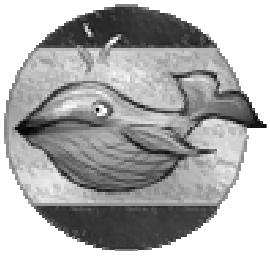


Mystery Meat

By Jennifer A. Collins and Allen G. Collins

http://www.paleobio.org/MysteryMeat/

Teacher Directions

Lesson Overview

In this lesson students follow the steps of scientists Steve Palumbi (Stanford University) and Scott Baker (University of Oregon), who used DNA data ("Barcoding") to reveal the species identifications of *kujira* (whale meat) from foreign markets. Their study used DNA data to identify unknown organisms in order to investigate whether whales are being illegally hunted and sold as meat. By going through the lesson, students practice various aspects of the process of science by asking scientific questions, collecting and analyzing data, comparing their results with those of the real researchers, and finally determining possible next steps.

Students begin by reading some brief background information on cetaceans and International Whaling Commission (IWC) laws protecting them, and on DNA barcoding techniques. They come up with their own investigation questions(s) and alternative hypotheses about what the meat they test could reveal. Students then use a set of web pages to examine several DNA sequences from meat samples taken from the market. These web pages are set up to mirror the exact process that researchers use when conducting this type of study. When students "submit" their unknown barcodes two outputs are produced: 1) a phylogenetic tree showing how close the unknown barcode clusters with barcodes taken from known species, and 2) a data table showing percent similarity to known species. Students record their results, then use these results as evidence to test their hypotheses about the hunting status of each organism revealed. Finally, students compare what they found with Baker and Palumbi's discoveries, and suggest ways this research is important or could be expanded upon.

National Standards:

- Biological classifications are based on how organisms are related. Organisms are classified into a hierarchy of groups and subgroups based on similarities which reflect their evolutionary relationships.
- Understanding basic concepts and principles of science and technology should precede active debate about the economics, policies, politics, and ethics of various science- and technology-related challenges. However, understanding science alone will not resolve local, national, or global challenges.
- Science often advances with the introduction of new technologies. Solving technological problems often results in new scientific knowledge. New technologies often extend the current levels of scientific understanding and introduce new areas of research.
- Science and technology are pursued for different purposes. Scientific inquiry is driven by the desire to understand the natural world, and technological design is driven by the need to meet human needs and solve human problems. Technology, by its nature, has a more direct effect on society than science because its purpose is to solve human problems, help humans adapt, and fulfill human aspirations. Technological solutions may create new problems. Science, by its nature, answers questions that may or may not directly influence humans. Sometimes scientific advances challenge people's beliefs and practical explanations concerning various aspects of the world.

Grade Span: 9-13

Materials:
Mystery Meat Student Directions handout
Computers with Internet access
Background Information pages: The Status of Whales, DNA barcoding, Whale Status Data
Optional: *Which Whales Are Hunted?* 1994. Science, vol. 265: 1538-1539.

Advance Preparation:
- Students should have a basic understanding of 1) how to read and use phylogenetic trees, and 2) basic understanding of DNA.

Time: One - Two class periods.

Grouping: Individuals, partners or small groups

Vocabulary: DNA barcoding**,** PCR amplification, genome, phylogenetic tree.

Teacher Background:
Among the mammals that live in the sea (seals, sea lions, walruses, sea otters, manatees, dolphins, and whales), whales may be the most familiar. Whales are very closely related to dolphins. As a result of their close relationship, scientists classify species of whales and dolphins together in the group *Cetacea*.

In 1982 the International Whaling Commission (IWC) voted to suspend commercial whale hunting, making it illegal to hunt whales for the purpose of selling their products. This moratorium was intended to help reestablish rapidly disappearing whale species that had been under threat due to decades of large-scale whaling.

Under the IWC treaty, hunting a limited number of whales of specific species for scientific study and for subsistence use by aboriginal peoples (those who have lived in the same region for many generations) allowed. Though the suspension went into effect in 1986, fish markets in Japan and Korea, where there has been a long tradition of eating whales, continued to offer whale meat. In addition to legal hunting since 1986, a number of instances of illegal whale poaching have come to light.

Given that whale meat is being sold while both legal and illegal whale hunting continues, one might wonder what are the sources of whale meat sold in Asian markets? Is the IWC treaty being upheld? If whale meat from illegally caught whales is sold in these markets, then whale populations might be impeded from returning to healthier numbers.

DNA sequencing techniques became prominent in the early 1990s and provided a novel avenue for monitoring the sale of whale meat (and other natural products). In the absence of an entire animal, genetic data, dubbed “barcodes” can still reveal what species the meat actually comes from. Two researchers, Scott Baker and Steve Palumbi were determined to use DNA data in order to identify what was actually being sold as whale meat, or *Kujira*, in local markets in Japan to get at whether the meat came from legal hunting practices.

These intrepid researchers faced one big problem. Namely, it is illegal to transport whale tissue across international borders because whales are covered by the Convention on International Trade in Endangered Species (CITES). In order to obtain molecular sequence data from meat

sold as whale at Japanese markets, these scientists carried portable equipment that allowed them to extract DNA and PCR amplify target gene sequences right in their hotel room.

After taking tissue samples of *Kujira*, extracting DNA, and using PCR techniques to amplify targets regions of DNA, these researchers were able to compare the sequences to a database of known whale species. These DNA barcodes can not only show what species the product likely comes from, but in some cases can identify the specific region where the animals were taken. Baker and Palumbi concluded that some whale meat comes from whale species that are illegal to hunt. Moreover, they determined that some of the meat did not actually come from whales.

Such studies have now become routine and the technique, now commonly known as DNA barcoding, has been applied to a variety of problems, from identifying agricultural pests to the identities of bird smudges left after collisions with airplanes.

Procedure:

I. Begin the Investigation

1. Pass out the **Student Directions** to individuals or teams**.** Students should be aware that in order to proceed in an investigation, seeking out background information to help make decisions on the direction to take is important. Begin by having students read the introduction, then brainstorm questions they need to answer before they can begin their investigation. Below is a list of several questions that students should be encouraged to come up with, though many others may be relevant. Pass out the **Background Information** and **Whale Status Data Sheets** or any other resources you have to help students answer the questions. Students should then come up with investigation question(s) they will pursue. A brief class discussion will allow students to see a variety of ways groups posed the questions and will assure that each group is headed in the right direction.

Background Questions

What kinds of laws protect whales from being hunted? *The International Whaling Commission has laws that protect all whale species from being hunted for commercial use. There is some permission for scientific catch and aboriginal catch.*

What kinds of whales are legal to sell as meat? *There are currently no whale species that are allowed to be hunted and sold for commercial use. Some species, such as Fin or Minke whales can be hunted for scientific purposes in limited numbers and sold commercially. These species and some others can be hunted for aboriginal use, but may not be sold commercially. Students should notice that none can be hunted commercially. It is useful to point out the small numbers that can be caught for scientific purposes.*

How can DNA help you identify the source of meat being sold as whale? *Since all organisms have unique DNA sequences you can compare unknown sequences with those that have been identified. A close match suggests close relatedness. This is called DNA barcoding.*

Investigation Question(s)

What is/are the scientific question(s) you will be investigating?
Answers will vary. Some possibilities include: Is meat being sold as whale legal or is it coming from whales that are protected by international law? Are whales being illegally hunted and sold in markets?

II. Form Alternative Hypotheses

Students should be aware that good science involves devising multiple alternative hypotheses that are testable. This allows researchers to have several ideas of possible outcomes that they can then use collected data to test. A good scientist tries to disprove their hypotheses. In this investigation, four main hypotheses can be tested:

Hypotheses for each piece of meat being tested:

1. *The meat comes from a whale species that is legal to hunt and sell for commercial use.*
2. *The meat comes from a whale species that is illegal to hunt and sell for commercial use.*
3. *The meat comes from a whale that was hunted before the law went into effect.*
4. *The meat comes from a source other then whale.*

Students should come up with at least one opposing hypothesis to the one given. They are most likely to come up with the #2 hypothesis listed above. Hypothesis #3 may require a class discussion based on what students learned from the background information. Hypothesis #4 is very relevant, but it is OK if students do not come up with it. In fact by leaving #4 out, it give them an opportunity to see how hypotheses are sometimes made after rather than before data are collected and analyzed.

Based on what you learned from your background investigation, what evidence would you expect to find to support each hypothesis you listed.

Hypothesis 1: *Only whales hunted under scientific permit are also legal to sell commercially on a limited basis. However, a review of the Whale Status chart indicates that the number of whales allowed to be killed for scientific research should be in numbers so low, that it would be difficult to find on the market, except for Minke whales. There is a large number of Minke whale caught under scientific permit so finding this meat on the market could be legal. However, it comes into question whether this large number is really being killed for scientifically valid research.*

Hypothesis 2: *Meat found from any of the whale species could support this hypothesis since all are protected from commercial use. There is no question that meat found from Pilot Whale or Blue Whale is illegal since there are no scientific, aboriginal, or commercial use granted. Some Minke may be legal if hunted with a scientific permit and those whales were indeed used for science.*

Hypothesis 3: *Only whales hunted before 1985 when the International Laws went into effect are legal to sell. Any meat assumed to come from such whales, would have had to remain frozen in storage for over 20 years.*

Hypothesis 4: *The DNA barcode from a non-cetacean would indicate a different source.*

III. Collect and Analyze the Data

Students need access to computers and the Internet to collect the Data by going to http://www.paleobio.org/MysteryMeat/MysteryMeat-Unknowns.html. Students will record all data in **Data Table 1: Identification of Market Samples Sold as Whale Meat**

Data Table 1: Identification of Market Samples Sold as Whale Meat

Unknown Meat Sample #	*Common Name of Closest Species Inferred from DNA Analysis*	*Specimen Similarity*
1	Northern Minke Whale	100
2	Blue Whale	100
3	Fin Whale	100
4	Horse	100
5	Gray Whale	100
6	Atlantic Spotted Dolphin	100

IV. Interpret the Data

Students should use their previously written hypotheses to analyze the data. They should either write or explain their analysis for each sample.

Data Table 2: Hypothesis Analysis

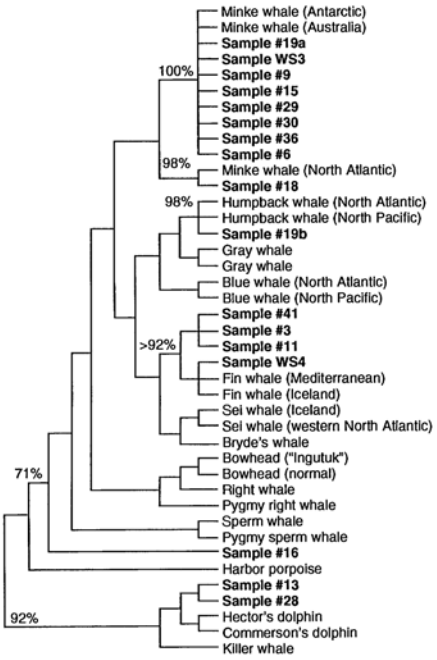
Identified Meat Sample #	*Identified Meat Sample Name*	*Which hypothesis does your data support? If the data do not support any of your hypotheses, write a revised or alternative hypothesis.*
1	North Atlantic Minke Whale	***The meat comes from a whale species that is legal to hunt and sell for commercial use.*** Though it might be important to discuss with students that the number of Minke whale being caught under "scientific permit" is suspect and the actual meat may not be from a Minke whale that was legally caught.
2	Blue Whale	***The meat comes from a whale species that is illegal to hunt and be sold for commercial use.*** ***The meat comes from a whale that was hunted before the law went into effect.***
3	Fin Whale	Most likely ***the meat comes from a whale species that is illegal to hunt and be sold for commercial use***. In 1989 sixty eight fin whales were caught under scientific permit. That meat would have had to been frozen for a long time before being sold. In 2005 only 10 were caught with scientific permit. It would be unlikely to find this meat in the market, though possible.
4	Horse	***The meat comes from a source other then whale.***
5	Gray Whale	***The meat comes from a whale species that is illegal to hunt and be sold for commercial use.*** ***The meat comes from a whale that was hunted before the law went into effect.***
6	Atlantic Spotted Dolphin	***The meat is illegal.*** Dolphins are protected from hunting.

1. What is your reaction to your findings? *Answers will vary.*

2. Use the data you collected and analyzed to write a conclusion for each **Investigation Question** you posed **in Part I.**
Answers will vary. Students should point to the fact that much, if not all of the meat being sold is illegal. They can use specific data, such as position on the phylogenetic tree or % Specimen Similarity.

V. Analyze and Compare the Results

The phylogenetic Tree students analyze comes from Baker and Palumbi's published paper "*Which Whales Are Hunted? A Molecular Genetic Approach to Monitoring Whaling" Science* New Series *(1994).*



1. What can you conclude from their data about the meat being sold as whale? *Students should recognize that this data supports the data they worked with and reveals that meat being sold as whale is coming from illegal sources.*

2. How do Barker and Palumbi's data compare to what you found? Give at least two similarities and two differences between your results and theirs. *Students should notice that Baker and Palumbi collected many more tissue samples then the six the students used. Minke whale is the most common type of food sold, followed by fin whale. Baker and Palumbi also found a close relative of pygmy sperm whales and porpoises. Like them, the students should recognize that they found similar meat being sold, including humpback. The students should also notice that one of the samples of Kujira was actually from Horse, which is something that a study found subsequent to Baker and Palumbi's initial research.*

3. Does their data support your conclusions? Explain. *Students should recognize that their data, though more detailed does in fact mirror what they collected. Have students look at specific claims they made and see if the published phylogenetic tree supports the same conclusions.*

VI. Apply What you Learned

The results of many investigations often lead to ideas for further investigations. Results can also be used to inform policy, solve problems, address societal issues, develop technology, and/or to simply build upon our basic understanding of the natural world.

1. What new questions or ideas would you pursue if you could continue this investigation?
Answers will vary. Some possibilities include: using DNA barcoding to test the source of other types of food being sold, if illegal whale meat is being sold elsewhere in the world, figuring out how DNA barcoding can be used to monitor whaling practices.

2. Explain at least two potential benefits and/or outcomes of the investigation.
Answers will vary. DNA barcoding can be a tool useful for monitoring the markets in search for illegally sold products, it could aid in recovery efforts of the whale populations or make the public more aware of possible laws being broken.

Extension Ideas
Read Baker and Palumbi's "*Which Whales Are Hunted? A Molecular Genetic Approach to Monitoring Whaling" Science* New Series, Vol. 265, No. 5178 (Sep. 9,*1994), 1538-1539*. (this is a short article, readable by high school students possibly with some help). Discuss their conclusions and background information they used.

Write letters to government officials explaining their position on the illegal hunting and selling of whales or other protected species.

Research other practical uses of DNA barcoding such as identifying agricultural pests or identities of bird smudges left after collisions with airplanes.