

# Summer Research Fellowship Proposal

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## Page 1

Summer Research Fellowship Proposal (no more than 4-pages double-spaced = 1,200 words in length).

### Introduction/Background:

We are trying to determine whether we can use an aging assay in *Caenorhabditis elegans* as a model for the effects that nutritional supplements might have in humans. The specific question that we are trying to answer is whether nutritional supplements alter the lifespan of the organism *Caenorhabditis elegans*. Other researchers have used *C. elegans* in studies to monitor the effects of compounds on lifespan with the idea of using *C. elegans* as a model for human aging. Wilson and colleagues (2006) found that blueberry polyphenols caused increased lifespan in *C. elegans*. Harrington and Harley (1988) showed that certain concentrations of vitamin E extended lifespan. *C. elegans* works well for these experiments because they have a relatively short lifespan, many offspring, and are easy to maintain in the lab (Wood, 1988). There are also genetic mutations we can test to determine what cellular pathways are required for any affects we observe. For example, the insulin signaling pathway has a known role in aging (Murphy and Hu 2013). This pathway is also highly conserved in humans (Murphy and Hu 2013). Dr. X has been working with *C. elegans* for many years, more recently studying insulin signaling. I have been working with Dr. X since last fall through the Research Apprenticeship Program and believe that SURF is a good transition point to continue my research. Since last October I have been trying to replicate aging assays of wild type (N2) and *daf-2(e1370)* mutants from previous literature (Chang et al., 1993). For example at 20°C, the wild type worms had a mean lifespan of 20 days and the *daf-2* mutants had a mean lifespan of 42 days (Chang et al., 1993). Our wild type aging assay gave similar results, however, the *daf-2* mutants did not live as long as expected. We suspect that the mutants got mixed up with another mutant, *daf-7*, that does not show increased lifespan but otherwise has a similar phenotype to *daf-2*. This would explain why there is no significant difference between the wild type and suspect *daf-2* worms. We are in the process of using fresh *daf-2* worms to replicate the assays.

Objectives:

In our project we will be looking at lifespan and health span. Lifespan consists of how many days the worm is alive. We will determine whether nutritional supplements cause *C. elegans* worms to have a longer mean lifespan than untreated controls. Health span is concerns how long the worm is active. To detect this, we count how many pharyngeal pumps a worm has in a given period of time.

We will determine whether the rate of pharyngeal pumping is increased in worms treated with nutritional supplements compared to untreated controls. If we identify nutritional supplements that increase mean

lifespan or pharyngeal pumping rates, we will set up assays with

Reviewer 2: No title

Reviewer 2: Good citations.

Reviewer 2: Need an introductory statement. The first sentence should not state the objective of the study. This comes a little later in the first paragraph.

Reviewer 4: Need to provide some introductory, background material for readers with less-than-great scientific knowledge...

Reviewer 2: Need a common name too.

Reviewer 3: This is good for showing a precedent that other researchers have learned useful information about human health by studying the model organism *C. elegans*.

Reviewer 1: It is good that you included sources

Reviewer 2: Give the year.

Reviewer 2: Nice transition from RAP to SURF.

Reviewer 3: It helps to show evidence that the student has experience working with the kind of research they are proposing.

Reviewer 4: Like to see a clear, concise statement of purpose before beginning an enumeration of steps that will be taken to execute the project.

Reviewer 1: Since not everyone who will read this are familiar with this, you might to briefly explain what these are and how they are significant.

Reviewer 3: Sometimes, like in this instance, it can be helpful to define something that might otherwise seem very straightforward (or insignificant), such as how to tell if a worm is living a long and a healthy life.

**mutant strains of C. elegans to narrow down what signaling pathway the supplement is affecting.**

Approach/Methodology:

We are currently using five plates per worm strain. Each plate has about 20 worms on it. The plate is made of NGM agar with E. coli as a food source. To start the assay we first need an abundant amount of worms that are all approximately the same age. In order to achieve synchronization, we obtain hermaphrodites that are full of eggs and expose them to a hypochlorite solution that will kill the adults but leave the eggs. We wash the eggs several times and incubate them overnight, letting the eggs to develop into L1 larvae. We then distribute them to plates and put them in a 15°C incubator until they are adults. Once they are adults we put them into a 20°C or 25°C incubator. The plates are then counted and worms are transferred every other day. They need to be transferred so we do not mix the adults with their offspring. When we are counting and transferring the worms, we may encounter some that appear dead. Poking the worm with the pick will determine if they are still alive or not. If they are dead we will remove them from the plate and make note of it. In total, the experiment could take upwards of 80 days depending on what temperature we hold them at. First, we will test

Vitamin E will first be tested as a positive control. Certain concentrations of vitamin E yield prolonged life for this organism (Harley and Harrington, 1988). After this experiment is concluded we will work with Standard Process to identify candidate supplements to test. It may take substantial time to figure out how to administer the supplement into the food of the worm. The dose of the vitamin is also a key factor. If the concentration is too low or high, it may harm the worms. Another procedure we are looking into is using an automated system to count the worms. The system is a scanner that takes images of the worms and recognizes if the worm is alive or dead. There have been previous experiments done with this setup compared to doing it by hand and the results are identical

(Stroustrup et al., 2013). This machine will increase efficiency by being able to have a greater quantity of worms with various concentrations of the supplement within a single experiment. Over this period of time I hope to learn new techniques and machines to use to perform this experiment. I hope to master the technique of worm picking and using the results to predict what the next step is. The results could give us information relevant to aging processes in humans. All of the supplies we will need we already have, except some of the strains of mutant worms. Tools that we use are: microscope, worm picks, agar plates, E. coli bacteria, vitamin supplement (from Standard Process), and access to a computer. The only traveling that may be necessary would to pick up the supplement from

Standard Process. **Timetable:**

Weeks Planned Activities

- 1-2 Determining how to deliver supplements to worms. Setting up preliminary experiments with supplements.
- 3-4 Installation of automated system. Continue data collection on initial experiments.
- 5-6 Analyze data from first experiment and set up experiments with automated system.
- 7-8 Monitor automated system. Set up other experiments with different genetic backgrounds.
- 9-10 Collect and analyze data and plan out next experiments.

References

Harrington, L., Harley, C. (1988). Effect of Vitamin E on Lifespan and Reproduction in Caenorhabditis elegans. Mechanisms of Ageing and Development, 43, 71-78.

Reviewer 2: Probably better off in methods.

Reviewer 4: Can we present the methodology in small sections: procedure, measurement, data analysis, etc. rather than quickly moving from one area to the next with no transitions....Here again, help out the novice reader to some degree.

Reviewer 2: State who them is.

Reviewer 1: So, this is the nutritional supplement that you will be testing for? Are there any others?

Reviewer 3: Positive controls are helpful to make sure that researchers are conducting their experimental methods properly.

Reviewer 4: This is the only mention of a direct benefit to the student....need more on what will be learned and how it will transfer to future career plans, etc. as per evaluation criteria.... All of this should come after discussion of supplies, travel, etc.

Reviewer 2: Very detailed.

Reviewer 2: Did not leave time to make poster.

Reviewer 4: Overall, solid proposal with a bit more organization needed to novice readers can understand it.....more needed on benefits to student both short-term and long-term.

Reviewer 1: Good that you included your references

Reviewer 2: Good references

Kenyon, C., Chang, J., Gensch, E., Rudner, A., Tablang, R. (1993). C. elegans Mutants that Lives Twice as Long as Wild Type. Nature, 366, 461-464. A

Stroustrup, N., Ulmschneider, B., Nash, Z., Lopez-Moyado, I., Apfeld, J., Fontana, W. (2013). The Caenorhabditis elegans Lifespan Machine. Nature Methods, 10(7), 665-673

Murphy, C., Hu, P., (2013). Insulin/insulin like growth factor signaling in C. elegans. WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbok.1.164.1, http://www.wormbook.org

Wilson, M., Shukitt-Hale, B., Kalt, W., Ingram, D., Joseph, J., Wolkow, C. (2006). Blueberry Polyphenols Increase Lifespan and Thermotolerance in Caenorhabditis elegans. Aging Cell, 5(1), 59-68.

Wood, W.B. (1988) Introduction to C. elegans biology. In: Wood, W.B. and the Community of C. elegans Researchers (Eds.), The Nematode Caenorhabditis elegans. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY pp.1-16.

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Applicants may not upload any other file nor may applicants use this upload feature for additional text/information related to the proposal narrative. Files uploaded that include additional text as part of the narrative will not be forwarded to the reviewers.

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**KEY TERMS:** assay, nutritional supplements, Caenorhabditis elegans, lifespan analysis