What can I learn from worms?

Regeneration, stem cells, and models

Lesson 3: How do planarians regenerate?

I. Overview
Students are introduced to the use of planarians as a model for neural regeneration through a historical overview about planarians as a model organism and a basic exploration of planarian biology. In this lesson, students design and carry out an experiment focused on developing their understanding of regeneration. Students do this by cutting planarians in different ways based on an explicit hypothesis they develop. Over the course of several days, students record a range of observations about their planarians’ regeneration.

In the conclusion of Lesson 3, included as a separate lesson plan, students analyze their data in order to evaluate their hypothesis and draw scientific conclusions about planarian regeneration. Groups of students present their findings to the rest of the class, which effectively concludes the regeneration experiment.

Connections to driving question
The students collect evidence and conduct their own research to learn about the regenerative capacity of the flatworm. The students will use this landmark experiment to collect data throughout the unit, which will delve deeper into the cellular mechanisms of regeneration.

Connections to previous lessons
After observing the purposeful behaviors of planarians, such as chemotaxis, phototaxis and mechanotaxis, students can hypothesize how environmental stimuli may affect regeneration. In addition, the previous lesson taught students that simple creatures like planarians have complex behaviors; these traits have benefitted their species to enhance their survival.

II. Standards

National Science Education Standards
- Content Standard A: Understandings about Scientific inquiry. Scientists usually inquire about how physical, living, or designed systems function. Conceptual principles and knowledge guide scientific inquiry. Historical and current scientific knowledge influence the design and interpretation of investigations and the evaluation of proposed explanations made by other scientists. (9-12 A: 2/1)
- Content Standard A: Understandings about Scientific inquiry. 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 knowledge (9-12 A: 2/5)

- Content Standard C: The Cell. Cells can differentiate, and complex multicellular organisms are formed as a highly organized arrangement of differentiated cells. (9-12 C: 1/6)
- Content Standard C: The behavior of organisms. Multicellular animals have nervous systems that generate behavior. ... In sense organs, specialized cells detect light, sound, and specific chemicals and enable animals to monitor what is going on in the world around them. (9-12 C: 6/1)
- Content Standard C: The behavior of organisms. All organisms must be able to obtain and use resources, grow, reproduce, and maintain stable internal conditions while living in a constantly changing external environment. (builds on 6-8 C: 3/1)
- Content Standard G: Historical perspectives. The historical perspective of scientific explanations demonstrates how scientific knowledge changes by evolving over time, almost always building on earlier knowledge. (9–12 G: ¾)

**Benchmarks for Science Literacy**

**Common Themes: Systems**

- Even in some very simple systems, it may not always be possible to predict accurately the result of changing some part or connection. 11A/H4

**The Nature of Science: The Scientific Worldview**

- No matter how well one theory fits observations, a new theory might fit them just as well or better, or might fit a wider range of observations. 1A/H3a
- In science, the testing, revising, and occasional discarding of theories, new and old, never ends. This ongoing process leads to a better understanding of how things work in the world but not to absolute truth. 1A/H3bc
- In matters that can be investigated in a scientific way, evidence for the value of a scientific approach is given by the improving ability of scientists to offer reliable explanations and make accurate predictions. 1A/H3d

**The Nature of Science: Scientific Inquiry**

- Investigations are conducted for different reasons, including how to explore new phenomena, to check on previous results, to test how well a theory predicts, and to compare different theories. 1B/H1
- Sometimes, scientists can control conditions in order to obtain evidence. When that is not possible, practical, or ethical, they try to observe as wide a range of natural occurrences as possible to discern patterns. 1B/H3

### III. Learning Objectives
## Learning Objectives

**Identify basic distinguishing anatomical and physiological traits of planarians.**

Acceptable responses include:
- photoreceptors/eyespots, for sensing light
- pharynx, for ingesting
- gastrovascular system, for digestion and respiration
- nervous system, for sensing environment and locomotor behaviors

**Explain the use of planarians as a model system, with specific attention given to their regenerative abilities.**

Acceptable responses include discussion on:
- extraordinary ability of planarians to regenerate
- simple model system for complex biological behaviors

**Use historical documents in the design of an experiment.**

Acceptable designs will vary based on which hypothesis they would like to test. However, their designed experiment should appropriately test their hypothesis.

**Collect data by making basic observations about regenerating planarians.**

Acceptable answers will vary depending on how planarians were cut.

Data should include:
- drawings of the planarians day to day
- observations on changes in anatomy or function

**Propose, test, evaluate and revise a hypothesis based on data collected and observations made over several days of an experiment.**

Acceptable answers will vary but should include:
- use of collected data to confirm or reject hypothesis
- restructured hypothesis based on collected data
- observations showing regeneration of cut planarians across days

## Assessment Criteria

**Location in Lesson**

Hypothesis development and Cutting of planarian (planarian anatomy handout)

Opening of lesson

Pre-readings and Hypothesis development

Subsequent days in unit

End of unit
IV. Adaptations/Accommodations
Students who are opposed to or otherwise incapable of slicing planarians should be allowed to observe the procedures conducted by other members of their group. Such students will still be expected to participate fully in other aspects of the activity including planning for the cuts and developing hypotheses. For students who have a high interest in this activity, more cuts can be made with more planarians to test out a group’s multiple hypotheses. For students who may have difficulties articulating their scientific explanations for hypotheses, give examples during class as to what constitutes a scientific explanation and what does not using their planarian cut ideas.

Safety
Students need to take extra care and caution when using sharp objects to cut the planarians.

V. Timeframe for lesson

Opening of Lesson
- Introductory Discussion – 15 minutes

Main Part of Lesson
- Activity 1: Demonstrating the Procedure and Designing the Investigation – 10-15 minutes
- Activity 2: Carrying Out the Investigation and Initial Observations – 10-15 minutes
- Activity 3: Daily Regeneration Observation and Data Collection – 10 minutes in subsequent class periods

Conclusion of Lesson
- Summary discussion – 5-10 minutes

VI. Advance prep and materials

Activity 1: Demonstrating the Procedure and Designing the Investigation

Materials:
- “Cool Scientists: Morgan & Randolf” Pre-Reading
- Overhead projector or document camera
- Spring water
- 1 planarian
- 1 plastic coverslip
- 1 plastic pipette
- 1 small ruler
- 1 thermometer
- 1 flashlight
• 1 Petri dish with frozen spring water
• 1 piece of filter paper (coffee filter works well)

Preparation:
• Freeze a Petri dish full of spring water to use as during the planarian cutting demonstration.
• Have document camera or overhead set up before the start of the lesson in order to project the planarian cutting demonstration.
• Collect a coverslip, ruler, thermometer, piece of filter paper and flashlight for use during the demonstration.

Activity 2: Carrying Out the Investigation and Initial Observations

Materials:
• Each group should have the following items:
  o 1 plastic pipette
  o A minimum of 2 Petri dishes w/ covers (these can be purchased at carolina.com)
  o 2 Petri dishes with frozen spring water
  o 1 black permanent marker
  o 1–2 plastic coverslips
  o 1–2 magnifying glasses or microscopes
  o 1 small ruler
  o 1 thermometer
  o 1 flashlight
  o Appropriate number of planarians for each group. (This number will vary based on the investigations that are designed.)
• “Planarian Investigation” Student Packet, 1 per student (U2_L3_StudentPacket_PlanarianInvestigation.docx)
  o “Planarian Anatomy” handout (pg. 1)
  o “Hypothesis Development and experimental design” handout (pg. 2)
  o “Sketch Your Cuts” handout (pg. 3)
  o “Planarian Data and Observation Sheet” handouts (pg. 4-9)
• For the entire class:
  o 2–4 one-gallon bottles of spring water
  o 1 pre-chilled stock container of ~50–100 planarians
  o Filter paper

Preparation:
• The day before the lesson, place an appropriate number (i.e., 2 X the number of groups) of Petri dishes half-filled with spring water in a freezer
• About 20 min. before the beginning of the period, place the planarian stock container on a bed of ice.
• Place all the group materials and spring water in an accessible area of the classroom.
• Designate a somewhat darkened area of the classroom for the storage of the student groups’ Petri dishes at the end of the lesson.
• Make certain students have Pre-Reading document.
• Make copies and distribute student packets at the beginning of the lesson.

Activity 3: Daily Regeneration Observation and Data Collection

Materials:
• Each group should have the following items:
  o 1–2 magnifying glasses or microscopes (or one larger magnifying glass and stand)
  o 1 small ruler
  o 1 thermometer
  o 1 flashlight
• Approximately ten minutes should be set-aside during every day of the unit to allow students to collect their daily data. The data collection should be recorded under “Day 0” at the end of Lesson 3 (see “Lesson Implementation” section).

Preparation:
• Place all the group materials in an accessible part of the classroom.

Homework and Assessments

Materials:
• “Journey to Neoblast Division” pre-reading (U2_L4_Homework_JourneyToNeoblastDivision.docx)

VII. Resources and references

Teacher resources

References
VIII. Lesson Implementation

Opening of Lesson:
Briefly review what was done in the previous lessons by asking students to describe the types of behaviors seen in planarians. If students don’t mention regeneration as an observable behavior, remind them that regeneration, which they read about in Lesson 1, is another behavior they can observe.

Ask students if they can think of instances of regeneration or where regeneration would be helpful beyond what they read about. Make certain that students understand the range of regeneration potential that specific animals have. Briefly review what students read about in lessons 1 and 2.

Teacher Content Knowledge
The biological ability to regenerate is an important concept in science. Regeneration is the key to stopping human degeneration from aging, accidents or illnesses.

Overview of main ideas from Lesson 1 readings:
- Some chemicals (BBG) can restore the ability to walk following spinal cord injury in mice
- Humans cannot regenerate lost limbs, but following limb loss the neuronal connections can change to mimic the presence of the lost limb (phantom limb syndrome)
- Salamanders have the remarkable ability to regenerate the correct limbs following amputation, because the cells at the amputation site revert to stem cells specifically destined for leg cells.
- Flatworms have the amazing ability to regenerate all of their tissue and neural circuitry because almost 20% of the cells in the worm are stem cells.

Overview of main ideas in lesson pre-reading:
- Cells that regenerate “know” what they need to turn into, but these cells do not have a pre-determined fate.
- If you split an embryo in half, twins are formed

Explain that in today’s lesson, students will cut the planarians and observe how they regenerate their body tissue. Ask students:
What do you know about planarians based on the readings from Lesson 1, the observations in Lesson 2, and the Pre-Reading?

What were Harriet Randolph and Thomas Hunt Morgan interested in studying? How did they study it?

Why do you think you read about Harriet Randolph and Thomas Hunt Morgan?

Explain that planarians are an evolutionarily ancient invertebrate with a very simple body design found in the phylum Platyhelminthes, or the flatworms. Ask students:

Why does this body structure make the planarian an attractive model organism for researchers to use?

**Scientific Practices: Developing and using models**

To study the basic mechanisms underlying complex processes such as regeneration, scientists prefer to use flat worms as model organisms so they can control their environment and study the effects of different manipulations on their regenerative capacity. Students should be encouraged to develop an appreciation of why planarians and other organisms are used in basic science research.

Draw a simple image of a planarian on the board or display the picture of one from the student handout on an overhead projector or document camera. Using the “Planarian Anatomy” handout, explain that planarians are **bilaterally symmetric**, meaning that there is an imaginary line that can be drawn which splits the animal into two equivalent halves.

Ask students if humans and other mammals are externally bilaterally symmetric.

- Students might initially say yes since it appears that humans are from the outside but the internal anatomy is not so humans are not considered bilaterally symmetric.
- Have students describe heart, digestive system etc. to highlight lack of internal symmetry.

**Student Misconceptions**

Students may think that humans are symmetrical. However this is only true, for some people, externally. Internally, our organ systems are not symmetrical. For example, our heart rests toward the left side, and even our brains aren’t symmetrical in function. The human brain is thought to be asymmetrical because of language areas.

Although the planarians have functionally similar organ systems to humans, they are organized very differently, allowing for bilateral symmetry within the organism. Also, because planarians are simple organisms the function of these systems is far less
complex than humans.

Ask students if there are organ systems that the planarian has that humans have as well. Students should be able to identify from the image that planarians also have a nervous system that allows them to interact with the world, though it is far less complex.

- Point out that the two eyespots found on the head can distinguish light from darkness but do not form images as human eyes do.
- Explain to the students that planarians additionally possess an excretory and reproductive system, as well as a combined gastric and cardiovascular system (known as a “gastrovascular system”).

Using the Pre-Reading and images included in the student materials, discuss initial thoughts about planarian regeneration. Ask students:

- Why might regeneration be beneficial to planarians’ survival from an evolutionary standpoint?

Take a few minutes and have students describe what is depicted in some of the different diagrams and sketches included from the classic papers, using the text excerpts as a guide.

Teacher Pedagogical Knowledge
This activity could be used as homework readings or alternatively they could be used as a short jigsaw activity similar to what was done in Lesson 1. By introducing students to historical research, you are providing a context for students of how science is done and progress made.

Have students describe how the different worms were cut. Be sure students identify that in some cases, Morgan, Randolph, and others did not slice worms into separate pieces, but instead cut the animal so that everything in the body remained attached; this is how, for example, a “double tail” could be generated. Ask students:

- Why is the worm capable of regenerating?
  - Specialized cells known as neoblasts begin to divide to replace tissue that has been lost.
- Are there any limitations to planarians’ regenerative abilities?
  - A single planarian can be divided into 279 individual parts and still have each of those parts regenerate into an entire worm.
  - Different parts of the worm regenerate at different rates due to differing densities of neoblasts within different parts of the body.
Main Part of Lesson

Activity 1: Demonstrating the Procedure and Designing the Investigation

Tell the students that they will be starting an experiment to explore planarian regeneration. Explain that they will divide into groups of 3–4, and each group will develop a hypothesis to test in a planarian cutting experiment. They will then devise appropriate cuts they want to make to test their hypothesis. Students will need to get approval for both their hypotheses and proposed cuts before making the latter. Before having students begin to design their investigation, ask students:

- How many worms will you need to test your hypothesis?
  - A minimum of two—one to cut and one to observe.
- Why would it be beneficial to repeat each kind of cut multiple times?
  - Replication of 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.

Remind students that the cuts they decide to make can yield large or small, symmetrical or asymmetrical pieces of the worm being separated, or they can choose to cut the worm in such a manner that everything in its body remains attached.

Students will also want to include a control in their experiment—an uncut planarian. Use this as an opportunity to discuss what a control is, why it is essential for the scientific process.

Scientific Practices: Planning and carrying out investigations

Scientists Morgan and Randolph learned about the regenerative capacity of planarians by testing the limits of the organism through experimentation. However before starting their experiments Randolph and Morgan formed a hypothesis to guide their experimental design. In this experiment students will use evidence from the pre-reading and handouts to formulate a hypothesis about planarian regeneration and design an appropriate cutting experiment to test this hypothesis.

Demonstration of Activity:

Using an overhead projector, demonstrate the cutting activity to the students while clearly outlining each step:

1) Take two different Petri dishes with lids, and explain that each of the two dishes should be used to house the worm segments that result from one kind of cut. For example, if the worm were
bisected to yield head and tail segments, the first dish could be labeled “Cut 1, Worm 1—Head,” and the second should be labeled “Cut 1, Worm 1—Tail,”

- Remember to write on the bottom (so they can easily observe planarians day to day) of the dishes and to place the group members’ initials on each of the dishes.

2) Place a damp coffee filter on a single-chambered Petri dish filled with frozen spring water to use as a dissection station. Using a plastic pipette, place a worm from the chilled planarian stock solution onto the coffee filter. Cut each worm using a plastic coverslip. It is possible to use either a microscope or a magnifying glass to see what types of cuts are being made. Demonstrate how the coverslip can be cut or bent to yield curved or jagged cuts if desired.

- Use pre-chilled planarians if possible as part of the demonstration.
- If a magnifying glass is being used when students do the cutting, one student should hold it in position for the student who is doing the cutting. Alternatively a larger magnifying glass and stand can be used.

3) Remember that Cut 1 should be replicated in two worms.

- For the demonstration, cut a single worm in half widthwise on the overhead projector, separating the head from the tail. This will make for a quick cut and eliminate the need to magnify the worm, as the procedure will be magnified on the overhead. Emphasize caution when using the coverslips to cut the planarians.

4) After the worms have been cut, transfer the worm segments to the appropriately labeled Petri dishes.

5) Explain that for the next few days, including today, students will be collecting daily data on their planarians as they regenerate. Have them follow along on their “Planarian Data Sheet” handout. Demonstrate the collection of the following sorts of data with the worm that was just cut; ask the students why each of these data may be relevant:

- Use a small ruler to measure the total length of each cut segment or any other relevant lengths (e.g., the width of the cut)
- Take the temperature of the room using a thermometer (leave a thermometer in central location)
- Note the coloration of each segment or the coloration around the cut
- Note the general mobility of each planarian segment; how much and how well does it move?
- Shine a flashlight on each of the cut segments; how do they respond to light? If any of the cut segments stands to regenerate one or both photoreceptors, how visible is/are the photoreceptor(s)?
  - Review with students the need to have a darkened section of the petri dish for the planarian to move towards. The flashlight should only be a small light.
- Encourage students to note other aspects of the planarians

Before students break off into groups, discuss the generation and testing of hypotheses. Instruct students to complete the “Hypothesis Generation and experimental design” handout immediately after they break off into groups. Remind students that hypothesis should be based on previous scientific
understandings, encourage students to review the readings and previous day’s activities as they begin to develop their hypothesis.

Use the demonstration that was just done to illustrate an example hypothesis and experimental protocol for students to use when they are developing their own hypothesis and experiments.

- An example of a student hypothesis could be: When we cut the planarian in half, the head portion will grow a new body and the body portion will grow a new head. I think this will happen because I have read that other scientists have tried this experiment and the planarians regrew these body parts.

**Scientific Practices: Planning and carrying out investigations**

Scientists develop their hypotheses using scientific reasoning. Using evidence from prior experiments or observations to fuel scientific inquiry. In thinking about a hypothesis, students need to be encouraged to go beyond the “if...then” statement and “educated guesses” that they might be familiar with. Students need to understand that a hypothesis is a testable statement that often makes a prediction that is based on previous observations, experiments or scientific reasoning.

As a class discuss what makes a good hypothesis. Emphasize that a good hypothesis should be testable; that which cannot be proved or disproved is outside the realm of experimental science. In addition, a hypothesis should be developed using scientific reasoning. In the model hypothesis, the reasoning could be linked to the readings that were done.

If students have difficulty in thinking about a possible hypothesis to test, ask them to consider how the following might impact the regeneration:

- The removal of one or both eyespots from the rest of the body
- A lengthwise bisection of the head or tail that leaves everything attached
- The removal of circular, triangular, or rectangular segments from various parts of the body
- Various kinds of non-linear (i.e. curved or V-shaped) cuts that bisect or trisect the worm
- Any cut that creates a discontinuity in some feature of the internal anatomy (e.g., the pharynx or cephalic ganglia). Encourage students to reference the “Planarian Anatomy” handout if they decide to do this, since they might have to use the diagram to make these more specific cuts.

Possible questions that could be used to engage students in thinking about variables that might impact regeneration include:

- Will certain parts of the body regenerate faster than others? Why do you think this may be?
- Do you think the progress of regeneration will be affected by the shape of your cut (i.e., whether it is straight, curved, etc.)?
• What sort of environmental factors do you suppose might affect regeneration?

Once each group has developed at least one hypothesis, tell students they should begin to plan their specific cuts aimed around testing one hypothesis.

• Instruct the students to complete the “Sketch Your Cuts” handout once their experimental cuts have been decided.
• Remind groups that they need to receive approval before they start the experiment.
• Students should use the experimental protocol that was demonstrated at the start of the class to help structure their own investigation.

Activity 2: Carrying Out the Investigation and Initial Observations
Allow students to carry out their experiment after they have had it approved. Instruct students not to remove the planarian stock container from the refrigerator or bed of ice; each group should collect its worms while they are still chilled and proceed to cut them as quickly as possible thereafter.

Once groups have completed their cutting, remind them to collect the preliminary data using the Planarian Data Sheet handout for Lesson 3, found in the Student Packet, starting on pg.4.

Once students have completed their cutting, have them clean up. Students should return the magnifying glasses to where they found them and place their labeled planarian Petri dishes in the designated area.

The plastic pipettes and coverslips should be disposed of in the appropriate containers.

Activity 3: Daily Regeneration Observation and Data Collection
Remind students that they will continue to make observations and collect data on a daily basis until the end of the unit.

Conclusion of Lesson
Before they leave, as a whole class discussion or exit slip, ask the students:

• How do the planarians regenerate? How will they grow new body parts?

Encourage the students to state not only their claim, but also piece(s) of evidence and reasoning to support their claim. This can easily be done using an exit slip or, the students can discuss the evidence and reasoning using their classmates’ ideas to ponder about the mechanisms of planarian regeneration. The discussion can be used as an early informal assessment on students’ understanding of planarian regeneration. Their responses will not be as sophisticated as later in the unit, where the students learn more about the mechanisms of cell division and migration as well as gene and protein function.

Tell them that tomorrow’s lesson will explore how planarian cells divide in greater detail. Each student should receive the “Journey to Division” reading with the guided reading questions for homework completed before the start of Lesson 4. Ask the students:
• What do you think is happening to your planarians’ cells now that you have made these cuts? What will its body begin to do?
• Why do you think scientists want to study regeneration at the cellular level? Why not just watch what happens to the planarians with a simple microscope like you all, as well as Randolph and Morgan, did?

The “Journey to Neoblast Division” and Reading Questions should be completed outside of class before the start of Lesson 4, found as U2_L4_Homework_JourneyToNeoblastDivision.docx.

Assessments
From the student packet:

“Hypothesis Development and Experimental Design” handout: Students create a hypothesis that they test in their cutting experiments.

“Sketch Your Cuts” handout: Students will sketch the proposed cuts they wish to make on their worms in order to test their hypothesis.

“Planarian Data Sheet” handouts: Students will complete the first of five data sheets, making observations about the length, coloration, response to light, photoreceptors and mobility of the different worm segments, as well as the ambient temperature.