**Investigating the Effects of Disease on Predator-prey Dynamics
in a Protozoan/Bacterial Model System**

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Abstract

The relatively new field of eco-epidemiology investigates how diseases spread in relation to fundamental ecological topics. Knowing how diseases affect predator-prey dynamics, an important topic in ecology, may have beneficial applications in both conservation biology and biocontrol. However, most work in this field up to the current date has been purely theoretical (especially involving mathematical modeling). Experimental and observational evidence may or may not actually support mathematical models in the real world. In this experiment, we created microcosm communities with the ciliated protist species *Tetrahymena pyriformis* (predator), the bacterial species *E. coli* (prey), and bacteriophage T4 (pathogen) in order to determine how a pathogen affects predator-prey dynamics at the community level in a laboratory setting. The results indicate that T4 populations are benefited by the presence of the predator *Tetrahymena*, which may be due to the emergence of *E. coli* strains resistant to T4. If this is the case, *Tetrahymena* could be both hurting T4 on an ecological scale while helping T4 on an evolutionary scale.

Introduction

The relationship between the densities of a predator and its prey is an important topic in ecology. The incorporation of disease into this predator-prey relationship is an important factor to be investigated in the relatively new field of eco-epidemiology, which includes aspects of both ecology and epidemiology, the study of how diseases spread (Auger et al. 2009). Predator-prey dynamics are fundamental to ecology because knowing how species interact in these roles can potentially help scientists to generate solutions to such extensive problems as controlling invasive species and conserving threatened or endangered populations. The role of disease in these relationships is also important when noting that pathogens are sometimes used in an attempt to control invasive species (such as the introduction of the *Myxoma* virus for the purpose of controlling rabbit populations in Australia) and that disease is sometimes the cause of a population's threatened or endangered state, such as in the case of the Iberian lynx, which is currently being threatened due partially to parasites that infect their kidneys (Berman 2006, Millan et al.2009).

Many studies have examined how diseases spread with variations of the classic "SIR" model (including populations of susceptible, infected, and recovered or resistant individuals) first proposed by Kermack and McKendrick (1927). Some of these studies have been devoted to the interactions between predator, prey, and pathogen or parasite, such as the models proposed by Auger et al. (2009), Xiao and Chen (2004), and Roy and Holt (2008), which combine the SIR model of infectious disease with the Lotka-Volterra model of predator-prey dynamics. However, most studies up to the current date on the effects of pathogens on predator-prey dynamics have

been theoretical or observational in nature, many involving mathematical modeling (e.g. Chattopadhyay et al. 2003; Braza 2005; Auger et al. 2009), while few have been experimental.

Though mathematical models are useful tools for predictions of population sizes under certain conditions and can be used to effectively predict how species interactions will affect compositions and population dynamics, many of these models are accepted based on faith that they are correct rather than being supported with experimental evidence. Experimental tests of these models could also disprove rather than support specific theoretical models, and thus could be beneficial to the field of population biology. Experimental studies could also be beneficial to this area of research in the sense that not all mathematical models are applicable to real-world situations, and especially not to all communities when vastly different species are involved. This experiment will aim to create experimental, ecological communities including predator, prey, and pathogen species in order to determine the patterns of population density over time through experimentation rather than through theoretical modeling.

A relatively simple way to create communities for ecological studies involves microcosms—small, controlled habitats containing bacterial species and sometimes protists; the proposed experiment aims to utilize such techniques to create a community for interactions between a predator, its prey, and a pathogen inflicting the prey. The first aim of this study is to perform a microcosm experiment using a protozoan/bacterial system (predator-prey), with the addition of a bacteriophage acting as a pathogen (affecting only the bacterial prey).These microcosms would be regarded as a model ecosystem to concretely display how the presence of a pathogen will affect the predator-prey dynamics. The second aim of this study is to determine if the species densities recorded over time conform to the expected outcomes of existing theoretical mathematical models of such systems, such as the models mentioned in Xiao and Chen (2004)

and Roy and Holt (2008) (which combines the SIR model of disease transmission with the Lotka-Volterra model of predator-prey dynamics), or if the results predict that a different pattern of population densities is followed, maybe due to a different mode of transmission in bacteria in liquid culture. The conditions of this experiment align with a model that assumes a specialist predator (since only one prey population will be present, forcing the predator to act as a specialist) and that also assumes the predator is not affected by ingesting infected prey (as bacteriophages do not infect protists). An advantage to using microcosm communities for this research is that these communities can easily be created and manipulated, and the population densities can be estimated with sampling methods.

Knowing how the presence of a pathogen affects the population densities of a predator and its prey will be useful to ecologists and conservation biologists trying to solve such problems as conserving threatened species (such as the lynx) and controlling invasive species (such as the rabbit). In the case of controlling an invasive species, possible combinations of increasing natural predator abundance and introducing a pathogen could be attempted once the knowledge of how the interactions of these species will affect the prey population size is auditable. While mathematical models are currently being used to make these predictions before action is taken, these models may not be applicable to each particular system or may not be accurate in their predictions; this is where experimental evidence might be beneficial to this field. This experiment aims to explore how the population sizes of predator and prey species are affected by a pathogen infecting the prey in a model system in the hopes that the model system accurately represents at least some large-scale predator-prey systems that are too difficult to experiment with directly.

I. Materials and Methods

II.1 Species and Model System

This experiment aimed to create microcosm communities that would represent an isolated predator-prey-pathogen system with populations that could be estimated and tracked in the laboratory. The model system used in this experiment was composed of the ciliated protist species *Tetrahymena pyriformis* as the predator, the bacteria *Escherichia coli* (ATCC) as the prey, and bacteriophage T4 (ATCC) as the pathogen. The bacterial and T4 stock cultures were made using LB medium (Difco) and were incubated at 37.0°C to obtain optimal growth conditions. The *Tetrahymena* stock culture, which had been grown up on peptone (a bacteria-free medium treated with antibiotics to ensure that no other bacteria were introduced into the system), was kept at 23.0°C.

II.2 Microcosms and Chemostat Set-up

The microcosms used in this experiment were 250-mL Pyrex glass jars, each containing 100 mL of sterile protozoan pellet medium (abbreviated PPM) filtered twice. The microcosms were set up for a chemostat experiment, meaning that thin glass tubes were added to the rubber stoppers in order to add fresh medium and remove waste medium via a pump, reducing the risk of contamination. The stoppers were covered with Parafilm in order to stop air flow from occurring with the outside atmosphere. A pump (Bioreactor) was connected to a carboy containing 10 liters of sterile PPM and to the 12 jars by rubber tubing and needles which were stuck into each individual jar. Each microcosm also contained a filter. The samples were taken from the medium being removed from the system on its way to the waste beaker, and another beaker was present between the flask of fresh medium and the tubes in order to observe for

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possible outside bacterial contamination of the medium. At the beginning of the experiment, *E.coli* was introduced to all 12 jars in a clean bench workspace ~14 hours before *Tetrahymena* and T4 were introduced to their respective jars so that the *E.coli* population could establish itself before being attacked by both either a predator, pathogen, or both. The dilution rate—the rate at which the medium was replaced—was 0.05 turnover per hour, meaning that 5% of the medium in each jar was replaced each hour that the chemostat ran; thus, the entire volume of medium in each jar was replaced every 20 hours. The sterile medium source was changed to replenish the fresh medium four times throughout the course of the experiment.

Twelve chemostat microcosms were set up for this experiment—four treatment groups (see Table 1) with three replicates in each treatment group. Community #1, the experimental group, contains all three species in order to observe the effects that a bacteriophage has on the predator-prey dynamics between a protist and its bacterial prey. Community #2 does not include the bacteriophage in order to observe how the predator and prey populations change over time without a pathogen in this *Tetrahymena*/*E. coli* system (this is a control for the pathogen presence in community #3). The *E. coli* and bacteriophage system is present to observe how only the phage affects the bacterial population densities in the absence of any predators. In commnity #4, the *E. coli* monoculture is present as a control for the effects of the bacteriophage presence on the bacteria.

II.3 Data Collection

To obtain a sample from the chemostat, a small volume of medium (approximately 0.5 mL) was allowed to flow into a small flask connected by rubber tubing to the microcosm (one flask was connected to each jar) before the medium reached the final waste beaker. From these samples, the bacterial densities were estimated by plating the liquid medium onto LB agar plates

at five different dilution factors (10^{-3}-10^{-7}). The plates were incubated at 35ºC for 24 hours before the colonies were counted. The T4 densities were estimated by mixing 100µL of the serially diluted samples with 300µL of a host *E. coli* population and 4.5 mL of top agar, which consisted of LB agar mixed to half the standard concentration. This mixture was poured onto an LB plate and incubated at 35ºC for 24 hours, at which point the "plaque-forming units," or "holes" in lawns of bacterial growth on the plate were counted to estimate the number of phages present in the sample. The *Tetrahymena* population was estimated by pipetting about 0.3 mL of medium to be observed under a stereomicroscope. The number of individuals present in that sample was divided by the volume of sample medium counted to estimate the number of individuals present per mL of medium. Population densities were sampled every day for a period of 14 days.

Community Number	Species Composition
1	*Tetrahymena, E. coli, T4*
2	*Tetrahymena, E. coli* (no phage)
3	*E. coli, T4*
4	*E. coli*

Table 1: A list of the species compositions to be set up in microcosm communities: Community #1 is the experimental group of all three species. Community #2 does not contain the bacteriophage as a control of the predator-prey dynamics. Community #3 contains only infected bacteria in order to see how the bacterial population itself is affected by only pathogen presence, and a monoculture of bacteria is present as a control for these effects (Community #4).

II. Results

The results from the experiment are shown as graphs of species densities over time, with species densities shown on a logarithmic scale. *E. coli* seems to approach a stable carrying capacity in replicates 3, 4, 5, and 6, while showing some oscillations in replicates 2, 8, 10, 11, and 12. *Tetrahymena* shows some oscillating population cycles in all microcosms in which it is contained (replicates 8, 10-12). The T4 patterns are more difficult to determine due to difficulties with accurate data collection. Replicates 1, 7, and 9 could not be sampled for the entire course of the experiment due to complications with the rubber tubing connecting the pump to the jars and complications with the filters; the results from these replicates are thus not shown.
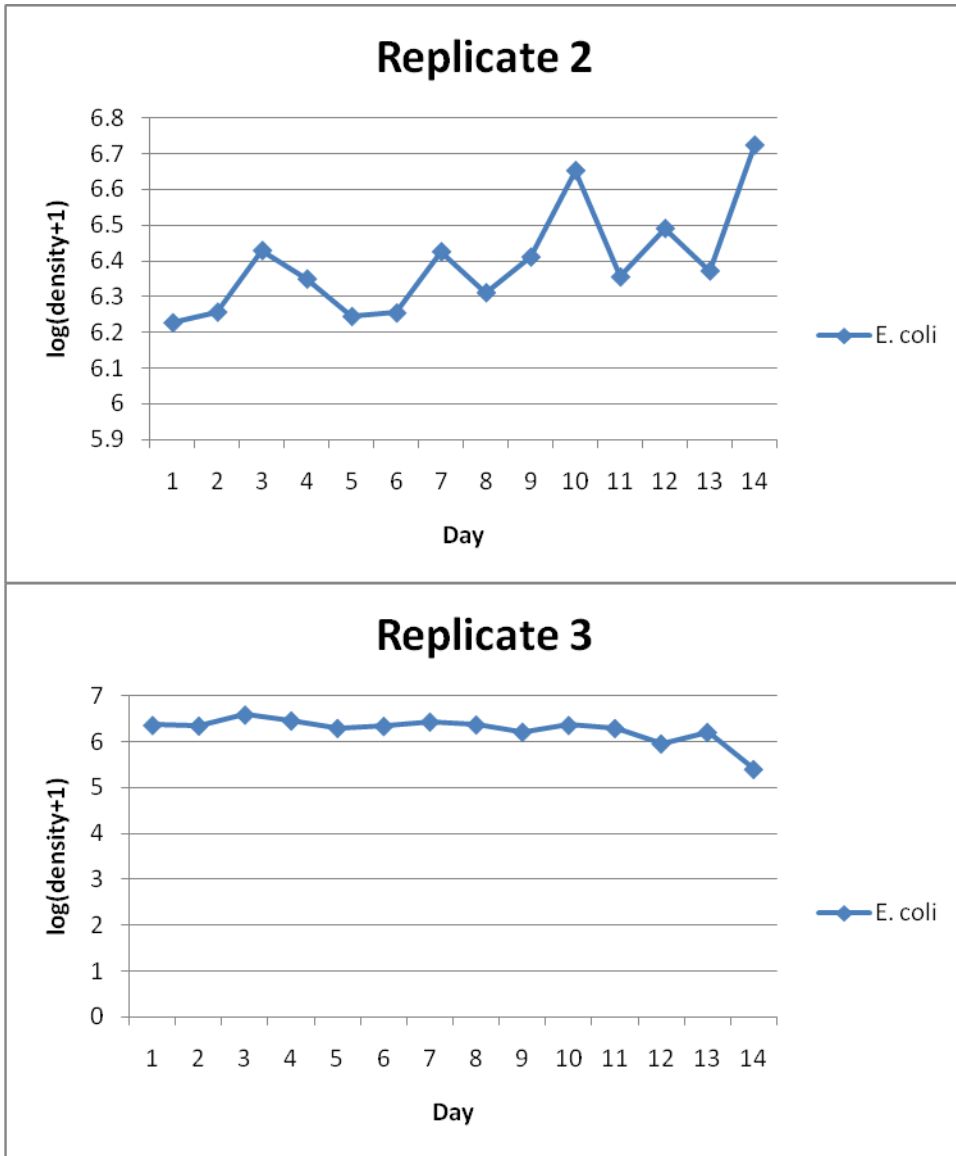
Figure 1: Replicates 2 and 3 show the population patterns of *E. coli* in the the isolated prey condition. Replicate 1 was lost due to problems with the chemostat tubing.
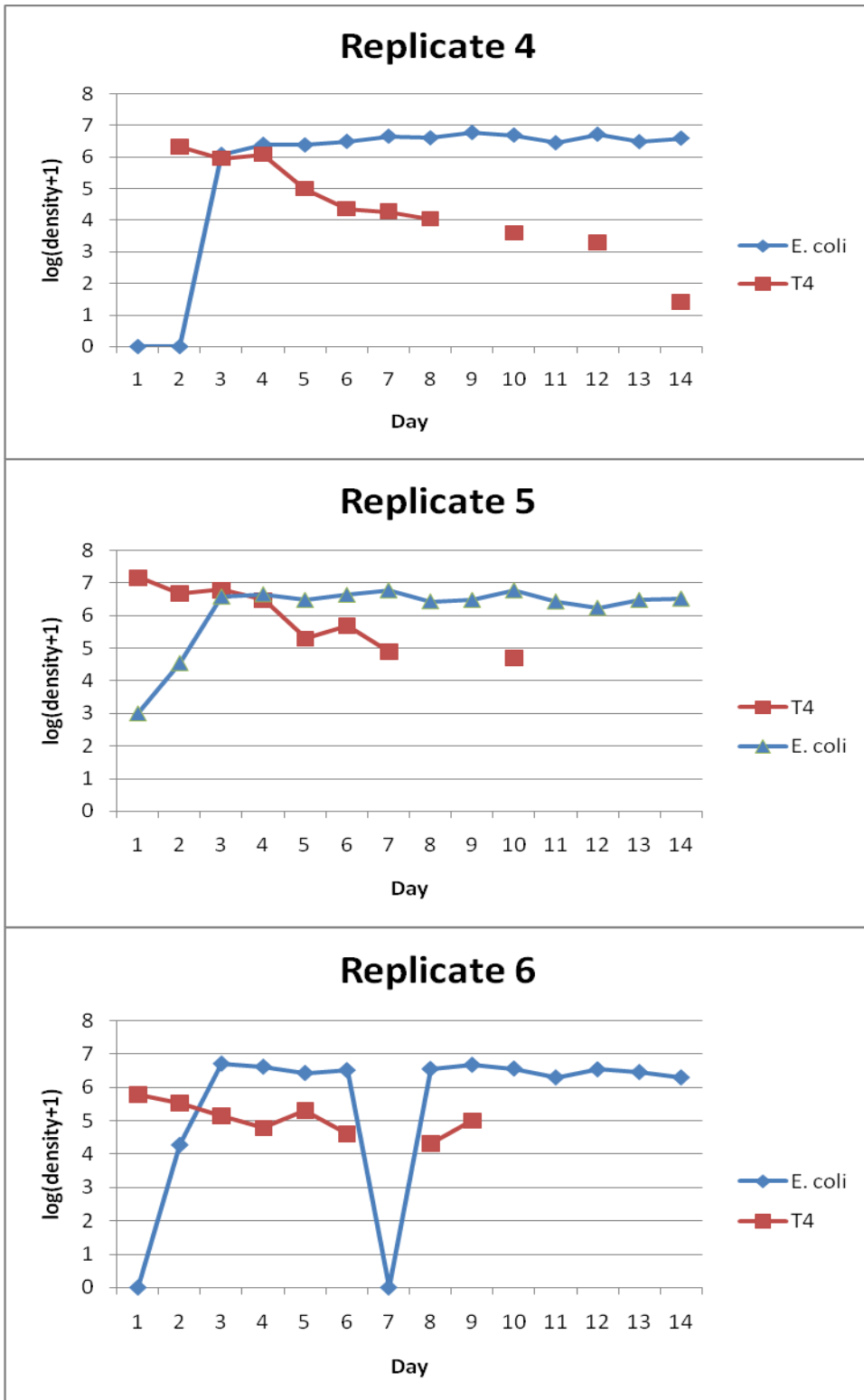
Figure 2: Replicates 4-6 show the population dynamics of *E. coli* and T4 in the prey-pathogen system. The dates missing data reflect days on which sampling could not be completed due to complications with agar. The uncharacteristic *E. coli* population in Replicate 6 most likely represent sampling error.
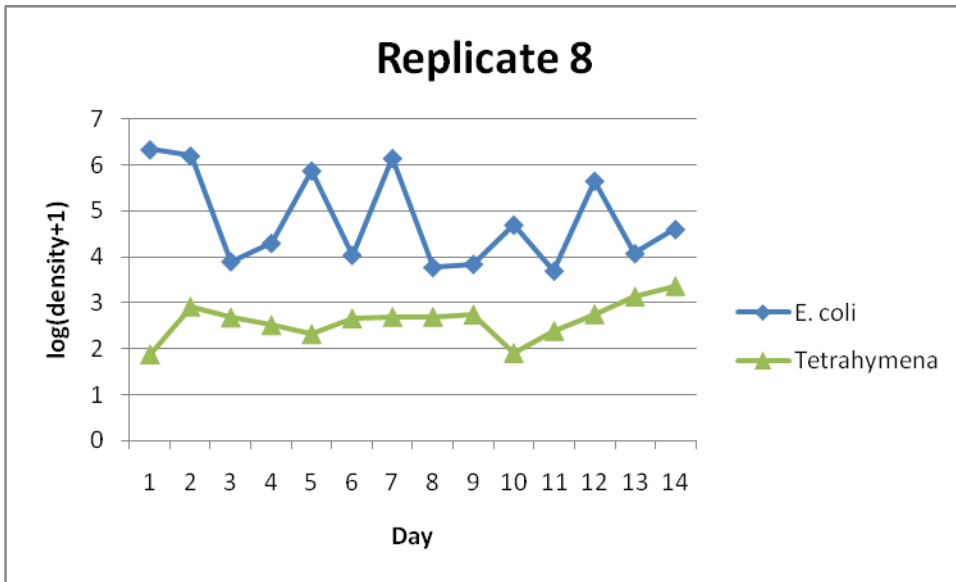
Figure 3: Replicate 8 shows population dynamics of *E. coli* and *Tetrahymena* in the predator-prey system. Replicates 7 and 9 were lost due to problems with the chemostat tubing.
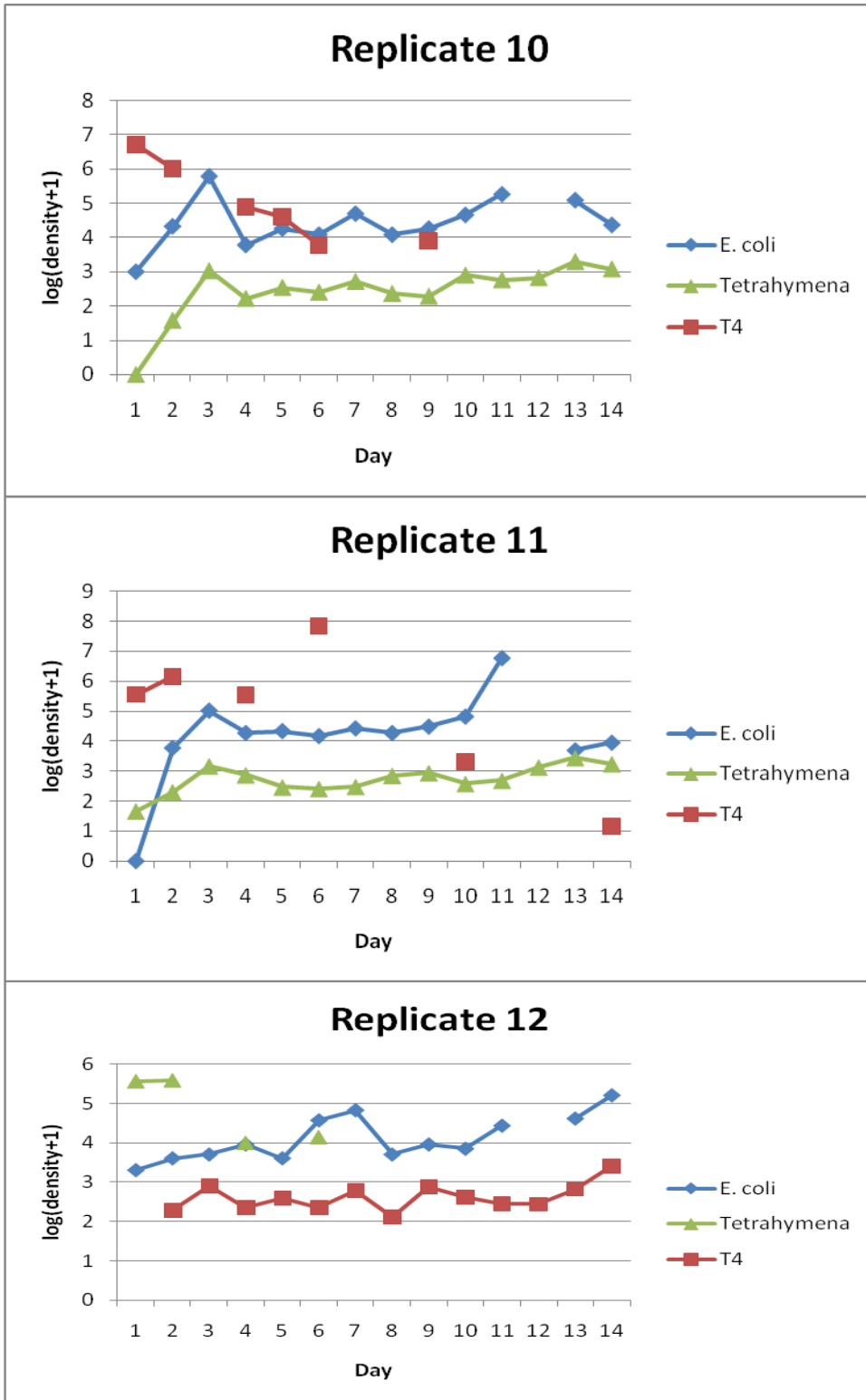
Figure 4: Replicates 10-12 show the population dynamics obtained in the predator-prey-pathogen condition. The dates which are missing data reflect days on which sampling could not be completed due to complications with agar.

III. Discussion and Conclusions

Although the population dynamics in the microcosms are not completely clear, some trends can be ascertained from the figures. In the microcosms containing only *E. coli* and T4 (replicates 4-6), *E. coli* seems to reach the same carrying capacities as those reached in the *E. coli* only treatments (replicates 2-3), indicating that T4 is not having a very great effect on the *E. coli* populations. This could be an indication that the *E. coli* populations in replicates 4-6 are evolving resistance to T4, causing T4's [apparent] approach to extinction. The longer survival of T4 in the microcosms containing all three species (replicates 10-12) may be an indication that *Tetrahymena*, the predator, may be aiding T4's survival by keeping the *E. coli* populations low, and thus the mutation rate low. A low mutation rate could decrease the likelihood of the emergence of resistance to T4 in the *E. coli* populations, helping T4 to survive in the ecosystem for a longer time period than in the microcosms without *Tetrahymena*. This phenomenon may contradict Roy and Holt's (2008) prediction that when predator density approaches a certain threshold, the pathogen population will approach extinction (however, the exact value for this kind of threshold is unknown for *Tetrahymena* in this system). It is also possible that the dilution rate (0.05 turnover/hour) may have been too high, causing T4 particles to be flushed out of the system too rapidly. The oscillation seen in the control population (isolated *E. coli*) in replicate 2 may be due to variations in the time of day that samples were taken or may be due to sampling errors.

Several difficulties with data collection occurred throughout the experiment that can be addressed in future works. As previously mentioned, three replicates were lost (replicates 1, 7, and 9) due to problems with the rubber tubing not delivering adequate medium to the

microcosms as well as complications with filters. This is especially problematic because these problems eliminated two replicates from the same treatment group, leaving only one replicate left in the group with only *E. coli* and *Tetrahymena* (replicate 8); it is impossible to draw concrete conclusions from only one replicate of the community. Also, the data collection of T4 is very inconsistent and sparse because the top agar did not consistently solidify in all replicates; the agar usually only solidified in one or two of the serially diluted samples, which is difficult to explain because the exact same procedure was employed for every sample. (The plates that did solidify correctly were often the 10^{-6} and 10^{-7} serially diluted replicates, which showed zero phage growth and therefore were not useful in ascertaining accurate population estimates). With so few data points, it is difficult to extrapolate the trends in T4 population densities with certainty, so the trends can only be estimated.

The *Tetrahymena*/*E. coli*/T4 system is a special system for studying the effects of a pathogen in that there is no acquired resistance to T4 (the phage lyses all infected cells instead of any recovery at all). Also, the short generation time of *E. coli* most likely allows mutations to accumulate in the population, which leads to increased resistance to T4 in the *E. coli* populations. This could mean that the presence of *Tetrahymena* is suppressing T4 populations in an ecological time scale (by depleting the *E. coli* population, thereby making it more difficult for T4 particles to find bacterial hosts) while at the same time helping T4 in an evolutionary time scale (by keeping the *E. coli* mutation rates low enough to reduce the frequency of resistance from evolving). The predator in this system therefore has the potential to impose both detrimental and beneficial effects on the pathogen population.

Possible future work includes repeating this experiment with different dilution rates (a lower dilution rate may keep T4 from approaching extinction as quickly) and possibly with a different

method of estimating T4 densities. These results, if they show clearer population trends, could then be compared with more existing mathematical models to see whether the trends are consistent or not. The experiment could also be repeated with a different model system, possibly one with a pathogen that can confer acquired immunity. A model system with a pathogen that could infect the predator or both predator and prey could also be studied to see what differences arise from these two variations on the system.

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