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DNA Detectives: Outreach Activity Teaching Students to Identify Fish Eggs Using DNA Barcoding†

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INTRODUCTION

Fish are a valuable worldwide resource important in many coastal economies. About 17% of people around the world rely on fish as their main animal protein source (1). Since fish are so important to our economy (2), there are many measures in place to protect them, including fishing regulations. While adult fish are easier to catch and regulate, all stages in a fish's development must be protected for effective species management. Most fish start out as eggs, then hatch into larvae, grow to become juveniles, and live out the rest of their lives as adults (3). As they grow and move, many species of fish use different habitats to live in and feed from. For example, salmon spawn in rivers but live most of their lives in the ocean (4). The locations critical for reproduction and development of early life stages are still unknown for many of the thousands of fish species worldwide. To find spawning locations, scientists can collect fish eggs and identify them based on DNA. By the end of the lesson presented here, students will understand an example of fish reproduction, how fish eggs can be collected, and how scientists can determine what species they belong to using DNA barcoding.

Many fish are broadcast spawners, releasing their eggs and sperm into the water column, where the eggs are fertilized and float to the surface. Scientists can collect these eggs using surface plankton tows, which involve dragging a large net (typical mesh size, ~300 μm) either from shore or from a ship. Plankton, the small plant- and animal-like organisms that drift with ocean currents, are concentrated and collected in a jar at the end of the net.

Once the plankton sample is taken back to the lab, fish eggs are picked out from the wide variety of planktonic organisms using microscopes and forceps (5). Since fish eggs are usually clear spheres with few distinguishable characteristics, accurate visual identification of the species is difficult and often unreliable (6), leading scientists to use DNA barcoding to identify the eggs down to species level. The most common region of DNA used for barcoding fish is a portion of the mitochondrial cytochrome *c* oxidase I (COI) gene (7). This region of the genome is variable enough to have a unique code for most fish species but conserved enough to be found in all animals. To extract the fish egg DNA, scientists use a sterile toothpick to pop open the egg and release its DNA, which is then extracted through heating and chemical treatment. The COI gene is amplified using PCR, then Sanger sequencing (8). The sequence of this gene serves as a barcode, like the ones you find on grocery store items, to differentiate fish species based on comparison with sequences in the Barcode of Life Database (9). Instead of the numbers of a barcode, four letters—G (guanine), T (thymine), A (adenine), and C (cytosine)—are used to characterize DNA. This activity teaches students how DNA barcoding can be used to identify fish eggs from plankton samples. By accomplishing this activity, students become DNA detectives, deciphering a series of colored beads to sequences, and then matching these to a database, thus solving the case of the unidentified fish egg.

The "DNA Detectives activity" has been completed at multiple events from 2019 to 2020, including the St. Petersburg Science Festival, the Great American Teach-In, Localtopia, and a Taste of Science event. While this activity is designed for students in grades 2 to 5, it has been successfully completed by people of all ages as a fun, educational tool. To enable broad participation, this hands-on activity has also been adapted to a web-based format (https://sites.google.com/view/dnadetectives/home).

PROCEDURE

Preparing the materials

Find the full materials list in Appendix 1. Using beads and string, make strands of DNA or print 20 paper bead strands. Enclose one strand in each plastic egg (Appendix 2) to represent the fish eggs. Make sure to mark one end of the string with a red mark to indicate the starting position for reading the DNA code. Place all the eggs filled with DNA into a large bin or small plastic pool. To make the eggs more difficult to

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FIGURE 1. Fishing for fish eggs.

FIGURE 2. Deciphering the code.

capture with the nets, add stuffed animals of marine life or other obstacles. Print out enough DNA decipher sheets (Appendix 3) and coloring pages (Appendix 4) for the number in your group. Print out the fish matching guide (Appendix 5) on large paper or make your own poster boards using the codes and fish drawings. Optionally, stickers with the DNA Detectives logo (Appendix 6) can be produced as a prize for finishing the activity (e.g., www.stickermule.com).

Teaching lesson and outreach activity

Begin the lesson by talking about how fish are important ecologically and as a protein source for the world, asking how many students eat fish. Explain the different life stages of a fish (Appendix 7), going from egg, to larvae, to juvenile, to adult, and their predominant form of reproduction—broadcast spawning. Describe how fish eggs are collected from the surface of the ocean using a plankton net. Show students the pictures of fish eggs (Appendix 8) and ask if they can tell which fish species each of them came from. The response should be "no," because they all look the same. Next, ask students how scientists determine which species the eggs belong to if they cannot tell by looking at them. Define DNA and describe how it can be used like a grocery store barcode to differentiate between species, as described above in the introduction.

Review the entire activity before allowing students to begin. Using a fish net (or their hands if nets are not available), have students collect one fish egg from the pool (Fig. 1). They will open the egg to release the DNA strand (beads or paper) and begin to decipher the DNA code using the worksheet (Fig. 2). Instruct them to start by putting the red mark of the DNA string, or the number for the paper version, on the left side of the worksheet and change each bead color into its matching letter using the guide. There are 20 beads for each strand and 20 lines on the worksheet, so every line should be filled. Once they have completed the DNA code, have students match the code with the fish guide to determine which species the fish egg belongs to. As a reward for correct identification, give each student the coloring page belonging to their fish species, which also contains a fun fact.

Once everyone has completed the activity, ask them the following questions: What is DNA? How do you identify fish eggs? Why is this important?

There are no safety issues associated with this activity.

CONCLUSION

This activity allows students to understand how fish eggs are collected in the field and identified in the lab, while learning about the life stages of a fish and practical applications of DNA barcoding. Students will become DNA detectives of the sea and be rewarded with coloring pages and stickers. These items will allow students to teach their families and friends about the activity and remember the concepts more clearly.

SUPPLEMENTAL MATERIALS

Appendix 1: Materials list

Appendix 2: DNA codes

Appendix 3: DNA decipher worksheet

Appendix 4: Fish coloring pages

Appendix 5: Fish matching sheet

Appendix 6: DNA Detectives logo

Appendix 7: Fish life cycle and broadcast spawning

Appendix 8: Pictures (fish eggs and plankton net)

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