LIFE HISTORY RESPONSES TO VARIATION IN BACTERIAL FOOD SOURCES IN THE NEMATODE *CANORHABDITIS ELEGANS*

by

ALEX SMITH

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Obtaining nutrients from the environment is an activity shared among many organisms. The effect of these nutrients on life history traits of organisms can vary depending on their composition or their source, though the metabolic pathways that mediate these responses are highly complex are not fully understood. The nematode *Caenorhabditis elegans* is a candidate model to study the varying effects that changes in bacterial food sources might have on development, reproduction, lifespan, and other life history traits. I assessed whether a panel of natural isolate bacteria species produced any identifiable changes to life history traits of a population of nematodes and discovered that species *Comamonas aquatica* and *Comamonas testosteroni* produce the most dramatic change to development time. Further investigation of these two bacteria species and their effect on nematode offspring counts showed that they both produced reduced offspring numbers, and the pattern of change in response to environmental temperature is not consistent with the pattern observed in standard laboratory conditions. These results suggest that changes in diet can have implications to development, but that this change comes with a tradeoff to reproductive capability. Changes in nutrient composition will likely be a factor in tradeoffs in life history traits of organisms of varying complexity.

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Introduction

Nutrient acquisition is a necessary function of both simple and complex lifeforms. Feeding is an activity that has direct impact on quality of life. Changes in the nutrient composition of consumed food can affect complex metabolic processes in the body, and many life history traits can be drastically altered by a change in diet. For instance, the Dutch famine of 1944-45 allowed scientists to measure the effects of extreme nutrient deprivation on health, with the major findings including higher chance of obesity, diabetes and cardiovascular disease in the children of pregnant women exposed to the famine (Painter et al, 2008). Optimal nutrition produces the most profound effects in early brain development; increases or decreases in Iron, Thiamine, Vitamin B6, Vitamin B12 (and others) during prenatal and early postnatal development can have drastic impact on the individual's cognitive development (Cusick and Georgieff, 2016). While extreme cases are useful for demonstrating the degree of importance of diet to life history traits, the specificity of changes and the mechanism of these changes is tremendously complex. Indeed, much of the complexities of diet and their link to life history traits are not yet fully understood.

Feeding is an activity that is shared among most organisms despite substantial differences in their evolutionary history. One example is filter feeding, a strategy of feeding practiced by a plethora of aquatic organisms, from clams, to sponges, and even whales. Despite drastically different evolutionary lineages, many animals in aquatic environments have experienced convergence to this similar form of feeding. This phenomenon emphasizes the importance for all organisms to retrieve certain nutrients present in the environment. Understanding the effects that changes in

diet, or the effects of specific diets can have on an individual is important to understanding which foods and nutrients support different aspects of life. Within humans, deficiency and overabundance of dietary nutrients have clear effects on developing children and have consequences that are present throughout that individual's lifetime (Georgieff, 2007). Therefore, a better understanding of the complex links between diet and life history traits is essential to fully understand human health. Studying the effects of various foods on simple organisms can provide a greater understanding of nutrient allocation in the metabolism of more complex organisms.

I found during this work two novel bacteria species that show to increase the developmental rate of nematodes. While these bacteria species, *Comamonas testosteroni* and *Comamonas aquatica,* appear to act as a super food that will increase individual fitness, further investigation shows evidence otherwise. Nematode offspring counts appear to be significantly lower than expected when they are given a diet solely of *Comamonas testosteroni* or *Comamonas aquatica.* This pattern is similar to one induced by increased temperature, in which nematode development rate increases and total offspring decreases. This suggests that there is a tradeoff between developmental rate and offspring count that is induced by the diet. This gives evidence that super foods may not exist, and that life history traits are intricately linked and there will always be tradeoffs.

Background

Caenorhabditis elegans as a Model Organism

C. elegans are transparent nematodes that normally live in temperate rotting vegetation (Schulenburg and Félix 2017) The small, free living roundworm lacks a circulatory, skeleton, and respiratory system (Wallace et al, 1996). Despite its differences from more complex organisms such as humans, its simplicity is part of what makes it so advantageous. Sydney Brenner first proposed the nematode as a model in 1963 and began experimenting of his during the same decade (Brenner, 1974). Since then *C. elegans* has been used extensively as a biological model to study development, aging, neurobiology, and more. Its relevance as a model is evident in that it was the first multicellular organism to have its full genome sequenced (The *C. elegans* Genome Consortium, 1999).

C. elegans presents many features making them a useful species for analyzing biological principles. Existing as one of the simplest organisms with a nervous system, it was originally suggested by Sydney Brenner as a model to study neural development and structure. The transparency of the organism allows for straightforward study of cellular differentiation and organogenesis in great detail. For instance, the developmental fate of each somatic cell in an adult nematode has been mapped (Sulston and Horvitz, 1977). *C. elegans* also possesses the advantageous ability to be frozen and subsequently thawed for use. This allows for a fixed reference when performing long term studies and has made the organism an attractive model for evolutionary research.

Of particular note, nematodes are an attractive model organism due to their ease in maintenance and ability to grow in large populations. Thousands of individuals can

be kept on a single agar plate. *C. elegans* are bacterivores, so the only major food source they require is adequate quantities of accessible bacteria. The bacteria of choice for most laboratory settings is *E. coli* OP50, an uracil requiring bacterium that will not overgrow on agar plates due to its deficiency (Brenner, 1974). OP50 also does not call for any major safety precautions because it is non-pathogenic.

Reproduction and Development of *Caenorhabditis elegans*

C. elegans has been particularly used as a model to understand organismal development, and its development has some unique aspects that are important to understand. *C. elegans* exist naturally in populations dominated by hermaphrodites. Embryonic development occurs inside hermaphrodite individuals after self-fertilization. Hermaphrodites produce all their sperm during a single larval stage, the L4 stage, then sperm development halts and is followed by oocyte production and fertilization. However, males also have the ability to inseminate hermaphrodites, which hermaphrodites preferentially use for fertilization (Ma et al, 2012). Once adults, hermaphrodite nematodes begin to lay fertilized eggs. After the embryonic stage, hatched larvae develop through four distinct larval stages, L1, L2, L3, and L4, in about 3 days at 20°C. Each stage is distinguishable by features the *C. elegans* gonad, cuticle, or vulval development. The stages are punctuated by a molt of the nematode's transparent cuticle. At 20°C, eggs hatch about 9 hours after being laid, giving way to an L1 nematode. The first molt follows after 12 hours, leading to an L2 nematode, followed by the second molt after 8 hours (L3), a third molt after another 8 hours (L4), and the final molt after another 18 hours. Following the final molt, the nematode is considered an adult, and reproduction commences ~9 hours later. A reproductively

mature adult will continue to lay eggs as long as it is healthy and has fertilized eggs to lay.

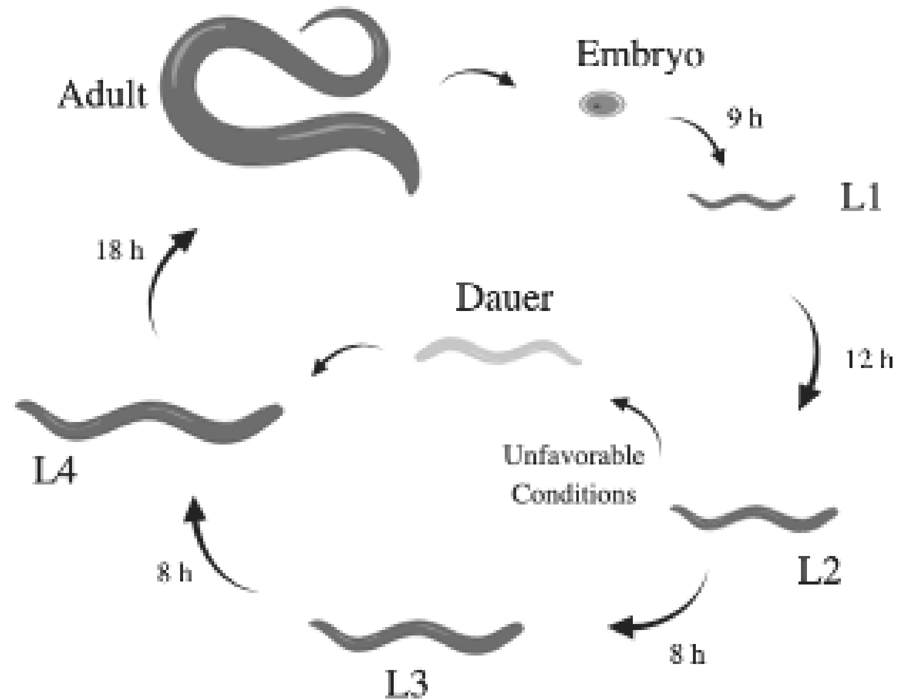

Figure 1. Life Cycle of *Caenorhabditis elegans* at 20°C. Animals pass through development in 3 to 4 days under favorable conditions at 20°C when they are given a diet of *E. coli* OP50. In response to harsh environments, such as low food or changes in temperature, animals can enter an arrest state known as dauer.

Development of *C. elegans* can measured in a number of different ways. For instance, molt time provides a measure of the amount of time between each developmental stage. Molts are a definitive indicator in breaking down the time nematodes spend in each stage as a developing organism. Measuring this process is demanding, however, as it requires the experimenter to observe the exact moment at which the shedding of the previous cuticle occurs. When molting occurs, animals enter a lethargic state where motion is minimal. This is because it takes time for the cuticle to shed from the organism but gives the experimenter a period of time in which to catch

the molt. Comparative developmental assays provide another means of measuring relative developmental rates. By comparing the relative abundance of worms at different larval stages at defined time points, the difference in developmental rate between two experimental conditions can be determined. Finally, Alpha (α)-time can also be used to measure reproductive development for *C. elegans*. α-time is defined as the time from when an individual egg is laid to when it becomes a reproductive adult laying its own eggs. This measurement provides a timeline for how long the nematode progresses through larval development before reaching adulthood. The procedure is significantly less burdensome than measuring molt time.

Caenorhabditis elegans Feeding Behavior

Diet is largely the result of an organism's feeding behavior. *C. elegans* are bacterivores, meaning they obtain their nutrients primarily from the consumption of bacteria (Avery and You, 2005). There are multiple aspects of feeding behavior that are important to food acquisition: the physical mechanism required to consume bacteria, the rate at which the bacteria are consumed, and the act off seeking and choosing bacteria. The nematode has a tubular pharynx that specializes in pumping and sucking bacteria into the worm from the environment, concentrating the bacteria, and then grinding it up (Albertson and Thomson, 1976). To concentrate bacteria, the nematode's pharynx acts as a size filter. Upon pharyngeal contraction the pharyngeal lumen opens, creating a suction that pulls in bacteria that are in liquid suspension and are also small enough to enter the mouth. This establishes an upper size limit of consumed bacteria (Avery and Shtonda, 2003). Upon relaxation, the pharyngeal lumen collapses and expels the bacterial suspension. Due to physical constraints imposed by the sequential muscle

relaxation, bacteria larger than 1 micron are preferentially retained, while smaller bacteria exit with the expelled liquid. This places a lower limit on the consumed bacteria size. (Fang-Yen at al, 2009; Avery and Shtonda, 2003). The pharyngeal muscles are then able to transport the bacteria where it is metabolized in the nematode's primitive intestine (Seymour et al, 1983). Feeding motion and behavior is regulated by the food in the immediate environment.

Nematodes have the ability to seek out and distinguish among food types that best support their growth. Food quality is often defined as the ability to support nematode growth, and higher quality foods are identified by the growth rate they produce in *C. elegans* (Avery and Shtonda, 2003). Food seeking behavior in *C. elegans* even in the earliest larval stage illustrates their ability to seek out higher quality foods. L1 nematodes that are placed on agar plates with two foods will distribute such that more worms are actively feeding in the higher quality food (Avery and Shtonda, 2006). This phenomenon has been suggested as a consequence of difference in leaving probability. If nematodes are satisfied with the bacteria they are feeding on, they are less likely to leave the lawn of food and venture out in search of a higher quality food. Thus, more nematodes are found within lawns that contribute to growth rate.

Development in Altered Environmental Conditions

Most experiments with *C. elegans* are performed under standardized laboratory conditions. For example, they are typically incubated at 20°C on agar plates seeded with *E. coli* OP50 as a food source. However, changes to these conditions can have some significant impacts on the development, fecundity, and lifespan. A common

environmental condition to change is temperature, and its effects on *C. elegans* have been well documented. Nematodes
can readily propagate in a temperature range of 15°C to 25°C. At temperatures higher than 20°C, development through each larval stage is accelerated, though the total eggs laid and overall lifespan both decrease. At temperatures lower than 20°C, nematode development through each larval stage increases, though lifespan increases as well (Byerly et al, 1976; Lewis and Fleming, 1999). Different natural isolates of *C. elegans* display different thermal preferences. Whereas some isolates will seek warmer temperatures to favor population growth rate, others will favor lower temperatures that favor reproductive success (Anderson et al, 2011). All values are relative to the standard laboratory temperature of 20°C.

Changes in standard laboratory conditions are not limited to temperature. Though often raised on *E. coli* OP50 lawns, these bacterivores will eat almost any bacteria available to them. This change in food source is another factor that can significantly change life history traits. Because much of the behavior of *C. elegans* is driven by chemotaxis, changes in the food source of their environment can change their activity and feeding behaviors. Extensive activity or dietary restriction can both impact *C. elegans* health and longevity (Szewczyk et al, 2006). Changes in bacterial availability can also lead to changes in nutrient composition with the nematode's diet. Individuals will feed on the bacteria available to them and are thus reliant on the nutrients provided by that bacterial species, especially if their environment is limited to a single bacteria species as it often is in the laboratory. It is likely that these changes in nutrient composition are also what can lead to the observed increase or decrease rate of

larval development, abundance of offspring, and lifespan (Watson et al, 2014). One trend that has been observed in different bacterial food sources is that there is an optimal size range of bacteria that determines food quality. That is, species of bacteria that exceed the size threshold, as well as species that fall below the minimal size threshold produce slower growth rates in nematodes (Avery and Shtonda, 2003). This is, however, an overly simplistic model of determining good and bad foods. A competing model claims that nutrient composition of the bacteria actually plays a greater role in determining if it is a good food. One such example is vitamin E, which when supplemented in the environment of the nematode during the development prolongs survival. It has been suggested that this may be due to slowing development and is also linked to decreasing nematode fecundity as well as delayed timing in reproduction (Harrington and Harley, 1988).

Hypothesis

This study seeks to uncover some of the complexities underlying the relationship between diet and life history. The central hypothesis addressed here is that the bacterial food source provided to developing nematodes will cause strong shifts in the pattern of life history traits within those individuals, including development, reproduction, longevity. Because different life history traits are often connected, I tested whether the pattern of bacteria-driven changes in life history traits were similar or different among key bacteria species. I also tested whether the influence of bacterial food source on nematode life history changes with other environmental factors, such as temperature.

Methods

Bacteria Culture and Maintenance

Microbiological cultures of all bacteria species were initial grown on agar in petri dishes. Agar was given a thin layer of LB growth medium before exposed to bacteria. Plates were incubated at 30°C for ~16 hours, or until sufficient bacterial growth was observed. After growth was achieved, bacteria cultures were refrigerated and preserved before use in experiments. Culture plates were freshly made every month.

Large cultures of bacteria were necessary to seed the agar plates used for nematode feeding and experiments. To obtain concentrated bacterial populations we propagated liquid cultures. Flasks with LB broth were inoculated with the desired bacteria by colony selection from an LB agar plate. Liquid cultures were stored at 30°C for 15 hours on shaker to ensure uniform bacteria growth. Bacteria from liquid cultures was filtered into 50mL conical tubes and refrigerated for future seeding of agar plates.

Nematode Culture and Maintenance

Nematodes were cultured on nematode growth medium (NGM) seeded with *E. coli* OP50 (Brenner 1974), *Comamonas Testosteroni,* or *Comamonas Aquatica DA1877* (Avery and Shtonda 2003) at 20°C unless otherwise stated. Populations were transferred to fresh NGM plates every 72 hours. A N2 hermaphrodite strain, regarded as a wild type *C. elegans,* was used in all aspects of this study.

Synchronization of nematodes was accomplished through egg lays or hatch offs. Egg lays were performed by isolating gravid hermaphrodites and allowing to lay eggs on a fresh NGM plate and allowing them to lay eggs for a short time. Egg laying plates

were seeded with the same bacteria as was present in the adult nematodes previous condition. Adult hermaphrodites were removed from the plates after 1-2 hours. Nematode offspring would then be used in the subsequent experiment after incubation and development to the appropriate stage. Hatch offs were performed by standard procedure (Sulston & Hodgkin, 1988). Nematodes and eggs were moved to a conical tube by liquid transfer and were suspended with 4 mL water and 750 uL of NaOH and Bleach solution. Solution was mixed periodically until adult nematodes were no longer visible in the conical tube. Remaining eggs were rinsed with 10 mL water three times and were then resuspended in 4 mL M9 in 15 mL conical tube. Eggs were then incubated in the conical tube at 20°C on a rotator. After 24 hours arrested L1 nematodes could be used for experiments.

Bacteria Screenings

To identify bacteria strains that that influence nematode development and reproduction, candidate bacteria strains were screened (Table X). Nematodes were synchronized by hatch off and plated onto NGM plates seeded with various natural isolate bacteria strains. Nematodes were allowed to grow to adult over the course of one week, and observations were made every 24 hours. Differences in nematode development, population size, and overall health were noted. Comparisons were made to Nematodes growing on plates seeded with *E. coli* OP50.

Developmental time

Development time was measured by α- time and a developmental staging assay. For α-time**,** individuals were synchronized by a one-hour egg lay that marked the alpha

time start. After adults were removed from egg laying plates, offspring were allowed to grow on the plate for 24 hours. Nematode larvae were then isolated onto individual small NGM plates that were seeded with the same bacteria as the plate that the adult was originally taken from. Nematodes were allowed to grow until they reached the L4 stage. At this stage nematodes were observed periodically under microscope in order to see when the first egg is laid. Once an egg was observed on a plate, the alpha end time was recorded for that nematode. Total α-time was calculated as the difference between alpha start and alpha end times.

Nematodes on *E. Coli* OP50, *Comamonas testosteroni,* and *Comamonas aquatica* also had development timing measured through a developmental staging assay in a separate experiment. To perform the assay, nematodes were synchronized by hatch off. The concentration of nematodes in M9 solution was calculated and equal numbers of nematodes were plated onto small NGM plates. Plating of arrested L1s was recognized as day one. Each subsequent 24 hours the number of nematodes at each larval stage as well as number of adults was identified on each plate. This process continued until all nematodes on every plate were recognized as adults, or adult nematodes on the plate began to lay their own eggs. Data for each stage was converted to a percentage of the total nematodes on all plates of the same condition.

Egg Count Assays

Nematodes were synchronized by a 1-hour egg lay. 24 hours after the parent adult was removed, L1 larvae were isolated onto individual small NGM plates. Nematodes were allowed to grow until adulthood on the same plate. Once egg laying began adults were transferred onto fresh NGM plates every 24 hours, until egg laying

ceased. Living larvae were counted on plates 24-48 hours later in order to ensure that all living offspring were visible under microscope. Unhatched embryos were not counted as offspring. The number of living larvae from each day was summed to achieve a total offspring count for each individual nematode. The procedure was performed with nematodes incubated at 20°C, 25°C, and 15°C.

Statistical Analyses

For appropriate sets of data, Non-parametric Wilcoxon tests were used to determine if differences exist between any of the groups of data. If the Wilcoxon test resulted in a ChiSquare approximation that was deemed significant (Prob>ChiSq <0.05) the further nonparametric multiple comparisons tests were performed. A Wilcoxon test of each individual comparison was performed, as well as a Steel-Dwass test to protect the overall error rate of a data set.

Table 1. Bacterial Species Used

Strain Designation	Species	Reference
OP50	*Escherichia coli*	(Brenner, 1974)
N/A	*Comamonas aquatica*	(Avery and Shtonda, 2003)
C3B	*Comamonas testosteroni*	
C3A	*Pseudomonas putida*	
C5B	*Acinetobacter lwoffii*	
C6A	*Acinetobacter lwoffii*	
N/A	*Bacillus subtillus*	
HT115	*Escherichia coli*	(Virk et al, 2012)
C6A	*Stenotrophomonas maltophila*	
C6B	*Stenotrophomonas maltophila*	
C6B	*Serratia proteamaculans*	
Gut C5D	*Serratia proteamaculans*	
C5D	*Acinetobacter baumannii*	
Gut C5A	*Enterobacter aerogenes*	
Gut C3E	*Ochrobactrum anthropi*	
C3D	*M. opportunism*	

Results

Caenorhabditis elegans Growth Observations on Various Bacteria Diets

Diet can have dramatic effects on growth and development. We therefore sought to measure the effects of varying diet of the bacterivores *C. elegans* by culturing the nematodes on a variety of bacterial species. In a laboratory setting, the only available diet for bacterivores nematodes in the bacteria lawn presented to them. In brief, a panel of bacteria. species were selected based upon their availability. Small populations of synchronized L1 wildtype nematodes were plated onto petri dishes seeded with a single bacteria species from the panel of available species. Control plates with the lab standard *E. coli* strain OP50-1 were always seeded and plated with nematodes alongside experimental bacteria species so that all observations could be made in reference to a laboratory control.

Observations were made regarding the developmental state of the nematodes. Observations focused in particular on the development stage, activity, and overall health of the nematodes relative to nematodes growing on *E. coli* OP50-1. Most bacterial strains produced no gross-level phenotypes within the nematodes upon initial observation regarding their development and overall health. The exceptions were nematode populations that appeared to develop on *Comamonas aquatica* and *Comamonas testosteroni.* These nematodes noticeably developed faster and showed egg laying activity before nematodes growing on *E. coli* OP50-1.

Bacteria Diet Effects on α-Time

Because bacterial diet appeared to change the developmental rate of wildtype nematodes, we measured the alpha time of *C. elegans* grown on each of the bacterial strains in the panel (Table X). α-times were collected for individual nematodes from a population habituated through multiple generations of growth on that bacteria species. This growth is important because α-time, being measured as the time between being laid as an embryo to the time when the nematode is an egg laying adult hermaphrodite, represents the inverse of the sum developmental rate through embryonic development all 4 larval stages. While larvae will be exposed to the bacteria of interest, it is vital to note that the impermeability of the egg shell makes embryonic development dependent on maternal diet

Several bacteria species produced a longer α-time, and therefore slower developmental rates, than nematodes on *E. coli* OP50-1 (Figure X). The greatest fold change was seen in *Stenotrophomona maltophilia, Acinetobacter baumannii,* and *Acinetobacter iwoffii.* Anecdotally, nematodes on these bacteria species generally experienced a lack of activity when compared to nematodes on *E. coli* OP50-1.

Comamonas aquatica and *Comamonas testosteroni* produced the most significant alpha time change from nematodes on *E. coli* OP50-1. Nematodes on both experimental bacteria species had a significantly lower α-time (Figure X). The dramatic reduction in α-time produced by these bacteria species prompted us to focus on those two species for further characterization on their effect on nematodes.

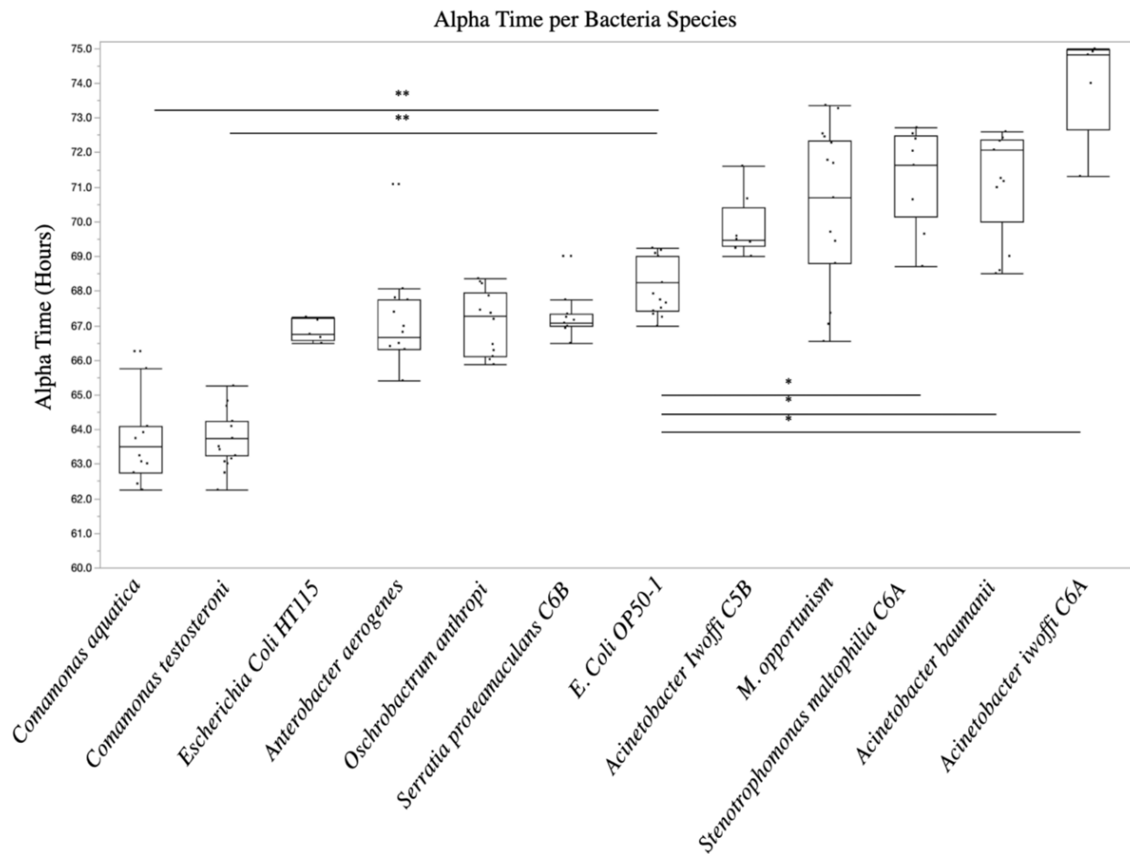

Figure 2. α-Times for Nematodes Raised on Bacterial lawns of Various Species. α-times were measured for individual nematodes as the time from when the organism was laid to the time it lays the first egg as an adult hermaphrodite. Each point represents the time of a single individual to reach egg laying adulthood. Nonparametric Steel-Dwass comparison showed that animals raised on *Comamonas aquatica* and *Comamonas testosteroni* lawns had the greatest reduced alpha time from standard *E. coli* OP50-1. Nematodes on bacterial lawns that produced increased alpha time generally exhibited poor health and premature death.

*indicates p-value <.01

**indicates p-value<.001

Development Acceleration by *Comamonas aquatica* and *Comamonas testosteroni*

While α-times give a gross measure of the rate of passage through all embryonic and larval stages, it lacks information on whether changes in developmental rate occur in all stages or are stage specific. We therefore examined the developmental acceleration for nematodes feeding on *Comamonas testosteroni* and *Comamonas aquatica* by performing a developmental staging assay to track progression of nematode development from when they were laid as eggs until adulthood. Egg lays were performed on three different bacteria species; *Comamonas aquatica, Comamonas testosteroni,* and *E. coli* OP50-1 served as a reference for normal development timing. Nematodes were observed every 24 hours after egg lay and categorized based on larval stages. Staging was determined by the development of the germ line.

Looking at the percentage of nematodes at each larval stage after each 24-hour segment shows differences between nematodes on the different bacterial species (Figure X.). The effect is most pronounced at 48 hours after initial egg lay. Nematodes on *Comamonas testosteroni* and *Comamonas aquatica* were proportionally higher developmental stage than nematodes on *E. coli* OP50-1, suggesting faster larval development during the first 48 hours. Interestingly, proportional differences in nematode larval stage did not appear dramatically different at 24 hours or 72 hours. At 24 hours after egg lay most nematodes were still L1 stage, though the only nematodes at any later stage were found on *Comamonas testosteroni* and *Comamonas aquatica*. At 72 hours after egg lay most nematodes were mature adults. By 96 hours all nematodes were mature adults on all bacteria species.

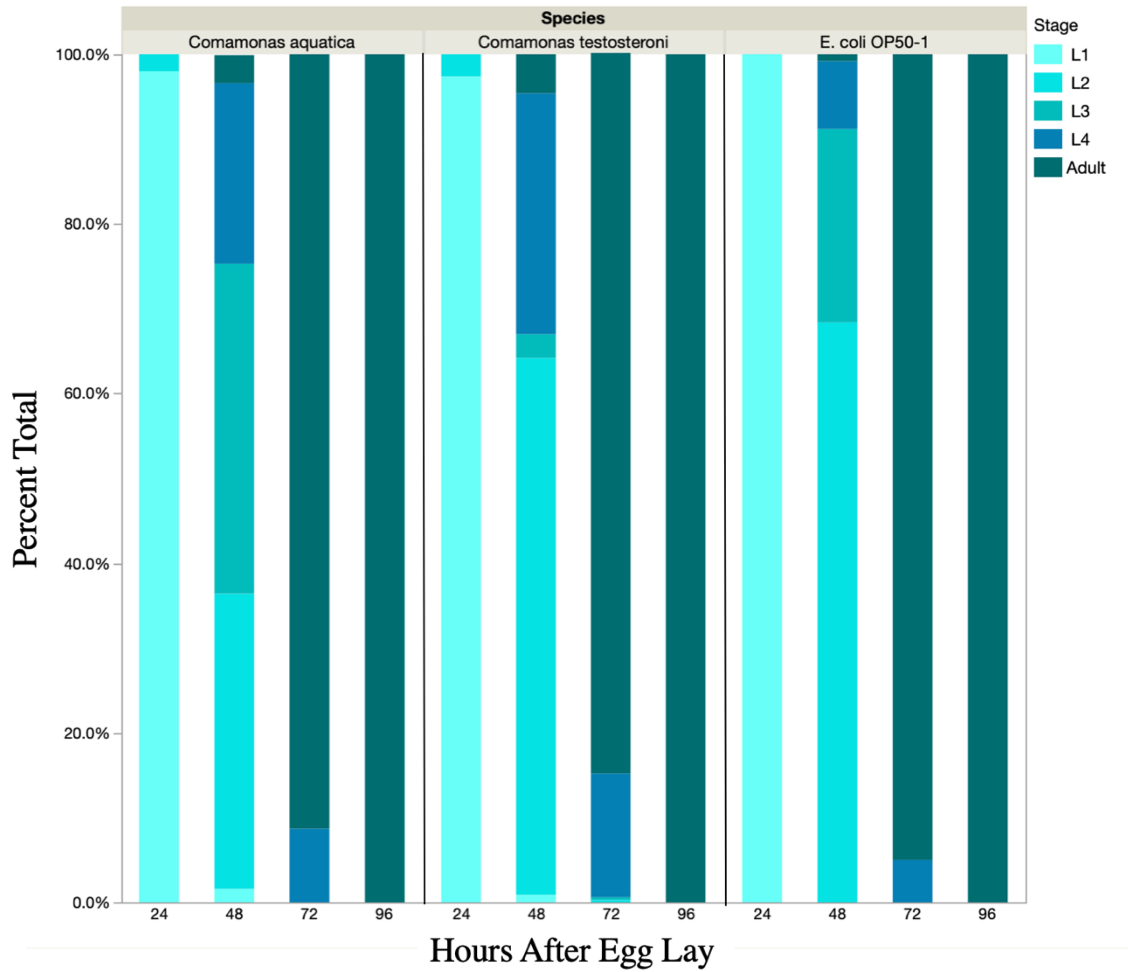

Figure 3. Progression of Larval Stages of Nematodes on Different Bacteria Species. Stage was determined based on germ line development under a dissection microscope every 24 hours after nematodes were laid embryos. At 48 hours, a larger portion of nematodes on *Comamonas aquatica* and *Comamonas testosteroni* had progressed to later development stages than nematodes on *E. coli* OP50-1. Percentage differences were most noticeable for nematodes in the L3 and L4 stage.

Egg Laying Changes Caused by *Comamonas aquatica* and *Comamonas testosteroni*

Reproduction is an essential life history trait of all organisms. For *C. elegans* hermaphrodites, the L4 larval stage is important for sperm development, as all sperm is made during this stage of the nematode's life. In the absence of males, the amount of sperm created by hermaphrodites during this stage becomes a driver in the total reproductive output potential of the organism. We suspected that changes in the amount of time spent in the L4 stage could impact the total reproductive capability of the hermaphrodite. Because *Comamonas aquatica* and *Comamonas testosteroni* produced accelerated larval development, we decided to see if these bacteria also produced any changes in the egg laying of adult hermaphrodite N2s. Populations were grown on bacterial lawns of either *E. coli* OP50-1, *Comamonas aquatica,* or *Comamonas testosteroni,* and maintained over 3 generations to ensure that any residual bacteria present in the gut of the nematodes was not a confounding variable.

We isolated hermaphrodite larvae after an egg lay onto individual small petri dishes seeded with one of the three bacteria. Nematodes developed on the same bacteria strain as the previous ancestors over at least three generations. Nematodes developed on a single plate until egg laying began. Once egg laying began, nematodes were transferred to new plates every 24 hours for 5 days. Progeny was counted from each plate and added up to measure the total progeny count for an individual nematode. At 20°C, nematodes raised on *Comamonas aquatica* and *Comamonas testosteroni* both produced a reduced number of offspring compared to the standard *E. coli* OP50-1.

Wild type nematodes on *E. coli* OP50-1 lawns produce a reduced number of offspring when grown in increased or decreased environmental temperatures. This

effect is exaggerated as temperature increases. This may be a consequence of altered spermatogenesis, which is a temperature sensitive process. However, we also suspected that this may be a result of reduced time spent in the L4 stage leading to less sperm produced by the hermaphrodite. We decided to see if this pattern of progeny decrease is conserved for wild type nematodes raised on *Comamonas aquatica* and *Comamonas testosteroni* bacteria lawns. At 15 °C, nematodes did not produce significant differences in their overall progeny count between bacteria species (Figure 4). At 25°C, nematodes on *Comamonas testosteroni* produced a significant decrease in progeny count compared to nematodes on *E. coli* OP50-1. Interestingly, nematodes on *Comamonas aquatica* did not mimic this decrease and instead produced a comparable number of offspring compared to nematodes on *E. coli* OP50-1 (Figure 4).

Nematodes on *E. coli* OP50-1 had a lower progeny count at 25°C when compared to the other environmental temperatures. Interestingly, Nematodes on *Comamonas testosteroni* had a higher overall progeny count at 15°C compared to the other environmental temperatures. Nematodes on *Comamonas aquatica* did not produce significant changes in progeny count as environmental temperature increased or decreased. The pattern of progeny count change was different for wild type worms raised on each of the bacteria species.

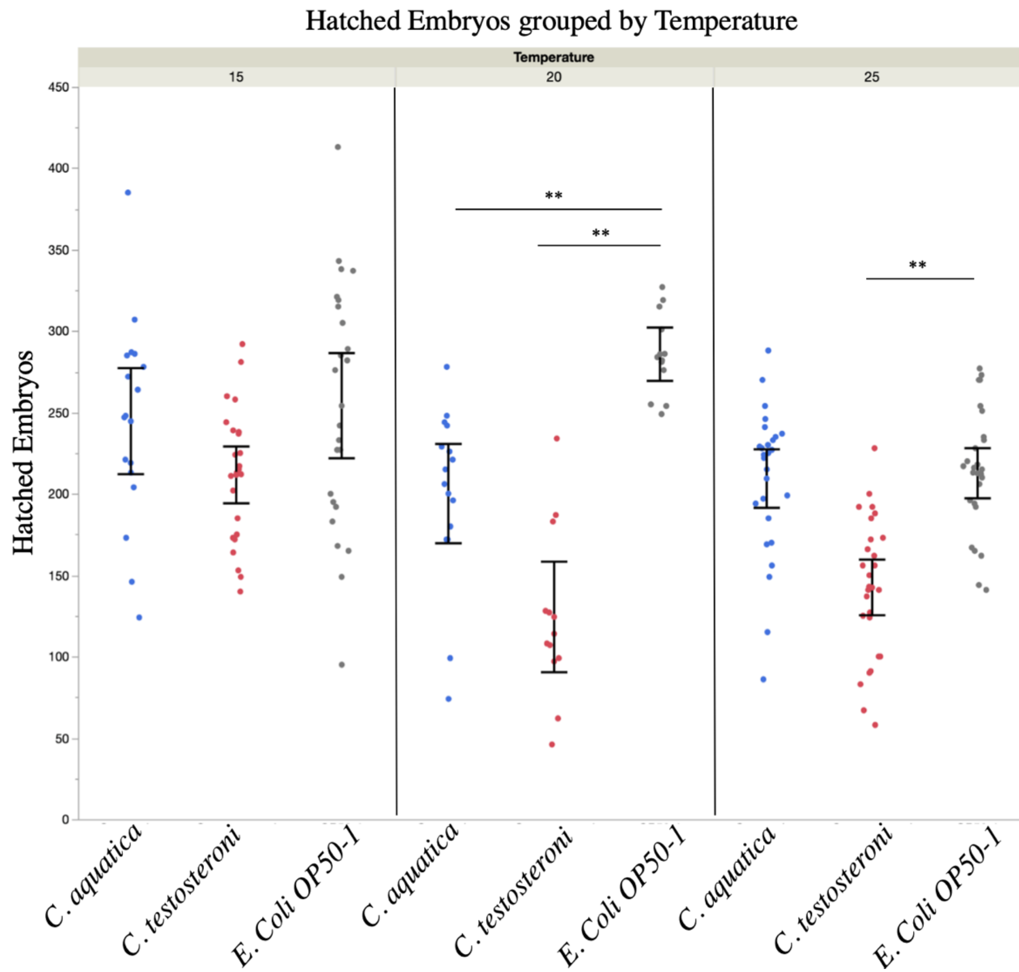

Figure 4. Hatched Embryos Grouped by Temperature for nematodes living on *C. aquatica, C. testosteroni,* and *E. coli* OP50-1. Nematodes are raised on the bacteria lawn for several generations before experiment. Adult hermaphrodites are isolated from a population and allowed to lay eggs for 5 days. Adults are moved to a new plate every 24 hours to ensure that plates are not overpopulated. Progeny are counted 24-48 hours after adult removal. (A) Grouped by Temperature. Nonparametric comparison using Steel-Dwass method showed no differences between bacteria species at 15 C, significant differences between both experimental bacteria species from OP50-1 at 20 C, and differences between *C. testosteroni* and OP50-1 at 25 C. Error bars represent 95% confidence interval from the mean.

* indicates p-value <.05

** indicates p-value <.01

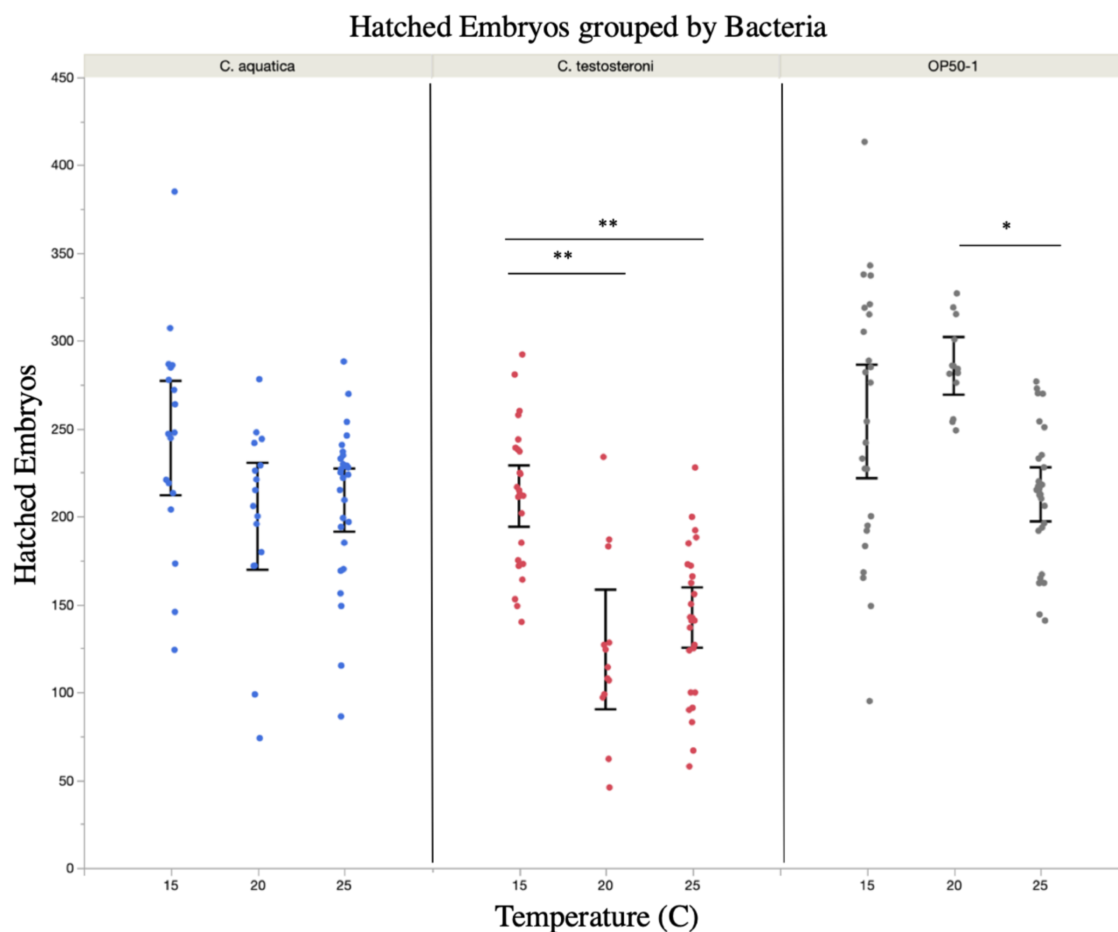

Figure 5. Hatched Embryos Grouped by Bacteria Species for nematodes living on *C. aquatica, C. testosteroni,* and *E. coli* OP50-1. Nonparametric comparison using Steel-Dwass method showed no differences between nematodes on C. aquatica at different temperatures, significant differences between nematodes on C. *testosteroni* at 15 C compared to nematodes at 20 C and 25 C, and difference between nematodes on OP50-1 at 20 C and 25 C. Error bars represent 95% confidence interval from the mean.

* indicates p-value <.05

** indicates p-value <.01

Discussion

Changes in diet can have a multitude of effects on *C. elegans*, including both increases or decreases in development rate. Various natural bacterial isolates can alter nematode health metrics, suggesting that differences in the bacteria themselves matter for the development of nematodes. Several of the bacteria species that we tested produced an increase in α-time. This could be the consequence of a number of factors, including size of bacterium or the metabolic components that may be present or missing relative to the standard diet. While some bacteria species produced little or no changes to the development of *C. elegans,* this does not outweigh their potential as dietary options. Bacteria can differentially affect *C. elegans* response to chemotherapeutics (García-González, 2017) and therefore new potential diets that have limited effects on other life history traits could be explored to enhance *C. elegans* longevity under stressful conditions.

Comamonas aquatica and *Comamonas testosteroni* produced the most dramatic response to development when used as the sole bacteria species fed to wild type *C. elegans.* Both bacteria species increased the rate of development for wild type nematodes. *Comamonas aquatica* has been characterized as a bacteria species high in vitamin B12 relative to the standard laboratory diet of *E. coli* OP50. Vitamin B12 is a metabolite linked to changes in gene expression in *C. elegans* that affect fertility and development (Watson et al, 2014). The presence of B12 in *Comamonas aquatica* is the most likely driver of changes in life history traits that we observed. *Comamonas testosteroni* is not a bacteria species that has been characterized as thoroughly as *Comamonas aquatica.* However, since it is a closely related species, it is possible that

vitamin B12 is also the major driver of its effects on *C. elegans.* While this may be the case, egg laying numbers were comparable but not identical between wild type nematodes on a diet of *Comamonas aquatica* and *Comamonas testosteroni,* suggesting that there is an unidentified player that may also be contributing.

More complete knowledge of the effects of *Comamonas aquatica* and *Comamonas testosteroni* on life history traits of *C. elegans* can be accomplished through further tests. The priority of these tests includes lifespan assays. Accelerated development as influenced by the environment is often linked to a decrease in survivorship for the nematode. This is well demonstrated in increased environmental temperature (Klass, 1977) but is also a pattern present in mutants that have increased metabolic rates (Voorhies and Ward, 1999). The natural prediction based on this pattern would be that bacteria species that increase rate of development, such as *Comamonas aquatica* and *Comamonas testosteroni,* would also lead to a decrease in lifespan. If either or both of these bacteria species were shown to retain the lifespans of wild type C. elegans grown on an E. coli OP50 diet, or were shown to increase longevity, they would be outliers. Teasing apart this lifespan relationship would therefore have something more to offer in terms of investigating links between environmental conditions and life history traits.

Food preference is another trait that could be measured between *Comamonas aquatica, Comamonas testosteroni,* and the standard *E. coli* OP50. If given the choice, do nematodes have a preference as to which bacterial food source they would feed on, and what life history strategies do those sources of food correlate with? Generally, wild type *C. elegans* prefer *E. coli* over other bacteria species, however there are several

bacteria that are an exception. These include *Bacillus mycoides* and *Bacillus soli*, which also produce extended lifespan in the organism (Abada et al, 2009). Correlations between life history effects and nematode preference in bacteria species could prompt further investigation as to whether the nematode itself has capabilities in determining what it deems as a good food, and what life history strategies are favored through that food preference.

Diet is clearly a factor that contributes to the observable and measurable life history traits of the *Caenorhabditis elegans.* Changing from one sole food source to another is a dramatic change that cannot be understated, however even changes between bacteria of the same genus can produce measurably different patterns in traits like offspring count. This may suggest that small changes in nutrient composition can have effects that cascade up trophic level. The importance of the connection between diet and life history is something that can be stressed outside the realm of the nematode as well. Organisms of all complexity share a need for nutrients that must be acquired in the environment, and the lack or in balance of those nutrients over time could impact features like post-natal development, reproductive capability, or overall lifespan.

These results emphasize the importance of understanding the complex interactions between metabolism and life history traits. This knowledge will aid in understanding whether there is an optimal composition of nutrients that will lead to favorable more conditions. It will also help to explain why certain feeding behaviors are shared and why some differ, as well as why there are differences in the life history strategy of organisms that uptake a similar balance of nutrients.

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