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Density Dependent Growth of Pseudomonas fluorescens in the Presence and Absence of C. elegans Predation in Liquid Media

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DENSITY DEPENDENT GROWTH OF *PSEUDOMONAS FLUORESCENS* IN THE PRESENCE AND ABSENCE OF *C. ELEGANS* PREDATION IN LIQUID MEDIA

By

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DEDICATION

This thesis is dedicated to my parents, Roger and LeeAnn, and to my brothers, Cole and Luke. Thank you for the endless encouragement and support.

ACKNOWLEDGEMENTS

Thank you to my research advisor, Dr. Peter Zee, for his guidance and support throughout this project. I also want to thank Tyler Bacon, Alex England, and Elizabeth Whitaker for lending a helping hand throughout and making each day in the lab enjoyable.

ABSTRACT

The purpose of this study was to understand the predator-prey relationship between *C. elegans* worms and *P. fluorescens* bacteria in liquid media. We were aiming to create a predation model of the relationship between these two species.

This study was performed through three experiments. Using 96-well plates, we were able to measure the change in optical density (OD) of these wells and calculate the growth rate. This growth rate was then graphed and analyzed. The first experiment contained *P. fluorescens* and KB liquid media, this study demonstrated that the bacterial population has a carrying capacity. We were also able to use this study as a baseline metric of bacterial growth in this environment. The second experiment measured the growth of *P. fluorescens* in the presence of a predator, *C. elegans*. Varying initial densities of P. fluorescens were grown in wells with a standard amount of *C. elegans*. The third and final experiment was similar, but instead had varying initial densities of *C. elegans* with standard *P. fluorescens* in each well.

Overall, we found that *P. fluorescens* had a carrying capacity in their wells, and that the presence of *C. elegans* effectively lowered the growth rate of the bacteria. Because of inconsistencies in *C. elegans* population density after the bleach synchronization process and contamination during the process, we were unable to parameterize the Lotka-Volterra predation model with this data. Further studies and troubleshooting would be required to finish this experiment.

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LIST OF ABBREVIATIONS

KB	King's B (liquid media)
SDP	Super dense population or super dense worm treatment
OD	Optical density
NGM	Nematode growth media

Introduction

Predation can be quantified between two populations to better understand and predict outcomes. These paired equations of the Lotka-Volterra predator-prey model allow us to see how populations change over time between the predators and the victims.

$$\frac{dN}{dt} = rN - aNP \qquad \frac{dP}{dt} = baNP - mP$$

Victims *Predators*

As the victim population changes over time, $\frac{dN}{dt}$, we can see that it is influenced by several variables. The instantaneous rate of increase, *r*, can be calculated by subtracting the deaths from the births within a given population (Gotelli 4-5). It essentially acts to tell us how a population is growing (or declining) in any given moment. *N* is the number of individuals in a population. *P* is the number of predators. The capture efficiency, *a*, is the effect of a predator on the victim population. As the capture efficiency become larger, we see a bigger decrease on the victim's per capita growth rate with the addition of a single predator (Gotelli 126-127). Some of these variables are included in the predator equation. $\frac{dP}{dt}$ is the output that gives how rapidly the predator population changes over time. Conversion efficiency, *b*, is the measure of how much the predators increase their per capita growth rate for each individual victim. Conversion efficiency is expected to be higher when the victim is of more value to the predator, such as a moose to a wolf (Gotelli 128). The death rate of the predators, *m*, is the only contributor to their population decline.

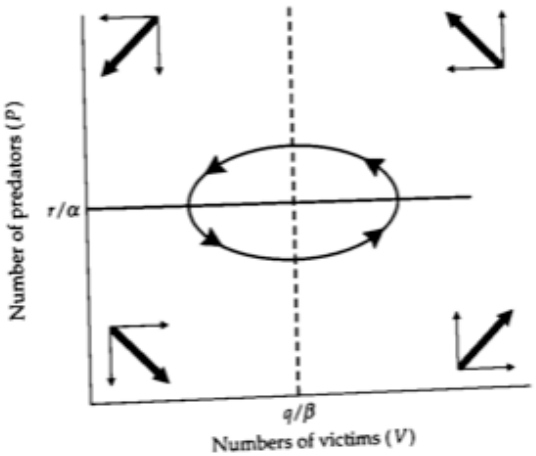
If we take the two formulas above as a system of equations, we can solve them to see when their populations stop growing or declining (i.e., reach equilibrium).

$$\hat{P} = \frac{r}{a} \qquad\qquad \widehat{N} = \frac{m}{ba}$$

Victims Equilibrium Eq. *Predators Equilibrium Eq.*

It may seem backwards that the victim equilibrium is in terms of *P*. In essence, there is a fixed number of predators, $\hat{P}$, that can stop the victim population from growing or declining. This value is then directly proportional to the instantaneous rate of increase, *r*, and inversely proportional to the capture efficiency, *a*. Similarly, the equilibrium of the predatory population is dictated by only by a fixed number of victims, $\widehat{N}$. The number of victims required to maintain equilibrium in the predator population increases when the death rate of the predators, *m*, increases. Likewise, $\widehat{N}$ decreases when the conversion efficiency, *b*, or the capture efficiency, *a*, increases (Gotelli 129).

We can visualize these equilibria of the Lotka-Volterra predator-prey model as shown in Figure 1.

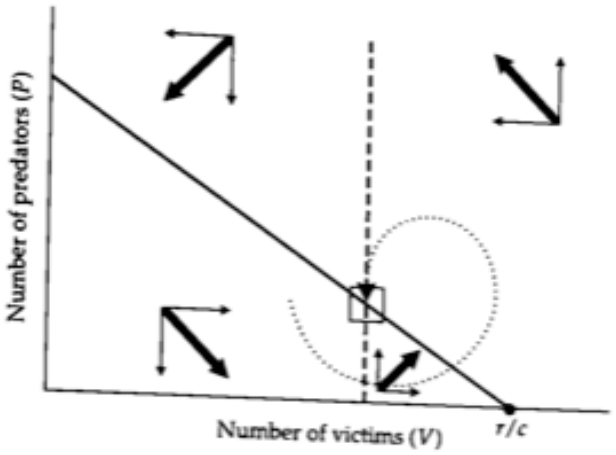


Lotka-Volterra Predation Model

Figure 1. The predator and prey isoclines are represented by horizontal and vertical lines, respectively. The ellipse in the middle shows that the populations will cycle indefinitely if no factors affect their population growth, apart from the number of individuals in each population (Gotelli 132).

This model shows the expected dynamics of a predator-prey system with known parameters. The x- and y-axes are population sizes of victims and predators, respectively. The isoclines in the middle denote the population equilibrium values described above. The vectors represent where we expect the system of both species populations to move depending on what region the point is. In the top left quadrant, there are a lot of predators, but not enough prey to sustain them. This results in a decline in predators, moving us into the bottom left quadrant. The ellipse in the middle represents how these two populations would never stabilize, because the increase of decrease of one will cause a change in the other and vice versa. However, if the populations are precisely where the isoclines intersect, the two will stay at that point. If one population is so low that the intersection is close to an axis, that population will be unable to recover (Gotelli 131-133). This exact model has assumptions that do not match the biological specifics of our experimental system.

As we do not have the potential for density-independent population growth of prey in this experiment due to limited resources and space, it is important to introduce a carrying capacity for the prey population (i.e., density dependent growth of the victim), resulting in the modified prey isocline as seen in Figure 2.



Lotka-Volterra Model with Victim Carrying Capacity

Figure 2. The prey isocline has a negative slope now that the carrying capacity is incorporated. This signifies that as the prey population gets larger, it has a harder time sustaining itself. This can be due to overcrowding, lack of resources, etc. The dotted line shows that the populations will stabilize around the intersection of the isoclines (Gotelli 135).

It is expected that this model will be a better representation of the populations we are using. Seeing that *P. fluorescens* will only have a finite volume to grow in, with a limited amount of resources. It is impossible for this population to demonstrate long-term exponential growth, and it is unlikely that the populations would cycle indefinitely. Instead, it is more appropriate to anticipate that they will show damped oscillations into a stable equilibrium, as seen in the model above (Fig. 2) (Gotelli 135).

In this study, I measured the population growth rate of *P. fluorescens* in the presence of *C. elegans* predators. The predator-prey interaction was measured in liquid media, as opposed to the solid media typically used for *C. elegans* studies. Using 96-well microtiter plates and an optical density plate reader, I was able to measure the OD of *P. fluorescens* over a 24-hour period, as the populations grew. This established a base growth rate for several initial population sizes of *P. fluorescens* in King's B (KB) liquid media. The next two experiments included standardizing initial population sizes of *P. fluorescens* and *C. elegans* across all wells, respectively. This was done to better understand the effects of the presence of *C. elegans* on the

growth rate of *P. fluorescens*. With these results, the goal is to estimate parameter values for the key parameters of a Lotka-Volterra competition model. More specifically, this experiment has the potential to tell us whether a particular density of predators is able to cause a negative population growth rate in the prey (i.e., on the right side of the prey isocline in Figure 2). The purpose of this experiment was to better understand predator-prey relationships between *C. elegans* and *P. fluorescens* in liquid media. In essence, I was able to determine the growth rate of *P. fluorescens* in KB liquid media. But the question I wanted to answer was, how many *C. elegans* worms would need to be added before this growth rate was noticeably changed? And what would this change look like?

Materials and Methods

The general experimental method used in this study was to measure *P. fluorescens* population growth rate in the absence of predators, and also in a range of predator densities. The population growth rate of *P. fluorescens* was measured at varying starting population sizes. The predator for this experiment was *C. elegans* (wild type lab strain N2). We measured predation in liquid media (KB modified with M9 buffer) in 96-well microtiter plates. The growth metric was optical density (OD) measured at an absorbance of 600 nm. These readings were taken at three intervals within 48 hours, and then used as estimates of population size for calculating growth rate (see below). From these we were able to make inferences on the impact that *C. elegans* pose on P. *fluorescens* population growth.

Prey: *Pseudomonas fluorescens* (Strain SBW25)

SBW25 is a strain of *P. fluorescens* that has been studied extensively in regard to its aiding in plant growth (Trippe, Kristin, et al., 2013). However, there is a lack of literature as to how this species reacts in the presence of a predator without time for it to evolve. This strain was chosen because it was readily available in the lab. To the best of our knowledge, a density dependent predation study had never been done between this bacterium and *C. elegans*.

Predator: *Caenorhabditis elegans* (Wild type lab strain N2)

C. elegans is a soil-dwelling, free-living nematode that feeds on bacteria and other microbes. The worm grows to about 1 mm in length. It has been extensively studied since it is

one of the smallest organisms that still provide anatomical complexities, such as a nervous system (*What is C. elegans?*, 2021). Bacterial intake by *C. elegans* can measured by change in optical density at 600nm, which is the metric that this study used (Gomez-Amaro, Rafael L, et al., 2015). Knowing that *C. elegans* predate on bacteria and that there are several treatments available to manipulate their populations, it was a suitable predator for this experiment.

Media Recipes: M9 and King's B (liquid)

M9 buffer consists of KH_2PO_4, Na_2HPO_4, NaCl, H_2O (DI), and $MgSO_4$. This buffer is then autoclaved at 121ºC for 20 minutes (M9 Buffer for Worms, 1970). King's B liquid media is made from proteose peptone, glycerol, K_2HPO_4, H_2O (DI), and $MgSO_4 \bullet 7H_2O$. This media was then autoclaved for 45 minutes at 121ºC (Allison, 2008).

Bleach Synchronization of *C. elegans*

C. elegans (N2) are a species of nematode that are grown on nematode growth media (NGM). These plates are first seeded with *E. coli* (OP50), which the worms use for nourishment. As the *C. elegans* mature, they lay eggs on the plate which later hatch into juvenile worms. The bleach synchronization process produces a population of *C. elegans* that are the same age. It does so by removing *E. coli* (OP50) and *C. elegans* that have already hatched. Having a culture that solely consists of eggs allows us to remove confounding variables that might happen from having worms at different stages of maturity. For example, if gravid worms were added to the plate, they could have offspring and a burst of new worm individuals would artificially increase the effect of predation. Removing the *E. coli* also removes the confounding variable of two bacterial populations in the experiment.

This process kills all worms on the plate, while leaving laid eggs unharmed. First, a population of *C. elegans* (strain N2) were grown on an NGM (nematode growth media) plate that was initially inoculated ('seeded') with *E. coli* (strain OP50). Plates were washed with 3-4 mL of M9 buffer. A sterile pipette tip was gently scraped over the surface to free any stuck eggs and increase the yield. The plate was then tipped to the side in order to pool the M9, eggs, and worms to be pipetted in 1mL increments into 1 mL centrifuge tubes. After checking to ensure there were no eggs stuck to the plate, these tubes were then centrifuged at 3000 rpm for 30 seconds. The supernatant was removed from these tubes. If this supernatant was not clear, then 1 mL of M9 was put in, and the tube was centrifuged again. This part of the process is repeated until the supernatant is clear, which indicates a significant reduction in bacteria cells. Next, 200 µL of 4% bleach solution was added to each tube, and then these tubes were vortexed for approximately 30 seconds. 800 µL of M9 buffer were then added to help neutralized the bleach. These tubes were centrifuged at 3000 rpm for 30 seconds. After this, the supernatant is removed from each tube, and 1 mL of M9 was added. The tubes are centrifuged again, the supernatants are removed, and fresh M9 is added to each tube. This "washing" process is repeated 3 times at this part of the process. This serves to wash out any residual bleach, so the larval *C. elegans* are not killed after hatching from eggs. The tubes are then treated with 20 µL of gentamicin (and later kanamycin, see below). This antibiotic treatment serves to prevent growth of *E. coli* within the tubes. These tubes are then placed on a rotator for 48 hours, and result in a population of *C. elegans* that are synchronized in the L4 larval stage life stage. The L4 stage of the *C. elegans* lifecycle is the final larval stage before the worm molts and enters adulthood (*Introduction to …*, 10-14).

Experiment 1: Variable starting population sizes without predation

P. fluorescens was grown in KB liquid media in a 96-well microtiter plate. OD was measured at hour 0 and hour 24. *P. fluorescens* (strain SBW25) was inoculated from a freezer stock into 6 mL of KB liquid media in a shaking incubator at 150 rpm and 28ºC and allowed to grow for 24 hours.

Samples of this culture were then pipetted into 11 different "columns" of the plate, each column having 5 replicates. The first column contained 0 µL of the *P. fluorescens* culture, and 200 µL of KB liquid media. The second column contained 20 µL of the *P. fluorescens* culture and 180 µL of the KB liquid media. This pattern continued in 20 µL increments until the 11th column, which consisted of 200 µL of *P. fluorescens* and 0 µL of KB liquid media. This resulted in 5 replicates of 10 different initial population densities and 5 control replicates. All wells had a total volume of 200 µL. The plate was kept in static conditions at 20 ºC in an incubator, and only removed in order to measure the OD of the wells. This data was then analyzed using Excel and RStudio.

Experiment 2: Variable starting *P. fluorescens* densities with predators

A culture of *P. fluorescens* was prepared and grown in the same conditions and manner as Experiment 1. A petri dish of *C. elegans* was chunked onto a new NGM plate that was seeded with *E. coli* (OP50) the day before. These worms were then allowed 3-5 days to mature and lay eggs, until many unhatched eggs were clearly visible on the plate. Three of these plates of worms were then bleach synchronized (see Method above), and the unhatched eggs were placed on a rotator for 48 hours. After rotating for 48 hours, ten drops of 100 µL of hatched worm solution

were placed under a microscope to find the average number of juvenile worms per 100 µL. An average of 22 *C. elegans* per 100 µL were observed.

This experiment used a 96-well microtiter plate, and the changes in OD were measured as a proxy for population size changes. 11 columns were used, and each dilution had three replicates. 100 µL of *C. elegans* was standard across all wells. The first column contained 0 µL of *P. fluorescens*, and 100 µL of KB liquid media. Each subsequent column contained 10 more µL of *P. fluorescens*, and 10 less µL of KB liquid media. The final column contained 100 µL of *P. fluorescens*, and 0 µL of KB liquid media. The resulting plate had variable initial densities of *P. fluorescens* and a constant number (~22) of juvenile *C. elegans*. The OD was taken at hour 0, hour 24, and hour 48. The plate was kept in static conditions at 20 ºC in an incubator, and only removed in order to measure the OD of the wells. These values were recorded and then analyzed with Excel and RStudio.

Experiment 3: Constant initial *P. fluorescens* population size and variable number of worm predators

C. elegans and *P. fluorescens* were prepared and grown in the same manner as the prior experiments, with one exception. We had reason to suspect contamination (see Results for Experiment 2 below) with our standard gentamicin treatment during bleach synchronization, so we used kanamycin, another commonly used antibiotic. This experiment had an average of 2.5 *C. elegans* per 100 µL. Four columns and three rows were used in a 96-well microtiter plate for this experiment. Each well contained a standard 50 µL of *P. fluorescens* and 50 µL of KB liquid media. The first column contained 100 µL of M9 buffer solution (i.e., no worms). The second

column contained 10 µL of *C. elegans* worms, and 90 µL of M9 buffer solution (~0.25 worms). The third column contained 100 µL of *C. elegans* worms (~2.5 worm). The final column contained a dense population of worms (termed 'super dense population' or SDP). This was prepared by centrifuging bleach synchronized worms at 1000 RPM for 30 seconds. Once a pellet of worms had formed at the bottom of the centrifuge tube, 50 µL was pipetted to gather the entirety of the pellet. This 50 µL, and 50 µL from a second, similarly treated centrifuge tube were placed in each well. This created a 'super dense population' (SDP) of worms. The OD was taken at hour 0, hour 24, and hour 48. The plate was kept in static conditions at 20 ºC in an incubator, and only removed in order to measure the OD of the wells. These values were recorded and then analyzed with Excel and RStudio.

Results

At the conclusion of Experiment 1, we were able to approximate the per capita growth rate of *P. fluorescens* using this formula as:

$$r = \ln\left(\frac{OD_{24}}{OD_0}\right)$$

This can be derived from the exponential growth equation. The value, *r*, is the instantaneous per capita growth rate, and the subscripts of the OD values correspond to the hour at which they were measured.

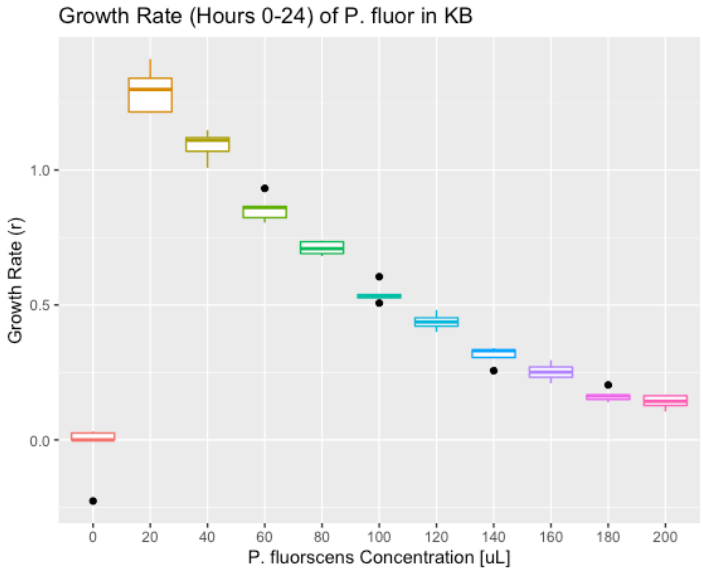


Figure 3. Growth rate of *P. fluorescens* in the absence of predators over 24 hours.

Figure 3 shows that the highest growth rate ($r = 1.295$) occurred when the wells contained 20 µL of *P. fluorescens* and 180 µL of KB liquid media. The lowest growth rate occurred in the control group and was approximately zero. The second lowest ($r = 0.141$) occurred in the column that contained 200 µL of *P. fluorescens* and 0 µL of KB liquid media.

This suggests that per capita growth rate declines with increasing density, an expectation of density-dependent population growth.

The second experiment used the formula listed above for growth rates within one day intervals (hours 0-24 and 24-48). To calculate the growth rate from hour 0 to hour 48, the above equation is weighted by (1/2) so that the time-scale is comparable across experiments, and is used for the growth rate estimates shown in Figure 4:

$$r = \frac{1}{2}\left(\ln\left(\frac{OD_{48}}{OD_0}\right)\right).$$

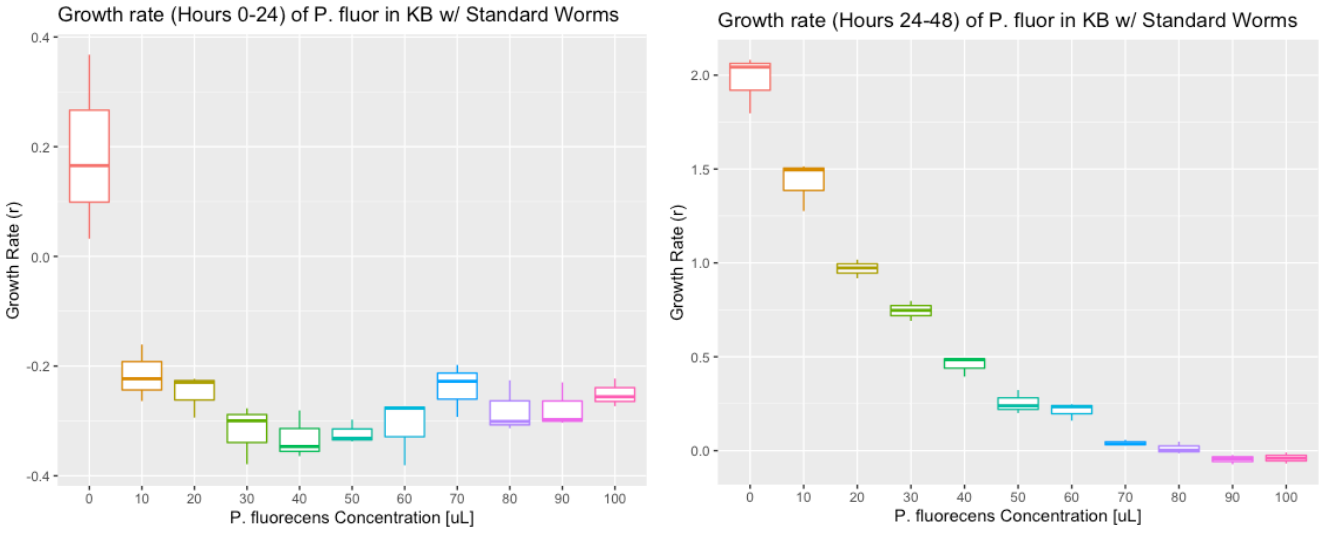


Figure 4 (Left). Growth rate of varying starting densities of *P. fluorescens* with 100 µL of *C. elegans* from hour 0 - 24. **Figure 5 (Right)**. Growth rate of varying starting densities of *P. fluorescens* with 100 µL of *C. elegans* from hour 24-48.

Within the first day, we can see that the presence of any bacteria in the wells decreased the growth rate. The only group that demonstrated growth within the first 24 hours was the control group, which only contained *C. elegans*. All other groups had lower OD's after 24 hours than their initial measurement (Figure 4). The second day shows growth rate decreases with initial *P. fluorescens* concentration in a cascading manner (Figure 5). As a larger initial *P. fluorescens* populations were present, the subsequent growth rate from hours 24-48 decreased as in Experiment 1.

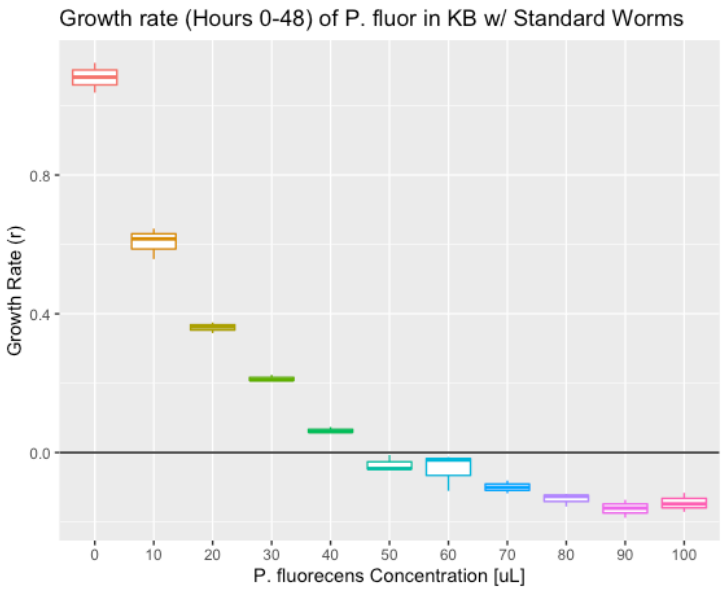


Figure 6. Growth rate of varying starting densities of *P. fluorescens* with 100 µL of *C. elegans* over 48 hours.

Looking from hour 0 to hour 48, it can be seen that the growth rate trends in a decreasing manner as greater amounts of *P. fluorescens* were added initially. The highest growth rate seen over the two-day span was in the control group, in which no bacteria were present (see below for Discussion of contamination). As the initial concentration of *P. fluorescens* was 50 µL or greater, the OD decreased, indicating a decrease in *P. fluorescens* population size.

Growth rate were estimated for Experiment 3 with the same methods as Experiment 2.

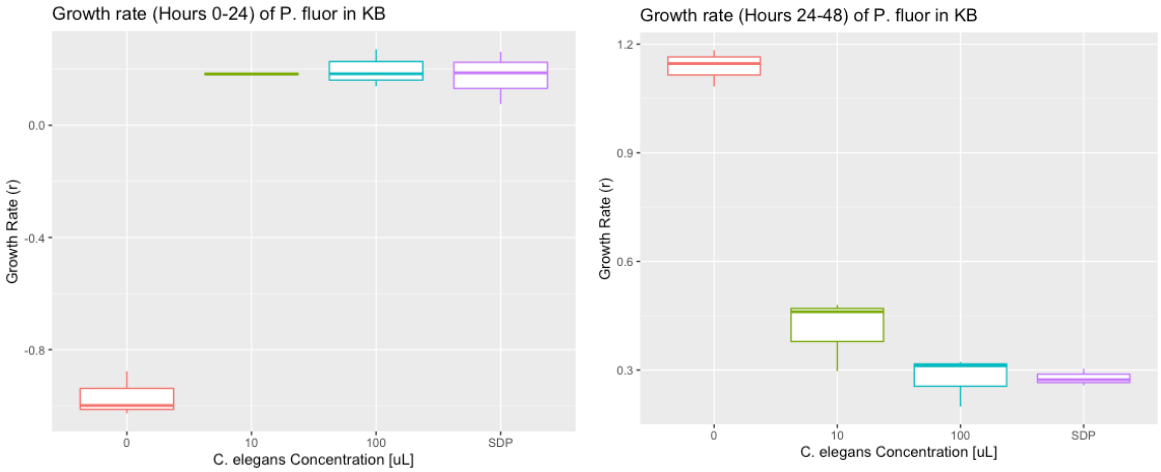


Figure 7 (Left). Growth rate of varying starting densities of *C. elegans* with 50 µL of *P. fluorescens* and 50 µL of KB liquid media from hour 0 - 24. **Figure 8 (right)**. Growth rate of varying starting densities of *C. elegans* with 50 µL of *P. fluorescens* and 50 µL of KB liquid media from hour 24 - 48.

In the first 24 hours, the growth rate was negative ($r = -0.968$) for the control group. The other groups all showed growth rates that were similar to one another. The growth rate for the 10 µL group was 0.183. The 100 µL group and the super dense worm treatment (referenced as the "SDP" or super dense population) were 0.198 and 0.175, respectively. For hours 24-48, it was seen that the control group had a growth rate of 1.138. The group with 10 µL of *C. elegans* had a mean growth rate of 0.412. The group with 100 µL of *C. elegans* had a growth rate of 0.277, and the "super dense worms" treatment had a growth rate that was also 0.277.

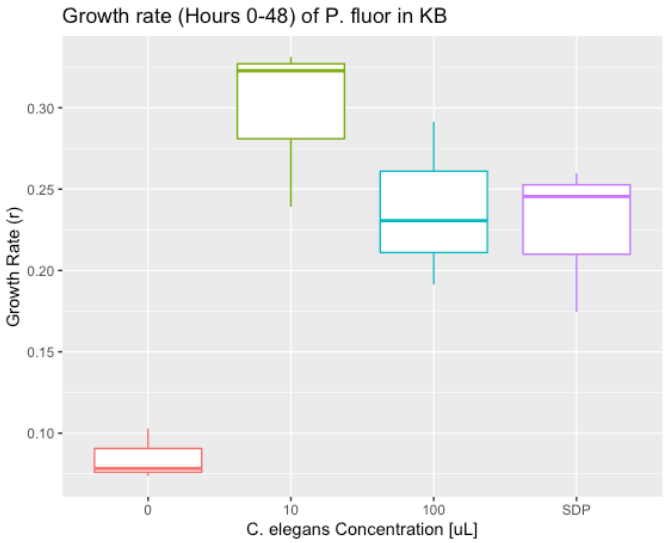


Figure 9. Growth rate of varying starting densities of *C. elegans* with 50 µL of *P. fluorescens* and 50 µL of KB liquid media over 48 hours.

The lowest overall growth rate was observed in the control group and was 0.085 over a 48 hour span. The highest growth rate was in the group with 10 µL of *C. elegans*, where $r = 0.298$. The group with 100 µL of *C. elegans* had an r value of 0.239. The super dense population (SDP) had an r value of 0.227.

Discussion

From the first experiment we were able to discern that *P. fluorescens* grows in a density dependent manner. Since there are limited resources and the well has a finite volume, it can be expected that these populations will grow rapidly and then slow their growth rate as they reach the carrying capacity.

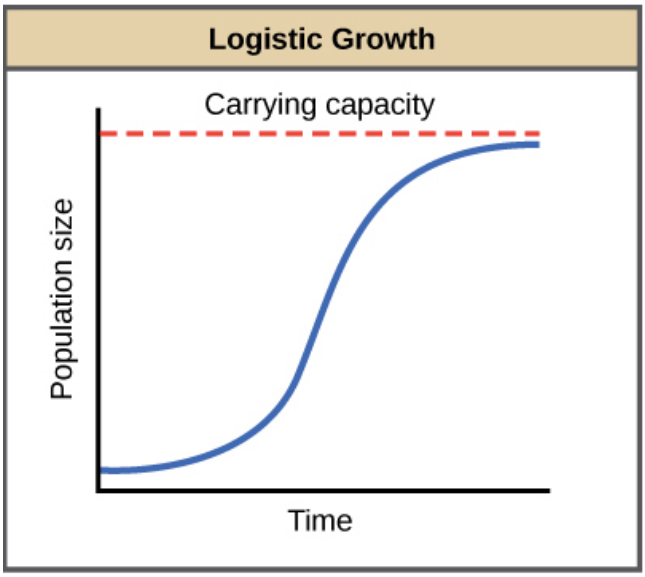


Figure 10. Logistic growth model of a population over time.

Figure 10 (Rye et al.) shows the dynamics of the traditional logistic growth model that demonstrates that as the number of individuals, *N*, increases over time, *t*, it reaches a carrying capacity, *K*, which is demonstrated by the dashed red line. In Fig. 1, we see that the growth rate diminishes as the initial number of individuals increase. As the well becomes more saturated with individuals, there is less room to grow, and fewer resources available for each cell. This is why exponential growth was not what we expected, nor what we observed in the data. As expected, we also observed no growth in our control column, which only contained KB liquid

media. The rate of growth is initially expected to increase with time (e.g., the beginning of the logistic curve), but our initial densities were likely too high to sample this section of the logistic curve.

In the second experiment, we did see that the presence of *C. elegans* had reduced the population growth rate of *P. fluorescens*. The most interesting observation seen in Figure 6 was that groups with initial densities of 50 µL or greater showed negative growth. This is what we expect from groups with high initial starting densities in the presence of predators based on the density-dependent Lotka-Volterra model (Figure 2). In a retest, we would look at this range between 40-50 µL of *P. fluorescens* closer to see if it is near the equilibria we were looking for in this experiment. However, we also observed that the group with the most growth over 48 hours was the control group that only contained *C. elegans*, M9 buffer, and KB liquid media. This is not what we were expecting. Instead we believed that the group with no *P. fluorescens* would demonstrate little-to-no change in OD because we did not inoculate a bacterial population that could grow. Initially, we thought that the *C. elegans* themselves might have some contribution to the OD that we were not anticipating. However, it became apparent later that the antibiotic (gentamicin) that we had been using may have been ineffective. Within the first 24 hours, every group had a negative growth rate apart from the control group. It is possible that the gentamicin might not have prevented the growth of *E. coli* OP50 that was present on the plates that the *C. elegans* were grown on. The presence of both *E. coli* and *P. fluorescens* in the wells might have caused competition between the two populations that stabilized after 24 hours. This could explain why our control group had shown growth while the other groups did not. Considering this, it can still be seen that the presence of worms seemed to decrease the growth rate compared to the values we found in the first experiment. We also see that as more *P.*

fluorescens was added initially, the growth rate decreased. This may be because the worms were able to better predate on the bacteria, because there were more bacteria readily available to them. This could also be attributed to the lack of resources available to the bacteria, as they were approaching their carrying capacity.

In Experiment 3, with constant *P. fluorescens* and KB liquid media across all wells, we found unexpected results similar to those we found in Experiment 2. In the first 24 hours, the control group had a growth rate of $r = -0.968$. Seeing as this group only contained bacteria, media, and M9 buffer, a negative growth is not consistent with the results of our first experiment. The three other groups, with 10 µL, 100 µL, and the super dense population (SDP) of *C. elegans*, demonstrated growth rates of 0.183, 0.198, and 0.175, respectively. All of these result in positive growth of the population. On the second day, the control group had a growth rate of 1.138, which is more consistent with our results from experiment one. The group with 10 µL of *C. elegans* (r = 0.412) showed slightly more growth than the other two groups, and the SDP showed the least growth. This is consistent with what we anticipated – the wells with less predators demonstrated more bacterial growth. Looking at the growth rate in the overall 48-hour span, we see the smallest growth rate in the control group, and the largest in the group with 10 µL of *C. elegans*. The SDP and 100 µL groups showed similar growth rates. Seeing as the control group contained no predators, we anticipated to observe the most growth. Additionally, we expected to see significantly less growth in the SDP group, assuming that the worms were effective predators. Despite using a different antibiotic (kanamycin) for this experiment, we found that it still was ineffective at preventing unwanted bacterial growth. This may help to explain some of the results we found from this experiment. This experiment had an average *C. elegans* density of 2.5 worms per 100 µL, which is smaller than that from our prior experiment nearly by a magnitude of 10.

This may also be responsible for some of the unanticipated results we observed. Follow-up experiments with more consistency in the number of predators are necessary.

If we had more time to study this problem, we would troubleshoot the bleach synchronization process in order to understand its inconsistencies. The density of worm populations after this process varied greatly throughout our experiment. The second experiment had a density of 22 *C. elegans* per 100 µL, while the third experiment had a density of 2.5 *C. elegans* per 100 µL. Unfortunately, due to time constraints, we were unable to grow a new population of *C. elegans* and bleach synchronize them. These densities could have had an effect on the predation rate and thus the growth rate. The bleaching process also does not remove the bodies of the worms who did not survive it, which may lead to an effect on the measured OD. Another possibility is that the *C. elegans* themselves have a greater effect on the optical density metric than we believed, or perhaps they are ineffective predators in liquid media due to locomotion issues. We also experienced issues with the antibiotics we were using, which did little to prevent bacterial growth. This certainly had an effect on the results, and it was evident across several experiments in the lab by other research assistants.

In total, our studies of *C. elegans* predation in liquid media need further work to better parameterize models of predator-prey interactions. However, our studies to suggest that there is negative density dependence in *P. fluorescens* growth rate, which provide clearer hypotheses about how variable predator densities should influence prey population growth rates. To better understand the population dynamics of *P. fluorescens* in the presence of varying numbers of *C. elegans* predators, we would repeat these experiments to test different starting conditions on the phase planes shown in the Lotka-Volterra models. Time constraints secondary to COVID-19 prevented us from exploring all the avenues of this experiment that were initially intended. Upon

a retest we would hope to observe significant predation by *C. elegans* in these wells. Ideally, we would find out if the population stabilizes as predicted in the Lotka-Volterra predation model with a carrying capacity. Because of these inconclusive results, we were unable to parameterize a Lotka-Volterra model of this species interaction.

References

Allison, Steve. *Reagents and Procedures for Pseudomonas Culturing*. Apr. 2008, allison.bio.uci.edu/protocols/pseudomonasculturing.pdf.

Gomez-Amaro, Rafael L, et al. "Measuring Food Intake and Nutrient Absorption in Caenorhabditis Elegans." *OUP Academic*, Oxford University Press, 21 Apr. 2015, academic.oup.com/genetics/article/200/2/443/5936166.

Gotelli, Nicholas J. *A Primer of Ecology*. 4th ed., Sinauer Associates, 2018.

Introduction to C. Elegans Anatomy. Worm Atlas, 20 Nov. 2014, bio.as.uky.edu/sites/default/files/C%20elegans%20anatomy%20and%20lifecycle.pdf.

"M9 Buffer for Worms." *Cold Spring Harbor Protocols*, 1 Jan. 1970, cshprotocols.cshlp.org/content/2014/3/pdb.rec081315.full?text_only=true.

Rye, Connie, et al. "Environmental Limits to Population Growth - Biology." *OpenStax*, 21 Oct. 2016, openstax.org/books/biology/pages/45-3-environmental-limits-to-population-growth.

Trippe, Kristin, et al. "Pseudomonas Fluorescens SBW25 Produces Furanomycin, a Non-Proteinogenic Amino Acid with Selective Antimicrobial Properties." *BMC Microbiology*, BioMed Central, 20 May 2013, bmcmicrobiol.biomedcentral.com/articles/10.1186/1471-2180-13-111.

What Is C. Elegans? | College of Biological Sciences, University of Minnesota, 22 Mar. 2021, cbs.umn.edu/cgc/what-c-elegans.