Continuous Manufacture of Homogeneous and Graded Rods by Low-Temperature Extrusion

Claire R. Adner

Freeze casting, the directional solidification of solutions and slurries, is a straightforward technique for the manufacture of porous materials. However, it has reached its limitations for the manufacture of long and slender components with its current mold-based approach. A process is needed with which slender materials and structures longer than 50 mm and 1-4 mm diameter can be made for applications such as peripheral nerve repair or ureteral stents. A low-temperature extrusion system has been developed for this purpose. Slender rods were extruded from chitosan solutions to demonstrate the principle of this continuous processing approach for the preparation of rods. The microstructural features achieved are currently unobtainable by other methods. Using our method, two types of samples with uniform dimensions have been created, either having homogeneous properties, or having carefully-controlled layered and graded properties, both through-thickness and along the length. Here, we present a manufacturing process and the principles of structure formation, as well as the results of the systematic structural and mechanical characterization of the resulting homogeneous and graded rods.

Using Object Detection Machine Learning to Count and Categorize *C. elegans*

Matthew Adner and Celine Tan

C. elegans worms are a very powerful model organism for conducting research: they are easy to cultivate, have consistent life cycles, and their genome has been fully mapped. As a result, they are used by labs around the world to conduct research. Counting *C. elegans* is tedious and time consuming, especially for those who are new to using this model organism. To combat this problem, we used Apple's ML-Kit to train an object detection model to identify worms and their developmental stages. Object detection is a type of computer vision that is used to detect the location and number of certain objects or classes. Our model uses a deep learning algorithm called a Region-based Convolutional Neural Network (R-CNN) for this. To create training data for the model, we manually annotated images, marking regions of an image containing worms, with MakeML. The algorithm was then trained and tested with CreateML. The annotated pictures were taken at various magnifications through an Olympus SZ4045 stereozoom microscope with an Olympus PEN Lite E-PL7 camera. The algorithm was trained, using these annotated pictures, to box and classify any worms it sees in a picture. We compared the results from pictures annotated by the algorithm to the results of a mediocre human counter (a novice *C. elegans* researcher) using an excellent human counter (an experienced *C. elegans* researcher) as the accepted value. The algorithm's requirements include high contrast images, well-focused images, and images that are not overcrowded. If an image meets these requirements, the algorithm can outperform the moderately-experienced human counter by 3% on accuracy of the total number of worms and 8% on the correct identification of the worms' developmental stages.

The Effects of Cannabidiol Oil on the Egg Hatching Rates of *C. elegans*

Gabrielle R. Anzalone, Esme E. Kimber

Cannabidiol (CBD) oil, is a substance whose use and popularity has increased significantly, as it has become a $270 million industry within the last few years. Despite the popularity of CBD oil, little research has been conducted on the safety and effects it has on our bodies. One of the few scientific studies on CBD oil was conducted by Emilio Perucca from the University of Pavia, suggesting CBD oil can help prevent seizures. Although lacking thorough scientific studies, CBD oil is believed to reduce inflammation, improve heart health, calm the nerves, and reduce pain. Some negative side effects associated with CBD oil include nausea, anxiety, dizziness, and mood changes. The goal of this study is to contribute to the understanding of the use of CBD oil for medical purposes and how CBD oil affects the body's response to stress. The specific factor examined in this study was whether CBD oil triggered a change in the hatching rates of the model organism *Caenorhabditis elegans* (*C. elegans*). *C. elegans* were the ideal model organism to use in this study because of their short lifespan, which allows for conducting an experiment that studies short-term effects of a substance on the body. The study monitored egg hatching rates because variations in egg hatching suggest the worms are under stress. In this study, the worms exposed to CBD oil had a hatching rate of 33%. The worms exposed to the control, MCT oil (coconut oil), had a hatching rate of 27%. The plates exposed to CBD oil showed no significant difference in egg hatching when compared to the control plates (p-value = 0.435). The data collected is preliminary, but suggests that CBD oil does not change the egg hatching rates of *C. elegans.* More research needs to be conducted on whether CBD oil may cause other stress responses in *C. elegans* or humans.

The Effects of Sunscreen Chemicals on Chicken Embryological Development

Eden Anne Bauer

We studied the potential teratogenic effects of popular chemical sunscreens. Teratogens are substances that can harm the development of a fetus. The embryos of the common chicken *Gallus gallus domesticus* were used as a model system because of their short incubation period and well-characterized embryologic development. With an incubation period of ~22 days, this allows for short experiments of less than a week. Embryos were incubated in their eggs for ~2 days before being transferred to sterile nutrient media in petri dishes. Two substances were tested: Coppertone SPORT Continuous Sunscreen Spray Broad Spectrum SPF 50 and Banana Boat Ultra Sport Sunscreen Spray SPF 50+; both were diluted to a 0.0001% solution with sterile isotonic saline and compared to a control of sterile isotonic saline. Two methods of sunscreen exposure were used: in vivo (in an egg) and in vitro (in a petri dish). In vivo exposure was accomplished by drilling a hole in the eggshell and injecting either the test or control solutions either into the air sac or directly into the albumen of the egg. The in vivo exposure groups were injected immediately before initial incubation. Two days later, all embryos were removed from their eggs and placed on agar. For the in vitro groups, sunscreen dilutions or control saline were pipetted onto embryos that had been transferred to sterile media in petri dishes. All embryos of both in vivo and in vitro groups were photographed with an SMZ-U Nikon stereo zoom light microscope at 75x mag. during the same time slots for the next four days. Embryos were monitored for lifespan, peak length, and peak 2D area determined from photo analysis in Image J. We hypothesized that embryos exposed to sunscreen would have shorter lifespans, shorter peak lengths, and smaller 2D areas than embryos not exposed to sunscreen. All embryos were euthanized on day 4. Embryos exposed in vivo to Coppertone reached a peak 2D area 53% as large as embryos exposed to the control saline in vivo, and embryos exposed to Banana Boat in vivo reached a peak 2D area 48% larger than control embryos. Controls subjected to either method of exposure lived ~0.1 to 1.3 days longer, on average, than embryos exposed to a chemical sunscreen. This suggests that Banana Boat and Coppertone spray sunscreens may be harmful to chicken embryos in their early stages of development, however further investigation is needed to elucidate their effects.

Creating an Economical Interferometer Through CAD Engineering

Joseph Blackburn

The considerable cost of optical hardware is a limiting factor for pursuing optical research and development. For schools, even relatively basic demonstration experiments may require a considerable investment. For instance, the cost for a basic interferometer, an instrument that uses the interference of two beams of light to measure precise distances, can cost over $3,000. The idea of implementing optical systems with 3D printed components has been done before, including at Onsabrück University, where a Physics Research Group created a LEGO Mach-Zehnder model interferometer. This project was introduced to our NHAS lab to study the trade-off between cost and functionality of 3D printed optical systems for use in a school lab environment. This study proposes that, by 3D printing mechanical components, affordable optical systems may be created with little compromise in terms of functionality and precision. To achieve this goal, the factory-machined parts were both replicated and completely redesigned as 3D printed components. The process included breaking each factory-made piece into several simple parts that could be easily made individually by sketching and then 3D printing. It was accomplished with the help of 2 key programs: Fusion 360 (CAD/CAM design tool) and Cura (3D Printing Slicing Program). To demonstrate the potential of this approach, a Michelson interferometer served as a model. The original factory-made parts were estimated to cost between $3,000 - $5,000. In comparison, the 3D printed version cost $378.95. To put this in perspective, the cheapest item (lens holder) which was designed and 3D printed with material expenses of less than $3 replaces the factory version priced at $80. With mechanical parts less than $5, the greatest expense is lenses, mirrors, and beam splitters, since they can currently not be replaced by a cost-effective 3D print process. The project was able to demonstrate successfully a white light Michelson interferometer at a substantially reduced cost. All parts are both durable and well-suited for a classroom environment. The reduced cost has to be weighed against the limited precision of the 3D print process. Commercially available 3D Printers such as our LulzBot Taz 6 are not as precise as factory-grade metalworking. Future work will focus on facilitating optical setups with both movable and adjustable parts, and more extensive experimentation with different print parameters.

Development of a Modular Multi-Material Multistable Linkage

Avery Clowes

Compliant mechanisms move via the deflection of flexible members allowing them to be designed with reduced part count, fewer points of failure, and increased durability. Bistable devices are compliant mechanisms characterized by two stable fixed positions separated by an unstable equilibrium position. Complex bistable devices can be developed by modularizing mechanisms into individual components, improving testing timelines, and technology accessibility. With the advent of commercially available additive manufacturing technologies, chains of bistable mechanisms (modular linkages) have created the opportunity for more complex actuation profiles. In this investigation, a modular, multi-material, multistable mechanism was designed and tested. This bistable mechanism was investigated because of its ease of manufacture and reduced design-iteration timeframe. The mechanism acted as a spring with a nonlinear spring constant, such that it had several stable positions along its travel length. A linear actuator attached to a force sensor was used to measure the restoring force of the mechanism at a given displacement from the mechanism's natural length. Restoring force as a function of distance was measured for several devices with different spacings between flexible units (defined as the intermediate arm length). Testing revealed that restoring force increased as intermediate arm length increased. The modular system was durable, with force vs. displacement profiles remaining similar over more than three hundred tests. Bistable linkages require no energy input when not in the actuation phase, and the units developed during this study were flexible and durable. Therefore, these systems could be used for robotics applications, providing low energy movement and the capability to navigate complex terrain.

Monitoring Chicken Embryological Development with Machine Learning

Elizabeth Dixon

Teratogens are agents that cause malformations in embryos during development. With 3% of live births having a malformation and the cause of 65-75% of malformations in human newborns being multifactorial or unknown, it is a common yet complex problem. There is currently no teratogenic screening test, so determining what drugs and other products are safe for a developing fetus can be difficult. Our goal is to create a teratogenic assessment method for clinical and research use. In this study, chicken embryos were used since they are an ideal model for embryonic and developmental studies. They can be observed during their development outside of the mother and, with modern practices, be removed from the eggs to better visualize development over time. In this study, we extracted about 15 embryos from their eggs at approximately 50 hours of development and maintained their development in vitro on individual nutrient agar plates. All embryos were photographed twice a day for the subsequent five days using a Nikon SMZ-U at a magnification of 75x. Using the machine learning program MakeML, boxes were drawn on the images by hand around five prominent anatomical parts of embryos: the spine, the end of spine, somite 16, the telencephalon, and the eye cup. The data annotated by hand was transferred to the CreateML program to train the computer to identify those objects. After 25 pictures were annotated and the first training session was completed, the program had a loss of 1.4. Loss is a measure of how often the computer is incorrect when it checks itself against the answer key of the human-annotated boxes, and a value of 2 or below is considered acceptable. The algorithm also had 100% confidence in all areas except somite 16. This was understandable since all of the somites appear identical. As we build this database of healthy embryo images, the computer will learn what is normal at various stages of development. In the future, we will train the computer to recognize developmental defects caused by known teratogens. This will lay the foundation for a teratogen screening system that may one day be used in clinical and research applications.

Developing a CAR T cell Based Therapy for Graves' Disease

Katherine Duan

Graves' disease is an autoimmune thyroid disorder that results in elevated plasma levels of the thyroid hormones T3 and T4. Thyroid hormone overproduction is caused by pathogenic B cells producing IgG antibodies that bind to and stimulate the thyrotropin receptor (TSHr). These anti-TSHr B cells are activated and maintained by helper T cells that bind to linear epitopes of TSHr. Without these anti-TSHr helper T cells, the pathogenic B cells would remain inactive and normally die by apoptosis. Therefore, the elimination of anti-TSHr helper T cells could be a treatment method for Graves' disease. Chimeric antigen receptor (CAR) T cell therapy offers a highly specific and effective method for targeting and eliminating pathogenic immune cells in autoimmune diseases. CAR T cell therapy works by engineering T cells to target a given region, or epitope, that it would come across in the body. Here, we designed a plasmid for an extracellular CAR domain that could target the pathogenic anti-TSHr helper T cells found in Graves' disease. This extracellular domain is based off of an MHC class II molecule presenting a linear epitope of TSHr in order to mimic the complex formed between anti-TSHr T helper cell receptors and the MHC class II molecules found on professional antigen presenting cells. The ten most cited linear epitopes for TSHr in Graves' disease were selected from the Immune Epitope Database as candidates for the plasmid. HLA-DR3 and HLA-DR5 were selected as the MHC class II molecules for presenting the epitopes. Five additional epitopes with high binding affinities to the selected HLAs were generated using the NetMHCIIPan server. We reasoned that the fifteen selected epitopes would provide a broad coverage of the disease associated epitopes in Graves' disease. To mimic the natural structure of the HLAs, the sequence for the epitope was inserted between the two subunits of the HLA and tested in silico for correct protein folding. Future work will include assessment of the binding affinities of the various constructs to anti-TSHr T helper cells and selection of internal domains for therapeutic CAR T cells.

Persistence of live fluorescent *E. coli* in the *C. elegans* gut microbiome

James Eiler

C. elegans is a common model organism and, because of its transparent anatomy, is easily used for fluorescent imaging. The *C. elegans* gut microbiome has been found to play an in important role in its behavior and stress resistance. What is not certain and not well examined is whether bacteria inside the *C. elegans* remain as stable, living colonies for a prolonged period of time after the *C. elegans* stops consuming the bacteria. To answer this question, a fluorescence assay was performed in which the *C. elegans* were fed *E. coli* expressing a teal fluorescent protein. Control *C. elegans* were fed *E. coli* of the same strain expressing a red fluorescent protein that does not fluoresce under the light settings used for the teal fluorescent protein. After eating fluorescent bacteria for two days, the worms were observed over a period of three days on plates without bacteria. Images of the *C. elegans* were recorded on a Nikon SMZ-U microscope with a NIGHTSEA fluorescent light and analyzed for the level of green fluorescence expressed by the teal fluorescent protein. The fluorescence of the day 1 group, morning and afternoon day 2 groups, and morning and afternoon day 3 groups were analyzed and found to be significantly different from each other and the control group of worms not fed teal fluorescent bacteria. The average fluorescence increased from day 1 to day 2 by 0.250 arbitrary fluorescence units (afu), but then decreased between the morning and afternoon of day 2 by 0.562 afu and only decreased by 0.055 afu between the end of day 2 and the end of day 3. All groups had significantly higher fluorescence than controls (p-value<0.05). This suggests that the bacteria maintained a significant presence in the *C. elegans* gut over the examined period. To confirm the presence of living fluorescent bacteria in the gut after two days on a starvation plate, worms, independent of those photographed, were crushed and plated onto bacterial nutrient plates. Two days after plating the fragmented *C. elegans*, the plates were examined and fluorescent bacteria colonies were observed. These experiments indicate that that bacteria can survive and maintain a presence in the *C. elegans* gut for at least two days after the *C. elegans* stops consuming the bacteria. This prolonged presence indicates that bacteria establish long-term colonies in the *C. elegans* gut and that this stable microbiome could play a role in the functioning of the organism.

The Effects of Glucose Exposure on *C. elegans* in the Context of the Obesity Paradox

Emily Gaw

Lifelong ingestion of glucose has been proven to significantly shorten lifespan in *C. elegans*, a worm with an evolutionarily conserved insulin signaling pathway, similar to that of humans. This study examines the effect of periodic high-glucose exposure on the lipid levels, fecundity, and lifespan in *C. elegans*. It was hypothesized that increases in glucose administration after a certain unknown point would decrease the lifespan of worms, increase lipid levels, and decrease fecundity. To administer the glucose-shocks, the worms were periodically transferred to glucose and OP50 *E. coli* seeded plates throughout their two-week lifespan. A counterintuitive, positive correlation was observed between glucose exposure and survival rates. The worms given a constant supply of glucose lived a statistically significant 14.5% longer than the control groups fed only OP50 ($p=0.004907$). Lipid levels and fecundity followed the expected trend, offering assurance that the worms were ingesting the glucose. This unexpected correlation with survival rates is a supporting data point for the “Obesity Paradox” theory, which argues that there is a positive correlation between excess weight and increased life expectancy, especially during illness. This study further adds to the Obesity Paradox by suggesting that the correlation between excess weight and life expectancy extends to organisms exposed from the earliest stages of life, prior to any illness. Further studies could aid in understanding what is driving the Obesity Paradox – whether the advantage comes from the excess body fat maintaining homeostasis (e.g. absorbing and reducing free radicals) or whether the excess body fat itself is positively influencing specific biological functions (e.g. actively disrupting harmful chemical reactions).

Genetic Analysis of *Cypripedium reginae*

Alisa Halchenko and Garima Rastogi

Showy lady's slippers orchids (*Cypripedium reginae*) are critically endangered in New Hampshire, existing in less than 5 places in the wild. The intent of this experiment was to develop an approach for using PCR and DNA sequencing of microsatellite regions to assess genetic diversity of wild *Cyp. reginae* populations. A lack of genetic diversity can be a contributing factor for a population's decline especially when facing environmental challenges such as global climate change. We performed PCR on DNA samples extracted from plants in an artificial fen located in Lyme, NH. These plants were grown in axenic seed culture from seeds collected from the Eshqua Bog in Vermont. We utilized PCR primers that had been previously used to target variable microsatellite DNA regions in another species of *Cypripedium*. We targeted these specific microsatellite regions because they are likely to change between two distinct *Cypripedium reginae* populations. Our experiments identified a set of primers that could be used for our *Cyp. reginae* samples and yielded PCR products for one microsatellite region from 16 plants that were submitted for DNA sequencing. This produced sequencing results for the very short microsatellite segments of DNA. The obtained sequences were analyzed for quality and aligned to determine the amount of differences among individuals. There were few differences, with the sequences generally aligning. This suggests our analytic approach works for *Cyp. reginae* and can be used to analyze the genetic diversity of this orchid species. Based on these results, the plants within the Lyme fen were not substantially genetically different from one another, but comparisons to populations in the wild are needed to fully confirm this suspicion. We would like to analyze the genetic diversity of plants from wild fens to determine if they have robust genetic diversity within and among different fens.

Developing an Improved Procedure for Monitoring the Heart Rate of *D. magna*

Alexander Kish

Daphnia magna is an aquatic invertebrate and a well-established model organism for toxicological studies. It is transparent, allowing heart rate monitoring to be used as a toxicological assay. Monitoring the heart rate of *D. magna* is complicated by the movement of the organism and the speed of their heart rate, making it nearly impossible to count reliably with the naked eye. This study presents a procedure for video capture of *D. magna* heart rate as well as the design of instruments to aid the researcher in this procedure. To restrict movement of *D. magna* for video capture, a round bottom 96-well microtiter plate was used with one *D. magna* per well. The majority of the liquid in each well was pipetted out, leaving the *D. magna* stationary so that a 10-second video could be captured. Video was originally captured at 120fps or greater using a modified dissecting scope and an iPhone. To improve image quality, a dissecting scope with a darkfield base and a Sony a6500 digital camera were used. To improve ease of pipetting for video capture, a specialized tip and automated pipetting system were developed. The tip was formed from a glass Pasteur pipette melted to a 90˚ angle and a lifter that could actuate the tip's movement in and out of the well was designed and 3D printed. This lifter was actuated by a micro servo motor. To further automate the pipetting process, a device to control a 100-1000µl micropipette was designed and prototyped with 3D printing. This device allows the plunger and volume setting of the pipette to be controlled with stepper motors. The proposed method of heart rate assay provides a reliable quantitative measurement of *D. magna* heart rate, while allowing the organism mobility during the course of the experiment, only restricting movement for 10-second observations. The use of servo and stepper motors allows for customizable control over pipetting functions with a microcontroller. Future directions include automation of microtiter plate movement, a plug-and-play solution for control of the proposed devices, and developing an integrated system for easy deployment in research and laboratory settings.

The Impact of JUUL E-Liquid and E-Cigarette Flavorings on *C. elegans* Using Chemotaxis

Leanna Kish

E-cigarettes/vapes have become popular in the past decade due partly to marketing as alternatives to traditional cigarettes. Flavor varieties and availability have made them popular and they are now the most common nicotine delivery device among youth in the US. There are currently few FDA regulations on these products and manufacturers are not required to list their ingredients. One of the most prolific E-cigarette brands is JUUL with Mango and Mint as the most popular flavors. Our previous study investigated JUUL flavors on *C. elegans*. This study wished to determine the effects of JUUL E-liquid and non-JUUL E-cigarette flavorings on the survival of Daf-9 and Egl-4 mutant *C. elegans.* Daf-9 mutants are stress-resistant and Egl-4 mutants are olfactory-defective. We used chemotaxis and divided the worm plates into four concentric sections, with the test solution placed in the center. Mango and Mint were used as both the JUUL and non-JUUL flavors. The concentrations tested were 1%, 25%, 50%, and 100%, made by diluting the substances in *E. coli* bacteria or sterile water. Chemotaxis and survival were monitored for 6 hours. The results showed that almost every flavor tested resulted in dose-response patterns. JUUL Mango did not cause Daf-9 mutant death in either the water or *E. coli* dilutions. The Egl-4 mutants exposed to JUUL Mango showed a death dose-response pattern for dilutions in *E. coli* or water. The JUUL Mint 100% concentrations caused death for both the Daf-9 and Egl-4 mutants. There was no death in the 50% concentrations diluted in *E. coli* for both Daf-9 and Egl-4, although there was death in the 50% concentrations diluted in water for Daf-9 and Egl-4 mutants. No death was seen with the non-JUUL Mango flavoring. No death was seen with the Daf-9 mutant for the non-JUUL Mint flavor. Ninety-percent of the Egl-4 mutants died in the 100% concentration of the non-JUUL Mint flavor diluted in *E. coli* and, for the dilutions in water, death followed a dose-response pattern. These data suggest that the JUUL brand products have more toxic impacts on *C. elegans* than the non-JUUL flavorings. The JUUL products caused more death in the Egl-4 mutants than with the Daf-9 mutants. The results vary between Mint and Mango, suggesting that the ingredients used to flavor the JUUL E-liquids may be part of the cause of the varied effects.

Toxicological Effects of Aluminum and Titanium Nanoparticle Exposure in *C. elegans*

Casey McGuire

Nanoparticles, ranging in size from 1-100 nanometers, are potentially toxic to animals. Aluminum oxide nanoparticles (Al_2O_3 NPs) have been linked to brain inflammation and dopaminergic neuron loss. Titanium dioxide nanoparticles (TiO_2 NPs) can increase oxidative stress in the brain resulting in neurodegeneration. We used *C. elegans* as a model organism because their neurological system is mapped, they have an Insulin/IGF-1 Signaling (IIS) pathway that helps control inflammation, and there are relevant mutant strains. We investigated potential neurotoxic effects of Al_2O_3 NPs and inflammatory effects of Al_2O_3 and TiO_2 NPs through the IIS pathway. Our hypotheses were that both Al_2O_3 and TiO_2 NPs cause inflammation and that Al_2O_3 NP exposure would lead to dopaminergic neuron loss. Wildtype (WT) *C. elegans* were used in addition to two mutant strains for comparison. Daf-9 mutants are resistant to inflammation because of a mutation in the IIS pathway and cat-2 mutants are deficient in dopamine. An ethanol preference test was used to test the effect of Al_2O_3 NPs on dopaminergic neurons. Previous research shows that, following exposure to ethanol, WT *C. elegans* develop a preference for ethanol. Cat-2 mutants were used since they do not develop this preference to ethanol due to their dopamine deficiency. When exposed to 10 g/L Al_2O_3 NPs, WT worms had a preference index that was found to be statistically different from the control group ($P<0.05$) while the Al_2O_3-exposed *C. elegans* preference index was not statistically different than the cat-2 mutants ($p>0.05$). WT and daf-9 mutants were exposed to 1 g/L and 10 g/L of Al_2O_3 and TiO_2 NPs. The eggs laid per nematode was measured and a lipid stain was used to observe differences in inflammation. The WT and daf-9 *C. elegans* exposed to 10 g/L Al_2O_3 or TiO_2 NPs had laying rates that were statistically different from the non-exposed nematodes throughout the reproduction period ($P<0.05$). The WT and daf-9 *C. elegans* treated with 1 g/L Al_2O_3 and TiO_2 NPs had more lipids than the control treatments. The daf-9 mutants had lower lipid levels than the WT for both control and experimental groups. This study suggests Al_2O_3 NPs could have neurodegenerative effects on the dopaminergic neurons of *C. elegans*, and that Al_2O_3 and TiO_2 NPs cause inflammation in *C. elegans*. Al_2O_3 and TiO_2 NPs are used increasingly in medicine, cosmetics, manufacturing, and food industries. These particles should be tested further due to possible neurodegenerative and inflammatory effects they may have on humans.

Counting Showy Lady's Slipper Orchids with Machine Learning

Theodora Montague

Showy lady's slipper orchids (*Cypripedium reginae*) are a critically endangered species in New Hampshire and much of the northeastern United States. They live in temperate regions and are highly adapted to fens, a type of wetland. The label of "critically endangered" indicates that there are less than five distinct populations in a given state. The New Hampshire Academy of Science seeks to monitor showy lady's slipper populations in the wild for conservation purposes. We are particularly interested in being able to identify the distribution of showy lady's slippers in a given fen. The goal of this project was to design an efficient and accurate automated method to assess wild lady's slipper populations. Because of the potential damage caused by walking through a fen, we chose to use a drone to photograph the lady's slippers. The drone, fitted with a digital camera, was used to take high-resolution images of all parts of a fen located in Strafford, Vermont. Images were taken at heights ranging from approximately 3 to 8 meters with an average height of about 6 meters. We used a version of YOLOv2, an image recognition system, that had previously been translated into Python, to create an object detection model that could count lady's slippers in a photograph. The images from the drone were human-annotated to mark lady's slipper flowers and used to train and test this model. When tested with images taken at about 6 meters, similar to the majority of the ones it was trained with, the model averaged 4.8 true positives, 0.2 false positives, and 90.4 false negatives after 100 epochs of training. An epoch is when the machine learning model looks at each image in the training set once in order to learn what constitutes a lady's slipper. When tested with pictures taken at about 3 meters above the ground, the model averaged 1.4 true positives, 0 false positives, and 11.6 false negatives, after the same number of epochs. This could perhaps be improved by training the model with more and higher quality images. This model, although imperfect, is a substantial first step toward the goal of being able to precisely map the locations and distribution of individual showy lady's slippers in a fen. The next steps will be to improve the accuracy of the image recognition and modify the program to perform the same computation on videos.

Colorful or Colorfool: The Effect of Artificial and Natural Food Dye on *C. elegans*

Deetya Nagri

Food colorants are common in our diets. They are found in candies, beverages, and medicines. Recent studies suggest a link between artificial food colorants and negative behavioral patterns in children. Natural food colorants such as red beet powder, turmeric, and blueberry powder may provide a suitable alternative to artificial colors such as Red #40, Yellow #6, and Blue #1. *Caenorhabditis elegans* is a non-parasitic worm with a completely mapped genome that is often used as a model organism in toxicity and behavioral experiments due to its short life span and easily measurable behavioral traits. This study investigated the behavioral impact of artificial and natural food colorants on *C. elegans* and it was hypothesized that artificial colorants would have more negative consequences on the behavior, mortality, and fecundity of the worms. A chemotaxis experiment was used to test the tendency of the worms to migrate to or away from yellow and red artificial and natural food colorants. The chemotaxis response to varying concentrations of each dye, as well as sterile deionized water, was tested. A dose response experiment at 1%, 0.1%, and 0.01% each of artificial Red #40, natural red beet, artificial Yellow #6, and natural yellow turmeric food colorants was also conducted. Worm behavior, mortality, and fecundity were recorded over a five-day period to test if the colorants had any adverse effects. Chemotaxis data did not suggest any preference or avoidance when exposed to any artificial or natural colorant since worms were evenly spread through different concentrations of each colorant. Dose response experimental data was inconclusive due to missing worms and difficulty ascertaining egg and worm position due to colorants, although it was noticed that worm egg-laying when exposed to the artificial and natural red colorants was lower than that of the control. Future work will include repeating the experiment to test for reproducibility and statistical significance, testing other artificial and natural food colorants, and testing the effects of exposure over multiple generations of worms.

The Pursuit of Happiness: The Effect of Herbs on Serotonin-Deficient *C. elegans*

Shreya Nagri

Serotonin is a neurotransmitter that is thought to affect mood in humans. A common class of prescription drugs used to treat symptoms of depression are selective serotonin reuptake inhibitors (SSRIs). These drugs are thought to act by increasing serotonin levels, but they do not work for some individuals and can have debilitating side effects. Herbal remedies, such as lavender and St. John's Wort, are thought to be natural alternatives to these drugs, but they may not have the same efficacy as prescription medications. This study was conducted to test the efficacy of these herbal remedies in increasing serotonin production in *C. elegans*. *C. elegans*, a non-parasitic nematode, was used for this experiment because it is easily propagated and has a completely mapped genome. Both wild type and tph-1 mutant (serotonin-deficient) *C. elegans* were used. For the herbal remedies, decoctions were made of either lavender (*Lavandula stoechas*) and St. John's Wort (*Hypericum perforatum*). A chemotaxis experiment was used to determine the preference of living area of both tph-1 and wild type *C. elegans*, given an option between a control and three concentrations of each decoction (lavender and St. John's Wort). A dose response experiment with 2%, 0.2% and 0.02% dilutions of the original decoction was conducted to test the effects of herbal remedies on the worms. Fecundity, behavior and mortality were monitored for two days for control and experimental groups. These worms were then harvested into worm pellets and analyzed using quantitative real-time PCR to determine the genetic efficacy of these herbal remedies. Results with the lavender decoction and St. John's Wort decoction suggested an increase of serotonin levels as observed by increases in *tph-1* mutant fecundity when compared to the untreated *tph-1* worms. Both solutions also showed effectiveness at the genetic level when analyzed with qPCR because both caused an upregulation of the *tph-1* gene. The effectiveness of these solutions suggests ramifications for humans who are affected by the side effects or ineffectiveness of SSRIs and other serotonin targeting medications because it suggests that there may be viable alternatives with less risks.

The Effects of Prozac [Fluoxetine] on Chicken Embryological Development

Saia Patel

The use of antidepressants during pregnancy is estimated at 15% of the pregnant population in the United States. The most widely-prescribed antidepressants for pregnant women are called selective serotonin reuptake inhibitors, or SSRIs, and are found to increase the risk of embryonic malformations. Fluoxetine [trade name – Prozac], is the most prescribed antidepressant for pregnant women. Studies have found fluoxetine in the amniotic fluid and cord blood in humans, but little is known about the drug's effects on embryonic development. The aim of this investigation was to use chicken embryos as a model to evaluate whether exposure to fluoxetine affects three variables in development: embryo length, somitogenesis, and survival. Embryos were surgically removed from the yolk and placed on sterile nutrient agar. Two solutions of fluoxetine (0.1 µM and 10 µM) were administered to the chicken embryo by pipetting the solution over the cultures. All control and experimental embryos were monitored and photographed through a Nikon SMZ-U microscope at 1.5x and 2x magnification for up to four days from transferring the embryo to the agar. The lower dose of fluoxetine had an observable effect in all three variables studied. The higher dose of fluoxetine had a more profound effect on embryo development in all of the physical categories studied, and exposed embryos exhibited abnormal torsion of the body. Both experimental groups fell short of the control average values for the experimental variables for the duration of the experiment. The chicken embryos that were exposed to the 10 µM dilution had 13.59% fewer somites, on average, than the control group throughout the experiment. The chicken embryos that were exposed to the 0.1 µM dilution had 7.54% fewer somites, on average, than the control group throughout the experiment. The chicken embryos exposed to the 10 µM dilution had 63% percent less survival, on average, compared to the control group throughout the experiment. These results suggest fluoxetine does affect the development of chicken embryos in terms of embryonic length, somitogenesis, and survival rate.

Effects of Wheat Gluten and Its Hydrolysate on *Caenorhabditis elegans*

Anshul Rastogi

Wheat gluten is a common ingredient of commercial foods. This study observed how exposure to wheat gluten and its hydrolysate (WGH) affects stress in the nematode *Caenorhabditis elegans* (*C. elegans*). Due to several evolutionarily conserved systems in *C. elegans*, it was purported that the results may be representative of the effects of consuming wheat gluten and its hydrolysate on humans. It was hypothesized that wheat gluten exposure would reduce brood size and lifespan under heat shock, which is indicative of increased stress, whereas exposure to WGH would increase both brood size and lifespan under heat shock, indicative of decreased stress. For exposure, the substances were combined with the food source of the *C. elegans*, the noninfectious OP50 *Escherichia coli* (*E. coli*) strain. Wheat gluten hydrolysate was acquired by digesting wheat gluten with pepsin. Two experiments were conducted: a pilot experiment with wheat gluten exposure, and an experiment with both wheat gluten and WGH exposure. The pilot experiment had 4 groups: the control, 0.1 mg/mL wheat gluten, 0.5 mg/mL wheat gluten, and 1 mg/mL wheat gluten, with one plate per group. All heat shock groups were exposed to 37 degrees Celsius and checked at intervals of 15 minutes until death. Results of the pilot experiment suggest that wheat gluten was associated with adverse concentration-dependent effects for both lifespan under heat shock and brood size. The second experiment had three groups: the control, 1 mg/mL wheat gluten, and 1 mg/mL WGH with 3 plates per group. Data suggested that, whereas wheat gluten had clear adverse effects on the *C. elegans* in both the brood size and heat shock assays in comparison to the control, WGH exposure had no clear effects. This supports the part of the hypothesis regarding wheat gluten, but does not support the hypothesis regarding WGH. Additionally, the extent of the adverse effect of wheat gluten appeared to be concentration-dependent. This indicated that higher exposure to wheat gluten was associated with increased stress. Future directions for this experiment include repeating it with a much larger sample size.

A Simple Qualitative Test for Surfactants in Glyphosate-based Herbicides

Sora Shirai

In 2016, 286 million pounds of glyphosate were used on crops in the United States. Glyphosate is the active ingredient in glyphosate-based herbicides (GBHs), such as Round-up® and Rodeo®. Only 2% of the commercial final product applied to crops is glyphosate, with the other 98% composed of “undisclosed “or “other” ingredients. To allow the glyphosate to get past the cuticle of plants, many GBHs contain surfactants formulated in these undisclosed ingredients. A surfactant can be defined as a substance which reduces the surface tension of a liquid. Surfactants disrupt cell membranes and are known to be toxic to aquatic organisms. Considering the broad use of GBHs, this presents a potential ecological threat. This investigation devised a simple method to determine whether there was surfactant present in the GBHs Round-up and Rodeo. The presence of surfactant in herbicides and controls was tested using a qualitative loss of surface tension. For each test, six milliliters of pure reverse osmosis water were placed on a standard microscope slide cleaned with isopropyl alcohol. This was tested to be the maximum amount of water that could be held on a slide when 5µL of pure water as a negative control was added to the slide. If the surface tension broke after the addition of 5µL of a test herbicide, it was recorded to contain a surfactant. Triton X-100 was used as a positive control and pure reverse osmosis water was the negative control. The Triton X-100 was diluted to concentrations of 100, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625% and the Round-up and Rodeo were diluted to concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625%. It was found that the surface tension did not break at any levels of concentration tested of Rodeo while the surface tension broke for high levels of concentration (100, 50, 25%) of Round-up. All trials of the Triton X-100 broke the surface tension at a concentration of 0.625% and above, and some trials of the Triton X-100 broke the surface tension at concentrations of 0.3125 and 0.15625%. All trials of the negative control did not break the surface tension. These results suggest that there is little to no surfactant in the herbicide Rodeo but there is some surfactant in the herbicide Round-up. We hope this method can be used to quickly determine whether any of the many GBHs contain surfactants. These results also suggest that the high amounts of GBHs being applied to crops may present an ecological threat to water supplies.

How Fluoxetine May Affect the Egg Laying and Development of *C. elegans*

Lian Snow

Prenatal depression affects up to 23% of pregnant women in the US. Depression has been shown to be associated with birth defects, premature birth, and stillbirth. Fluoxetine is one of the only antidepressants in the selective serotonin reuptake inhibitor (SSRI) class to be prescribed to pregnant women. Consuming fluoxetine in the last trimester of pregnancy has been shown to increase pre- and post-natal health risks to both the mother and the baby. Long-term effects in infants that were exposed to fluoxetine in utero are unknown. To further determine if the fluoxetine is acting through serotonin, two different strains of *C. elegans* were used: Tryptophan hydroxylase mutants (TPH-1, genetically modified to have reduced serotonin synthesis) and wild type (WT, genetically similar to *C. elegans* in the wild). L4 *C. elegans* (the life stage where reproductive organs develop) were exposed to fluoxetine for 19 hours and were then transferred to non-exposed petri dishes where egg laying was monitored. The egg laying and larval development was monitored to adulthood. Although it was hypothesized that WT *C. elegans* would produce a greater number of viable offspring than the TPH-1 mutant, it turned out to be the opposite. Exposure to fluoxetine did not significantly change the number of eggs laid for WT or TPH-1 *C. elegans*. While fluoxetine seems to palliate some of the manifestations of serotonin deficiencies/irregularities, the data suggests that fluoxetine may not be fully compensating for the lack of serotonin in the TPH-1 mutant *C. elegans*. For example, the data shows that the fluoxetine-exposed parent TPH-1 and WT *C. elegans* laid fewer eggs than the control group. There were no noticeable impacts on the larval development in the progeny of fluoxetine-exposed *C. elegans*. The results of this experiment point to associations between *C. elegans* maturation, egg laying, and the physiological changes accompanying controlled serotonin regulation. Future experiments could include viewing how much fluoxetine the *C. elegans* are intaking in this experiment. This could be done by attaching a fluorescent dye to fluoxetine and observing the drug inside of *C. elegans* with a fluorescent microscope.

The Genetic Diversity of *Cypripedium reginae* in a Fen in Strafford, VT

Yuyang Sun

This experiment examines the genetic diversity of showy lady's slippers (*Cypripedium reginae*) growing in the wild in a fen in Strafford, Vermont. *Cyp. reginae* is a critically endangered lady's slipper orchid species in much of the northeast United States. Lady's slippers are capable of reproducing by sexual or vegetative reproduction. There is speculation whether, in the few fens where showy lady's slippers exist, the population is genetically diverse due to sexual reproduction or largely clonal through vegetative reproduction. A clonal population may be more susceptible to environmental stresses than a genetically diverse population. We had two hypotheses: 1) we could use primers on *Cyp. reginae* that had been used on another orchid of the same genus, *Cypripedium tibeticum*, and 2) the showy lady's slippers in the same fen would largely be genetically similar. After performing PCR and gel electrophoresis on showy lady's slipper DNA using different primers, it was found that the primer set M209 from the studies on *Cyp. tibeticum* also worked for showy lady's slippers. This primer set targets a variable microsatellite region of the lady's slipper genome. The post-PCR DNA samples were sent for DNA sequencing and the returned chromatograms were analyzed to assess the genetic diversity. No two samples analyzed showed high genetic similarity for the genomic region sequenced, and therefore none of the plants sampled, some of which were growing in close proximity with one another, were completely identical. The fact that variation is seen in a single ~120 base pair region suggests that the showy lady's slippers have genetic differences throughout their genome. This would allow the population to be more resilient to environmental changes. Further analysis of additional genomic regions and with a larger plant sample size across multiple fens will be needed to acquire a more complete picture of genetic diversity in wild showy lady's slippers.

Genotoxicity and Reproductive Toxicity of Cobalt Nanoparticles in *C. elegans*

Luke Young-Xu

Cobalt nanoparticles, valued for their magnetic and catalytic properties, are employed in a growing number of industrial and medical applications. Workers in industries involving cobalt nanoparticles are at risk of coming into contact with these particles through inhalation or physical contact. As the production of nanoparticles increases, the amount released into the environment has also risen, causing increased risk of exposure for wildlife. Most studies exploring the effects of nanoparticle exposure use direct administration into the tissue or bloodstream of subjects. This study investigates the potential genotoxicity and reproductive toxicity of cobalt nanoparticles through environmental exposure to better simulate how wildlife or industry workers may encounter them. *C. elegans* were exposed to cobalt nanoparticles an average of 28nm and 200nm diameter at concentrations of 250mg/L, 100mg/L, 50mg/L, with 0mg/L as a negative control. The egg laying rate, egg hatching rate, and living rate of the worms were measured over 5 days to determine if cobalt nanoparticles presented any toxic effects. A comet assay was performed on the exposed *C. elegans* to test for DNA damage. Exposure to the 2 most concentrated suspensions of a small nanoparticle size (28nm) was found to cause significant decreases to the egg laying rate, egg hatching rate, and living rate when compared to the control ($P=0.032$, 2.598×10^{-5}, and 0.0014 for 250mg/L, respectively). Exposure to a larger particle size did not induce significant changes to the 3 measured variables at any concentration. Exposure to the most concentrated suspension of 28nm particles for over 24 hours resulted in increases to the tail DNA percent in the comet assay, suggesting increased DNA strand breaks. The findings from this study suggest that cobalt particles of a nano-size (<200nm) on the surface of the *C. elegans* surroundings can be taken up and interfere with the organism's reproduction. Larger cobalt particles are less of a threat to the organism's normal functions, suggesting a larger size may limit uptake and/or the particle's ability to enter tissue and cells. These results suggest the reduction of cobalt particles to a nano-size significantly increases the danger they pose to organisms that come into contact with them, and can have pronounced effects on the health of organisms exposed to relatively large concentrations of cobalt nanoparticles that may be present in or around an industrial setting.