What makes me tick...tock?

Circadian rhythms, genetics, and health

Lesson 2: Why do scientists study fruit flies to understand what makes us “tick”?

I. Overview
Following the introduction of basic principles and key terminology of circadian rhythmicity in Lesson 1, this lesson begins with students discussing model organisms and how scientists use them; Drosophila melanogaster is used as a model for the study of circadian rhythms. Students use several NetLogo simulations to examine how light, temperature, and genetic mutations can affect a fly’s behavior. Students make connections between these simulations and their own circadian rhythms, developing models throughout the lesson of how three different factors (light, temperature, and genetic mutations) can affect both the flies’ activity levels and their own throughout the day.

Connections to the driving question
This lesson allows students to use a model to address the driving question, collecting data to draw conclusions on what drives our circadian rhythms. The created models allow students to analyze how environmental influences and genetics can impact an organism’s circadian rhythm. This information is then applied to their own lives in a concluding discussion at the end of the lesson.

Connections to the previous lesson
In the previous lesson, the students generated ideas and hypotheses regarding what impacts the circadian rhythms as well as what the circadian rhythm impacts in daily life. Also, they use an experimental model, the fruit fly to examine how light/dark cycles impact circadian rhythms, similar to the experiment Michel Siffre performed on himself in the cave.

II. Standards/Benchmarks

National Science Education Standards

Content Standard A: Abilities necessary to do scientific inquiry
- Identify questions and concepts that guide scientific investigations. Students should formulate a testable hypothesis and demonstrate the logical connections between the scientific concepts guiding a hypothesis and the design of an experiment. They should demonstrate appropriate procedures, a knowledge base, and conceptual understanding of scientific investigations. (9-12 A: 1/1)
- Formulate and revise scientific explanations and models using logic and evidence. Student inquiries should culminate in formulating an explanation or model. Models should be physical,
conceptual, and mathematical. In the process of answering the questions, the students should engage in discussions and arguments that result in the revision of their explanations. These discussions should be based on scientific knowledge, the use of logic, and evidence from their investigation. (9-12 A: 1/4)

- Recognize and analyze alternative explanations and models. This aspect of the standard emphasizes the critical abilities of analyzing an argument by reviewing current scientific understanding, weighing the evidence, and examining the logic so as to decide which explanations and models are best. In other words, although there may be several plausible explanations, they do not all have equal weight. Students should be able to use scientific criteria to find the preferred explanations. (9-12 A: 1/5).
- Scientific explanations must adhere to criteria such as: a proposed explanation must be logically consistent; it must abide by the rules of evidence; it must be open to questions and possible modification; and it must be based on historical and current scientific knowledge. (9-12 A: 2/5)
- Results of scientific inquiry—new knowledge and methods—emerge from different types of investigations and public communication among scientists. In communicating and defending the results of scientific inquiry, arguments must be logical and demonstrate connections. (9-12 A: 2/6)

**Content Standard C: The Behavior of Organisms**

- Organisms have behavioral responses to internal changes and to external stimuli. Responses to external stimuli can result from interactions with the organism's own species and others, as well as environmental changes; these responses either can be innate or learned. The broad patterns of behavior exhibited by animals have evolved to ensure reproductive success. Animals often live in unpredictable environments, and so their behavior must be flexible enough to deal with uncertainty and change. Plants also respond to stimuli. (9-12 C: 6/2)
- Behavioral biology has implications for humans, as it provides links to psychology, sociology, and anthropology. (9-12 C: 6/4)

**Content Standard G: Nature of Scientific Knowledge**

- Scientific explanations must meet certain criteria. First and foremost, they must be consistent with experimental and observational evidence about nature, and must make accurate predictions, when appropriate, about systems being studied. They should also be logical, respect the rules of evidence, be open to criticism, report methods and procedures, and make knowledge public. Explanations on how the natural world changes based on myths, personal beliefs, religious values, mystical inspiration, superstition, or authority may be personally useful and socially relevant, but they are not scientific (9-12 G: 2/2).

**Benchmarks for Science Literacy**

**The Nature of Science: Scientific Inquiry**

- Investigations are conducted for different reasons, including to explore new phenomena, to check on previous results, to test how well a theory predicts, and to compare theories. 1B/H1
- Hypotheses are widely used in science for choosing what data to pay attention to and what additional data to seek, and for guiding the interpretation of the data (both new and previously available). 1B/H2
- To be useful, a hypothesis should suggest what evidence would support it and what evidence would refute it. A hypothesis that cannot, in principle, be put to the test of evidence may be interesting, but it may not be scientifically useful. 1B/H9** (SFAA)

Common Themes: Models
- The usefulness of a model can be tested by comparing its predictions to actual observations in the real world. But a close match does not necessarily mean that other models would not work equally well or better. 11B/H3*
- The behavior of a physical model cannot ever be expected to represent the full---scale phenomenon with complete accuracy, not even in the limited set of characteristics being studied. The inappropriateness of a model may be related to differences between the model and what is being modeled. 11B/H5** (SFAA)

III. Learning Objectives

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<tr>
<th>Learning Objectives</th>
<th>Assessment Criteria</th>
<th>Location in Lesson</th>
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<tr>
<td>Explain what a model organism is and why they are often used in experimental biology</td>
<td>A model organism is an organism, other than a human, used to study biology processes and/or questions. They are used for a range of reasons, including but not limited to: rate of reproduction and growth, number of offspring, ease of genetic manipulation, external fertilization and ease of care.</td>
<td>Opening of Lesson</td>
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<tr>
<td>Discuss why fruit flies are a good model organism for circadian rhythm studies</td>
<td>Fruit flies are a model organism used to study circadian rhythms for a number of reasons. They are relatively cheap and easy to care for and have large numbers of offspring. Their life cycle is relatively short so scientists can quickly see the effect of mutations on phenotypes. Genes have been identified that impact certain traits/behaviors. Their genome has been sequenced which allows scientists to compare the sequence of a fly gene to other organisms, such as humans.</td>
<td>Opening of Lesson and Activity 1</td>
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<tr>
<td>Develop a model for what influences circadian rhythms</td>
<td>Based on previous experiences and knowledge, students develop a model of what impacts circadian rhythms, including but not limited to what they</td>
<td>Activities 2 and 3</td>
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believe will happen under certain light/dark conditions, temperature changes, or genetic mutations to the flies’ activity levels.

<table>
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<th>Task</th>
<th>Description</th>
<th>Activity</th>
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<tbody>
<tr>
<td>Develop a hypothesis testing the proposed models.</td>
<td>Based on previous experiences and knowledge, students develop a hypothesis to test their model of circadian rhythms.</td>
<td>Activity 2</td>
</tr>
<tr>
<td>Manipulate a simulation and collect data to test a hypothesis</td>
<td>The students work with NetLogo simulations to collect data. They create data tables and graph representing their data. Students need to determine how frequently data needs to be collected to They should collect data at least once every hour.</td>
<td>Activities 2 and 3</td>
</tr>
<tr>
<td>Develop a scientific explanation for what impacts circadian rhythms using the claim, evidence and reasoning framework.</td>
<td>Students develop a revised hypothesis, or add evidence to their accurate hypothesis, based on the data collected from the NetLogo simulation. In their explanations, they state their claim (revised hypothesis), evidence that supports their claim, and scientific reasoning that explains why the evidence is sufficient to support the claim. They can also have a rebuttal where they state why another claim is not valid based on their evidence.</td>
<td>Activities 2 and 3</td>
</tr>
<tr>
<td>Revise their initial circadian rhythm model based on new information.</td>
<td>Students use the evidence from the NetLogo simulation to either support or refute their initial circadian rhythm model (hypothesis). Their revised model should contain more specific information than their initial model.</td>
<td>Activities 2 and 3</td>
</tr>
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**IV. Adaptations and Accommodations**

While it would be ideal for students to be able to work in groups of two while using the models, this lesson may be done as a whole class demonstration/discussion if only one computer is available. If this is done, it is important that students are able to see the models as they are run. Alternatively, it would be possible for groups of 3-4 students to work together throughout this lesson.
V. Timeframe for activity

Opening of Lesson
- Review of Siffre Video – 2-3 minutes
- Model Animals – 5 minutes
- What are models? Why use them? – 5 minutes
- Flies as models of circadian behavior (including fly activity video) – 5-10 minutes

Main Part of Lesson
- Activity 1: Investigating live fruit flies’ activity levels
  - Live fly observations – 20-25 minutes
- Activity 2: Environmental effects on fruit flies’ activity levels
  - Introduction to NetLogo models and experiment – 10 minutes
  - Discussion of experimental design – 10 minutes
  - Using NetLogo to Collect Fly Data – 25-30 minutes
- Activity 3: Genetic influences on fruit flies’ activity levels
  - Collect data – 20-25 minutes
  - Data analysis and sharing with group---how mutants compare/contrast in behavioral effects – 5-10 minutes

Conclusion of Lesson
- Sharing of scientific explanations – 5 minutes
- What other variables might we test? – 2-3 minutes
- Making connections to previous lesson: males/females; genetics – 5 minutes
- Introduce Lesson 3 – 2-3 minutes

VI. Advance prep and materials

Opening of Lesson

Materials:
- Computer with projector
- Internet access for all computers

Preparation:
- Before class begins, connect a computer to the classroom projector and access “Fly Activity” video at the following URL: http://www.hhmi.org/biointeractive/media/activity_pattern-pattern-lg.mov

Activity 1: Investigating live flies’ activity levels

Materials:
• Student Reading on Drosophila (U3_L2_Reading_Drosophila411.docx) (Optional)
• Live Fly Observation Sheet, (U3_L2_StudentSheet_InitialFlyObservations.docx)
• 1-2 vials of flies per group of students. ~6-12 vials per class.
• Box of parafilm (optional) to cover vials when students trace flies’ paths.
• Timers
• Sharpie markers
• Floss or string
• Rulers

Preparation:
• Distribute the flies evenly across the number of vials needed. Each vial will have Drosophila medium and a stopper so the flies cannot escape.
• If desired, print Drosophila 411 student reading for students to complete in class before working with fruit flies.

Activity 2: Environmental effects on fruit flies’ activity levels

Materials:
• Computers for students (1 computer per 2-3 students)
• NetLogo program loaded onto computers
• “Fly Composite” Circadian NetLogo model available through Project NEURON: Fly A is 14:10 L/D; Fly B is 12:12 L/D; Fly C is 10:14 L/D; Fly D is 8:16 L/D (L/D= light/dark hours) while X= 18°C, Y=25°C, and Z=29°C.
• It is necessary that the NetLogo application be loaded onto the computers or that the computers be able to access the NetLogo models on the internet found on the Project NEURON website (neuron.illinois.edu). If accessing the NetLogo models on the Internet, no additional application is needed.
• NetLogo Temperature/Light Investigation sheet (found as U3_L2_StudentSheet_TempLightInvestigation.docx)
• Graphing paper (optional)

Activity 3: Genetic influences on fruit flies’ activity levels

Materials:
• Computers for students (1 computer per 2-3 students)
• NetLogo program loaded onto computers
• “Fly Mutant” Circadian NetLogo model available through Project NEURON
• It is necessary that the NetLogo application be loaded onto the computers or that the computers be able to access the NetLogo models on the internet found on the Project NEURON website (neuron.illinois.edu). If accessing the NetLogo models on the Internet, no additional application is needed.
• NetLogo Genetics Investigation sheet (found as U3_L2_StudentSheet_PerGene.docx)
• Graphing paper (optional)

Preparation:
• Make certain that all NetLogo models are working and understandable. NetLogo and NetLogo models need to be loaded on the individual computers or all computers need to have Internet access. The NetLogo application is freely available from Northwestern and can be downloaded http://ccl.northwestern.edu/netlogo/

Conclusion of Lesson

Materials:
• Computer with internet access
• Mouse actogram found on the Project NEURON website on the Games and Media page

Homework and Assessments
The Drosophila 411 (U3_L2_Reading_Drosophila411.docx) reading can be assigned in class or as homework. The students can also complete the results and conclusions sections of the student investigation sheet at home.

The student investigation sheet should provide the instructor with the ability to gauge how well the students are able to develop a hypothesis, collect data, organize their results and draw conclusions. The per gene mutant observations sheet will also allow students to practice their data collection and graphing skills further, and analyzing data to draw a conclusion on how a genetic mutation influences circadian rhythms. Also, the guided reading questions from the Drosophila 411 reading should enable students to discuss the importance and relevance of model organisms in scientific research.

VII. Resources and references
VIII. Lesson Implementation

Opening of Lesson:
To open the lesson, ask students to recall the video they watched in Lesson 1 on French scientist Michel Siffre. Use the following questions to help guide the discussion:

- What were the major conclusions they drew from the video?

Emphasize the use of circadian terminology, as developed in Lesson 1, to describe the video: for example, when Siffre was in the cave, he had no zeitgebers available to him. Therefore, he was on a free running schedule based on his endogenous bodily rhythms. Since the human body’s endogenous rhythms are not quite in sync with the 24-hour day and absent entrainment to the sun, Siffre’s circadian rhythms were phase-shifted over the time he was in the cave.

- Why don’t more people live in caves now to help us learn more about circadian rhythms?
- How long do they think Siffre would have been willing to live in the cave?
- What could a researcher do if he/she wanted to study the circadian rhythms of an animal for its entire life cycle in the cave?
  - This question should be used to introduce the concept of model organisms.
- What is a model organism? How is this organism different from a model?
  - Students should have a basic idea what a model organism is based on the Lesson 1 homework reading that they did.
- Why might a scientist use a model organism as a research tool?
  - There are many reasons as to why a scientist might use a model organism. Reasons include (but should not be limited to): ethics, abundance of the organism, cost of maintaining in a research facility, ease of manipulation (genetically and sometime physically), rate of reproduction, rate of growth (life cycle), external fertilization (in some cases).
  - Model organisms often have characteristics that fit well with the research question or approach.

Provide the class with one common model organism they might be familiar with, the mouse. Explain to the class that mice share many things in common with humans; our nervous systems, internal organs, and life cycles are very similar. Because of this, the mouse is often used by many researchers who study human diseases and medicines.

- Ask the class if they can think of other model organisms. What could each be used to study?
  - Mice, rats, fish, Xenopus (frog), flies, c. elegans (worms) and planarians are just some examples of model organisms used in scientific research. One of the recipients of the 2012 Nobel Prize in Physiology or Medicine, John Gurdon, did his work using Xenopus.
- What sort of organisms might be suitable for studying circadian rhythms?
  - In general, any animal whose circadian activity can be well-monitored and easily manipulated by environmental cues in a confined environment.
Tell students that in this lesson, they will examine data from an experiment similar to the one Siffre ran on himself but uses flies as an experimental organism. Ask students:

- Why might scientists have selected flies to use instead of humans?
  - They’re cheap to obtain, easy to observe, reproduce prolifically, and do not pose the same ethical concerns that working with vertebrates does.

Ask the students what they suppose a day in the life of a fruit fly is like; what may its circadian activity involve.

- To help students answer this, show the HHMI video on fly activity at this point. [http://www.hhmi.org/biointeractive/media/activity_pattern-lg.mov](http://www.hhmi.org/biointeractive/media/activity_pattern-lg.mov)

Use this video to motivate a discussion about using flies in research. Emphasize the computerized fly in the video has its activity mapped on an actogram, a tool discussed in Lesson 1. Ask students: when was the fly’s greatest activity?

- Students should be able to identify that flies were the most active at dawn and dusk, with the activity at dusk greater than that at dawn.

Ask students if they know the term for an animal that displays its greatest activity at dusk and dawn (crepuscular). If there is interest, have students brainstorm other animals that are crepuscular (e.g. deer, rabbits, cats and dogs…) and why (avoid predation, heat).

In the discussion, highlight that when the fly is kept on a 12-12 light/dark cycle, its activity occurs at fixed times every day. Have students hypothesize why this is the case. This level of activity is due to entrainment, where the light in the vial is the zeitgeber, acting as the sun would act.

- Ask students what happens when the fly is kept in perpetual darkness.
  - Flies go to a free-running schedule, as Michel Siffre was in the cave.

Explain that there is a phase shift in the fly’s rhythms under these circumstances. Because the fly’s endogenous rhythms are on a 24.5-hour cycle, slightly greater than the solar cycle of 24 hours. In the absence of lighting, the fly’s activity patterns depend on its endogenous rhythms.

Ask students what they would predict if the flies were entrained to a different length of light, how would the fly’s behavior change? What would happen if the amount of light was shortened (8 hours or 10 hours) or if it was lengthened (14 hours)?

Have students draw and describe this initial model of what controls the flies’ circadian rhythms under the specific conditions on a separate piece of paper, that they will refer back to at the end of the lesson.
Scientific Practices: Developing and Using Models
In this lesson, students articulate their initial models of what they believe will happen to the flies in certain environmental conditions as well as with certain genetic mutations. Scientists almost always have a model of what they believe will happen as a result of an experiment, even if they do not have specific previous experimental results to fully support their models. However, just as the students will do, scientists always base their initial models on what they currently know about the subject, no matter how much or how little they know about the topic. Just as scientists constantly revise their models based on new evidence from experiments, students will go through cycles of revising their models based on new evidence collected after each NetLogo investigation.

Main Part of Lesson

Activity 1: Investigating live flies’ activity levels
After students have developed their initial model, ask them:

• How might they test their model of fruit fly activity under different entrainment schedules?
  o Design and carry out an investigation.
  o Manipulate a simulation.

Turn the students’ attention to the Initial Fly observation sheet.

Demonstrate how to measure fly activity using floss in front of the whole class on the whiteboard. Make sure that students understand how to take accurate measurements.

In their student groups (3-4 students) distribute the fly vials, Sharpie markers, stopwatches, parafilm (optional), floss or string, and rulers. Allow students to investigate fly movements before moving on to NetLogo models so they can observe their subjects first-hand.

Teacher Pedagogical Content Knowledge
Students have the opportunity to measure the flies’ activity in a hands-on manner, using the string to measure the distance in a 30 second time period. If they were to actually test their models, this method of data collection would take many weeks, since they would need to collect multiple trials, set up multiple entrainment schedules, etc. Thus, the NetLogo simulations allow the students to collect the flies’ activity level data, and continue their experiment, in a more time efficient way using data previously collected in a research laboratory.

Tell students that they will be using several different NetLogo models that will allow them to test their predictions of what happens when the fly is exposed to a shortened or lengthened amount of light over a 24-hour period.
Ask students why they think that scientists might be interested in studying these types of questions.

- Do these sets of simulations have any connection to human lives?
  - Day lengthening and shortening depending on the seasons. Careers where humans have to change their circadian rhythm depending on when they need to be active.

Explain to students that before they can measure circadian activity of the fly, they must first define what circadian activity is.

Ask students what might constitute circadian activity in flies. Remind students that fruit flies are crepuscular, exhibiting their greatest activity at dawn and dusk, although they are known to exhibit activity at all times of day. Make a list of these activities on the board, evaluating the choices for accuracy as students present them.

- Examples might include walking, flying, foraging and mating. Of these, movement will be readily observable to the students in the NetLogo models.

**Activity 2: Environmental effects on fruit flies' activity levels**

Explain to students that they will be working in groups of 3–4, with each group examining all of the NetLogo models in order to determine how changing the amount of light/dark impacts the activity of the flies.

Explain that students will be using the NetLogo models to test the hypothesis of how the amount of light and temperature influences the behavior of flies.

Ask students, what makes a successful experiment? Tell students that they need to consider these issues even though they will be collecting data from a simulation. Tell students that the simulation is modeled after data collected by scientists using flies that have been entrained to specific light dark cycles. It will be their (the students’) responsibility to identify what light/dark cycle the flies have been entrained to.

As students volunteer information, make a list on experimental design elements on the board. Using the list, have a discussion about key elements to include in the design of an experiment. Make certain to include the following:

- A hypothesis
- Variables and constants
  - What factors in the experiment will be changing? What are independent and dependent variables?
    - **Independent variables** are those directly manipulated by the experimenter such as amount of light in our fly experiments or the temperature changes. In the first experiment, the amount of L/D will be kept same, 12:12, while the temperature will change from 25°C to either 18°C or 29°C.
    - **Dependent variables** generally represent measures taken in an experiment, such as amount of activity expressed by the flies. Experiments are often designed to
detect changes in dependent variables resulting from a change in independent variables.

- Which variables will be kept the same? Why is it important to keep certain variables constant or the same throughout the experiment?

- Methods of data collection
  - What are some different methods of collecting and expressing data?
    - Making observations, taking explicit measurements, drawing pictures, etc.
  - Discuss the concept of a sample. It is impossible to collect information from all members of a group so we sample that group. Highlight real-world example; e.g. quality control for food
    - Oftentimes in life science research data are converted to numbers so results can be best expressed in a graph

Scientific Practices: Analyzing and Interpreting Data
A key scientific practice for students to develop proficiency in is collecting and representing their data appropriately. When collecting data from NetLogo, students may choose to pick timepoints at every five hours and then graph their results. Their graph will appear astonishingly different from another group who chooses to collect data every hour. Depending on the class, it may be of interest to assign students different intervals of time to collect data of which to graph, to show the importance of collecting enough data to more appropriately accept or refute one’s hypothesis. This can also be seen in the scientist’s lab, where taking too little data may draw inappropriate conclusions.

- Awareness of potential errors and experimental limitations
  - Can any experiment be implemented perfectly? Why is it important to anticipate possible errors before attempting an experiment?
  - No experiment is ever “perfect.” Scientists often run the same experiment multiple times and get different results. Biological systems in particular are extraordinarily complex, and scientists are never able to account for all the variables within an organism or its environment that can produce effects on independent variables. By designing an experiment with attention to the ways in which it might possibly deviate from your hopes or expectations, you are better equipped to confront unexpected results, explain variability between observations and measurements, and subsequently refine your experimental design as necessary.

In the discussion, stress that while scientists approach experimental design in many different ways, they often generate experiments directly from hypotheses.
Teacher Pedagogical Content Knowledge

It would be helpful to use examples that could scaffold the discussion about what makes a good experiment. Examples should be related to either class activities and experiences or real life experiences. By having a hook, students will be more engaged in the discussion about elements of an experiment.

Hand out the NetLogo Temperature/Light investigation sheet. Ask students how they would define or describe what a hypothesis is.

- The technical definition is “a tentative explanation for an observation, phenomenon, or scientific problem that can be tested by further investigation.”
- How is a hypothesis different from a simple guess or a prediction?

However students answer, emphasize that hypotheses do not arise in a vacuum, as a guess might. In fact, hypotheses are based off of previous experimental observations and conclusions and/or theoretical models.

Emphasize that a good hypothesis should be testable; that which cannot be disproved is outside the realm of experimental science.

- What might be a good hypothesis to test about circadian rhythms using the NetLogo models?

Tell students that the variables that change in these models is the length of light per 24 hour period and the temperature, either room temperature (25°C), 18°C, or 29°C. In this first activity, they will actually make three hypotheses, explaining how activity levels will change based on the temperature with the 12:12 L/D entrainment kept constant.

Remind students to put a reason why they believe their hypothesis to be correct based on their understanding of circadian rhythms in humans, since they have had the most experience with their own sleep/wake cycles. Ask the students:

- How would your sleep/wake schedule respond to changes in light/darkness in your environment?
- How would your sleep/wake schedule respond to changes in temperature? (Think about winter and summer sleep habits!)

Students should be told that they will be able to access a number of different models where the length of light varies over a 24 hour cycle and at different temperatures. Their hypotheses reflect their initial understanding of fruit fly entrainment on a 12:12 L/D cycle, with changes to temperature addressed.

- How might they collect data to evaluate their hypothesis?
  - Point out that when scientists typically study an animal’s circadian rhythms, they measure activity frequently or constantly over a 24-hour period.
Tell students that they will be able to collect data over a 24-hour cycle from each model.

- Ask students how will they want to organize their data? How many flies should they sample? Should they average the data that they collect?
  - Data should be collected from a number of different times across the 24-hour day. Students need to decide how many time points they need to collect.
  - In the NetLogo simulation, students can choose how many flies they wish to collect data from. They should collect data from 3-5 flies, as the “average distance travelled” number in the simulation already has averaged the distance travelled for the 3-5 flies. Thus, students will not have to collect multiple trials, this has already been built in to the simulation.

Ask the class why it might be beneficial for each group to work with more than one fly while collecting data from their models.

- Explain that a large sample size in an experiment is important in scientific research; if one individual in a population is an outlier (is not representative of the general population in some respect), averaging over many individuals minimizes the impact of the outlier. Replicating the same experiment multiple times also increases the significance of collected data. Given the extent to which individual flies move, having more than two flies in a single vial could make measurements of their activity prohibitively difficult.

If there is sufficient time, students can collect data from multiple runs of the NetLogo models.

Hand out the Student Investigation Sheet, and optional graphing paper so that students can begin developing their hypothesis and creating their data tables. This should be completed in their groups with the instructor walking around to make sure students understand what is asked of them.

- Data tables need to include at least 12 different times throughout the day so students can generate appropriate graphs.
Have students open the NetLogo Model “Fly Composite.” The students should access Fly B, entrained on the 12:12 L/D cycle and choose the 25°C option first.

Before allowing the students to explore the model, explain a few key points about the NetLogo interface. Do this by having the students place 5 flies on the “world”; moving the slider to 5 hours; pressing set up and clicking go.

Questions to ask the students:

- What are the “flies” on the screen doing? Have students describe what happens.
- What behavior does this mirror with the real fly?
- What would happen if you changed the hours?

Point out to the students that you can speed up or slow down the movement of the flies by moving the slider at the top of the model (“normal speed”). Trails can be cleared by clicking the “clear trails” button. Flies can be cleared by pressing the “clear flies” button. This allows students to take measurements of the path length that a fly moved. Ask students why this might be important.

Allow students to continue to explore the model interface. Make sure to tell students that to start and stop the model in NetLogo, they click the “go” button.
To modify the parameters of the model, change the variable by moving the slider (i.e. the number of flies or the hours and then click the “setup” button. If the button “setup” is not clicked, the old variables are maintained. Ask students to think about the following questions:

- In carrying out their data collection, should students change the number of flies? Should they change the hours?
- How are the different cycles influencing the flies’ behaviors?

Students should be encouraged to manipulate the hour slider and to collect data in a systematic manner. Allow students to open the other simulations and have them identify what light-dark cycle is represented in each.

Students should construct data tables and graph for the other simulations. It might be easier for students to graph their plots using graphing paper.

**Activity 3: Genetic influences on fruit flies’ activity levels**

After students have become familiar with wild type fruit flies entrained on various L/D schedules, have students open the “Fly Mutant” simulation and explore how per gene mutations in fruit flies affects their response to entrainment.

- The per gene family is found in mice, flies, and humans - so the mutations represented in the simulation are mutations that could potentially be found in humans!

Hand out the *U3_L2_StudentSheet_PerGene* document and have students work in groups to complete the activity. Here, each student will choose one or both mutants (*perA, perB*) and compare it to the *perG* mutant which confers a normal phenotype. Have students collect data and graph the results to share with the other group members. Thus, each student will record how each mutation affected the flies’ circadian rhythms.

After students have collected and graphed their data, have them re-visit their conclusions from the end of Activity 2. At the end of Activity 2, students will have strengthened their models of how the flies’ activity levels change due to temperature and light. Now, students should add to that model and explain how the various mutations in the per gene affect the activity levels of the flies (Question 1 of the Conclusion section of the Per Gene Mutant Observations student sheet).

The last conclusion question can be asked to the whole class:

- How would your life be affected if you had the same genetic mutation as the fly you studied?
  - This question links to Lesson 4, where students will investigate how one young man’s circadian rhythm is affected, which negatively impacts his school, family, and social life.

**Conclusion of Lesson**
After students have created their scientific explanation asked for on the student sheet, have them share it with the rest of the class. Have a discussion regarding how their hypotheses were supported or refuted based on the evidence they collected in the two activities.

In addition, ask students:

- How have you models about the sleep/wake cycle changed due to the NetLogo simulations?
- Are flies a suitable model organism to test your ideas regarding changes in circadian rhythms? Is there a better model out there to answer your questions?

It is important in this activity to stress that the NetLogo model is a model and, as such, has limitations — this is the very nature of a model and so there might be multiple “correct” explanations. What is important with the scientific explanations is that students present their evidence and reasoning.

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<td>After the students have collected their data, they will revise their initial models to include more specific evidence to support their initial model with more detail or to refute their initial model to make it more aligned with the collected data. While their initial model was based on their own experiences with circadian rhythms and information from Lesson 1, they now have a more sophisticated model based on empirical research data collected from a research laboratory.</td>
</tr>
</tbody>
</table>

Make certain to make connections back to the hypothesis and how these models can be connected to understand the processes in a living fly and by extension to other organisms as well. Open the mouse actogram located on the Games and Media page on the Project NEURON website [http://neuron.illinois.edu](http://neuron.illinois.edu) and have students look at the 12-12 activity pattern. Ask the students:

- How does the activity of the mouse compare or contrast with that of the fly?

Run the full dark and then the full light. Ask students to comment on what patterns they see.

Ask students if this behavior is something that they would expect to see with the flies based on what they just did.

Have a discussion about what are the strengths and limitations of using these different types of models and model organisms that scientists use to study circadian rhythms in laboratory settings:

- How do the computer simulations help us understand what a circadian rhythm is and what controls these processes?
- How do the animal models help us understand what a circadian rhythm is and what controls these processes?
- Are there other variables that might be important in controlling circadian rhythms? What might these be? How could you test these additional variables?
Ask students to come up with an endogenous cue that could have affected their circadian rhythms in this experiment.

- Review the definition of “endogenous.” The students should think about Activity 2, how genetics influenced the flies’ circadian rhythms.

Have students return to the original model that they develop at the opening of the lesson. Ask students to now revise this model based on what they learned through the lesson. Allow students to revise this original model and have students provide a written explanation. This revision and explanation process is an important part of processing information. This revised model and explanation can be used as both a formative and summative assessment.

Tell students that they will be developing a model of the per2 gene in the next lesson and then transcribing and translating it to the protein PER2. PER2 is a key protein responsible for keeping an organism’s “clock” running smoothly. They will also observe what happens when the gene is mutated and how that affects the final protein structure.

**Assessment**

During this lesson there are ample opportunities for informal, formative assessments during group and whole class discussions. Additionally, monitoring students’ progress during the live fly and NetLogo fly simulation activities allows for evaluation of students’ abilities to perform the tasks. Formal formative assessments occur with the completion of the Fly Observations Student Sheet and NetLogo Investigation Sheets. Notably, evaluations of students’ abilities to conduct key scientific practices are found in the recording of their initial models, collecting data, and then analyzing their data to revise their models.

**Continuing with a hands-on extension activity:**

If students are interested, they should be encouraged to complete the Lesson 2 extension activity where they work with living flies to design an entrainment activity. The instructions for this activity are found in U3_L2_Extension_LessonPlan.pdf.