A hands on educational module to teach aspects of human dietary health using fruit flies as a model.

Student and teacher focus

For this educational module the students should be provided with the detailed protocols and links to web sites which have been constructed to explain the various components of the educational module. The module is produced specifically to relate human health issues to the exercises with using fruit flies as the animal model. The content is all open source. The open video sources also explain how to go about each of the activities while leaving a lot of leeway for new ideas of students to modify and improve techniques. This unit contains pedagogical support to assist teachers with facilitating open inquiry to acquire new knowledge (Abrams et al., 2007; Appleton, 1995; Nadelson, 2009).

The aims for the students conducting the module is to estimate the developmental time of larvae, survival of larvae and adults and behavioral changes in larvae and adults to various diets mimicking human consumption and therapeutic diets. In addition, the students should be gaining some knowledge about human health and the effects of diet on health. Integrating research experiences into undergraduate courses at a college level have a number of acronyms such as course-based undergraduate research experience (CURE) and active-learning laboratory undergraduate research experience (ALLURE) as well as undergraduate research experience (URE) which is more of the research focus as individuals or groups with in a class or an entire class (Rowland et al., 2012; Auchincloss et al., 2014). Having students involved in research hands on projects also helps students develop and apply scientific practices (Coll, France, & Taylor, 2005).

Content provided to Students

The fruit fly, *Drosophila melanogaster*, as a larval stage and as an adult are commonly used a model organism in research to understand biological principles. The fruit fly became well recognized as a model for studying genetics (Rubin and Lewis 2000; Morgan 1910) much like the genetic discoveries using the pea plant for botany (Ellis et al., 2011; Henig, 2000). There are various human genetically linked diseases which are being modeled using *Drosophila* since the genes which code for proteins are similar for basic biological function such as ones for ionic exchange, glucose metabolism, building contracting muscle and producing a functional nervous system (Reiter et al. 2001).

The life cycle of *Drosophila melanogaster* is relatively simple and easily followed. After males fertilize the eggs and the female lays them on a substrate in 1 day the larvae will emerge at room temperature (21^{o}C or 70^{o}F). Larvae develop through three stages which are easily identified by morphological changes in their mouth hooks used for feeding. In the late 3rd instar stage the larvae crawl out the food and find a place to become a pupa. After about 7 days the pupa eclose as adult fruit flies. The adults typically live for 2 to 3 weeks depending on the crowding and environmental conditions.

Using practical scenarios, such as focusing on a human health issues related to diet and a dietary problem which may be linked as an underlying cause of metabolic syndrome, can help unravel complex biological systems and provides one with an authentic context in which to learn and apply scientific concepts. This module starts out in providing an introduction about diets and health. Then we address why the fruit fly is used as a model organism for this particular educational module. Since our goal is for not only college level programs to be able to use this model and build on it but also for high school programs. Thus, some schools have very limited financial resources so we focus in how to conduct this module on a low budget with being as practical as possible while not compromising the level of understanding the science to be gained. There is a short description how to make a simple microscope for observing larvae so this step would not be a limiting factor for conducting the module with limited microscopic equipment. The exercise walks one through how to conduct various behavioral measures in larvae followed with how to measure developmental time as a larva to a pupa. In building on past published educational exercises, we introduce a means to blend in survival studies of the adults and population dynamics. Investigations in the effects of diet on adult behaviors are highlighted. In addition, a specific diet such as the ketogenic diet is a thematic focus for addressing the effects on behavior and function related to the suggested mechanism of action in mammals in treatment of epilepsy. Lastly, a well-developed physiological assay in monitoring the heart rate in larvae is discussed as a bioassay for health of the larvae exposed to various diets.

For starting out with a foundation to build various other experimental paradigms, we use diets consisting of a mix in various forms of fructose, glucose, peptones, coconut oil (i.e, fat) and a cornmeal mix (i.e, a standard mix for culturing *Drosophila*).

Procedures with larvae

1. Place about ten 1st instars or 2nd or 3rd instars into a dish containing the diet of choice for each condition to be examined.

2A. Determine the time period of exposure to the diets for behavioral assays as larvae.
2B. If using the larvae for developmental timing to pupation then observe every day and mark on the vial when pupa form.

3. Continue to repeat this procedure for each diet and make sure to label vials. Conduct assays below accordingly.

Procedures with adults

1. Know the age of the adults from the time of eclosion. If a vial has many pupa and adults, empty the adults out and then start experiments where one knows a window of time the pupa have eclosed to adults (within 1 day for example).

2. Determine the time period of exposure to the diets for behavioral assays.

3. If one is using the adults for survival assays then mark the vials with type of food, starting number of flies and record daily the viable flies (note if flies were stuck in food and died as a result or other such variables). Do not conduct behavioral assay on these flies as they may be harmed by the assays and a true survival just based on diets cannot be assessed.

4. Conduct the behavioral assays of choice taking note of the variables such as sex, time of exposure to the diets and type of diet.

Individual educational units for the module

Movies provided go along with introductory text, protocol text and sample graphs of data, as well as notes to instructors for each activity.

The YouTube movies listed are as follows:

1. Introduction to “A model for learning about aspects of metabolic syndrome” (LaShay Byrd) https://youtu.be/FZ1kB1_9QMM

2. Diets for metabolic syndrome (by LaShay Byrd and Jenni Ho) https://youtu.be/22Onri7mPdg

3. Stages of larvae (by Jenni Ho) https://youtu.be/ZVMnsA2o44U

4. Making a simple microscope (by Ruth Sifuma) https://youtu.be/wTSynwmyHBQ

5. Body wall movements (by Brecken Overly) https://youtu.be/smXe5axLZE8

6. Mouth hook movements (by Hunter Maxwell & Crysta Meekins) https://youtu.be/wynMJjyTt1s

7. Ethograms and why ethograms (by Brittany Slabach) https://youtu.be/8MMOJj4nMY0

8. The HAT behavioral assay for larvae (by Maddie Stanback & Emma Rotkis) https://youtu.be/fG7iFRF9HDg

9. Development of fruit flies as a measure of pupation rate (by Clare Cole & Kay Johnson)
https://youtu.be/qoFhLFie3K0

10. Anesthetize adult flies for transfering them (by Samantha Danyi)
https://youtu.be/ZfbN1GTu-Gg

11. Light and gravity sense in adults (by Sushovan Dixit)
https://youtu.be/zSJHN2NSrHk

12. Effects of ketogenic diet specifically (by Madan Subheeswar)
https://youtu.be/UiPDIEDa_mk

13. Measuring heart rate in larvae (by Ann Cooper et al.,2009)

Cooper, A.S., Rymond, K.E., Ward, M.A., Bocook, E.L. and Cooper, R.L. (2009) Monitoring heart function in larval *Drosophila melanogaster* for physiological studies. Journal of Visualized Experiments (JoVE) 32: http://www.jove.com/video/1596/monitoring-heart-function-larval-drosophila-melanogaster-for)

14. The effect of thermal stressors on larvae and adults fed various diets.
(by Alexandra Stanback)
TBA

15. A summary of this educational module (LaShay Byrd is working it up).

Detailed descriptions for each activity

1. Introduction to "A model for learning about aspects of metabolic syndrome"

Metabolic syndrome
NIH terms for it
http://www.nhlbi.nih.gov/health/health-topics/topics/ms
Refs:
http://care.diabetesjournals.org/content/34/2/497
http://www.ncbi.nlm.nih.gov/pubmed/18397447 rural kids
http://bmcpublichealth.biomedcentral.com/articles/10.1186/1471-2458-10-141

2. Diets for metabolic syndrome
D-glucose , fructose, and sucrose are used as sources of carbohydrate; synthetic soy bean extract is used as a protein source ; and 100% coconut oil is used as a lipid source. The concentrations of each of the above nutrients are able to be varied.

Owusu-Ansah, E. and Perrimon, N. (2014) Modeling metabolic homeostasis and nutrient sensing in Drosophila: Implications for aging and metabolic diseases. Dis Model Mech. 7(3): 343–350. doi: 10.1242/dmm.012989

3. Stages of larvae

https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=12&ved=0ahUKEwjL6Jvp54LYAhXDmOAKHb86AOcQFghYMAs&url=http%3A%2F%2Fcomp.uark.edu%2F~mlehmann%2Fpost.pdf&usg=AOvVaw0DAs1nw1-6hsry1oGXhrfW

https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=16&ved=0ahUKEwjL6Jvp54LYAhXDmOAKHb86AOcQFgh0MA8&url=https%3A%2F%2Fcarnegiescience.edu%2Fpublications_online%2FDrosophila_Guide2.pdf&usg=AOvVaw2D3ze9Hlt4ZuQLFWqQ9HYQ

4. Making a simple microscope

Explained in YouTube video (very easy)

5. Body wall movements

Open source file:
Majeed, Z., Koch, F., Morgan, J., Anderson, H., Wilson, J., and **Cooper, R.L. (2017)** A novel educational module to teach neural circuits for college and high school students: NGSS-neurons, genetics, and selective stimulations. F1000Research. F1000Research: Immediate & Transparent Publishing for Life Scientists. F1000 Research Ltd, Middlesex House, 34-42 Cleveland St, London W1T 4LB, UK. https://f1000research.com/articles/6-117/v1

6. Mouth hook movements

Open source file:
Majeed, Z., Koch, F., Morgan, J., Anderson, H., Wilson, J., and **Cooper, R.L. (2017)** A novel educational module to teach neural circuits for college and high school students: NGSS-neurons, genetics, and selective stimulations. F1000Research. F1000Research: Immediate & Transparent Publishing for Life Scientists. F1000 Research Ltd, Middlesex House, 34-42 Cleveland St, London W1T 4LB, UK. https://f1000research.com/articles/6-117/v1

7. Ethograms and why ethograms

Define Ethology: https://en.wikipedia.org/wiki/Ethology

https://www.britannica.com/science/ethology

Movie we made explains why we use ethograms.

8. HAT ASSAY:

Defined in this publication: Titlow, J.S., Rice, J., Majeed, Z.R., Holsopple, E., Biecker, S., and Cooper, R.L. (2014) Anatomical and genotype-specific mechanosensory responses in *Drosophila melanogaster* larvae. Neuroscience Research 83:54-63. doi: 10.1016/j.neures.2014.04.003. [PDF]

Locations and careful description

1. Side of head lateral
2. ½ way along side
3. Side of caudal end.

9. DEVELOP OF FRUIT FLIES-PUPATION RATE:

Defined in this publication: Potter, S., Krall, R.M., Mayo, S. Johnson, D., Zeidler-Watters, K., and Cooper, R.L. (2016). Population dynamics based on resource availability and founding effects: live and computational models. The American Biology Teacher 78(5): 396–403, ISSN 0002-7685, electronic ISSN 1938-4211. [PDF]

10. Anesthetize adult flies for moving them around

Movie above explains this procedure.

11. Light and gravity sense in adults

Open resource: Badre, N.H., and Cooper, R.L. (2008) Reduced calcium channel function in *Drosophila* disrupts associative learning in larva, and behavior in adults. International Journal of Zoological Research 4 (3):152-164.
http://web.as.uky.edu/Biology/faculty/cooper/COOPER-PUBLICATIONS.htm

12. Effects of ketogenic diet specifically

http://www.medicaldaily.com/role-diet-and-metabolism-epilepsy-fruit-fly-study-promises-hope-treating-seizures-269693

Why use this ketogenic diet?

- **Significant research with reduction in seizures (30% free, 60% reduced)**
- **The "ketone bodies" circulate in the blood and provide alternative fuel source for tissues and brain**
- **Promising future research**
- **Atkins diet: high fat, high protein, low carb**

13. Measuring heart rate in larvae.

Cooper, A.S., Rymond, K.E., Ward, M.A., Bocook, E.L. and Cooper, R.L. (2009) Monitoring heart function in larval *Drosophila melanogaster* for physiological studies. Journal of Visualized Experiments (JoVE) 32: http://www.jove.com/video/1596/monitoring-heart-function-larval-drosophila-melanogaster-for)

Materials for one set up

A list of equipment and supplies needed for a class of 24 students (8 setups with 3 students per station)

Forceps

Dissecting microscope (self-made ones are feasible)

Larvae crawling on a 2% apple juice agar plate made in Petri dishes (about 8.5-9 cm diameter).

Vials with cotton balls to grow larvae

Fine tipped Sharpe pens

4 Vials or tubes: about 9.4 height, 2.4 cm diameter (top), 2.25cm diameter (bottom).

For each student group:
6 small vials (25x95 mm, Cat#:32-120 https://geneseesci.com/)
1 Fine point permanent marker
6 cotton balls
Fly food substance
Wild type *Drosophila melanogaster* (6 males, 12 females
Means to anesthetize flies (carbon dioxide, ether, FlyNap, cold)
Hand lenses/ 1 dissecting scope
Paint brush/forceps (to move flies)
Fruit fly sex determining guide
Data table
Computer with JointPoint, Excel, or other spreadsheet software program

Relevance:

Throughout the module students will have an opportunity to engineer a range of models to demonstrate and test the impact of different variables on human systems using fruit flies as an example. The Next Generation Science Standards (NGSS Lead States, 2013) focus on exploration of natural phenomena through practices consistent with those scientists employ in the development of scientific knowledge. One of the practices that separates NGSS from previous national science standards and frameworks is the emphasis on using models as tools for thinking, visualizing, and making sense of phenomena and experiences (Krajcik & Merritt, 2012). Students can use models to make sense of what they observe and to make their thinking visible. Doing so allows them to construct and share their explanations with other students and refine their models as they continue to study a phenomenon in different contexts. NGSS recommends that models be used to generate data to support explanations, predict phenomena, analyze systems, and/or solve problems.

Focusing on metabolic syndrome as a theme provides students with an authentic context in which to learn and apply scientific concepts. Doing so can also help students develop and apply scientific practices (Coll, France, & Taylor, 2005). Using models to explore and construct scientific explanations promotes metacognitive thinking, communication skills, and creates opportunities for students to participate in the development of scientific knowledge (Gilbert, Boulter, & Elmer, 2000). The use of physical models with guided inquiry supports the conceptual nature of the topic (Coll et al., 2005; Ucar & Trundle, 2011). Further, in defining components of a complex biological problem, students and teachers can progress through increasingly complex objectives in a logical, stepwise progression.

Student assessment

In assessing student learning and the practically of the conducting the various components in implementation of the full module undergraduate college freshman group of students volunteered as non-identifiable individual participants. They provided feedback in a a pre- and post-survey on concepts related to diet and health as well as in the use of pedagogical tools such as models was implemented. They also worked through each module after taking the pre-survey. After completing the modules and sharing out amongst the entire class the various findings the students took a post survey. The responses on the surveys were analyzed and compiled as presented in the Appendix.

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Appendices

Include addresses of suppliers, instructions for the preparation of media and chemicals, rearing of animals, growing of plants, and any other special instructions and information.

1. List of supplies and suppliers for this exercise.
    TBA, This will be forth coming as I am also checking on prices to list.

2. To maintain adult flies for breeding and rearing. Cornmeal-molasses-agar culture for Drosophila

water 420 ml .
agar 4.5 gm
unsulfured molasses 60 ml
cornmeal 49 gm
brewer’s yeast 6.5 gm
cold water 145 ml
propionic acid 3.4 ml

Mix and boil water and agar 3–5 minutes.Add unsulfured molasses and heat to boiling again. Mix together cornmeal, brewer’s yeast, and cold water in a separate container until all lumps are removed. Add cornmeal-yeast mixture to molasses-agar mixture. Boil 5 minutes, stirring constantly. Cool mixture to 60°C. Add propionic acid (as mold inhibitor). Pour culture medium 1-inch deep into sterile culture jars with sterile plugs.

Add a sprinkle of active baker's yeast (from a salt shaker) to each jar before adding flies.

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Science supply warehouses, such as Bloomington Stock Center (http://flystocks.bio.indiana.edu/) offer established mutations that correlate to human diseases (Casci and Pandey, 2015; Taniguchi and Moore, 2014),

This unit contains pedagogical support to assist teachers with facilitating open inquiry to acquire new knowledge (Abrams et al., 2007; Appleton, 1995; Nadelson, 2009).