WHAT'S IN A CHOICE: LINKING FOOD PREFERENCE TO LIFE HISTORY OUTCOMES IN *C. ELEGANS*

by

BEN BLUE

A THESIS

Presented to the Department of Chemistry and Biochemistry
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

June 2015

An Abstract of the Thesis of

Ben Blue for the degree of Bachelors of Science
in the Department of Chemistry and Biochemistry to be taken in June 2015

What's in a Choice? Linking Food Preference to Life History Outcomes in *C. elegans*

Approved:

Patrick Phillips

C. elegans is a bacteriovore capable of consuming a variety of bacterial species. When presented a choice between bacterial foods *C. elegans* exhibits dietary preferences that generally correlate with the ability to support fast developmental rates. These preferred foods have therefore been described as "good quality" foods. However, it is not clear how well these foods mimic the worms' natural diet, nor whether these models are relevant to human diets for health research. Common lab practices skew experimental conditions even further to extremes by exposing worms to a vast excess of food which can have unaddressed consequences on their metabolism. The goal of the work presented here is to use different bacterial strains of variable quality to link *C. elegans* food preference with observed physiological outcomes. This dietary profile allows a better understanding on the internal calculus of resource acquisition behaviors and dietary choice as well as how resources become internally allocated. In order to create this profile we have developed an array of novel and traditional physiological assays that allow for fine resolution of life-history traits such as longevity, health, and reproduction.

Acknowledgements

I would first like to thank Patrick Phillips in whose lab I’ve had the privilege to work in for the last two years and who has been incredibly supportive as I work towards a career in science. Although there is no way I can make this dedication sufficient, I would like to thank Nadine Timmermeyer and Stephen Banse for all of the incredible support, mentorship, and motivation that they’ve provided. I would likely not still be involved in science if it weren't for the mountain of dedication they’ve shown. I would like to thank all the other members of the Phillips lab for all the help they’ve provided with a special thank you to Anna Crist, Joseph North and Katja Kasimatis. I would like to thank my other committee member, Samantha Hopkins as well as the CHC staff for their support in this process. Most of all I would like to thank my family for working so hard to allow me to attend UO. To my loving mother and father, my grandmothers Clara and Mary, and all of the aunts and uncles who’ve been there when I’ve needed them, this is for you.

Table of Contents

I. Introduction 1

- a. Aging and Experience 1
- b. Life-history and Diet 1

II. Background 4

- a. *C. elegans* as a Model Organism 4
- b. Mating and Reproduction 4
- c. Feeding Behavior 5
- d. Bacteria as Food 6
- e. Technology 8

III. Experimental Approach 10

IV. Materials and Methods 11

- a. Culture and Population Preparation 11
- b. Device Fabrication 12
- c. Preference Assay 13
- d. Longevity Assay 15
- e. Reproduction Assay 16
- f. Health Assay 17

V. Results 19

- a. Egg-counter 19
- b. Arena 21
- c. Electropharyngeogram 23
- d. Preference Data 24
- e. Reproductive Patterning 25

VI. Discussion 26

VII. Conclusion 29

VIII. Glossary 30

IX. Bibliography 31

Introduction

Aging and Experience

While aging is inevitable, the factors that determine the speed at which it occurs and the degree to which it affects an individual's health vary wildly between individuals. Within each organism's genetic code there are predispositions towards certain life-histories. These can take the form of increased or decreased capacities to process nutrients, fight off disease, or resist cancer. However, even genetically identical twins quickly become distinct as they develop because of the unique environmental factors around them. The huge degree to which these environmental factors can influence biology indicate that life-history, while built upon genetic predispositions, is alterable by individual environmental interactions. What remains difficult to unravel is how innumerable events that a person endures and the choices they make shape their ultimate life-history. In essence, many of the mechanisms that tie an individual's experience with their biology are either ill-defined or unknown. The motivating goal of this thesis is to provide linkages between cause and effect within the scope of life-history traits. However, the multitude of possible causes prevents a holistic analysis at this point. It was therefore necessary to limit the scope of examination to a single input while examining its effects upon an individual. As the human diet is one of the most discussed mediators of health in both popular and professional science, dietary input was an attractive candidate for analysis.

Life-history and Diet

The interaction between diet and health is often reduced some poor foods lead to poor health while others are good and lead to healthy individuals. However, in the last

century the medical and biological fields have generated an immense amount of data pertinent to how nutrient intake affects development, health, and aging. While in theory these advances should lead to consensus about the ideal diet, every passing year sees the advent of a dozen new nutritional fads. As the popularity of veganism, paleoism, gluten and vitamin supplements wax and wane, the distinction between healthy and unhealthy becomes more and more tenuous (Katz, 2003; Shtonda, 2006). How then does an individual choose their diet? While it is generally accepted that vegetables are healthy and fast-food is unhealthy, individuals often make poor choices. Furthermore, the broader scope of an individual's diet cannot be reduced to this simplistic good/bad dichotomy. It has been observed that both the makeup and quantity of nutrients consumed can play a large and complicated role in shaping the course of an individual's life (Akesson, 2014; Ruder, 2012). Broadly, the impacts of diet can be classified as involving physiological health, lifespan, and the patterning of reproduction. The general term for the sum of these linked metrics will be referred to as life-history. What is yet unknown is the degrees of effect and interplay between these factors.

Part of the complication of relating diet to individual choice and consequences is that modern humans have become somewhat removed from the historical context in which these factors evolved. Consciously and unconsciously, the dietary choices individuals make belie paths to different life-history goals (e.g. reproduction, preservation of healthspan), and these paths are of central relevance to human biology, as well as the biology of all organisms. Biologically speaking, one outlook might favor a dietary pattern in which growth rate to sexual maturity and subsequent reproductive patterns are maximized. However, since an individual has only so much energy to

expend, by partitioning it more towards childbearing, the mother has less available for her own physiological health. This means that increased reproduction occurs at the cost of lifespan and health. In fulfilling this goal, a potential mother might favor an excess of calorically rich and growth promoting foods at the expense of health-promoting alternatives. On the other hand, a potential mother might find it preferable to delay or limit reproduction in order to ensure that she can allot more of her resources towards her physiological health, thus extending her maximum lifespan and healthspan. This push and pull between opposing life-history goals and the resulting allotment of available resources is a large part of the internal calculus that mediates the coupled processes of development and aging.

By narrowing the scope of my investigation to dietary intake it becomes possible to experimentally measure how modification of a single, variable input leads to different life-history outcomes. However, in order to investigate the effects of diet upon aging and life-history, a system is needed in which the genetics and environments of the subjects remain standardized while their lifespan, reproduction and physiological health can be measured after being given a known diet. To explore these phenomena *C. elegans* was used as a model organism. However, since conventional techniques allow for too many variables and external sources of confounds, this thesis describes the design and development of several novel technologies that accurately measure life-history traits in a more controlled environment.

Background

Caenorhabditis elegans as a model organism

The nematode *C. elegans* is has been used as a model organism for the better half of the last century and has proved instrumental to the fields of genetics and aging (Brenner, 1974). As a near-microscopic roundworm, *C. elegans* adults measure little more than a millimeter long and are easily visible and recordable through conventional microscopes as well as novel imaging techniques. Additionally, adults only take about three days to develop from eggs and pass through several easily identifiable larval stages.

C. elegans' Reproduction

C. elegans are primarily hermaphroditic selfers, but populations occasionally produce rare males. Since each adult worm produces genetically identical larvae that are genetically identical it is easy to create clonal populations. This allows for a reduction in confounds that may arise from large amounts of genetic diversity and allow us to work with a population that has similar life-history probabilities. This, coupled with their three day life cycle, makes *C. elegans* an easy organism to raise, culture and prepare for experiments while maintaining a standardized environment. As their total lifespan is about two weeks, we have the capacity to observe them from birth to death on a timescale conducive to the experimental process. *C. elegans* eggs are also resistant to mild bleaching that otherwise kills and dissolves hatched worms. Common lab practices utilize the large scale bleaching of populations in order to isolate unhatched eggs. These eggs can then be placed in a buffer solution until hatched at which point they arrest growth at the first larval stage (L1). L1 arrest halts development until such

time as the larvae are reintroduced to food. From this technique we can assemble large populations of developmentally synchronized animals.

As a hermaphrodite, *C. elegans* produces both the sperm and egg necessary for self-fertilization (Brenner, 1974). In contrast to mammalian systems however, sperm is limited rather than eggs. It has also been observed that *C. elegans* reproduction is modulated by dietary intake. In extreme cases of starvation, adults can enter reproductive diapause and extend their reproductive span by up to 15-fold. However, what is not known yet is how more subtle variations in diet stemming from different food choices modulate reproductive rates.

Feeding Behavior

C. elegans is a filter-feeder that uses a neuromuscular organ called a pharynx to pump in food while expelling unwanted liquid (Avery, 2003). Like the human heart, the pharynx is a neuromuscular organ in which contractions and relaxations are triggered by electrical signals which in turn are mediated by serotonergic signaling (Shtonda, 2006). This organ consists primarily of a long hollow tract, or lumen, with muscles arranged around it in triangular symmetry. There are two larger sections, the anterior corpus and posterior terminal bulb. When feeding, the corpus and bulb contract in a syncopated pattern that slowly draws particles into a grinder that then breaks them down. The unique structure of this organ allows for particles of appropriate size to be drawn into the worm's gut while expelling particles that are too large or too small (Avery, 2003). This means that the ideal food for *C. elegans* is selected for not only by its nutritional load, but also its physical dimensions. Appropriately enough, the *E. coli* normally fed to *C. elegans* grown in labs falls within this range. It has also been observed that a

mismatch results in a greater negative effect than sporulation, a method of bacterial reproduction that causes them to be difficult for *C. elegans* to digest (Shtonda, 2006). It is therefore possible that some strains of bacteria which will be discussed further below are judged by *C. elegans* solely on their physical characteristics. *C. elegans* feeding is controlled by twenty different motor neurons of over a dozen varieties which are linked to the worms internal and external sensory systems.

C. elegans feeding behavior oscillates between cycles of roaming and dwelling. The former involves bursts of long straight movement that has been implicated in the search for food while the latter is comprised of slow movement and frequent stops and reversals (Avery, 2003). These frequent stops and reversals would allow worms to maintain relative location on a food source until it was consumed or they were sated. Furthermore, after roughly 30 min in the absence of viable food, *C. elegans* halts nearly all reversals and engages in roaming behavior. Roaming and dwelling have also been linked to *C. elegans* exposure to non-preferred and preferred food respectively.

C. elegans and bacteria

There is a small body of literature describing possible bacterial sources on which *C. elegans* can grow and some of the effects that these different strains have been previously examined (Albrecht, 2011; Avery, 2003; Bargmann, 1993; Shtonda, 2006; Whitesides, 2001). From these earlier works, we selected several possible food sources of what should be varying dietary quality. These food sources consisted of three *E. coli* strains (OP50, HB101, DA837), two *Bacillus* strains (DA1880, DA1885), and one species of *Comamonas* (DA1877). Previously, Avery and Shtonda observed which of these strains *C. elegans* preferred as food. It was observed that *C. elegans* preferred

DA1877 and one other strain of HB101 to all the other possible options. Next, they measured developmental rate on each food source and found that DA1877 and HB101 also promoted the fastest rate of growth from the first larval stage to adulthood. The most likely causes for the unpreferred strains promoting less growth was due to either bacterial pathogenicity, decreased nutritional content, or physical incompatibilities with the pharynx itself. Using a series of mutational analysis they determined that it was the size of the bacterial cells or cell aggregates that limited their consumption and *C. elegans* subsequent development (Shtonda, 2006).

The nutritional content of the bacteria obviously also plays a part in how *C. elegans* is affected by each strain. DA1877 has been shown to provide greater levels cyancobalamine and folate than other tested food sources (Lai, 2000). Folate is needed for the synthesis of nucleotides and the transfer of single carbon units while cyancobalamine acts as a coenzyme for several key metabolic processes. The intake of these essential vitamins likely played a part in increased developmental rate and preference shown but at a physiological trade-off. *C. elegans* grown on DA1877 show decreased fertility and shorter lifespan (Avery, 2003). Macronutrient composition also differs between strains and species and it has been shown that these differences can correlate to differences in fat storage levels and fatty acid composition within *C. elegans* (Brooks, 2009). These trends correlate to the degree of evolutionary divergence among strains and it is likely that the pattern of observed benefits and deficits conferred upon *C. elegans* will follow a similar pattern (Figure 1).

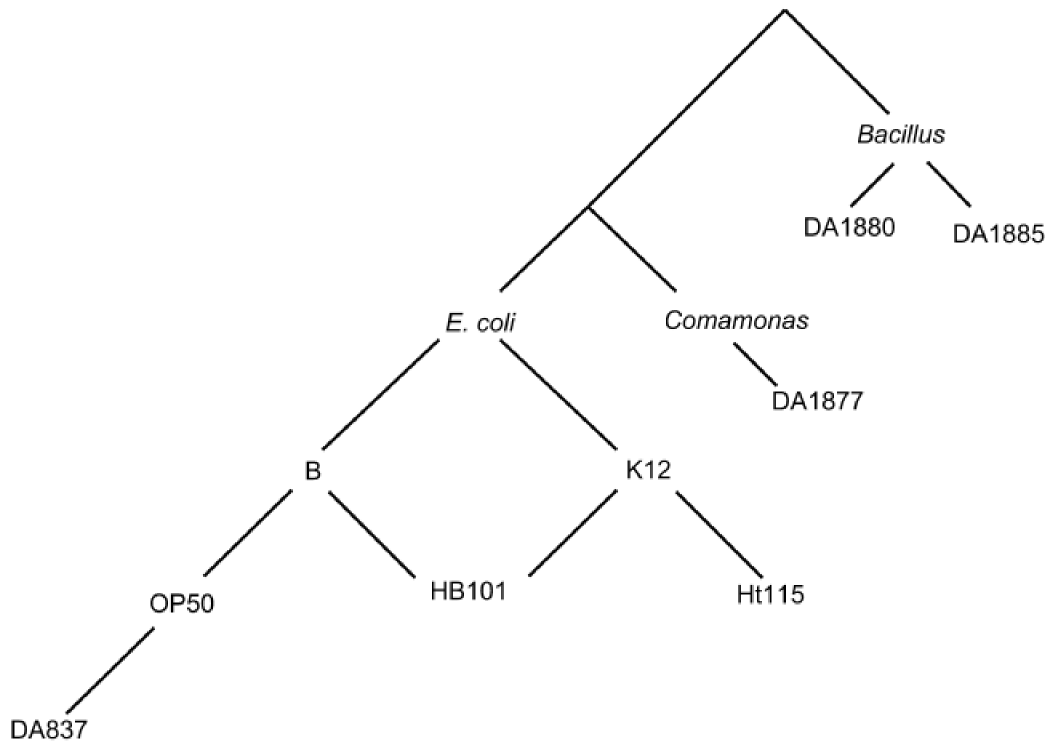

Figure 1. Ancestry of bacterial food sources. All but Ht115 were used in this study. OP50 is the strain most commonly used as a food source in labs (Brooks, 2009).

Technology

A large part of this thesis was the design and development of an array of microfluidic devices. Initial experiments were performed conventionally to determine food preference and reproductive response but it was soon observed that even within the relatively controlled environment of a petri plate, there were too many unknown or immeasurable interactions that precluded the drawing of definite conclusions. In order to control the environment more fully, it was necessary to limit the amount of physical and chemical stimulations that the worms could feel. To do so we primarily turned to microfluidics as a solution (Albrecht, 2011; Lockery, 2012).

A microfluidic device consists of a silicon polymer block bonded to a glass slide. Using photolithography, small channels less than 100 microns tall are made in the block before it is bonded to the glass. These channels are filled with liquid and provide a stable environment for the worms. At this scale, fluid physics becomes much less complicated. The flow of liquid becomes laminar and turbulence and vortices become essentially non-existent. The mathematics that underlie the fluid flow become essentially the same as those that determine the flow of electricity in a circuit. This allows the design of environments within which worms can be exposed to combinations of liquids and bacteria in a controlled manner. This allows the worms sensory perception to be limited to only what is flushed through the chip, enabling near-complete environmental control. Within the scope of this thesis I will address the design and use of three different microfluidic chips that measure preference to food, egg-laying rate, and the physiological health of the worm. These are respectively referred to as the Arena, the Egg-Counter, and the EPG chips.

The LifeSpan machines were developed by the Fontana lab and were adapted for use in the Phillips Lab. These modified scanners allow the automated collection of lifespan data from large populations of worms. An array of 35 devices was constructed by the author for use by the Phillips Lab. This allowed data acquisition to be completed with minimal help; a task that otherwise would have required several researches over the course of several months. Additionally, several devices were repurposed for integration with microfluidic devices.

Experimental Approach:

Nematodes underwent observation starting at adulthood and continuing until death (Figure 2). Until adulthood they were raised on the same strain of *E. coli* commonly used in lab to maintain environmental homogeneity. Our initial experiments were a series of choice assays using a microfluidic chip to determine the relative preferences *C. elegans* has for different types bacteria. In order to determine how the preferred foods were affecting *C. elegans* it was necessary to design several different devices that would allow controlled examination of *C. elegans* physiology. LifeSpan machines were built to allow high-throughput analysis of how *C. elegans* longevity was affected by each food. However, both malnutrition and an excess of food can shorten lifespan. In order to reveal if the nematodes were being affected positively or negatively by their food, their physiological health at each day of life would need to be determined. A microfluidic device was developed from an existing model that would take electrical recordings of the pharynx and then determine the frequency of their pharyngeal pumping. This newer model allowed for better signal resolution and higher-throughput screening of populations. Through supplementation of serotonin, it was possible to stimulate maximal pump rate and then measure how physically fit they are at each day of life. This is akin to a treadmill test wherein a human subject would be forced to run till exhaustion with the important data point being the speed and duration of the run. To understand how *C. elegans* partitions its resources on different foods we compared the amount of progeny produced upon initial contact with bacterial strains. This led to the development of a microfluidic automatic egg counting device that records each reproductive event of an individual.

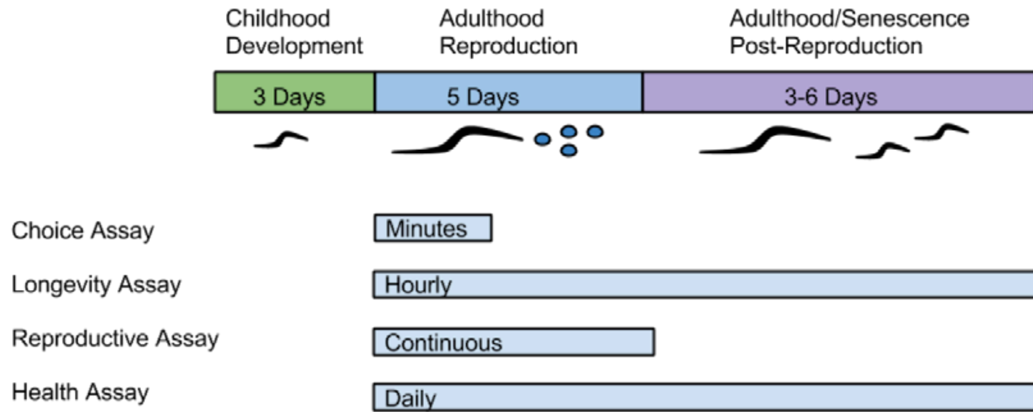

Figure 2. Timescale of experimental work within the lifespan of *C. elegans*

Materials and Methods

Cultures and strain preparation

Six previously described bacterial species (Table 1) were used as dietary input (Avery, 2013). Isolates obtained from the Lockery Lab as frozen glycerol stocks. Cells from frozen stocks were streaked out on LB agar. Once colonies were grown, a single colony is transferred to a container of filled with standard bacterial growth media. This mixture provides a rich environment for the bacteria to grow if set on a shaker for 24 hours at the proper temperature. Once the cultures reach stationary phase, the bacteria will be pelleted and rinsed. The remaining bacteria will then be re-suspended in S-basal buffer. The density of the bacterial suspensions was determined by measuring the optical density of the solution and comparing to standardization colony count curves determined through streaking out dilutions and measuring colony forming units. All suspensions of all bacterial strains used were then diluted to 10^9 CFU/mL.

Bacterial Name	Bacterial Species	Growth Temperature
OP50-1	*E. coli*	37
DA837	*E. coli*	37
HB101	*E. coli*	37
DA1880	*B. megaterium*	28
DA1885	*B. simplex*	28
DA1877	*Comamonas sp.*	37

Table 1. Bacterial strains used

Nematode strains were obtained from the *Caenorhabditis* Genetics Center and are maintained at 20°C. Age synchronized populations are obtained by bleaching plates with large numbers of adults and eggs. The bleach kills and dissolves the adults while leaving the eggs unharmed. The bleach is rinsed away and then the eggs suspended in S-basal buffer. After 18 hours the larvae hatch and arrest development due to a lack of food. When plates are prepared the larvae are pipetted onto bacterial lawns and begin developing in synchrony.

Microfluidic device fabrication

Microfluidic devices were fabricated using a standard photolithography process (Whitesides, 2001). Computer aided drafting software was used to create a two dimensional to scale outline of the desired device. Once designed using CAD program known as Vectorworks, a blueprint of the chip is sent to a printing company and they return a mask, or stencil, printed on a plastic sheet. This mask is then used to make a master on a round silicon wafer. First, a layer of photo-resistant polymer was spun-coat

onto the silicon wafer. Because the height left behind after rapid spinning is consistent if the viscosity and RPM are known, this layer is able to be made to any desired height. After the polymer coats the wafer, the mask was placed over it and intense UV light shone over it. Any place touched by the UV light hardened so all of the features of the stencil are left on the silicon wafer. Excess photoresist is then washed off. The remaining material resulted in the formation of raised sections that constituted the features on the final device. Next the master was coated with silane so that it became non-adhesive. A silicon-based polymer was poured over the master and allowed to harden. Because of the silane, the desired portions could be excised with a scalpel and gently peeled off the master. Inlet and exit ports were then punched in the silicon chip. Lastly the silicon polymer chip was placed with a clean glass slide in a plasma-bonder. Once removed, the glass and polymer covalently bonded upon being pressed together. Any areas that were blank spots on the stencil are then channels through which liquid and worms can flow on the finished device.

Preference assay

Initial preference assays were carried out on 35mm petri plates filled with NGM-lite agar. 60 uL spots of concentrated bacterial cultures were pipetted roughly a centimeter apart and then allowed to dry. Once dry, 10 worms were transferred onto each spot. The worms were taken from a synchronized population of day 1 adults grown on either OP50 or HB101. The worms were observed one and twenty four hours after their transfer and were scored according to which bacteria they were on or if they were outside bacterial spots.

Secondary experiments were performed using the Arena microfluidic chip (Figure 3). After fabrication the chip was flushed with a Pluronic F-127 treatment consisting of 5% Pluronic in S-basal buffer to reduce friction that the worms could experience from the walls of the chip. Next, 30 worms are loaded into either of the inlet ports and flushed inside. Two syringes are attached and diluted bacterial solution flushed through. By suspending the syringes in a shaker above the plane of the chip, gravity can act as an impetus to create flow. This apparatus is then taped to the glass of a modified Automated LifeSpan Machine (Figure 4) so that the chip remains in place once the shaker is turned on. The LifeSpan machine takes an image of the entire chip every 2 minutes. Afterwards, the images are converted into video files and the worms locations at each time point are recorded by hand. This data is then converted to a preference index that compares the number of worms in food A versus food B over the course of the experiment (Bargmann, 1993).

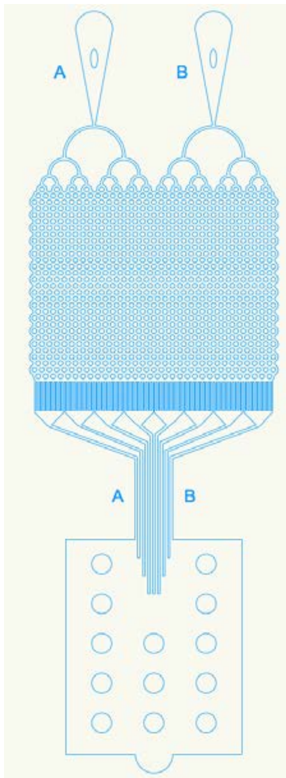

Figure 3. Arena chip.

Figure 4. Bank of 20 Lifespan machines.

Longevity assay

The longevity assay was performed using an array of LifeSpan Machines. These are modified computer scanners that image a set of petri plates once per hour (Stroustrup, 2013). After image acquisition, a program devised by Nicholas Stroustrup detects the location of each worm. At the conclusion of the experiment the program works backward to determine the time point at which each worm stops moving, a behavior indicative of death. The devices were prepared by pouring 16, 65mm, tight-seal petri plates with NGM-lite agar. Additionally, nystatin was added to prevent fungal contamination and FUdR is added to prevent the nematodes from procreating while on the scanner. Next, a 200 uL drop of the desired bacterial strain was added and then let dry. The plates then had 35 worms transferred onto them and were sealed onto a glass

sheet with silicone grease. Lastly, the glass sheet was transferred to the scanner and the run started. Typical run length was 30 days as that provides a large excess of time after the point at which all worms should have died.

Reproduction assay

Initial data was collected by measuring the number of eggs laid in a one-hour period on an agar petri plate. The worms were grown to adulthood on the common lab strain of *E. coli* and then removed from food and starved for one hour before being transferred to a small petri plate that had been spotted with one of the bacterial strains. After an hour we removed the nematodes and counted the number of eggs left behind. The number of eggs laid on bacteria mostly coincided with the strains they preferred more. However, some data for poorly preferred strains became artificially inflated as the worms would roam away from the bacterial spot and then release eggs as soon as they ran into the wall. Attempts to remove this boundary effect were performed by getting rid of the intervening space. Copper rings were affixed to new petri plates and their inner area filled with bacteria so the culture was flush against the ring. As copper repels nematodes, it was hypothesized that upon interaction with the copper perimeter they would return to the food. However, this resulted in much more sporadic egg laying overall and reduced fecundity. To fully reduce the boundary effects from the transition between bacteria, foodless agar, or copper we devised a microfluidic egg counter.

The egg counter device holds 16 worms in individual chambers through which a food solution is flushed and eggs can flow out (Figure 5). After treating the chip with Pluronic F-127, 16 adult worms were added to the inlet port and with the use of a syringe, pushed through a distribution network into individual channels. Once all

worms reside in chambers, flow containing food was allowed to flow through. As eggs were laid they were flushed out through exit channels. The exit channels converge at the imaging station and as eggs pass through channels a video camera takes an image and timestamps each event. These images were then analyzed and pixel discrepancies used to estimate each egg-laying event. As each channel only stems from a single worm, it is possible to trace back and measure the fecundity of each individual worm.

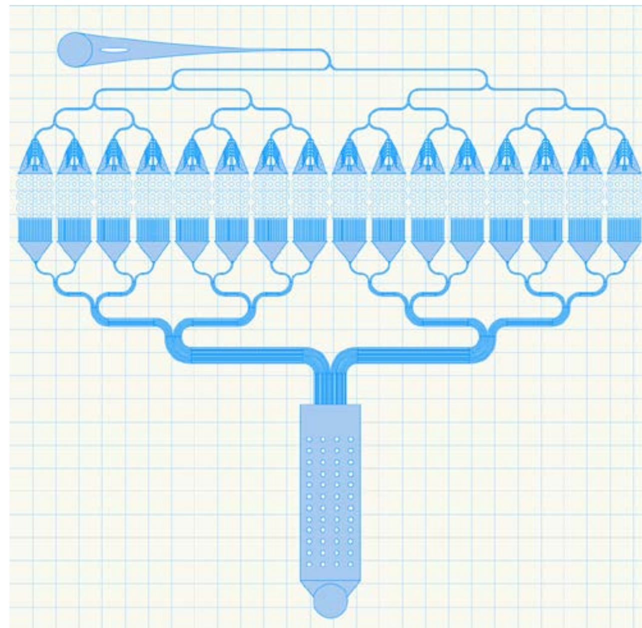

Figure 5. Schematic of egg counter. Lighter blue areas are the growth chambers in which worms reside. The inlet is at the top and the exit is at the bottom.

Health assay

Pharyngeal pump rate is used to as a metric of health and collected through electrical recordings of the neuromuscular organ itself. Traditionally methodology used a visual analysis of a single worms pharynx to determine pump rate. However, using a microfluidic device adapted from one developed in the Lockery Lab it is possible to simultaneously measure the pumping of 8 worms at once (Lockery, 2012). Worms were

picked from synchronous populations and 8 are placed in a glass wash dish containing several mL of M9 buffer with 10mM serotonin. After being exposed to serotonin for approximately 10 min, the worms begin pumping at their maximal rate. They were then loaded into the EPG chip through a distribution network similar to the one described previous. The EPG chip was then placed in its frame and the inlet valve is connected to a syringe pump that flows 10mM serotonin in M9 solution through the device at a rate of 6uL/min. This flow ensures both a constant treatment with serotonin and that the worms stay lodged in the constriction points. This flow was connected to a reference electrode. Next, electrodes are inserted on the opposite sides of the constriction points. The orientation of each worm in the chip is then noted as this can slightly affect the resulting signal. Once all these preliminary steps have been performed we begin data collection and allow it to continue for ten minutes, after which the chip is removed and the next one inserted. Once the raw data is collected, we can draw out the signals that mark the contractions and relaxations of the pharynx (Figure 6).

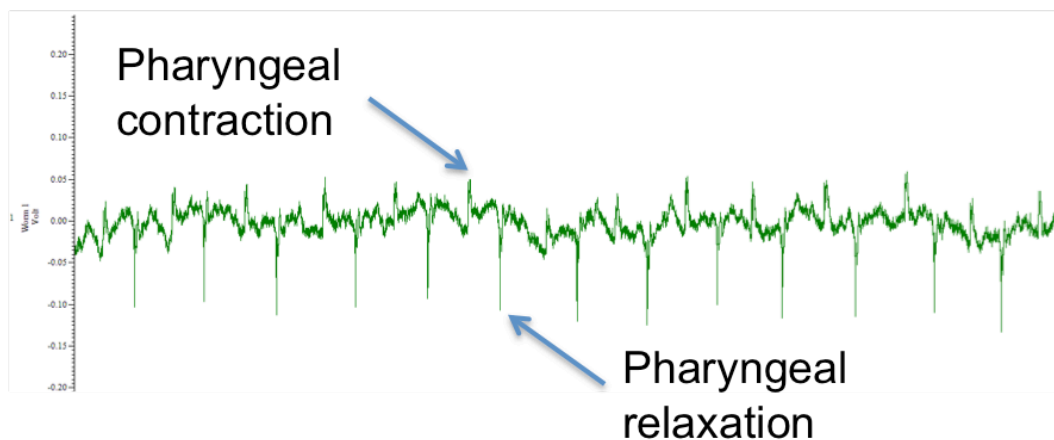

Figure 6. Example of EPG recording with annotations.

Results

Egg-counter

A major goal for the technology discussed herein was to create living chambers in which worms could grow, reproduce, and age under observation and in tightly controlled environments. The design and development of these microfluidic devices stemmed from chip developed by Elizabeth Hulme. This device allowed for an array of eight worms to be housed in individual chambers. Food could be channeled through the device and the worms would be allowed to grow, develop, and reproduce under complete control and observation. Initially, we attempted to fabricate our own but this device relied upon a failure-prone system of valves for loading and animals that proved difficult to replicate on the scale desired even with 3D printing and other modern methods of fabrication. We also attempted to provide each chamber with its own entrance and exit port so that worms could be loaded individually but this proved too time intensive and the amount of tubing required obscured viewing. The next iteration involved a series of contraction points that emanated from a larger channel. In this design, a population of worms could be flushed through under low pressure till each contraction point was filled. Then the excess worms would be flushed out the main channel while the ones in the contraction points would remain. A final high-pressure push would force the worms through the contraction points and into the chambers beyond. However, this design had not undergone mathematical analysis using equations for how flow is governed under microfluidic conditions and it soon became apparent that flow along the main channel decreased steadily so the majority of worms in chambers would be found in the ones first along the channel. Additionally, the

increased flow in the fore of the channel led to multiple worms getting pushed into single chambers. We then adapted a branching pattern of channels from a single entrance that allowed for worms to go into individual choke points while still keeping the path length of each channel the same. While the worms distributed evenly the chokepoints didn't allow easy access of the worms into the chambers. This was solved by loading through the exit port, a solution that led us to forego the choke point entirely. It was observed that while loading, back pressure was often needed to allow individual worms to go back a branch in the channel network but this often led to correctly loaded worms being pulled back out. In order to maintain the worms in the chamber we tried several different configurations of entrances so that worms could enter but not leave. We finally settled on an arrangement of pillars that would allow back flow but would block the worm from leaving.

The next aspect of design was the interior of the chip itself. The Hulme chip chamber was essentially a small bubble with smooth inner feature. Once loaded the worms would be essentially in a completely liquid environment with little physical stimuli except for interactions with the smooth walls surrounding them. However, in liquid culture, *C. elegans* thrashes rapidly and undergoes large stress from this exertion. Additionally, worms in liquid culture are more likely to "bag" and stop egg laying even though fertilization is still occurring. This leads to larvae hatching inside and killing the parent hermaphrodite. Both of these behaviors were observed in the Hulme design. In order to provide some physical stimulus and allow normal movement, pillars were added to the chambers that the worm could push off of. Several sizes and shapes were

tried and finally round posts spaced roughly 100 um apart were decided upon. Figure 7. shows the development of the Egg-Counter chip from the original Hulme design.

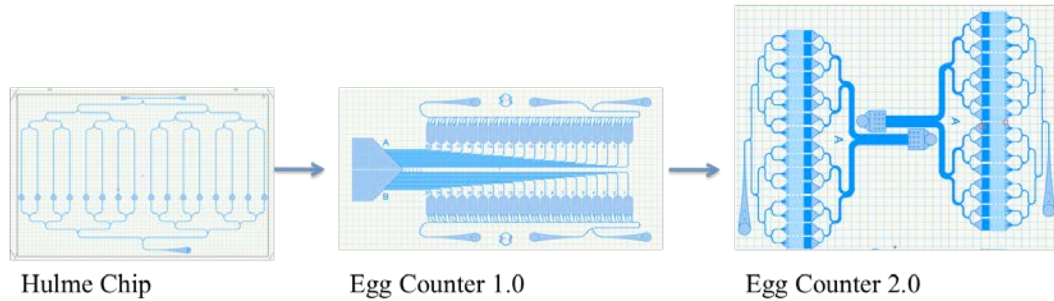

Figure 7. The development of the egg counter. The Hulme chip consisted of single empty chambers and a valving system for loading. Egg Counter 1.0 has arenas with pillars and a flowthrough distribution system. Egg counter 2.0 has a distribution network for loading, arenas with pillars, and sieve-like traps to keep worms in chambers.

Arena

While the previous device allows for high resolution of individual worms, we also wanted to observe large populations in a microfluidic environment. The Arena chip was a design through which 4 different solutions could be flowed and their individual runoffs collected. This was changed to a single exit port but found that worms were often leaving the pillars and flowing out with the runoff. By implementing long narrow filter channels flow was allowed but worms were kept inside the arena. It was at this point that the LifeSpan machines had been developed and we decided to integrate one with the Arena chip. By changing the focal plane of the scanner by the width of a glass slide the machine could then automatically capture images of the entire arena. While the scanning process normally takes 15 minutes per sample, by targeting many smaller areas for scanning, we were able to bring the temporal resolution down to a single minute. An additional problem was that the intense light of the scanner whitewashed the scanner images. Recreating shadowing effects that had originally been problematic for

LifeSpan machines actually increased the contrast and allowed clear destinction between worms and chip features. This was done by orienting the chips closer to the edge and raising the lid of the transparency unit, thereby increasing shadow effects and allowing for higher contrast in the images.

Our goal with the Arena chip was to observe where populations of worms would self-locate when different bacteria were flowed through different sides of the device. However, bacterial particles easily adhere to PDMS and can form blockages that can alter and even halt the flow of liquid. Our first steps in combating this were to initially flush devices with Pluronic F-127 to create non-stick surfaces inside the device, and to use flow through mixed with Triton-X, another surfactant. While this proved sufficient for loading, when under operation our devices are gravity fed and the bacterial particles sedimented and then flowed through in over-concentrated amounts that created micro-lawns inside the device. By keeping the inflow syringes on a shaker for the course of the experiment we were able to provide bacterial food in solutions and little to no aggregation was observed (Figure 8).

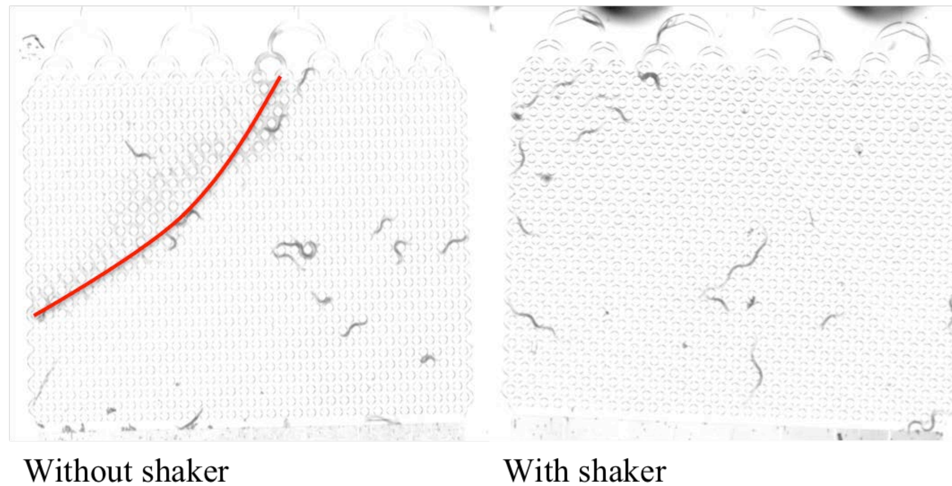

Figure 8. The effects of bacterial contamination on arena chips with and without shaker. Both images are from arenas that have had bacterial solutions flushed through for 3 hours. Red line highlights area in which bacteria have formed large aggregate clusters.

Electropharyngeogram

An aspect of the project for which design is still underway is the development of a high-throughput EPG recorder. By merging a traditional EPG recorder with a microfluidic worm sorter, we will be able to take EPGs of populations in a fraction of the time it would normally take with the EPG chip described in the methods section. One already observed benefit is that the EPG sorter will allow for clearer signal due to the decrease in path length. We originally observed this by modifying the Lockery lab's device with the reference electrode immediately adjacent to the worm rather than far away. Once this was done the signal to noise ratio increased dramatically (Figure 9). As some long-lived strains of bacteria have very faint EPGs, this will allow for proper capture of their physiological metrics.

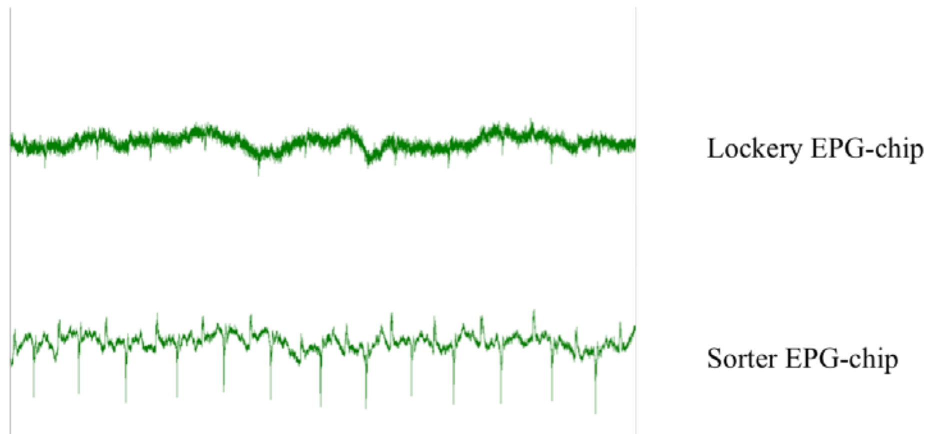

Figure 9. Comparison between Lockery and Sorter EPG chip signals. As indicated by the clearer peaks and valleys, the signal to noise ratio is much lower on the sorter chip.

Preference Data

Average preference data recorded by a Lifespan scanner of worms in an Arena chip showed that in this environment, DA837 was the most preferred food in relation to all the others (Figure 10). DA1877 and HB101 were both preferred over at least one food type and OP50 was not preferred in relation to any of the other strains. Temporal analysis looking at the trends over the total runs showed preferences were initially strong but then were often lost over time (Figure 11).

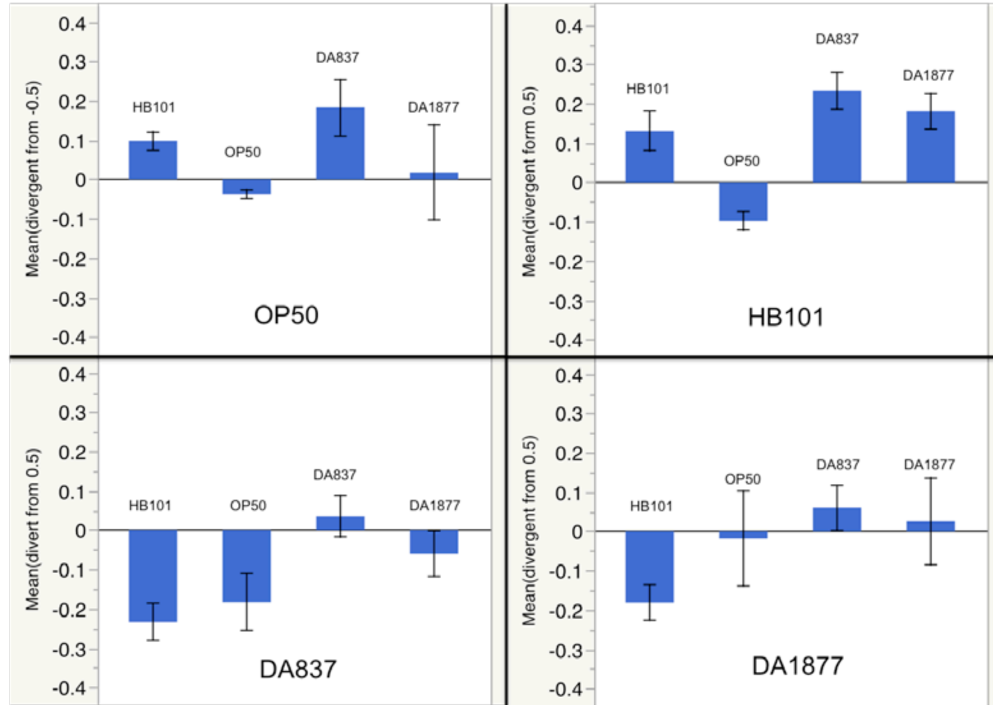

Figure 10. Average preferences for four strains as collected in an Arena Chip. Clockwise from top right are OP50, HB101, DA837, and DA1877. Midline of 0 represents no preference. Bars below show preference for base strain while bars above 0 show preference for comparison strain.

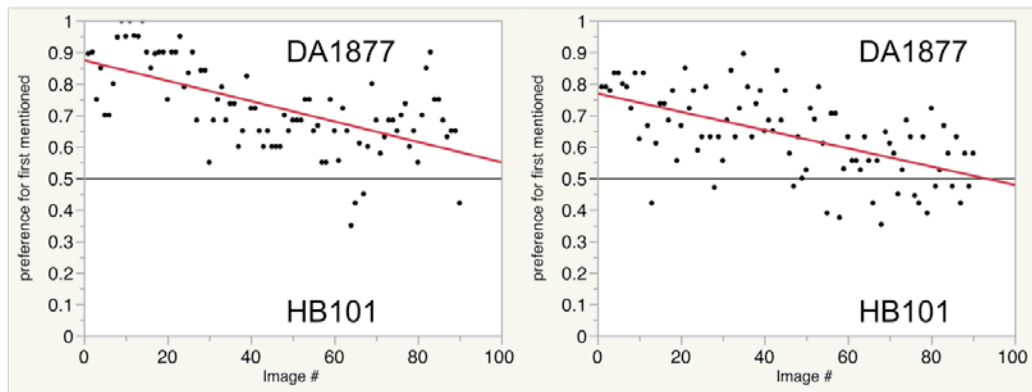

Figure 11. Change in preference over time. Midline at 0.5 represents an equal distribution of worms on each food presented. Red bar is fit for the distribution of worms as it changes over a three-hour period. Left and right graphs each show one replicate.

Reproduction Patterning

Egg laying data was collected from day 1 and 2 adults on all 6 possible bacterial strains (Figure 12). This data shows the induction of egg-laying as a sensory response to

initial contact with food rather than how well a solid diet of food impacts egg-laying through metabolic benefits. OP50 and DA1877 showed the greatest induction of egg-laying while HB101 and DA837 had much lower values. DA1880 and DA1885 showed artificially increased egg counts as this data also represents eggs lain through interaction with the petri plate which was heavily present in these two strains.

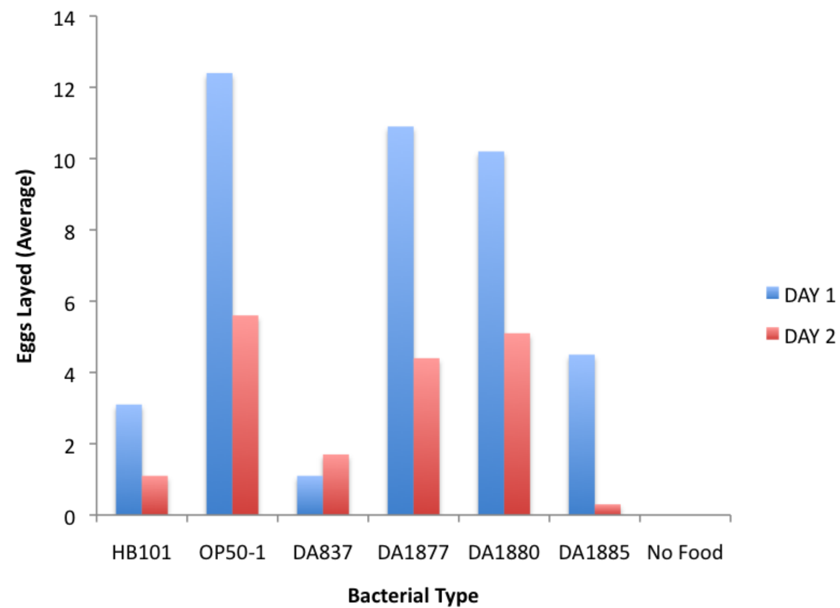

Figure 12. Egg laying response to bacteria in a one hour period shown by day 1 and 2 adults.

As this data represents the induction of egg laying from a sensory response it should be expected that familiar food such as OP50 would stimulate a large response. Since worms were not given an opportunity to metabolize the bacteria in question, how each strain effects total reproductive output is still unknown. As the Egg-counter becomes more fully operational it will be possible to collect such data.

Discussion

In order to accurately link cause and effect for a set of dietary inputs to life-history outputs it was necessary to develop several new technologies. Through the use

of the Arena chip, it was possible to increase the temporal resolution and reduce superfluous and confounding environmental inputs. This reduction of external signals, such as the aroma from each strain, could have led to the observation that the preferences observed using this method were different than those collected from preliminary experiments and the literature (Fig 13).

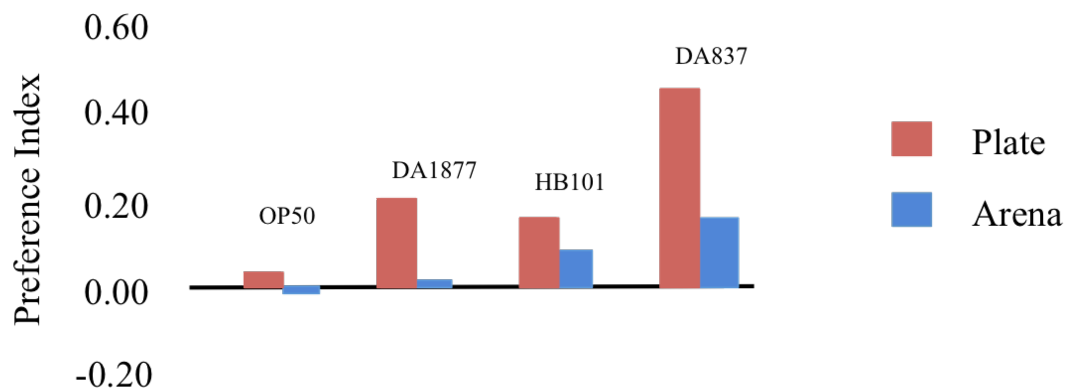

Figure 13. Comparison between data collected from plates and off the arena chips. All data is in comparison to OP50. Midline represents no preference while a positive value represents a preference for the option compared to OP50.

The biggest difference between our data and that of the literature was that DA837 was remarkably more preferred over normally preferred strains such as DA1877 and HB101. In the literature, DA837 was shown to be a poorly preferred and slow growth-promoting food because cells formed aggregates that were too large for *C. elegans* to digest easily. The marked preference for DA837 in the arena over HB101 and DA1877 could be due to both the agitation of the shaker breaking up aggregates through mechanical action, as well as the inclusion of the Pluronic F-127 and Triton-X detergents. Another possibility for these differences is that our assay was performed over the course of a three hour span rather than a full nine hours like those in the literature. In order to verify our preferences it will be necessary to repeat these experiments for longer durations.

Additionally, while it was expected that the controls experiments should trend towards no preference due to the same bacteria on either side, it was observed that most populations of worms trended towards no preference even when presented with combinations of bacteria. This suggests that dietary choices are not absolute and that a combination of food types could be healthier than a single option. However, to confirm this more physiological data, such as EPGs or reproductive patterning, will need to be confirmed. Another option would be to present individuals with either a single choice or a combination and observe how preference changes over time. If this were to be confirmed by future research it would suggest that the good food/poor food model espoused in much of the literature is actually far more complicated and that there is some benefit to individuals occasionally choosing "bad" foods.

To contextualize the decisions made by the *C. elegans* when presented with the aforementioned choices, several devices were designed in order to determine what the physiological outputs were for each of the given inputs. Using microfluidic technology, we developed experimental systems in which we could measure both reproductive patterning and health over the course of individual worms adulthoods. While data is not yet available for analysis here, both the EPG and Egg-counter devices are nearing stages at which large volumes will be able to collected in a high-throughput manner. However, the length of life conferred by growth on each strain of bacteria was collected using LifeSpan technology but due to rampant bacterial and fungal contamination, the computer assisted processing of images is still underway.

Conclusion

The central goal of this thesis was to examine how different life-history paths are governed by environmental interactions within the scope of an individual's food preference. The initial starting experiments involved common lab practices and conventional methods of data acquisition. It was soon observed that tighter control of the environment was needed than these methods would allow. To that end, several novel microfluidic devices such as the Arena and Egg-counter have been designed, developed and undergone testing. These devices minimize undesirable environmental factors and facilitate higher-throughput recording of physiological and behavioral traits. In the work presented above, the arena chip has already allowed *C. elegans* preferences to be determined with a better combination of temporal resolution and throughput than traditional methods. Expectedly, the elimination of variables such as food edges and aroma changed some of *C. elegans* preferences and showed that in a controlled environment many preferences are not absolute. As more devices reach the data collection stage of development, these established preferences will provide context for future research in which the life-history outcomes for *C. elegans* are linked to the strain of bacteria provided as food.

Glossary

Microfluidic- Term used to define how fluids operate when flow volume is less than 100 um.

PDMS- Polydimethylsiloxane. Polymer used to make microfluidic chips.

Cyancobalamine- Vitamin B12.

Folate- Vitamin B9

EPG- electropharyngeogram. waveform recorded derived from the change in electric potential of the worm as its pharynx pumps.

LifeSpan machine- Modified office scanner that automates the collection of lifespan data.

OP50- *E. coli* strain most commonly used in laboratory setting

HB101- *E. coli* strain produced through crossing B and K12 lines.

DA1877- *Comamonas* strain that provides increased micronutrients.

DA837- *E. coli* strain observed as low preference in Avery paper. Observed to form cellular aggregates.

DA1880- *Bacillus megaterium*. *Bacillus* strain that has cells too large for *C. elegans* to injest as well as a spore coat.

DA1885- *Bacillus simplex* strain that produces spores.

Bibliography

1. Albrecht DR, Bargmann CI (2011) High-content behavioral analysis of Caenorhabditis elegans in precise spatiotemporal chemical environments. Nature methods 8: 599–605.

2. Akesson A, Larsson SC, Discacciati A, Wolk A. Low-risk diet and lifestyle habits in the primary prevention of myocardial infarction in men: a population-based prospective cohort study. J Am Coll Cardiol. 2014;64(13):1299-306.

3. Avery L (2003) Food transport in the *C. elegans* pharynx. Journal of Experimental Biology 206: 2441–2457.

4. Bargmann CI, Hartwieg E, Horvitz HR (1993) Odorant-selective genes and neurons mediate olfaction in *C. elegans*. Cell 74: 515–527.

5. Brenner S. The genetics of Caenorhabditis elegans. Genetics. 1974;77(1):71-94.

6. Brooks KK, Liang B, Watts JL. The influence of bacterial diet on fat storage in *C. elegans*. PLoS ONE. 2009;4(10):e7545.

7. Katz DL. Pandemic obesity and the contagion of nutritional nonsense. Public Health Rev. 2003;31(1):33-44.

8. Lai CH, Chou CY, Ch'ang LY, Liu CS, Lin W. Identification of novel human genes evolutionarily conserved in Caenorhabditis elegans by comparative proteomics. Genome Res. 2000;10(5):703-13.

9. Leblanc JG, Milani C, De giori GS, Sesma F, Van sinderen D, Ventura M. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. Curr Opin Biotechnol. 2013;24(2):160-8.

10. Lockery SR, Hulme SE, Roberts WM, Robinson KJ, Laromaine A, et al. (2012) A microfluidic device for whole-animal drug screening using electrophysiological measures in the nematode *C. elegans*. Lab on a chip: 2211–2220.

11. Maier W, Adilov B, Regenass M, Alcedo J. A neuromedin U receptor acts with the sensory system to modulate food type-dependent effects on *C. elegans* lifespan. PLoS Biol. 2010;8(5):e1000376.

12. Ruder EH, Thiébaut AC, Thompson FE, et al. Adolescent and mid-life diet: risk of colorectal cancer in the NIH-AARP Diet and Health Study. Am J Clin Nutr. 2011;94(6):1607-19.

13. Shtonda BB, Avery L (2006) Dietary choice behavior in Caenorhabditis elegans. The Journal of experimental biology 209: 89–102.

14. Stroustrup N, Ulmschneider BE, Nash ZM, López-Moyado IF, Apfeld J, et al. (2013) The Caenorhabditis elegans Lifespan Machine. Nature methods 10: 665–670.

15. Turner BL, Thompson AL. Beyond the Paleolithic prescription: incorporating diversity and flexibility in the study of human diet evolution. Nutr Rev. 2013;71(8):501-10.

16. Walhout AJ. Genetic adaptation to diet preserves longevity. Cell Metab. 2014;19(2):177-8.

17. Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE (2001) Soft lithography in biology and biochemistry. Annual review of biomedical engineering 3: 335–373.

18. Yilmaz LS, Walhout AJ. Worms, bacteria, and micronutrients: an elegant model of our diet. Trends Genet. 2014;30(11):496-503.