Stages of zebrafish development

The goal of this laboratory is to learn the stages of zebrafish development, and to use dissecting and compound microscopes. Use these images to learn the different stages of zebrafish development.

1. Tools

You will use several tools for the experiment today:

Dissecting stereomicroscope and a compound microscope.

A pair of fine forceps. These forceps are fragile and expensive and should therefore be used only when working with zebrafish embryos. You can use these to dechorionate (take the shell off) the embryos and to push them around if you are careful.

Embryo loops. Embryo loops are for orienting and pushing the embryos around. These are made with fishing line, capillary tubes, and super glue.

Slides. Live embryos must be mounted on glass depression slides in methylcellulose before you can view them on the compound microscope.

2. Staging zebrafish embryos

The term "staging" means determining the developmental stage of a zebrafish embryo or larva. You will be given a petri dish with four to six stages of embryos. Your challenge is to separate embryos at different stages of development into different dishes, and to determine their stage. Please review the different stages of zebrafish development before beginning this experiment. *As you are reading, think about what differentiates zebrafish embryos at each different stage?*

Dissecting microscope

First look at the embryos you have been given under the stereomicroscope (low power). Put the petri dish on the stage and turn on the light underneath the stage. Adjust the mirror so you get even illumination across the field of vision. This is called brightfield microscopy.

As you look at the embryos, use the staging sheets to help you stage your embryos. Note characteristic features of each stage that help with identification.

An important skill in working with zebrafish is the ability to work with the embryo underneath the dissecting microscope. There are several things you can do even with the simple tools you have. Most of the stages you are looking at still have their chorion--can you take it off (dechorionate the embryo) with your fine forceps? *Does dechorionating make it easier of harder*

to see structures within the fish? When do you first see movement of the embryo? What kinds of movements can you see? When does it start to respond to touch?

As you are staging your embryos, think about the following issues and write about them in your laboratory report:

1. Staging identification

What distinguishes an embryo in the cleavage period from the blastula period? How can you differentiate different stages within the blastula period? In the gastrula period?

2. Microscopy

What are the limitations of an observational approach to developmental biology? What are some of its advantages? Think about developmental structures and processes that are easy and hard to observe. *What view is it easiest to observe embryos and detect what stage they are in?*

Play with the setting of the light and the mirror on your microscope. *Are there settings that make it easier or harder to see specific structures?* For example, try turning the mirror so light is not going directly through the embryo. This is pseudo dark field microscopy. *Is it easier or harder to see the somites? Can you count how many somites your embryos have?*

Compound microscope

Next take one of your embryos and look at it under different lenses of the compound microscope. You can use an embryo that is dechorionated, or one that is still in the chorion (but choose one with a clean chorion). Follow the directions for mounting the embryos using methylcellulose.

Adjust the compound microscope for bright field microscopy, and observe the embryos at different magnifications using the different objectives on the compound microscope. *How does what you see differ from what you saw using the dissecting microscope?*

Now adjust the microscope for dark field microscopy. In this method, put a coin on top of the light source-a big coin for a low power objective, and small coin for a high power objective. This will create a ring of light that is illuminating your embryo-this is called dark field microscopy. *How does this change the brightness and contrast of the image you see? Are their structures that are easier to see in dark field versus bright field, and vice versa?*

Discussion Questions:

What are some differences you noted between the dissecting and compound microscopes?
Is it easier to stage embryos with one microscope over the other?
Were any special techniques for light microscopy helpful in viewing embryos?
What techniques were most effective in the determination of the stage of a particular embryo?
How might you explain the variations between different stages to another student?