards extinction. At 24°C and 26°C, extinction occurred a week later than uninvaded populations, with no individuals found after day 77 at 24°C and day 70 at 26°C. However, 28°C is the exception where extinction occurred on the same day for invaded and uninvaded, with all microcosms dead by day 49.

In contrast, all invaded *Colpidium* populations had a later time to extinction than uninvaded populations (see figure 4.5 and 4.6). At 20°C on day 63, single species populations were starting to go extinct; however, all invaded *Colpidium* populations were still alive. They were also alive at larger population densities at a mean 13,000 cells compared to uninvaded populations at only 50 individuals on day 84. All populations at 22°C, 24°C and 26°C went extinct by day 63, up to 2 weeks later than some uninvaded populations. Finally, at 28°C all populations were extinct by day 49, again, two weeks later than the uninvaded populations in the same temperature.

Although *Tetrahymena* and *Colpidium*, invaded and uninvaded, both showed a very linear time to extinction in the data (refer to figure 4.7), it is worth noting that this is not reliable or representative. Several points plotted on day 84 are still not extinct; populations are alive and thriving well in high densities in some cases. Therefore, these populations may have a much later extinction date, distributing the data differently.

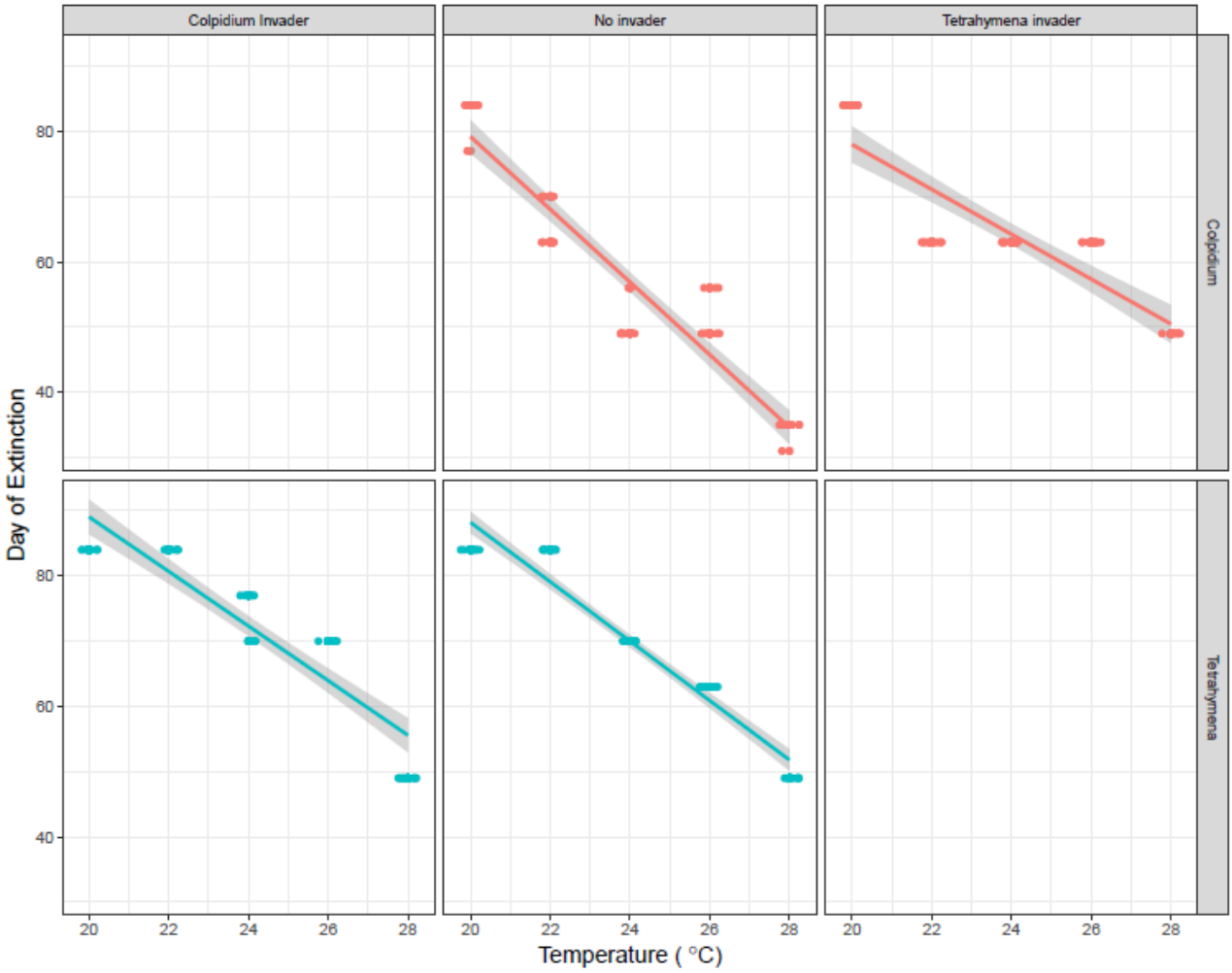


**Figure 4.7. Day of extinction as a function of temperature for resident *Tetrahymena* (blue) and *Colpidium* (orange) with and without invasion. Coloured lines represent a linear model fitted to the data and grey shading represents 95% confidence intervals. *Colpidium* were never invaded by *Colpidium* and *Tetrahymena* were never invaded by *Tetrahymena* so these were left blank.**

### 3.6. Testing the effect of rapid warming and simultaneous invasion on priority effects for both species

*Hypothesis 4 - Priority effects will be weakened when the invasion occurs simultaneously with warming towards and above the thermal tolerance of one species.*

Temperature had a smaller than anticipated effect on priority effects, with the strong priority effects in both directions overwhelming the effects of temperature. *Colpidium* was always excluded by resident *Tetrahymena*, with *Colpidium* never able to establish a population (See figure 4.6). In contrast, invading *Tetrahymena* was always able to invade resident *Colpidium* and establish a population, leading to a period of coexistence of many generations. But *Tetrahymena* then always went extinct before the resident *Colpidium* (see figure 4.6).

Throughout the period in which they coexisted, *Tetrahymena* never came close to reaching the population density of resident *Colpidium (*for example*,* at 20°C, *Colpidium* reached a mean maximum population density of 23,000 whereas *Tetrahymena* reached less than 4,000 cells). The closest the two populations became to equal densities was at 26°C where *Colpidium* reached a maximum of 26,000 cells and *Tetrahymena* reached 8000 cells. The population of *Tetrahymena* invading into *Colpidium* was always several orders of magnitude less abundant than where they were alone (for example, at 20°C, invading *Tetrahymena* reached a mean maximum population density of around 3,500 cells whereas *Tetrahymena* alone reached a mean maximum population density of 255,000 cells).

However, temperature did influence the rate at which priority effects occurred in the different treatments, either immediately or over a very prolonged period of time. When resident *Colpidium* was invaded with *Tetrahymena*, the warmer the temperature the sooner priority effects occurred. Coexistence occurred in all temperatures for a minimum of 19 days at 28°C up to 63 days at 20°C (see figure 4.6). In opposition, when *Colpidium* was introduced to resident *Tetrahymena* populations, the propagule could never establish a population and grow regardless of temperature. After day 14 when invasion was carried out, *Colpidium* never increase their density above the starting propagule size of 10 individuals and they immediately go extinct in all microcosms in all temperatures (See figure 4.6). This resulted in immediate priority effects, with resident *Tetrahymena* excluding all invaders instantly.

### 3.7. Testing the effect of temperature and simultaneous arrival on priority effects for both species

When arriving simultaneously in equal propagules, both species were able to grow and establish a population in all microcosms apart from at 28°C where *Colpidium* immediately went extinct (refer to figures 4.2 and 4.8). At all other temperatures both species were able to coexist for many generations before *Colpidium* was excluded by *Tetrahymena*. In contrast to where small propagules invaded populations at maximum population density (where coexistence was only possible where *Tetrahymena* invaded *Colpidium* and remained at a lower abundance), where both species arrive simultaneously, coexistence for long periods was possible with *Tetrahymena* having a much larger population than *Colpidium* (refer to figures 4.6 and 4.8).

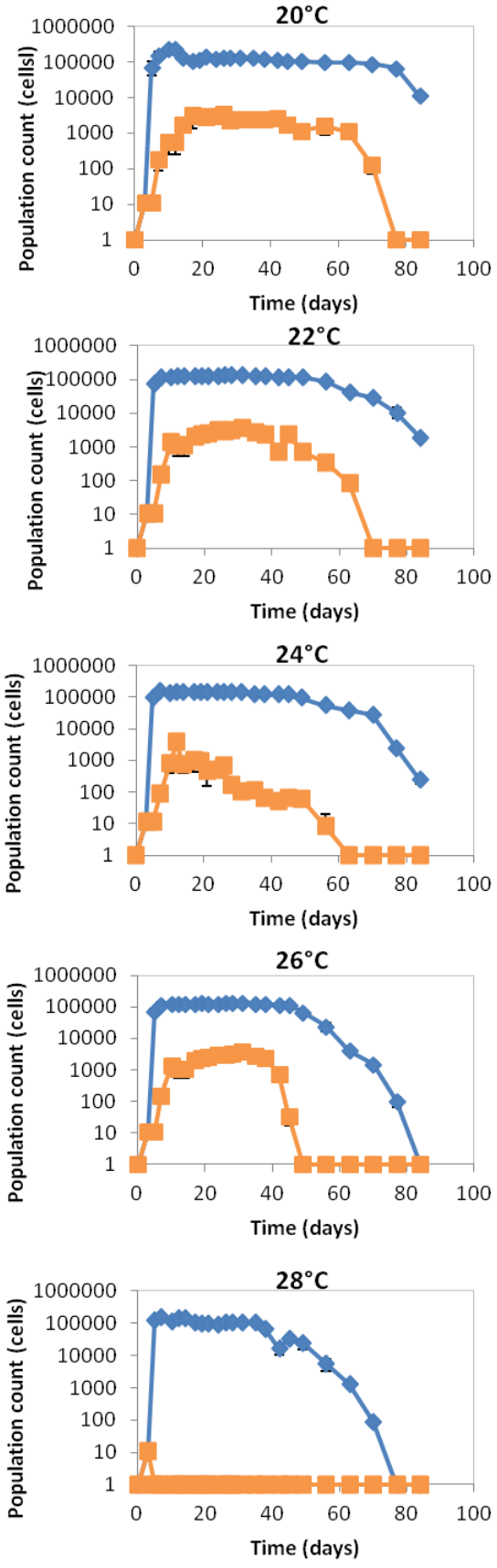


**Figure 4.8. Population density over time of *Tetrahymena* (blue) and *Colpidium* (orange) invaded simultaneously in equal densities immediately into each temperature. The Y axis is on a Log10 scale. Black bars represent one standard error.**

For example, at 20°C *Tetrahymena* reached a mean maximum population density of approximately 130,000 cells (the only exception is in the first week at 20°C where populations peaked at 230,000 cells but then return to 130,000 cells like all other microcosms). In contrast, *Colpidium* never established at 28°C and never reached a mean population density of more than 3,000 cells at 20°C, 22°C, 24°C or 26°C. Both species population densities never reached that of any of the species alone microcosms either. *Tetrahymena* alone attained a maximum mean population density of 255,000 cells at 20°C, double the population of *Tetrahymena* when simultaneously invaded alongside *Colpidium.* *Colpidium* alone populations reached 35,000 cells at 20°C, approximately 10 times more than populations arriving simultaneously with a competitor.

Temperature altered the length of coexistence and time to extinction, where warmer temperatures caused earlier extinction of *Colpidium*, which consequently resulted in shorter periods of coexistence (see figure 4.8). At 20°C, coexistence lasted until day 77 when *Colpidium* were excluded. *Tetrahymena* were still alive at the end of the experiment on day 84 in densities of a mean 10,800 cells. Coexistence occurred earlier still at 22°C, with *Colpidium* excluded by day 70, with *Tetrahymena* still alive on day 84 but in much lower densities of a mean 1,800 cells (six times less than those at 20°C). 24°C resulted in *Colpidium* extinction occurring on day 63, with *Tetrahymena* also on the verge of extinction, with only a mean of 200 cells left on day 84. However, 26°C was warm enough to cause eventual extinction of both species. *Colpidium* went extinct and ended coexistence on day 49 whereas *Tetrahymena* managed to survive an extra 5 weeks before going extinct by day 84.

The most interesting treatment was probably 28°C. Despite *Colpidium* being unable to establish (see figure 4.8), *Tetrahymena* densities never reached the same densities as when alone in the staggered arrival experiment (See figure 4.2), and extinction occurred earlier than those with *Colpidium* present in the other temperatures (See figure 4.6). Microcosms only reached a mean maximum population density of 157,500 cells (very similar to all other treatments in this experiment) which is smaller than *Tetrahymena* alone at 28°C at a mean 232,500 cells (nearly 1.5 times greater) (See figure 4.2 and 4.8). Additionally, extinction occurred on day 77, earlier than all microcosms at 20°C, 22°C, 24°C and 26°C with *Tetrahymena* and *Colpidium* arriving simultaneously. However, it was later than *Tetrahymena* at 28°C when alone. *Tetrahymena* alone at 28°C went extinct on day 63, 49 days after establishing and 4 weeks earlier than *Tetrahymena* in this experiment.

# 4. Discussion

## 4.1. Species Thermal Tolerances

Although there has been a considerable depth of research into priority effects and the factors that can alter community structure (Fukami, *et al.* 2016; Fukami, *et al.* 2015; Grainger, *et al.* 2018; Jiang, *et al.* 2011a; Jiang, *et al.* 2011b; Sarneel, *et al.* 2016; Chase, 2003), there is a large gap regarding the effect of temperature as a mechanism for manipulating relative fitness difference between species within a community and its effect on priority effects. Only a few papers, such as Clements, *et al.* (2013) and Grainger, *et al.* (2018) consider the effects of temperature and community assembly together.

The evidence derived from this experiment demonstrates that there were strong priority effects between *Tetrahymena* and *Colpidium*, with the resident species always being far more abundant than invading species and excluding the invader, regardless of temperature. Young, *et al.* (2017) describe a successful invasion as introducing a new species to a resident population, in which there is initial population increase established local dominance and then range expansion. Therefore, *Colpidium* is never able to invade *Tetrahymena* since they cannot establish at all but *Tetrahymena* can successfully invade *Colpidium* but this is only short term; priority effects still occur after the population has established and populations have coexisted for weeks.

From the initial pilot experiment, it was clear to see that each species had distinct thermal tolerances with different optimum temperatures, as 20°C resulted in the greatest maximum population density for *Colpidium* and 26°C for *Tetrahymena* (Frankel and Nelson, 2001).

Extending the temperature range to 28°C aimed to increase the relative fitness difference between both species to test Fukami's framework (2016). Arguably, the most interesting result was that with 28°C reaching beyond *Colpidium's* thermal threshold, they were consequently unable to invade or establish alone in such warm conditions. But surprisingly, when *Colpidium* were resident or were invaded simultaneously with *Tetrahymena*, both species could coexist together for a lengthy period of time in quite high densities and were relatively stable in some temperatures for up to a few weeks.

More astoundingly still, resident *Colpidium* could outcompete *Tetrahymena* and reached greater population densities despite them being unable to invade or survive when alone in this temperature. *Colpidium* populations unexpectedly repelled *Tetrahymena* invaders and resulted in *Tetrahymena's* extinction. Although this *Tetrahymena* species can survive in temperatures up to 32°C, in 28°C they still could not reach similar or higher population densities than *Colpidium,* despite *Colpidium* being unable to invade/establish in it when alone (Frankel and Nelson, 2001). All of this indicates that the order of species arrival could be one of the most vital fundamental aspects in determining community dynamics and composition (Fukami, *et al.* 2010; Ejrnaes, *et al.* 2006).

### 4.2. Effect of Temperature on Maximum Population Density

The effect of temperature on maximum population density of populations proved to be different for each species, with temperature having a greater influence on *Colpidium* than *Tetrahymena*. Similarly, Fjerdingstad, *et al*. (2007) identified that maximum population density is species and strain dependent. In this case, warmer temperatures decreased

*Colpidium* maximum population density, with populations at their greatest at 20°C but barely establishing at 28°C. In contrast, *Tetrahymena* populations all reached very similar densities with only slight variation between temperatures.

Similarly, Fox and Morin (2001) found that in both constant and warming conditions, temperature did not affect maximum population density and it remained high in all treatments. This is down to the fact that *Tetrahymena* have a much larger thermal tolerance rage than *Colpidium*; *Tetrahymena* are known to have an optimal growth temperature of 28°C with their growth beginning to be stunted at around 10°C (Sauvant, *et al.*, 1999). They are also known to show very little temperature dependence between 15°C and 27°, allowing them to grow and reach similar carrying capacities within this range (Hill, 1972). Some *Tetrahymena* strains are also able to withstand temperatures up to 40°C (Hill, 1972); therefore, 20°C to 28°C would never have restricted *Tetrahymena*'s ability to reach a high and consistent maximum population density.

In *Colpidium*, temperature had a stronger affect, where the warmer temperatures resulted in a decrease in maximum population density. 28°C was simply too warm for *Colpidium* to tolerate, with very little growth (any growth was probably as a result of cells already committed to division). However, from 20°C up to 26°C, warmer temperatures resulted in an increased metabolic cost, making populations more unstable and unable to reach higher population densities. Additionally, cooler temperatures increased population stability with reduced metabolic cost and slower predictable growth (Beveridge, *et al.*, 2010).

### 4.3. Effect of Temperature on Time to Extinction

Similarly, temperature affected the time taken for species to go extinct in all treatments. Extinction occurred earlier at warmer temperatures for single species invaded immediately into each temperature, when rapidly warmed and when warming and invasion occurred simultaneously. However, temperature affected the treatments differently; extinction was either the same or faster when moved from 20°C to warmer temperatures than when immediately invaded into each temperature. However, when warming and invasion occurred simultaneously, extinction actually happened later than when just warming occurred.

Temperature generally made extinction occur earlier due to the same reasons stated earlier for maximum population density. Warmer conditions resulted in increased metabolic cost (resources consumed faster) which resulted in the entire system running out of energy earlier. In contrast, cooler temperatures resulted in more stable conditions, with slower growth and slower resource consumption, which allowed systems to possess more energy for a longer period of time. Therefore, populations were able to survive for longer due to resources being available for longer.

When species experienced rapid warming, once in the new temperature, extinction occurred earlier than in populations invaded immediately into each temperature on day 14. When this is taken into consideration, both species went extinct faster than those immediately established into each temperature. The most likely reasoning for warmed populations going extinct earlier after warming was due to the sheer abundance of each population. The warmed populations were initially kept at 20°C to allow densities to reach maximum

population density. This meant populations were around 3,000 times larger for *Colpidium* and 30,000 times larger for *Tetrahymena* than the starting propagule of 10 for those invaded straight into each temperature. This could mean there is a greater chance for there being individuals present in the population who are more evolved or adapted to changing or warmer conditions, or there are just more individuals meaning populations will take longer to decline. In the single species microcosms invaded immediately into each temperature, there were only 10 individual cells that needed to be able to grow successfully over multiple generations in order to establish a population.

Simultaneous invasion and warming made extinction occur later for resident populations than when just warming occurred. This contrasts with Carrera, *et al.*'s (2015) findings that the more competition there is between species, the greater instability there is in the community. It is highly possible that the presence of a competitor caused the resident protists to adapt in order to enhance survival. For example, *Colpidium* are known to be able to alter their cell shape and size to become a much smaller cell volume under environmental pressures (Fyda, 1998). Therefore, requiring fewer resources in order to grow, prolonging resource availability and subsequently the populations survival. Additionally, cells could reduce their growth rate or feeding rate, stabilising populations to reduce the speed at which resources are consumed.

Alternatively, it could be possible that the presence of a new predator, or even a change in temperature, caused the *P. fluorescens* to adapt. This may have consequently reduced the protists ability to utilise resources as readily as they otherwise would have. *P. fluorescens* is a highly versatile bacterium which can easily adapt to any environmental changes and

pressures (Silby, *et al.*, 2011). Therefore, the bacteria could form more biofilms, altering the availability of free particulate chlorella and bacteria, preserving resources for longer with them being more difficult to utilise. The chlorella was dead due to the medium being autoclaved before bacterial inoculation so would have had no effect over the duration of the experiment.

Apart from the treatment where resident *Colpidium* was invaded by *Tetrahymena*, *Colpidium* always went extinct sooner than *Tetrahymena*, despite always reaching lower population densities than *Tetrahymena*. *Tetrahymena* are very small cells at only 50µm long and 30µm wide so would require less energy for their cell size and growth (Sauvant, *et al.*, 1999). However, they were always highly abundant and would have consequently consumed a large amount of resources very rapidly for their sheer population density, which resulted in the system running out of energy sooner and caused populations to go extinct.

In contrast, *Colpidium* have a much larger cell size so would have required greater volumes of nutrients to grow and divide, but their population densities were around ten times less than those of *Tetrahymena*, which resulted in fewer individual cells consuming a smaller quantity of bacteria. Nonetheless, *Colpidium* are recognised for their strong grazing ability; they can reduce bacteria density much more than *Tetrahymena* (Fox and Barreto, 2006). Therefore, even small populations of *Colpidium* could rapidly deplete resources leading to earlier extinction than other species.

Although *Colpidium* may be more successful grazers, *Tetrahymena* have their own advantageous characteristic. *Tetrahymena* thrive more in particulate material and it is

believed they can directly utilise nutrients in media (i.e. chlorella particles in the media) (Hill, 1972). This provides *Tetrahymena* with another source of nutrients for growth to enable populations to survive for a longer period of time. Another factor could be that *Tetrahymena* are better adapted to environmental pressures, such as limited resources, and are able to withstand any environmental changes and survive longer. *Colpidium* may be simply unable to tolerate any new conditions and are too sensitive to any changes that they are unable to withstand the pressures and consequently go extinct in a short period of time.

The only treatment where *Colpidium* outlives *Tetrahymena* is when *Tetrahymena* invade resident *Colpidium*. Temperature had no effect on the strength of priority effects, with the same result in all temperatures. All that temperature did influence is the time at which the invader and resident went extinct, with earlier extinction in warmer temperatures. I hypothesised that in temperatures where the resident was not at its optimal temperature and outside its thermal tolerance, or the invader was at its optimum, invasion would have been more successful and coexistence more probable.

### 4.4. Priority Effects

In both directions of the invasion (*Tetrahymena* invading resident *Colpidium* and *Colpidium* invading resident *Tetrahymena*) there were extremely strong priority effects. In both situations, the resident species always excluded the invading species. Although priority effects is not always the resident excluding the invader (it could simply be the resident being much more abundant and overwhelming the invader), in this case in both directions, the resident was present in greater densities and excluded the invading species. This was due to resident species having a large period of time before later arriving species were invaded,

which enabled them to freely utilise shared resources, grow, adapt and change the environment to suit themselves. This was then a disadvantage to late arriving species and hampered their ability to successfully invade and establish a population if resources were too scarce, amplifying competitive exclusion of late arriving competitors (Grainger, *et al.* 2018; Gray, *et al.*, 2015).

The more similarities the resident and invader have in their resource requirements, the stronger the competition between them (Jiang, *et al.* 2017). This means that if residents can freely deplete these resources, availability may be too limited to allow invading species to grow and establish a population within the community, resulting is strong priority effects. Nevertheless, there was clearly still a large amount of resources and energy in all the microcosms at the time of invasion due to resident populations being able to survive in some temperatures for the duration of the experiment. Therefore, it is likely that the resident was either too abundant that it overwhelmed invaders, or that residents had changed and manipulated the environment in a different way (as discussed earlier, it is possible that *P. fluorescens* can adapt to residents being present which allows it to be less available for invaders),

Nevertheless, despite these strong priority effects, when resident *Tetrahymena* were invaded by *Colpidium*, the invaders could not ever even grow or establish a population. Yet when resident *Colpidium* was invaded by *Tetrahymena*, invaders could grow and temporarily establish a population, there was a large period of time where coexistence was possible before *Colpidium* excluded *Tetrahymena*. This is logical as Sarneel *et al.* (2016) identified that species are less susceptible to priority effects if they are more successful when alone.

*Tetrahymena* alone reached greater carrying capacities than *Colpidium* alone and had a shorter generation time of only 3 – 7 hours (Hill, 1972).

Successful invaders are also more flexible in their ability to alter their growth rate in response to resource fluctuations or environmental pressures (Mata, *et al.* 2012). In theory, this implies that these stronger performing species, *Tetrahymena* in this experiment, will be able to more successfully invade a community and establish making priority effects much weaker. Also, Gray, *et al.* (2015) suggests that if a species enters an environment with no enemies, establishment should be straightforward. Therefore, *Tetrahymena* was able to establish as *Colpidium* is a weaker competitor and a minor threat to *Tetrahymena*.

Surprisingly, despite this, resident *Colpidium* still excluded *Tetrahymena* despite them establishing a fairly dense population. This is supported by Jones, *et al.* (2017) who discuss how if inferior species are more abundant than competitively superior species, then they can still cause extinction by simply overwhelming them in numbers. Invading *Tetrahymena* never reached as high densities as resident *Colpidium* and were simply outnumbered by them. However, this contrasts with Fox's (2001) findings where an environment could never be created where *Colpidium* could exclude all its competitors due to their slow growth rate. In support of this, Leiss and Dehil (2006) also found that *Tetrahymena* always excluded *Colpidium* but this involved different initial stock densities and populations were introduced simultaneously.

Chesson (2000) defines stable coexistence as populations always being able to recover from any pressures; therefore, this coexistence was not stable. With the one species population

being present in higher quantities than the other, and with both populations never being able to remain at a constant density (they always varied and always rapidly declined eventually), it must be defined as unstable coexistence where either population are never able to recover if densities begin to decline (Chesson, 2000). *Tetrahymena* always went extinct before resident *Colpidium*, which suggests that *Colpidium*'s presence was too great a pressure for *Tetrahymena* to stabilise. This occurred between *Tetrahymena* and *Colpidium* in this experiment due to the strong overlap in resource requirements, resulting in stronger competition (Jiang, *et al.* 2017). Both species had only *P. fluorescens* present in the microcosm as a resource, so competition became stronger as the bacteria became scarcer, leaving populations with no alternative but to decline.

The priority effects arguably occurred in very different timescales depending on the order of community assembly. For *Tetrahymena* invaded with *Colpidium*, effects were immediate with *Colpidium* never able to grow and residents instantly excluding the invaders. It is likely that *Tetrahymena* excluded *Colpidium* immediately due to their high population density compared to the 10 individual *Colpidium* invaders. By completely overwhelming *Colpidium, Tetrahymena* were able to consume a greater quantity of resources at a faster rate to hamper *Colpidium's* establishment.

In contrast, when resident *Colpidium* were invaded with *Tetrahymena*, priority effects did not take effect until weeks after invasion when only then did residents exclude the invaders. Resident *Colpidium* was still in high densities but it may have been low enough for *Tetrahymena* to still establish a population and remain present within the community for a few weeks. However, it is possible to argue that priority effects were present throughout

the whole duration of the resident *Colpidium* and invading *Tetrahymena* experiment, since *Tetrahymena* were never able to match the population density that *Colpidium* demonstrated and were never able to fulfil anywhere near the maximum population density they could reach when alone or as a resident species.

Priority effects tend to be studied over a short period of time to identify short term effects, but these effects can become stronger or more obvious over time, often over several generations after species have evolved and adapted to strengthen or weaken underlying mechanisms (Buckley, 2017; Faillace and Morin, 2016). Therefore, many studies could miss interesting prolonged results occurring at the end of the experiment when extinction occurs and the system runs out of energy (Fukami, *et al.* 2010).

### 4.5. Niche Modification and Niche Differentiation

One of the most likely mechanisms behind the extremely strong priority effects, in both directions, is niche modification. Originally the mechanism was thought to be niche pre-emption, but this would mean residents would use up energy and resources to hamper invasion. However, this is clearly not the case where populations were able to coexist for weeks, and the resident *Colpidium* was able to survive longer still, with populations still alive in 20°C at the end of the experiment. This demonstrates that resources were not drastically depleted, and instead the resident must have changed or modified the niches before the later arriving species was invaded.

Early arriving species have the opportunity to use the environment to their full potential when they are alone before invasion, filling as much of their fundamental niche as possible,

and possibly more than they would be able to with their realised niche if other species were present (Weisse, *et al.* 2016). Residents can then modify the environment to suit themselves freely, without any competition (Weisse, *et al.* 2016). This could be as simple as using up resources so that it would not be available to later arriving species. Therefore, species with more similar requirements and greater niche overlaps can struggle to coexist if the resources are limited (Little and Altermatt, 2018).

However, this does not mean that it is impossible; protists can often compete for the same bacterial resource but still coexist (Fox and Barreto, 2006; Holdridge, *et al*. 2017). However, the greater demand there is between species for a shared resource, the more rapidly it will deplete which can ultimately result in stronger priority effects, being strengthened with a greater overlap in similarities (Jiang, *et al.*, 2017). On the other hand, the Empty Niche Hypothesis, states that invading species might be more successful at filling any available niches that the resident has not fulfilled, to effectively utilise any available unexploited resources to allow themselves a greater chance of establishment and survival (Young, *et al.*, 2017). However, the results from the experiment presented here contrast with this, with *Tetrahymena* unable to grow to anywhere near the same density as *Colpidium*, and then being excluded. This suggests *Tetrahymena* could not grow, replicate, or consume resources at a fast enough rate to outcompete the abundant resident *Colpidium*.

Alternatively, there must be an essence of niche differentiation at play. In both treatments where coexistence occurred (*Colpidium* invaded by *Tetrahymena* and both invaded simultaneously into different temperatures) both species were able to survive together for weeks at fairly stable population densities. This can be explained by both species altering the

environment to suit them in order to survive despite the pressure of a competitor being present. Again, this could involve an interaction with *P. fluorescens* and that adapting to be more/less available for each species to prolong coexistence.

### 4.6. Phenotypic Plasticity as a Mechanism

When invading *Tetrahymena* populations peaked and resident *Colpidium* populations temporarily dipped, *Colpidium* appeared to reduce in cell size for a very short period of time (this was only observed during sampling, no data was collected for it). This was thought to be an adaptation as a consequence of competing *Tetrahymena* being present in increasing densities or resources depleting as this is known to trigger cell size changes in both species (Fox, 2001). However, this cannot be a known consequence of *Tetrahymena's* presence or resources depleting as other factors may be the cause (e.g. temperature). When a separate later attempt was made to photograph cell size and cells were measured, there was too much variance in the populations without any competitors present (10 cells were measured from each microcosm) to show any significant conclusions and patterns.

It is known that as a mechanism of defence, both species can alter cell size and shape to allow themselves a greater chance of survival, possibly by affecting their competitive ability (Mata, *et al.* 2012; Gray, *et al.* 2015; Luhring and DeLong, 2016; Young, *et al.* 2017; Faillace and Morin, 2016). This allows cells to improve their fitness; smaller cells require less energy to replicate and grow, which can allow species to gain more of an advantage over a competitor (Carrera, *et al.*, 2015; Glucksman, *et al.* 2010). This in turn, could also reduce the speed at which resources are depleted to allow longer survival.

The variability shown in the photographed cells (with some cells half the size of others) is most likely to be down to cells being in different stages of replication and growth. *Tetrahymena* can drastically change cell size throughout this process, where over several generations; cell size can reduce as much as 50%, resulting in stationary populations consisting of groups of large and small cells in the same environment (Hill, 1972). Small *Tetrahymena* or *Colpidium* cells may have only recently divided whereas large cells may be ones that have had time to grow and have not yet committed to a division. Therefore, it could not be determined whether the phenotypic plasticity was an adaptation to the pressure of temperature change or competition, or whether it was just a normal consequence of growth.

### 4.7. Experiment Application

Temperature was used as a mechanism to manipulate the relative fitness difference between the invading and resident species to test Fukami's (2016) conceptual framework for priority effects (changed the equalising mechanisms along the y axis on the model) (see figure 2.1). This, in theory, would alter the success of any invasions, with faster intrinsic growth reaching greater maximum population density in a species optimum temperature leading to the species being a more successful invader, or reduced ability to be invaded as a resident. However, despite temperature affecting the time to extinction of both species, and the maximum population density of *Colpidium,* this was not the result. Priority effects overwhelmed the effect of temperature (*Colpidium* could never invade *Tetrahymena* but *Tetrahymena* could always invade *Colpidium*, but eventually *Colpidium* excluded *Tetrahymena*) with the resident species always excluding the invading species, even in 28°C

which is past *Colpidium's* thermal tolerance threshold where resident *Colpidium* can exclude and repel invading *Tetrahymena* despite alone species of *Colpidium* never being able to grow or establish a population in this temperature. However, temperature did have a significant effect on the speed at which the system ran out of energy and at which species went extinct.

This experiment created a model to test Fukami's conceptual model for priority effects and highlighted that relative fitness difference between species is not as strong in this experiment as the actual priority effects, even when *Colpidium* are pushed towards and above their thermal threshold, priority effects are still stronger than the fitness difference created over a range of 8°C. The fitness difference and maximum population density or maximum population density difference between *Colpidium* and *Tetrahymena* was huge, yet priority effects always occurred in both directions. Instead, stronger destabilising mechanisms were at play (e.g. differential niche modification). For example, *Tetrahymena* modify the niche and consume resources enough so that *Colpidium* cannot invade successfully but *Colpidium* cannot do so enough initially to hamper invasion but are able to outcompete *Tetrahymena* when resources get lower in more unstable conditions. Or, while both species are coexisting, they both simultaneously modify and differentiate the environment to suit them and prolong survival and coexistence.

### 4.8. Conclusion

In conclusion, these results have evidenced that it is important when investigating priority effects, to consider more than just the basic mechanisms and interactions. My study has illustrated how species reactions to both climate change (rapid warming) and invasion can

be difficult to predict due to the complex species specific interactions organisms can have between themselves as well as with the environment (including resources). Although there were strong priority effects in both directions, with the resident species always being overwhelmingly more abundant than the invader, or allowing no possible invasion, the results also demonstrate the importance of monitoring long term effects on a community compared to just short term immediate effects.

Therefore, it is vital to observe the prolonged effects of community assembly as although some mechanisms can be present instantly, it can take several generations for them to strengthen and have more dramatic and obvious effects on the community. Community dynamics can change further and result in a different community climax altogether; even causing a coexisting community to instead result in competitive exclusion and species extinction. Therefore, in future models it should be vital to strongly consider species specific responses to pressures and the environment as well as long term dynamics.

It is fundamental to consider any species specific traits that could allow a species to gain a competitive advantage over the other species within the community. Again, this is important to consider long term as species will begin to adapt and evolve over many generations, with any results possibly not presenting until long after the start of the experiment. Future research should also incorporate the response of resources. The results from this experiment suggest that resources such as bacteria can evolve and adapt to the pressures and changes just as organisms can, and can consequently have their own species specific responses which will consequently affect the rest of the community as a whole. Monitoring

factors such as their growth rates, death rates and maximum population densities could give a fuller picture of the effect temperature can have on all aspects of a community as a whole.

It is important to understand how temperature can affect other factors of community structure rather than just assembly; with more interesting data surrounding time to extinction and how rapid warming can affect a population at the end of their lives. Understanding species specific extinction rates in varying conditions could help to make more accurate predictions of when priority effects and extinctions could occur in changing climates.

In order to create a more realistic idea of how species will react to climate change and possible invasions, as well as predicting priority effects, it will be vital to create more realistic experiments and models to forecast from. For example, using large scale experiments with more realistic changes (slower, gradual warming compared to an 8°C change within 2 hours). It would also be more rational to use actual field studies, with plants and larger organisms in climate controlled conditions. This will create a more realistic model of how certain species will react to the different pressures in real time in more genuine and authentic environments.

Additionally, combining warming and invasion within experiments will be vital for conservation models. This will help to identify if there are ways to encourage coexistence between species entering new environments n a warming world to remain within their thermal tolerance, whilst encountering new species and habitats. For example, where warming and invasion allowed temporary coexistence for resident *Colpidium*, it would be

important to investigate whether there are methods and opportunities to prolong the coexistence, or to increase its stability. For example, it would be interesting to examine whether repeated intervals of invasion (i.e. invade a new propagule monthly) could allow populations to remain stable for a longer period of time and reduce the chances of species extinction. This could allow better development of conservation methods in the real world to prevent mass species extinction.

# 5. Appendices

## UNIVERSITY OF BEDFORDSHIRE

### Research Ethics Scrutiny (Annex to RS1 form)

**SECTION A To be completed by the candidate**

Registration No: 1418524

Candidate: Emma Bright.

Degree of: Master of Science by Research

Research Institute: Institute of Biomedical and Environmental Science and Technology

Research Topic: The resilience of alternative community states driven by priority effects: a microcosm investigation.

External Funding: N/A

The candidate is required to summarise in the box below the ethical issues involved in the research proposal and how they will be addressed. In any proposal involving human participants the following should be provided:

- clear explanation of how informed consent will be obtained,
- how will confidentiality and anonymity be observed,
- how will the nature of the research, its purpose and the means of dissemination of the outcomes be communicated to participants,
- how personal data will be stored and secured
- if participants are being placed under any form of stress (physical or mental) identify what steps are being taken to minimise risk

If protocols are being used that have already received University Research Ethics Committee (UREC) ethical approval then please specify. Roles of any collaborating institutions should be clearly identified. Reference should be made to the appropriate professional body code of practice.

Community structure and function can be greatly affected by a new species invading an environment. Usually, resident species that establish first should become abundant and use resources with little restriction to hamper the establishment of later arriving species (Rillig , et al. 2015). The experiment will develop an experimental system using microcosms of only bacteria and ciliates (Tetrahymena, Colpidium and Pseudomonas fluorescens) to test Fukami et al's (2016) framework for priority effects and to then manipulate the environment (e.g. temperature) to test the mechanisms within the model.

The Home Office identifies that there are no ethical issues with the use of protists in research. Disposal of all protists, bacteria and other contaminated waste will involve autoclaving to destroy the organisms and avoid any health hazards. Data will be safely stored and backed up securely. Data will be communicated and presented honestly and professionally.

Answer the following question by deleting as appropriate:

1. Does the study involve vulnerable participants or those unable to give informed consent (e.g. children, people with learning disabilities, your own students)?
   ~~Yes~~ **No**

   If **YES**: Have/will Researchers be DBS checked?
   **Yes** **No**

2. Will the study require permission of a gatekeeper for access to participants (e.g. schools, self-help groups, residential homes)?
   ~~Yes~~ **No**

3. Will it be necessary for participants to be involved without consent (e.g. covert observation in non-public places)?
   ~~Yes~~ **No**

4. Will the study involve sensitive topics (e.g. sexual activity, substance abuse)?
   ~~Yes~~ **No**

5. Will blood or tissue samples be taken from participants?
   ~~Yes~~ **No**

6. Will the research involve intrusive interventions (e.g. drugs, hypnosis, physical exercise)? ~~Yes~~ **No**

7. Will financial or other inducements be offered to participants (except reasonable expenses)? ~~Yes~~ **No**

8. Will the research investigate any aspect of illegal activity?
   ~~Yes~~ **No**

9. Will participants be stressed beyond what is normal for them?
   ~~Yes~~ **No**

10. Will the study involve participants from the NHS (e.g. patients) or participants who fall under the requirements of the Mental Capacity Act 2005?
    ~~Yes~~* **No**

If you have answered yes to any of the above questions or if you consider that there are other significant ethical issues then details should be included in your summary above. If you have answered yes to Question 1 then a clear justification for the importance of the research must be provided.

*Please note if the answer to Question 10 is yes then the proposal should be submitted through **NHS research ethics approval procedures** to the appropriate **NRES**. The UREC should be informed of the outcome.

Checklist of documents which should be included:

| | |
|---|---|
| Project proposal (with details of methodology) & source of funding | |
| Documentation seeking informed consent (if appropriate) | |
| Information sheet for participants (if appropriate) | |
| Questionnaire (if appropriate) | |

(Tick as appropriate)

**Applicant declaration**
I understand that I cannot collect any data until the application referred to in this form has been approved by all relevant parties. I agree to carry out the research in the manner specified and comply with the statement of ethical requirements on page 1 of this form. If I make any changes to the approved method I will seek further ethical approval for any changes.

Signature of Applicant: ..... Date: 16th April 2018

Signature of Director of Studies: ..... Date: .....

*This form together with a copy of the research proposal should be submitted to the Research Institute Director for consideration by the Research Institute Ethics Committee/Panel*

**Note you cannot commence collection of research data until this form has been approved**

**SECTION B To be completed by the Research Institute Ethics Committee:**

Comments: The proposed research has no specific ethical issues to be addressed.

Approved

Signature Chair of Research Institute Ethics Committee:

Date: 20th April 2018

*This form should then be filed on the student's record*

If in the judgement of the committee there are significant ethical issues for which there is not agreed practice then further ethical consideration is required before approval can be given and the proposal with the committees comments should be forwarded to the secretary of the UREC for consideration.

**There are significant ethical issues which require further guidance**

Signature Chair of Research Institute Ethics Committee:

Date:

*This form together with the recommendation and a copy of the research proposal should then be submitted to the University Research Ethics Committee*

Reviewed by Dr Tham Jangaran,
Dr Toni Osborne,
Prof Pranee S. Sreeniwasprasad

*October 2014*

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