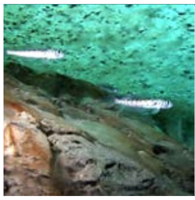

Icefish Blood Adaptations: Antifreeze Proteins

hhmi | BioInteractive

Activity
Educator Materials

OVERVIEW

This hands-on lab activity serves as an introduction to the BioInteractive short film *The Making of the Fittest: The Birth and Death of Genes*. It explores a key adaptation introduced in the film: antifreeze proteins in the blood. The lab serves primarily as an engagement exercise to motivate students into thinking about why blood that does not freeze in Antarctic waters might be an important adaptation for certain fish; they then watch a film that explains the process. For a similar activity exploring another adaptation discussed in the film, see "Icefish Blood Adaptations: Viscosity."

Most fish would freeze to death in the Southern Ocean around Antarctica, because the temperature of the water (–1.8°C/28.8°F) is below the freezing point of their blood. However, icefish and all other notothenioids (a group containing many Antarctic fish species) evolved "antifreeze" proteins that prevent their blood from freezing in this environment. As the antifreeze proteins circulate through the blood, they bind to ice crystals and prevent them from growing. The fish's blood thus does not freeze and continues to flow normally. As other species of fish died off in Antarctic waters, this adaptation allowed notothenioids to thrive and diversify.

In this activity, students create models of blood and explore their properties through two simple labs. In Lab 1, students explore the effect of putting two solutions in a cup of ice, one modeling notothenioid blood (with antifreeze proteins) and one modeling “normal” fish blood (without antifreeze proteins). In Lab 2, students explore the effect of adding ice crystals to two solutions. In both labs, students must determine which solution models which type of blood and answer the same analysis questions.

KEY CONCEPTS

- Some solutions freeze at lower temperatures than others.
- Some solutions freeze when exposed to ice crystals, but other solutions do not.
- Mutations can result in both the appearance of new gene functions and the loss of existing ones.
- Evolution does not ensure that organisms get what they need to survive. It can only affect the frequency of existing variations, which originate randomly from mutations.

STUDENT LEARNING TARGETS

- Explain the importance of different adaptations (in particular, antifreeze proteins) to the survival of notothenioids.
- Use models to make scientific claims based on evidence and reasoning.

CURRICULUM CONNECTIONS

Standards	Curriculum Connection
NGSS (2013)	HS-LS1-1, HS-LS4-4
AP Bio (2015)	1.A.1, 1.A.2, 3.C.1, 4.C.1, SP1, SP5
IB Bio (2016)	3.1, 3.4, 5.1, 5.2
AP Env Sci (2013)	II.C
Common Core (2010)	WHST.9-12.9
Vision and Change (2009)	CC1, CC2, DP1

KEY TERMS

antifreeze protein, evolution, freezing point, ice crystal, mutation, natural selection, trait, variation

TIME REQUIREMENTS

- One 50-minute class period is required for either Lab 1 or Lab 2. Additional time may be needed for watching the film in class and answering questions, which can be assigned as homework.
- See TEACHING TIPS below for alternative strategies.

SUGGESTED AUDIENCE

- High School: Biology (General, AP/IB)
- College-level: Introductory Biology

PRIOR KNOWLEDGE

Students should know that:

- a liquid freezes at a certain temperature (its freezing point), which can be changed by adding certain substances to the liquid
- proteins are encoded by genes
- mutations can have positive, negative, or neutral effects on the fitness of an individual
- due to natural selection, inherited traits that increase an individual's fitness (adaptations) tend to increase in frequency within a population over generations

TEACHING TIPS (for both labs)

- Students may ask whether the lab solutions contain actual antifreeze proteins. Explain that although the solutions do not contain antifreeze proteins (or, for that matter, most of the components of real blood), they can still be used as models for the specific properties of blood that the lab is investigating. You may wish to have students discuss what makes a "good" model, how realistic a "good" model needs to be, and how these criteria might change depending on the model's subject or purpose.
- Students do not need to do both Labs 1 and 2. You can pick one lab for the entire class to focus on. Alternatively, you can split the class in half and have each half do a different lab. Afterward, students can compare the results of the labs and discuss the pros and cons of each model.
- You can use the same questions at the end for both labs. However, some answers may differ slightly depending on which lab was used.
- The "Extension" questions can be assigned as homework. You may wish to assign different "Extension" questions to different groups of students and have each group present their answers to the class.
- Be on the lookout for the misconception that evolution always gives organisms what they need to survive, which may be evident in students' answers to Question 3c. Make sure that students understand that natural selection only affects whether the traits already in a population become more or less common, based on how advantageous those traits are in the population's environment. Some traits may never appear, even if they are advantageous, because no individuals have the mutations for those particular traits.

LAB 1

This lab models how "normal" and notothenioid blood respond to cold temperatures. The key concept demonstrated by this lab is that some solutions freeze at lower temperatures than others.

MATERIALS

For each group of students:

- Styrofoam cup
- crushed ice (enough to fill three-fourths of the cup)
- rock salt or coarse table salt (at least 1 teaspoon)

- teaspoon or electronic balance
- stirring rod or plastic spoon
- two small test tubes (no larger than 100 by 12 millimeters for best results)
- felt-tipped marker
- thermometer capable of reading below 0°C (e.g., an alcohol thermometer, a computer probe, or other digital thermometer)
- paper towels
- three small containers, for samples of solutions A and B and salt (optional)

For the entire class:

- two large containers (over 150 mL)
- 100-mL graduated cylinder or beaker
- water
- corn syrup
- large cooler or bucket of ice
- heavy-duty quart-sized plastic bag and hammer/mallet for crushing ice (optional)

PROCEDURE

1. Fill about three-fourths of the Styrofoam cup with crushed ice. Mix 1 teaspoon (about 8 grams) of salt into the ice with the stirring rod or spoon. This will help the temperature inside the cup get colder.
2. Using your marker, label one test tube "A" and the other test tube "B."
3. Get samples of solutions A and B from your teacher. Fill one-third of test tube A with solution A, and one-third of test tube B with solution B. Make sure that the test tubes are filled to the same level.
4. Put the test tubes in the cup of ice. Make sure that the liquids in both tubes are below the top of the ice.
5. Let the solutions cool for 10 to 15 minutes. Record any changes in the solutions over this time period.
6. With the clean and cooled thermometer, measure and record the temperature of solution A. Rinse and dry the thermometer with a paper towel, then measure and record the temperature of solution B.
7. When you're done, pour the solutions and the cup of ice down the sink.

TEACHING TIPS

- Have students work in pairs.
- To maximize class time, prepare solutions A and B ahead of time:
  - For solution A (models "normal" fish blood), pour 150 mL of water into a large container. Label this container "Solution A."
  - For solution B (models notothenioid blood), mix 90 mL water and 60 mL corn syrup in another large container. Label this container "Solution B."
    - Actual antifreeze is hazardous. If you wish to use antifreeze in place of corn syrup, it would require the implementation of a health and safety protocol.
    - Similar to antifreeze proteins, corn syrup lowers the freezing point of a solution. However, it uses a different mechanism. Antifreeze proteins bind to ice crystals and prevent them from growing. Corn syrup contains solutes such as sugars, which cause freezing-point depression; this is the same reason that ice cream stays soft at extremely cold temperatures.
  - You may want to aliquot samples of these solutions (several mL of each) and salt (at least 1 tsp) into smaller containers for each student group. Label these smaller containers "Solution A," "Solution B," and "salt" respectively.

- Prior to and between classes, cool the empty test tubes by keeping them in a cooler or bucket of ice. Thermometers should be cleaned and preferably cooled as well; since the volume of the solution in each test tube will be small, room-temperature thermometers could warm the contents and result in inaccurate readings.
- For best results, make sure to use *crushed* ice in Step 1 of the procedure. If you do not have pre-crushed ice, place ice cubes in a heavy-duty quart-sized plastic bag. Hit the bag (with the side of the head of a hammer or wooden mallet) on a wooden surface or thick plastic cutting board.
- The results of the labs should be as follows:
  - Solution A should partially or fully freeze.
  - Solution B should remain a liquid.
  - Solution B should be colder than solution A and likely below –1.8°C/28.8°F (the temperature of Antarctic water).
- After students complete Question 1, have them watch the short film *The Making of the Fittest: The Birth and Death of Genes* before doing Questions 2 and 3. The film and Question 2 and 3 can be assigned as homework.

LAB 2

This lab models how "normal" and notothenioid blood react to ice crystals. The key concept demonstrated by this lab is that some solutions "freeze" when exposed to ice crystals, but other solutions remain as liquids.

MATERIALS

For each group of students:

- two clear plastic cups or beakers
- felt-tipped marker
- 100-mL graduated cylinder
- ¼ teaspoon or electronic balance
- simulated "ice crystals" (¼ teaspoon of sodium polyacrylate or instant snow powder)
- plastic bag for disposal

For entire class:

- table salt
- water

PROCEDURE

1. Get cups of solutions A and B from your teacher.
2. Add ¼ teaspoon (about 0.58 grams) of simulated “ice crystals” to each cup and gently swirl.
3. Record any changes you observe in each solution.
4. When you're done, pour the solutions into a plastic bag and throw it in the trash. **Do not pour the solutions down the sink.**

TEACHING TIPS

- Have students work in pairs.
- To maximize class time, prepare solutions A and B ahead of time for each group of students.
  - For solution A (models "normal" fish blood), pour 100 mL of water into a cup. Label this cup “Solution A.”

- For Solution B (models notothenioid blood), mix ¼ teaspoon (1.61 grams) of salt with 100 mL of water in a cup. Label this cup "Solution B."

- Sodium polyacrylate is sold by science supply houses such as Carolina Biological. Instant snow powder is sold by some suppliers of novelties and toys. Both should also be available on Amazon.com.
- The results of the lab should be as follows:
  - Solution A will likely react rapidly. If using sodium polyacrylate, the solution will swell and form a solid gel-like substance. If using instant snow, it will form a large amount of snow-like powder.
    - Although these reactions look similar to ice crystal growth, they do not actually involve freezing. Sodium polyacrylate and instant snow are polymers that change structure when they absorb water. As a result, they will change appearance and grow when added to solution A.
  - Solution B may look cloudy and have some floating powder present. However, it should not react.
    - The salt in solution B attracts water molecules, which prevents sodium polyacrylate/instant snow from absorbing water.

ANSWER KEY

1. Remember that solutions A and B model different types of blood.
   a. Summarize the differences between the solutions that you observed.

Student answers may vary. Sample observations for each lab are shown below.

Lab 1: When the solutions were cooled, solution A formed some ice and solution B stayed liquid. But solution B ended up at a colder temperature than solution A did.

Lab 2 (sodium polyacrylate): When the "ice crystals" were added, solution A turned into a solid gel that looked like wet snow. Solution B got a little cloudy and had some floating "ice crystals" in it, but didn't really change overall.

Lab 2 (instant snow): When the "ice crystals" were added, solution A turned into a bunch of powder that looked like snow. Solution B got a little cloudy and had some floating "ice crystals" in it, but didn't really change overall.

   b. Would the blood modeled by solution A or B be more advantageous for an animal living in a cold environment? Support your answer with evidence from your observations.

Student answers may vary depending on their observations from 1a. Sample answers are shown below.

Lab 1: In a cold environment, blood like solution A might freeze, but blood like solution B would stay liquid. So the blood modeled by solution B would be more advantageous because it would keep flowing even when it gets cold.

Lab 2: In a cold environment with ice crystals, blood like solution A might turn solid like snow or ice, but blood like solution B wouldn't be affected. So the blood modeled by solution B would be more advantageous because it would stay normal even when exposed to ice crystals.

2. To learn more about notothenioid blood, watch the BioInteractive short film *The Making of the Fittest: The Birth and Death of Genes*. As you watch, pay attention to any mentions of adaptations that affect how blood freezes. Use what you learn from the film to answer the questions below.
   a. How do antifreeze proteins keep fish from freezing?

The antifreeze proteins bind to ice crystals in the blood and keep the ice from growing.

   b. Explain how antifreeze proteins are an adaptation to the Antarctic environment.

Antifreeze proteins prevent blood from freezing at Antarctic temperatures. This is an adaptation because it makes it more likely for fish to survive and reproduce in the Antarctic environment.

c. Make a claim about which solution, A or B, modeled blood from a notothenioid, and which modeled blood from a "normal" fish. Use the properties of the solutions and information from the film to provide evidence and reasoning for your claim.

Student answers may vary depending on their observations from 1a. Sample answers are shown below.

<u>Lab 1</u>: Solution A froze but solution B didn't, even though solution B was colder. This is similar to how notothenioid blood doesn't freeze even when the temperature is below the freezing point of "normal" blood. So solution B modeled notothenioid blood, and solution A modeled "normal" fish blood.

<u>Lab 2</u>: Solution A formed a solid like ice, but solution B didn't change. This is similar to how ice crystals can't grow in notothenioid blood. So solution B modeled notothenioid blood, and solution A modeled "normal" fish blood.

d. Predict what would happen if you put a fish *without* antifreeze proteins into Antarctic waters. Explain your reasoning with evidence from the lab model.

Students should base their answer on their observations of solution A, the solution that modeled blood from a "normal" fish.

<u>Lab 1</u>: Students may observe that solution A froze at a temperature above the average temperature of the water around Antarctica (–1.8°C/28.8°F). This result suggests that a fish without antifreeze proteins would die in Antarctic waters, because its blood would freeze and stop circulating.

<u>Lab 2</u>: Students may mention that solution A formed large amounts of "snow" or "ice" when exposed to ice crystals. Ice crystals would be common in Antarctic waters, so a fish without antifreeze proteins would probably develop large amounts of ice in its blood. This could damage the body or prevent blood circulation, killing the fish.

e. What are some properties of notothenioid blood or "normal" fish blood that were not captured in this model? Propose one or two modifications to the model that would help include these properties.

Student answers will vary.

<u>Lab 1</u>: Possible answers include that the solutions could have been swirled to simulate the circulation of blood or held in narrow tubes to represent blood vessels. Students may also note that the viscosities of the solutions should be reversed for icefish, the main notothenioid discussed in the film. Icefish have blood that is less viscous than the blood of "normal" fish; however, solution B (model of notothenioid blood) was actually more viscous than solution A (model of "normal" fish blood).

<u>Lab 2</u>: Possible answers include that the solutions could have been chilled to simulate Antarctic temperatures, swirled to simulate the circulation of blood, or held in narrow tubes to represent blood vessels. Students may also note that the viscosities of the solutions should be different for icefish, the main notothenioid discussed in the film. Icefish have blood that is less viscous than the blood of "normal" fish; however, both solutions A and B had similar viscosities.

3. Most fish, including the ancestors of notothenioids, do not produce antifreeze proteins.
   a. How did notothenioids "get" the gene for antifreeze proteins?

The gene arose from random mutations. Students may include additional details from the film, such as the fact that another gene was duplicated and several mutations occurred in the duplication. As these mutations accumulated, the gene eventually gained a new function: antifreeze protein production.

   b. Explain the evolutionary process by which the antifreeze proteins became common among notothenioids.

Student answers should reflect an understanding of natural selection. In an early population of notothenioids, individuals with antifreeze proteins were more likely to survive and produce offspring (had higher fitness) than individuals without them. The individuals with antifreeze proteins passed the antifreeze gene to their offspring. So, *each generation, the proportion of fish with antifreeze proteins increased in the population. After many generations (assuming that environmental pressures did not change over time), most of the population would have had antifreeze proteins.*

c. Does evolution always give organisms the traits they need to survive in an environment? Justify your answer.

Students should understand that evolution does not work "on demand" to give organisms what they need to survive. They may mention facts from the film (e.g., that many species of fish could not live around Antarctica because they were unable to evolve the adaptations needed to survive there). Students may also mention that natural selection can only act on traits that are already in a population. A population may die out because the traits needed for survival never appear, because no individuals in that population have the mutations for those particular traits. These mutations could also be lost through random chance (genetic drift), even though they are advantageous.

EXTENSION

Ask students to research and report on one of the following questions.

1. Penguins also live in the Antarctic, but they do not have antifreeze proteins. What adaptations make it possible for them to survive in this harsh, cold environment?

Student answers may vary. Penguin adaptations include special overlapping feathers, a layer of fat under the skin, circulatory adaptations in the extremities, and rounded bodies with a lower surface-to-volume ratio that decreases heat loss.

2. Do any animal species besides notothenioids make antifreeze proteins?

Student answers may vary. There are a number of other animal species that produce antifreeze proteins, such as many Arctic fish species, snow fleas (Collembola, or springtails), Antarctic krill, the Arctic carabid beetle, the Nebria beetle, and the emerald ash borer. Many insect species that overwinter in freezing environments also make antifreeze proteins.

3. Are any plant species able to make antifreeze?

Student answers may vary. A large number of plants make antifreeze proteins, such as carrots, rhododendrons, ryegrass, winter barley, and forsythia.

4. Antifreeze proteins work by binding to ice crystals. How does that compare with how the antifreeze used in cars works?

Car antifreezes do not bind to ice crystals. They work by adding solutes to fluid, which lowers the freezing point of the solution.

5. If students are comfortable with scientific papers, they may research the following question by reading the suggested reference:

   Scientists have hypothesized that there are possible disadvantages to having antifreeze proteins. What is one of these disadvantages, and how might notothenioids compensate for it? Suggested reference: Cziko, Paul A., Arthur L. DeVries, Clive W. Evans, and Chi-Hing Christina Cheng. "Antifreeze protein-induced superheating of ice inside Antarctic notothenioid fishes inhibits melting during summer warming." *Proceedings of the National Academy of Sciences* 40 (2014): 14583–14588. https://doi.org/10.1073/pnas.1410256111.

Student answers may vary. According to the reference, ice crystals don't melt as easily when bound to antifreeze proteins. Over time, many ice crystals bound to antifreeze proteins may build up in a fish's body. This could be disadvantageous if the accumulated ice crystals get stuck in blood vessels or tissues. Notothenioids might be able to *compensate for this by having other adaptations that block or remove the ice crystals from circulation. For example, certain cells (macrophages) might engulf and clear out the bound ice crystals.*

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