Investigating a Deep Sea Mystery

Investigating a Deep Sea Mystery is based on *Deep-sea mystery solved: astonishing larval transformation and extreme sexual dimorphism unite three fish families* by Johnson, et al. (2009)* published in Biology Letters, Royal Society. The deep sea fishes at the heart of the investigation and this activity were historically classified into three families or clades based on the obvious morphological differences between the members of each group. Over time, as new data was accumulated, a new hypothesis was generated; the three fish clades were really one. Johnson, et al. found patterns in collection data that supported an alternative relationship; that they are the males, females, and larvae of a single family or clade, and that the morphological differences are the result of extreme ontogenetic (developmental) metamorphosis and sexual dimorphism. In this activity students follow the steps of the science team to unravel the mystery of the fishes' classification by analyzing some of the same morphological and phylogenetic data as the science team.

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Grades 9-12

Objectives – Students will:

- Use external morphology to classify a group of deep sea fishes
- Analyze data to examine alternative hypotheses about the classification and evolutionary relationships of the organisms
- Use multiple lines of morphological and phylogenetic data to make inferences about the function of different features and test alternative hypotheses
- Learn how science is an ongoing and dynamic process of discovery

The activity is divided into six parts that should be completed in order. Below is a brief description, recommended time commitment, and list of related documents and other materials needed for each section.

Activity Sections and Materials

I. Introduction - What is a Fish? (45 minutes) – *Students devise their own definition of what makes an organism a fish, then test their ideas by reading about how scientists define "fish."*

Materials:
Student Research Notes KEY
Student Research Notes page 1
Fish Resource Guide

II. Classifying Some Unusual Fishes (30 minutes) – *To become familiar with the fishes in this activity, students sort a set of specimen cards into three clades based on their external morphology. This hypothesis will be referred to as the **Three Clade Hypothesis**. Students then check their classification scheme with the scheme historically proposed by ichthyologists. This will represent the first of two hypotheses that they will later test.*

Materials: Student Research Notes page 2
Deep Sea Fish Specimen Cards
Deep Sea Fish Specimen Key (also to be used for Part IV and V)

III. Looking for Patterns in Collection Data (45-60 minutes) – *Students look for patterns in data pertaining to the sex, collection depth, and stage of development that were compiled by Johnson et. al. From this data students will work through the alternative hypothesis the scientists came up with to explain the patterns they see. Students will refer to this as the* ***Single Clade Hypothesis****.*

Materials: Student Research Notes pages 3-4

IV. Testing Hypotheses – Using Morphology (60 minutes) – *Students test the alternative hypotheses by making observations of three sets of morphological features used by Johnson et al. For each morphological feature, students make inferences about the function of the features and how that might support or refute each hypothesis.*

Materials: Student Research Notes page 5-8
Deep Sea Fish Specimen Key (from Part II)
Morphology Cards (Gut, Muscle, Gill A&B)

V. Testing Hypotheses - Using Phylogenetic Trees (30 minutes) – *Students practice their skills at distinguishing how the topology of different phylogenetic trees support different hypotheses, then test the* ***Single*** *and* ***Three Clade Hypotheses*** *using one of the phylogenetic trees Johnson et al. referenced.*

Materials: Student Research Notes pages 9-10
Deep Sea Fish Specimen Key (from Part II)
Colored pencils, so each student or group has three distinct colors

VI. Wrapping it up (30 minutes) – *Students compile what they learned from the morphological and phylogenetic data to summarize which of the two hypotheses best represents the relatedness and classification of these fish. Students also generate new questions to investigate.*

Materials: Student Research Notes page 11

National Science Education Standards:
Life Science:
- Molecular basis of heredity
- Biological evolution

Science as Inquiry

Prerequisites and Recommended Resources
Below is a list of student prerequisites along with resources that provide background information and/or classroom activities that address each prerequisite.

Students should:

- *understand that a phylogeny, or evolutionary tree, represents the evolutionary relationships among a set of organisms or groups of organisms*
    - The Tree Room - What is an evolutionary tree? http://evolution.berkeley.edu/evolibrary/article/0_0_0/evotrees_primer_02
- *have a basic understanding of how to read and make inferences from phylogenetic trees, including an understanding of shared characters*
- The Tree Room - Primer on Trees
    - http://evolution.berkeley.edu/evolibrary/article/0_0_0/evotrees_primer_01
    - What Did T-Rex Taste Like? www.ucmp.berkeley.edu/education/explorations/tours/Trex/index.html
    - An Exercise in Tree Interpretation Page 9 of the Student Research Notes
- *understand that a clade is a group of organisms that includes an ancestor and all descendants of that ancestor*
- The Tree Room - Primer on Trees
    - http://evolution.berkeley.edu/evolibrary/article/0_0_0/evotrees_primer_01
- *have a basic understanding of how organisms are classified into clades*
    - Understanding Evolution - Using Trees for Classification http://evolution.berkeley.edu/evolibrary/article/0_0_0/phylogenetics_04

Other Recommended Resources

- *About the Johnson et al. research*
    - Deep-sea mystery solved: astonishing larval transformations and extreme sexual dimorphism unite three fish families http://rsbl.royalsocietypublishing.org/content/5/2/235.abstract
    - Museum Collections Solve Whalefish Mystery: http://ocean.si.edu/ocean-news/museumcollections-solve-whalefish-mystery/meet-suspects
    - An Overdue Family Reunion: http://vertebrates.si.edu/fishes/whalefish/index.html
- *More about how science really works*
    - Understanding Science: http://undsci.berkeley.edu/

Preparation – Prior to starting the activity, the teacher should:

- become familiar with the research of Johnson, et al. using the resources listed in the Recommended Resources section
- determine student research teams (recommended groups of 3-4 students/team)
- photocopy 1 per group of the following
    - Deep Sea Fish Specimen Cards (these will need to be cut in advance)
    - Fish Resource Guide
    - Deep Sea Fish Specimen Key
    - Morphology Cards (Gut, Muscle, Gill A, Gill B)
    - Student Research Notes (one per student)
- prepare 1 set of 3 different colored pencils or highlighters per group

The Activity:

I. Introduction - What is a Fish? 45 minutes

1. Pass out the Student Research Notes pages either as a packet or as individual parts as you move through the activity, and discuss the goal of the activity.
2. To get students thinking about what defines a fish, ask them the question; "What is a Fish?" then have them draw and label their answer on page 1 of the Student Research Notes. Encourage students to include features, behaviors and other traits they think all fishes have, and therefore that could be used to identify an organism as a fish.
3. Students should compare and discuss their fish drawings and labels to see how their ideas compare. Explain to students that defining "fish" is not very easy, even for ichthyologists.
4. Pass out Fish Resource Guide. This reading contains several sections that include information about the characteristics of fishes, classification, reproduction, and phylogeny that can be used as a reference throughout the activity. Students should read the first section only, *What is a Fish?* to learn how ichthyologists define "fish," then summarize the characteristics that define "fish" in the Fish Checklist on page 1 of the Student Research Notes. Students should also note exceptions to the general fish characteristics at the bottom of the page.
5. Discuss the following questions as a class. Students can take notes in the Student Research Notes on page 2:
   a. What characteristics did your team overlook? *Answers will vary.*
   b. After reading *What is a Fish?*, what surprised you most about defining a fish? *Answers will vary.*
   c. Of the features you listed and discussed, which ones do you think are useful for understanding the evolutionary history of fishes and how different fishes are related? *Discuss with students that not all features are useful for determining relatedness. Scientists look for synapomorphies, derived or "changed" character states shared by two lineages within a clade, to help them understand relatedness. Students might want to review the Evolutionary Classification of Fishes section in Fish Resource Guide to see examples of shared characters, contrast typical ideas about fishes with a definition based on synapomorphies, and emphasize the problems with focusing only on overall similarity. For more information visit: http://evolution.berkeley.edu/evolibrary/article/phylogenetics_06*
   d. What role does evolution play in defining something as a fish? *Students should be note that 'fish' does not represent a monophyletic group or clade (e.g. does not include the most recent common ancestor and all descendants), and typical definitions of fish are not based on phylogeny or shared evolutionary history.*
   e. What types of scientific questions do you think ichthyologists investigate? *A wide range of answers is possible. Studying the evolution of fishes and how different types of fishes are related should be part of the discussion.*

II. Classifying Some Unusual Fishes 30 minutes

1. Explain that classifying fishes into groups has been a challenge for ichthyologists for centuries.
2. Pass out a set of Deep Sea Fish Specimen Cards to each group. Explain that these are specimens housed in museum collections, such as those you might find at the National Museum of Natural History in Washington, DC. Challenge students to sort their cards according to which fishes they think are most closely related or are members of the same

clade. Students should identify and discuss features that unite members of each clade and that distinguish them from the other clades.
Note: Some of the specimens are damaged, a result of being brought in nets to the surface of the ocean from deep depths. Like the scientists who worked with these specimens, the students will need to make their best observations, given the state of the material.

3. Give students a chance to share their clades and explain the features they used to classify the fish. Then reveal how scientists over the last 100 years have grouped them using the Deep Sea Fish Specimen Key. Students should compare how they sorted the Deep Sea Fish Specimen Cards with the way the scientists did and discuss the names given to each group; Whalefishes, Bignose fishes, and Tapetails.
Note: Have students keep the Deep Sea Fish Specimen Key as a reference.
4. Discuss as a class:
    a. How did your classification scheme compare to that of the science community? *Answers will vary.*
    b. What features helped you group the Deep Sea Fish Specimen Cards? *Responses may include; body shape, number of fins, other. Students could revisit the Evolutionary Classification of Fish section in Fish Resource Guide.*
    c. What features presented challenges to classifying the fishes? *Some of the fishes may be challenging to classify because they are damaged. There is also no scale bar to show relative sizes of the fishes, which may present a challenge to students.*

    Students record what they learned and discussed about how these fishes are grouped into three groups on page 2 of their Student Research Notes.
5. Explain to students that Whalefishes or Cetomimids have been known since the 1890's, and it was not until the 1950's and 1960's that Tapetails and Bignose fishes were discovered. These fishes were initially classified into three families because of the differences in their morphology (what they look like), or where they live. Since then, ichthyologists have had access to a broader range of tools and technologies such as high resolution microscopy, staining techniques, and genetic analysis that allow them to investigate in more detail how different fishes are related, and therefore how they can be grouped into clades based upon their evolutionary relationships.
NOTE: In 1971 it was proposed that these fishes are all part of a single clade and this hypothesis has not been challenged since the 1980's. Johnson et al.'s work looked at different lines of evidence in this context to explain how it is possible that closely related fishes can show such extreme morphological differences. For the purposes of this activity, students are using the same features that Johnson et al. used, but in a slightly different way; to test two hypotheses (that the fishes form three clades or a single clade).
6. Explain to students that these classification schemes are hypotheses of relationships, and like all hypotheses, can be tested. Grouping these deep sea fishes as three separate but closely related clades will be known as the **Three Clade Hypothesis**. Grouping them as a single closely related group will be known as the **Single Clade Hypothesis**.

III. Looking for Patterns in Collection Data 45-60 minutes

1. Explain to students that lots of different scientists have been collecting individual specimens from each of these clades to learn more about them. Each time a specimen is caught some basic information is recorded such as their stage of development, sex, size,

and depth caught. Johnson et. al. compiled this data and noticed some interesting patterns that contradicted the **Three Clade Hypothesis**.

2. The students' challenge is to analyze this same data presented as the Fish Collection Data Table on page 3 of the Student Research Notes to look for interesting patterns that might suggest an alternative to the **Three Clade Hypothesis**. Guide students to look for similarities and differences in the data between and among the three clades. You can use the following questions to help students in their evaluation of the data:
    a. What is unusual about the Whalefishes that were collected? *They are all females, adults, and found in deep water.*
    b. What is unusual about the Bignose fishes that were collected? *They are all males, adults, and found in deep water.*
    c. What is unusual about the Tapetails that were collected? *They are all immature (larval forms), their sex cannot be determined, and they are caught the upper 200 meters of water.*
3. Students should record the patterns they observe below the data table. *Students should notice that the only Whalefishes caught are adult females, the only Bignose fishes caught are adult males, and the only Tapetails caught are immature. They should also see that almost all Tapetails were found above 200m, whereas individuals from the other two groups were all found at depths below 1000m. They may also observe that there were many more Whalefishes caught than the other two groups; that Tapetails have the smallest minimum size of all the groups, and that Whalefishes grow to be the largest.*
4. Students should discuss as a class their ideas and record responses to the following questions. Encourage students to consult the *Fish Reproduction and Life Cycle* section of Fish Resource Guide as a reference:
    a. How does this data impact the **Three Clade Hypothesis**? *It calls into question that these fishes are in three different clades. One would expect to see both adult males and females and immature forms, among the specimens of each group, if they were indeed separate families.*
    b. What is an alternative explanation or hypothesis to explain the patterns in the data? *The members of the different fish groups are really members of the same clade and represent different sexes and life stage.*
5. Share with students that they will call this alternate hypothesis the **Single Clade Hypothesis**.
    a. ***Three Clade Hypothesis****: The three types of fishes represent three different clades. The patterns in the data could be a result of incomplete sampling (i.e. we have not yet caught representatives of each of the sexes and growth forms).*
    b. ***Single Clade Hypothesis****: Tapetails, Whalefishes and Bignose fishes are all in the same clade. Whalefishes are adult females, Bignose fishes are adult males. The huge differences observed between Whalefishes and Bignose fishes are the result of extreme sexual dimorphism. Tapetails are the larval forms that transform into the adults.*
6. Have students write the two alternative hypotheses on page 4 of the Student Research Notes and generate ideas for how they could test the hypotheses. Ask students to think about what type of data they would collect. They can refer to the Fish Resource Guide to have them explore different features that might be useful for testing the hypotheses. This is a great opportunity to discuss what makes a good hypothesis and the importance of

alternative hypotheses (for more information, visit Understanding Science: http://undsci.berkeley.edu/index.php).

IV. Testing Hypotheses – Using Morphology (physical features) 60 minutes

1. Explain to students that they will investigate three different morphological features to test the hypotheses: gut morphology, muscle morphology, and gill-arch morphology. For each, students will follow the directions on pages 5-7 of the Student Research Notes and on the Morphology Cards to analyze and compare the structures of the three fish groups. They will record their observations and make inferences about the function of the features, which they can then use to determine the impact of the data on each of the hypotheses. Have students analyze each of the morphologies one at a time as outlined below. For more advanced groups, pass the cards out as a set, or have different individuals analyze different morphologies, then share. Be sure to review with students the directions, data tables, and questions on pages 5-7 of the Student Research Notes before they begin their investigations.
2. Pass out a Gut Morphology Card to each student group. Have students compare the internal features associated with feeding in the three fish groups: stomach, esophagus, liver, and intestine. Students record the presence and absence of each of these features in addition to noting any unusual sizes or shapes of these features in the data table on page 5 of the Student Research Notes. They then make inferences about each group's feeding habits. In addition to having students reference the *Fish Reproduction and Life Cycle* section of Fish Resource Guide it may be helpful to guide students using the following discussion questions:
    a. **Whalefishes:** Since Whalefishes have typical guts, what can you infer about their feeding? *Their feeding is typical of fishes. Food moves from their mouth, through the branchial basket, into the esophagus, and then into the stomach.*
    b. **Bignose fishes:** If a fish does not have an esophagus or gut, as is the case with the Bignose fishes, how could they get energy to survive? *Without a stomach or esophagus, they could not get energy from an external food source*. Go back to your earlier external anatomy descriptions. What is unusual about the mouth of Bignose fishes? *Bignose fishes have an immobile upper jaw and are thus not very effective at getting food into their mouths.* If a fish is not able to feed, how can they get energy resources to sustain them? *One way would be to store energy as an immature/larva that then gets used as an adult.* Would you expect a fish to live very long if they could not feed? *No.* Why might the Bignose fish have enlarged testes, but no ability to feed? *Reproduction is more important than feeding and long life span. The Bignose fish also have an enlarged nasal organ thought to aid in finding mates.*
    c. **Tapetails:** Why would a fish, such as a Tapetail, eat more food then it can use? *To store energy for later use*. How might it store this energy? *The liver is an organ that can store such energy*.

    Students should use their observations and inferences to determine how this line of evidence impacts each hypothesis. They should notice that this evidence best supports the claim that Tapetails are the larval form of Whalefishes and Bignose Fishes and therefore can support the **Single Clade Hypothesis**.

3. Pass out the Muscle Morphology Cards to each student group. Students should use the card to make observations about the proportion of red aerobic muscle and white anaerobic muscle present in each fish group. Students record the dominant form in the data table on page 6 of their Student Research Notes, and then make inferences about the swimming ability of each fish group. It may be helpful to guide students using the following discussion questions:
    a. Many organisms, such as yourself have both aerobic (red) and anaerobic (white) muscle fibers. The proportion of each influences an organism's endurance and speed. For example, long distance runners have a higher proportion of red aerobic muscles so lots of oxygen can get to the fibers for sustained effort. Sprinters on the other hand have a higher proportion of anaerobic fibers, which can act quickly but not for very long because of reduced blood flow. Based on your observations, which of the fishes would you infer have better endurance and so are able to swim for sustained periods? *Whalefishes and Bignose fishes.* Which fishes are less able to swim for long periods of time? *Tapetails.*
    b. Many types of fish have larvae. Larvae typically get around by drifting in the currents. Would you expect larvae to have lots of aerobic (red) muscle? *No.* What do you think happens to the proportion of red aerobic muscle as a larva grows into an adult? *The amount of red aerobic fibers increases.*
    c. Given what you inferred about Bignose fishes from the gut morphology, why would having a large proportion of red aerobic muscle be an advantage*? Since Bignose fishes can't eat and have large reproductive organs it was inferred that they focus on finding mates. Having a strong ability to swim could aid in this goal.*

    Students should explain how this line of evidence impacts each hypothesis. Though this evidence does not rule out the **Three Clade Hypothesis**, this evidence can support the claim that Tapetails are the larval form of Whalefishes and Bignose fishes, which would support the **Single Clade Hypothesis**.
4. Pass out Gill Arch Morphology Card A. Explain to students that the structures of the gills of the different fishes also showed extreme differences. The photos depicted in the Gill Arch Morphology Card are cleared and stained to make viewing the structures easier. As a class, have students compare the labeled structures and record the presence or absence of a **tongue**; the **orientation of bone 1** labeled in the diagram (horizontal, vertical, or the angle from vertical), and the **shape of the gill rakers** (thick, conical, toothed, or thin, forked, smooth of each specimen in the table on page 7 of the Student Research Notes. Students will not be able to determine the shape of the gill rakers in Specimen 3. Students should recognize that Tapetails have a tongue, but a tongue is missing in Whalefishes. Bone 1 of the Tapetail is horizontal, whereas the bone of the Whalefish is vertical. The gill rakers of the Tapetail are thin and forked. The gill rakers of the Whalefish are thick and conical.
5. Discuss the following:
    a. How do your observations impact each of the hypotheses? *The extreme difference between the two specimens suggests that they are part of different clades, therefore this data best supports the **Three Clade Hypothesis**.*

6. Explain to students that Johnson, et al. went back to the collections to search for transitional specimens as a way to test the hypotheses. Transitional specimens are individuals that are in middle stages of development. Ask students:
    a. What features would you expect to find in transitional forms if the **Single Clade Hypothesis** is supported? *Transitional forms would show the loss of a tongue, a movement of bone 1 from horizontal to vertical, and gill rakers that transform from thin, forked, and smooth to thick, conical, and toothed.*
7. Pass out the Gill Arch Morphology Card B that shows the gill region from two small "Whalefish" specimens Johnson, et al. found in the collections that they suspected could be transitional forms. Have students record their observations about the specimen's gill features in the same table on page 7 of the Student Research Notes. For the angle of the bone 1, students will need to record an estimated angle in the degrees from horizontal. Students should look at their data and explain if Specimen 3 and 4 (young Whalefish) could be transitional forms between Specimen 2 (Tapetail) and Specimen 1 (Whalefish) that would support the **Single Clade Hypothesis**.
8. Finally, students can revisit the fishes' external morphology using the Deep Sea Fish Specimen Key and the Fish Family Collection Data Table from Part III. They can incorporate their observations from the data in combination with the other lines of evidence and summarize their findings on page 8 of the Student Research Notes. Students can also generate new questions that they would like to explore to further test the hypotheses OR to extend their understanding about some aspect of the fish group(s).

V. Testing Hypotheses - Using Phylogenetic Trees 30 minutes

1. Explain to students that molecular technology allows researchers to use DNA data to produce phylogenetic trees that provide another way to test hypotheses. Johnson, et al. used molecular data to generate phylogenetic trees to test how closely related different specimens from the different fish groups were to one another. Students will analyze one of the same phylogenetic trees that Johnson et. al used, a Maximum Likelihood (statistical method that searches for the tree that has the highest probability of accounting for the observed patterns; best estimate of the relationships of the taxa included) tree based on partial 16s ribosomal DNA sequences.
2. To get started and help students understand how patterns in the phylogenetic trees can be used to test the two hypotheses, have students complete An Exercise in Tree Interpretation on page 9 of the Student Research Notes. Guide students to take a careful look at which specimens share the closest common ancestor. They should determine that Tree One best supports the **Single Clade Hypothesis** and Tree Two best supports the **Three Clade Hypothesis**.
3. Have students look at the Phylogenetic Tree on page 10 of the Student Research Notes. Explain that this tree is generated from DNA samples taken from individual species of fish including those that have been classified in the three groups. Students should select a color for each group of fish (Tapetails, Whalefish, and Bignose fish) to create their own color-coded key, and then use the Deep Sea Fish Specimen Key to color-code the Whalefish, Tapetails, and Bignose fish species depicted on the tree.
4. Students should analyze the patterns revealed in each tree to explain which of the hypotheses this tree best supports. They should discover that the individual species of each group are distributed throughout the branches rather than forming distinct and

separate groups (e.g. Tapetails are scattered throughout the tree). This pattern supports the **Single Clade Hypothesis**. The molecular evidence is consistent with the morphological evidence that these three groups of fish in fact belong to the same clade and represent females, males, and larvae.

VI. Wrapping it up 30 minutes

1. Have students review and discuss the process they went through to test the **Three Clade** and **Single Clade Hypotheses**. Students should have an opportunity to review all of the data they collected then summarize their findings in terms of how the different lines of evidence were used to test a hypothesis about the evolutionary relationships of deep sea fish. They can take notes on page 11 of the Student Research Notes before sharing and discussing their findings. Students can also discuss and record questions and ideas for future investigations.
2. Discuss the following:
    a. What kinds of evidence can scientists use to study relationships? *Different types of external and internal morphology and molecular data can be used to generate phylogenetic trees, hypotheses of evolutionary relationships.*
    b. How are these different lines of evidence useful? *It is important that different and multiple lines of evidence are used to test hypotheses when possible. Morphological features were the first types of evidence used to test hypotheses and so have been around the longest; new techniques and strategies have expanded this area of analysis. Molecular data is much more recent, and therefore less of it is available. External morphological data is often the first information that researchers recognize.*
    c. If you were on the Johnson science team, what would you want to investigate about these fish next? *Answers will vary. Students might be interested to know how such extreme differences evolve.*
    d. What have you learned about how these fish are adapted to the environment where they live? *Answers will vary.*
    e. What did you learn about how scientists investigate questions? *Answers will vary. It is helpful to highlight the problem solving and creativity needed for such investigations.*
3. Have students read and discuss the Johnson et al. research using *Deep-sea mystery solved: astonishing larval transformations and extreme sexual dimorphism unite three fish families* http://rsbl.royalsocietypublishing.org/content/5/2/235.abstract and/ or Museum Collections Solve Whalefish Mystery: http://ocean.si.edu/ocean-news/museum-collections-solve-whalefish-mystery/meet-suspects

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