Embryonic Development of Zebrafish

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1998

Grade-Level:

Elementary-High School

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Introduction:
The zebrafish is a common tropical fish found in pet stores in all corners of the world. Materials needed for this activity are fairly inexpensive and easy to access. This lesson is geared for a classroom with 15 or fewer students.

Purpose:
Allow students to observe, analyze and record the embryonic development of zebrafish.

Objectives:
Students will observe, record data, hypothesize, design experiments, conduct experiments, and draw conclusions.

Materials (per classroom):

1. 10 gallon fish tank
2. air tubing
3. sponge filter
4. fish net
5. chlorine cleaner
6. thermometer
7. turkey baster
8. black plastic garbage bag
9. bleach
10. scrub pad
11. dissecting microscope
12. petri dish
13. 1 liter of marbles
14. flake food
15. 10 female zebrafish
16. 15 male zebrafish

Preparation:

1. Zebrafish appear to do well in tanks with square corners.
2. Let tap water sit about two days before adding fish to the tank.
3. Add chlorine cleaner to the tank before adding fish to the water.
4. The temperature of the water should remain between 25-31 degrees Celsius.
5. When purchasing zebrafish, there are certain characteristics, which distinguish the male from the female. Males usually have a yellow belly and are long and thin, while the females are silver and heavier looking.

Procedure:

1. Add zebrafish to the prepared water.
2. Place two layers of marbles at the bottom of the tank to prevent the bigger fish from eating the eggs. The marbles should completely cover the bottom of the

tank. Eggs will settle to the bottom under the marbles, which will be out of reach for the bigger fish.

3. Feeding should take place three times a day. Give the fish an amount they will be able to consume within five minutes. Overfeeding may prevent the fish from breeding in a timely manner. While brine shrimp may enhance the breeding process, flake food is fairly inexpensive and sufficient for classroom purposes.
4. Sick fish need to be removed from the tank as quickly as possible to prevent contamination. If your fish are beginning to die, consider purchasing your next batch of zebrafish at a different pet store.
5. At night, cover the tank with a black plastic garbage bag. Remove it in the morning to simulate a night and day pattern. The light cycle should include 14 hours of light and 10 hours of darkness.
6. You may wish to set a timer to control the light in your room or in the aquarium. Usually zebrafish will breed about 30 minutes after light appears. If you have trouble finding any eggs it may be that you have all males or all females.
7. Siphon water below the marbles with a turkey baster and pour the contents in a petri dish. When siphoning, do not move the marbles around too quickly as the adult fish will eat the eggs that float around the tank.
8. The eggs should be visible; they look like a small soap bubble.
9. Place the petri dish with eggs under the dissecting microscope to determine what stage the eggs are in. A healthy egg will split every 20-40 minutes. Unhealthy eggs may have mold or other particles, which cloud its appearance. Students will also be able to see the development of various body parts and such actions as blood flow and the heart beating over the next 24 hours.

Stages:

1. There are seven stages, which provide accuracy in the development of an embryo. The stages are the zygote period, cleavage period, blastula period, gastrula period, segmentation period, pharyngula period and the hatching period.
2. At its inception, known as the zygote period, the embryo will have one cell that appears like a half- bubble. This period lasts for the first 45 minutes of life. The newly fertilized egg is .7 mm in diameter. The chorion (egg shell) swells and eventually lifts away from the fertilized egg.
3. After the first cleavage stage the cells divide at around 15 minute intervals.
4. The cleavage stage runs from 45 minutes to 2 ¼ hours. Nuclei are present and visible during the first half of each cycle during interphase.
5. The blastula stage lasts from 2 ¼ to 5 ¼ hours. Epiboloy begins at this time. Epiboloy is the thinning and spreading of both the yolk syncytial layer and the blastodisc over the yolk cell. The yolk is a nutrient store for embryonic development.
6. The gastrula period runs from 5 ¼-10 hours. Primary organs will begin to appear visible. The tail becomes more prominent and the embryo elongates.
7. The pharyngula stage occurs between 24–48 hours. This is the time of development when one can compare the embryos of diverse vertebrates. For the zebrafish, it is the second or third day of embryonic development.

8. The final stage is the hatching period, which runs from 48–72 hours. Individual eggs within a single developing clutch hatch sporadically during the whole third day of development and occasionally later. The embryo continues to grow at the same rate as it had earlier. During this time, the gills, jaws and pectoral fins are easy to see as they are developing very quickly.

Questions to ask:
Do zebrafish need light to release their eggs?
How do you tell the difference between a male and a female zebrafish?
Approximately what temperature is ideal for breeding zebrafish?
How long does it take for a zebrafish to reach the hatching period?

Where To Go From Here:
This lab activity would fit into a part of a curriculum dealing with the development of animals. Ideally a teacher would invite someone with background knowledge of how to breed zebrafish on a larger scale. The students will have the opportunity to take some of the zebrafish home to breed.

Safety:
This activity is very safe, as there aren't really any dangerous procedures involved. Following directions is an essential step in maintaining the quality of life for the zebrafish.

Suggestions for Assessment:
1. Students will be graded on these presentations.
2. Students will create their own board game with the main objective having to do with zebrafish development from the zygote period to the hatching period.
3. Students will read about, compare, and contrast different animal embryos.

Extensions:
1. The activities could be used as an introduction to a unit on the development of other life forms.
2. Invite an expert to speak on the topic.
3. Students will make a poster labeling two different stages of the embryo.

References and Resources:
Westerfield (1993). *The Zebrafish Book*. Eugene, OR: University of Oregon Publishing.

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Student Activity

Introduction:

You or someone you know may have an aquarium at home with many tropical fish including zebrafish. They are a very popular fish and are useful in observing embryonic development. We can control the life cycle of zebrafish by putting a black plastic bag over the tank and removing it in the morning.

Once removed, light appears and the female releases its eggs. A dark room with a light source can also be used. The light cycle should include 14 hours of light and 10 hours of darkness. You may with to use a timer in your room or attach it to the aquarium. The male follows the females and release sperm. Once the eggs are fertilized, we can watch the development.

With a partner, you will observe the egg development and determine what stage it is in.

Materials:

You will need:

- petri dish
- water siphon
- microscope

Procedure:

Place your water siphon at the bottom of the tank and begin squeezing. Empty your contents into the petri dish. You will be looking for an embryo shaped like a clear bubble. Collect a minimum of five embryos to observe. Place the embryos under the microscope and with your partner discuss the various shapes.

Draw a picture of each embryo on a sheet of paper. Next to the picture write down the name of the stage, which you believe closely, resembles that shape. You can use the attached picture handout to determine the stage.

Once this activity is completed, experiment with the pH level, water temperature, and different types of fish food (examples: microworms, bread crumbs, blood worms, hard boiled egg yolk crumbs, small insects) to see if these changes will inhibit or enhance the embryos development. Record your findings on a line graph. When you are done, present your findings in a 3- minute presentation to the class. After each presentation, the class will discuss your results.