Zebra Fish Embryology

http://zfin.org/zf_info/zfbook/zfbk.html
http://www.bio.umass.edu/biology/kunkel/fish/zebra/zebra.html

Information in this paper was compiled from the Zebra Fish Book online book from the University of Oregon and the University of Massachusetts, Amherst by Kunkel's lab.

Setting Up Your Aquarium

- Wal-Mart sells a 5-gallon tank set, which includes filter, heater and light all for around $20.00. The heater included does not work well so it might be beneficial to buy a heater that maintains the temperature at 28.5 degrees Celsius.
- Use a corner or side filter.
- Buy 6 males (longer, slimmer, and more yellow especially on the bottom side) and 6 females (plumper and more silvery). Females are fat when filled with eggs and do not usually have gold on their belly, if they do they have very little. Males tend to be the ones chasing the females, early in the morning, before they breed.
- Do not use gravel.
- Buy a programmable light timer at Wal-Mart also for about $5.00. You need to maintain a 15-hour light and 9 hour dark cycle. The fish will breed at dawn.
- If your classroom does not get entirely dark at night and lights come on then I would suggest covering your tank at night with a box lined with a black trash bag. I have found that this sometimes increases the temperature of your tank. You do not want an increase in temperature so be careful and watch for that.
- Place the egg collector on the bottom of the tank away from the filter. (How to make an egg collector is shown on a separate page).
- I would suggest setup multiple tanks so that you are sure to get a constant supply of eggs.

Successfull Breeding

- Zebra fish lay eggs every day.
- They eat their eggs to recycle the protein they lost from producing eggs, so you need to feed them a protein rich diet.
- The very best protein is live brine shrimp.
- Cichlid food is higher in protein than other foods for fish, too.
- Do not feed more than they can consume, because you will contaminate your tank.
- Feed them well for one week or until females get fat with extra eggs.
- pH needs to stay around 7.0 with a maximum range of 6.6-7.4.
- Watch out for high pH of your tap water. Some municipal tap water supplies are raised to 8.0 or more to reduce leaching of copper, etc. from the pipes.
- Water temperature should be 28.5 degrees Celsius (83° F). Above 31°C (88°F) or below 25° C (78°F), the zebra fish will not breed and development will be abnormal.
- Melafix has been observed to increase breeding. This is certainly and area that could be researched. Melafix is used for fungal diseases and can be purchased from most pet stores.
- Food: Tetramin Tropical, The Rich Mix Flakes, OSI Freshwater Aquarium Flake Food, Brine shrimp, Frozen Brine Shrimp. This should be given twice a day. If you use frozen brine shrimp dissolve the frozen chunk in some water from your tank and use a pipette to drop out the shrimp into the water. Watch the food intake because too much food is detrimental to fish. If you choose to use frozen brine shrimp watch for parasites. Make sure you do not get any in the egg-collecting container.
- Since fish are photoperiodic a physiological response to day length, they will breed at dawn, when your lights come on. I set my timer so that the light goes off at 9PM and turns on at 6AM. So there is 15 hours light and 9 hours dark. THIS IS THE MOST IMPORTANT STEP FOR SUCCESS IN BREEDING!! If you interrupt the light cycle you will probably not see eggs in the morning. On this cycle most of my eggs are laid by 10:30AM.
- Knowing they breed at dawn you can place your egg collector in the aquarium at night and when the light comes on in the morning watch the behavior of the fish in the morning.
- If you have any problems with algae or parasites, make a 10% bleach solution and clean your equipment.

Steps to Successful Breeding

- Obtain an aquarium preferably 5-10 gallons
- Add water
- Add de-chlorinator and wait 24 hours for fish insertion

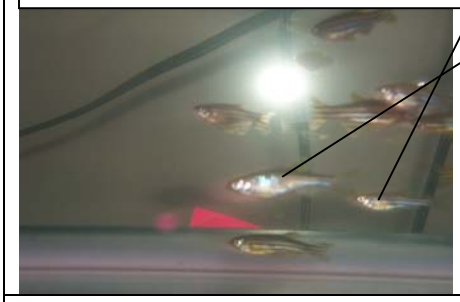
DIRECTIONS FOR USE OF ULTIMA:
ADD 1 teaspoon per 10 gallons of water

http://aquascienceresearch.com/ultimate.html

- I use a product from Aqua science called Ultimate, for dechlorination.
- AquaScience Research is located in NKC, Missouri 816-842-5936.
- This removes and detoxifies ammonia; chlorine, chloramines, copper and heavy metals, boosts alkalinity, adds essential electrolytes, adds 3-part skin slime replacer, reduces stress and instantly ages water.

Zebra fish

- ADD fish by floating the bag of fish for one hour without releasing them into the tank. The fish will acclimate to the water and temperature. I have read you don't have to do this but found that personally that is not true.
- I add 6 FEMALES AND 6 MALES
- You may want to add more females than males. When choosing fish try to choose younger fish, they will breed longer.

Sexing the fish **Hint:** I have had really good luck with Wal-Mart fish. They are on a light timer and you can really see the fat plump females. 	**Males:** Thinner and the older ones have a yellow color under their belly. They tend to be chasing the females and are much more active than females. **Females:** Fat, round and plump. The plumper the female allows you to determine whether they have released their eggs or not.
Squeezing sperm and eggs from fish so you know the exact parents.	http://zfin.org/zf_info/zfbook/chapt2/2.8.html#3 Visit this site if you are doing genetic crosses and need to have sperm and eggs from specific fish.
Breeding tanks 	Place your breeding tanks on the bottom of the aquarium. You may purchase breeding tanks from Aquatic Habitats http://www.aquatichabitats.com/btank4p.html Note the open ridges in the bottom that allows the eggs to fall through for easy retrieval. The price for these can be found at the following URL and are 10.00 dollars each. They are called 5 piece breeding tank. http://www.aquatichabitats.com/store.html
	

You can also make your own breeding tanks in which the instructions are on page 15. • All you need is a cheap plastic bowl cut out a circle in the bottom and hot glue a stainless steel screen to the bottom. 	 
Fish are PHOTOPERIODIC so you need to connect your aquarium light to a timer. • As shown in the picture, set the light to come on at 6:00 AM and go off at 9:00 PM. This will give you 15 hours of light and 9 hours of dark. Perfect for breeding! • **CORRECT LIGHTING IS ESSENTIAL IN BREEDING!** • **YOU MUST NOT ALLOW THIS TO GET INTERUPTED BY SOMEONE TURNING OFF AND ON YOUR LIGHTS.**	The timer costs approximately $5.00 at Wal-Mart. 
TEMPERATURE	Place a thermometer in your tank to assure the tank stays at around 82° F or 28° C. Read below to see how critical temperature can be.
pH	pH should be maintained to around 7.0 with a minimum and maximum range of 6.6-7.4

Feeding Hint: Watch out for overfeeding this will cause your tank to get dirty and unfit. This will cause parasites such as worms, which are hard to get rid of.	Food: Tetramin Tropical, The Rich Mix Flakes, OSI Freshwater Aquarium Flake Food, Brine shrimp, Frozen Brine Shrimp. This should be given twice a day.  OSI: 517-670-0888 or EECHO http://petsforum.com/aquascience/Associates/EECHO.htm Tetra flakes: any pet store
Algae	Sometimes I will come home to a green tank. Just go out and buy an algae eater fish, found at your friendly pet store or Wal-Mart. If you can stand it algae is great for your fish environment. One of the best fish supplier's tanks all contains tons of algae. They aren't pretty but the fish are healthy.
I place clear marbles in the bottom of my breeding tanks, which the fish seem to be attracted to.	These can be obtained at any pet store. Make sure they are safe for fish. 
After the lights come on the female will start swimming over the marbles and the male will chase her. This is the initiation	Do not marble your tank only marble your breeding tank. A marbled tank, allows eggs to escape predation from the fish. This is great so you can collect the eggs in the breeding tanks. If you have no marbles in your fish aquarium the fish can

of breeding behavior. DON'T MARBLE THE BOTTOM OF YOUR TANK!	no marbles in your fish aquarium the fish can easily see the eggs and quickly consume them. This is great for the fish because it can obtain protein lost from egg production and breeding behavior. By marbling a tank, the egg protein source is denied and energy loss to a tank is extensive, which is why fish should be put over marbles no more frequently than once per week. You need to give the fish a break and not place the breeding tanks in the aquarium every day. I only place them in the tank when I want to collect eggs. On days when the breeding tanks are not in there, they should be fed several times per day with protein rich foods to compensate for their energy loss.
How will I know I have healthy eggs? Observation by eyesight, of the eggs will allow you to tell if they are healthy or not. Zebra fish eggs are translucent and you can actually see through them. Put your egg- collecting device up to the light and you will see tons of eggs after about 1-2 hours after the lights go on.	Depending on health and unknown factors, females will produce: • *good eggs* (slightly yellowish, granular-looking eggs); **They are transparent and can be observed dividing every 20-40 minutes or so under a dissection microscope.** • *bad eggs* (eggs already broken down, whitish and rather like baby cereal) they will develop mold and fungus remove immediately. • *mixed eggs* (some good eggs mixed with bad eggs); and • *no eggs*
View your eggs using small petri dishes under a light microscope or dissection microscope.	If you use a light microscope be careful of the light getting to hot. This could hurt your eggs!
Developmental stages • This is a great site by Kunkel labs from the University of Massachusetts on the development of zebra fish.	http://www.bio.umass.edu/biology/kunkel/fish/zebra/devstages.html Use these pictures to decide what stage your embryo's are in.
Great Journal Articles:	
TRACES OF HOUSEHOLD PRODUCTS DISRUPT SEX GENES IN FISH BRAINS	http://www.umbi.umd.edu/nande/news/archive/02/080102_genes.html
Explorations taking place at medical schools using zebra fish	http://www.health.pitt.edu/pittmed/Jan_2003/Investigation.pdf
Internet Sites	**Zebra Fish Links Kunkel** **http://www.bio.umass.edu/biology/kunkel/fish/zebra/** **http://www.bio.umass.edu/biology/kunkel/fish/zebra/zebra.html**

	Fish Book **http://zfish.uoregon.edu/** **The Interactive Atlas of Zebra Fish Vascular Anatomy** **http://mgchd1.nichd.nih.gov:8000/zfatlas/Intro%20Page/intro1.html**

What do you do with the eggs when you get them?

What is the behavior of the fish when the female is laying the eggs and the male is fertilizing?	You will see the slim, active male chasing her around the marbles that are found in the bottom of the breeding tanks. This will not occur until one hour after the light has turned on. Sometimes it may take longer. The female will lay her eggs and the male will fertilize by spraying sperm on them, following her closely.
Breeding tanks **Note the fish will swim over the tanks when depositing eggs and fertilizing.**	
Egg Collection When you think you have collected eggs in your breeding tank, carefully lift it out of the water.	

Carefully take the top portion of the breeder out of the water shaking it to make sure you get all of the eggs.	 **Note:** **You can see the small ridges at the bottom of the top container. This is where the eggs fall through. If you look closely you can see the clear marbles. Plastic greenery comes with the breeder tanks for a great natural fish atmosphere!**
Now closely observe the eggs on the bottom!	It is now time to strain your eggs to keep the debris in the water away from the eggs.
Go to a coffee shop or kitchen gadget shop and by this strainer. They are around 5.00 dollars	
Open the strainer and pour the water containing the eggs into the strainer.	

Now you need to clean your eggs!	Pour tap water over your eggs found in your strainer!  • **Try to keep water on the eggs at all times!** • **Do not leave the eggs in the strainer with no water.** • **Make sure the water is warm, not hot or too cool.**
Cleaning continued!!	Now that all the dirty egg water is gone from the bottom-breeding tank, rinse it out and fill it to the top with tap water. (Do not leave the eggs without water.) Then take the strainer and swish it in the water to remove excess debris. This will ensure clean eggs.  **Hint:** **When doing this I keep the strainer above and the water below so the eggs stay wet.** Now Swish! 

Releasing the CLEAN eggs!	 **Pinching the handle opens the strain for release of the eggs.**
Make sure the eggs all come out of the strainer!	By immersing the strainer completely under water the eggs will be sure to fall out into the clean water. 
Should I use egg water?	If you want to use egg water the recipe can be found on page 14 and 15. Egg water contains ocean salt. Directions for egg water: Remove the eggs from the tank after they have been laid and place them in a container, containing egg water. Below is the recipe to make egg water. Make enough egg water so that you can clean your eggs with it and place the eggs into small petri dishes containing egg water. **Egg water recipe:** • **1.5 ml stock salts added to 1 L distilled water. Look below to see how to make stock salts.** **Stock salt recipe:** • **40 g "Instant Ocean" Sea Salts added to 1 L distilled water**

DO NOT USE AN AIR PUMP EARLY! JAVA MOSS AND DUCKWEED WORK GREAT FOR **DISSOLVED** OXYGEN! The tank shown in the picture is OK to use because it does not move the water so vigorously.	I personally have found it not to be necessary. I leave the eggs and larvae in the same water (aquarium water) until day 21. I then transfer them to a tank with an air hose, such as the one featured in the picture below. When using them for observation in the classroom I use the egg water recipe above.  **Note:** **This comes with an underground filter, air hose, plant, light and holds 2.5 gallons of water.** **The price is $9.95 for all and can be found at Wal-Mart.**
You could also use any small animal cages that are acrylic or any container as long as the air hose does not put out to much air.	
When larvae appear what do you do?	I use two types of food that has been really successful. The University of Oregon says paramecium which is the Best. I personally do not have time for all of that plus My lab set-ups. I simply use fry food. There are two types, Liquid and solid. It is suggested to use the liquid early and the solid after 14 days.

	The two are shown below:  Directions: Shake well. Use sparingly feeding 1 drop 3-4 times a day. Make sure you do not ruin your water.  Directions: Feed sparingly 3-4 times a day. Fry consume very small portions. In order for them to sustain healthy growth them must eat often.
How long do I feed them fry food?	• Feed the babies after they are **4 days old**. You may feed them live paramecia, small fry or liqui-fry that can be purchased at a local pet store. • **At 9 days** they can be moved to larger containers with more water. You can use liqui-fry, small fry or paramecia mixed with baby fish food or live brine shrimp larvae. • **At 14 days**, stop using Liqui-fry or paramecia and feed them only the baby fish food or the brine shrimp larvae. • At 21 days, **the fish can be transferred to a regular tank. Don't forget that big fish will still eat little fish at this stage, so they will need a separate tank.** I would continue to feed them fry food until they are able to eat normal fish food.

Temperature and Embryonic Development

You can actually speed up or slow down development by regulating temperature. Temperature needs to be controlled to some degree. I have seen that lowering the temperature, by placing the embryo's in the refrigerator slows down development. **Development is dependent upon temperature** and therefore can be controlled to some extent.

A simple formula for calculating stage times is:

HT=h / (0.055T - 0.57)

T = development temperature

h = hours of development to reach stage at 28.5 C

HT = hours of development to reach stage at temperature T

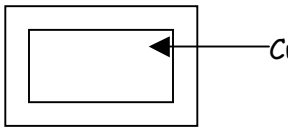
Stages for development at 28.5 C can be seen at the following link.

http://www.bio.umass.edu/biology/kunkel/fish/zebra/devstages.html

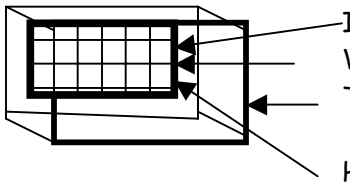
These stages were developed at 28.5 C and would not be seen at the same exact time if you varied the temperature. This would be a great research project to use a different temperature and take pictures at each time and compare.

How to Make Your Own Egg Collectors

- Buy 2 Tupperware (plastic) translucent or transparent containers that are approximately 15" x 8" depending on your tank size. For a 5-gallon tank this is perfect. I tried doing this with a white container and could not see the eggs as well. You might want to experiment with different colors to see if number one you can see the eggs on the bottom and two if the fish are more attracted to the colors.
- Cut a large hole or opening in the bottom of 1 of the containers.



  - Cut a piece of wire screen that is used for screen doors and found at your local hardware store that is a little larger than the hole you cut.
  - Hot glue the screen to the inside of the container like illustrated:



  - Now add clear marbles to your collection devise by pouring them on the screen collector. Make sure these marbles do not have anything in them that would contaminate the water.
  - Now insert your screen collector into your extra Tupperware container and place the collection devise into your tank.
  - To collect eggs wait until after dawn, watch the behavior of the fish. The males will swim after the females. The females will release their eggs and the males will follow those spraying sperm on the eggs.
  - When taking the egg collector out to check for eggs, slowly grab the entire devise and bring it out of the tank. The eggs are denser so they will migrate through the marbles to the bottom of the container. Slowly

take the wire collector out of the plastic collector. You will then find clear eggs in the bottom egg collector. HOPEFULLY!!

Feeding Your Adult Zebra Fish During Breeding Time

Fish need a high protein diet for breeding. To achieve this high protein diet feed your adult zebra fish, brine shrimp twice a day.

Brine Shrimp

- To establish brine shrimp culture, add 10 ml of shrimp eggs (Carolina Biological) to 2000 ml of salt water (Instant Ocean).
- Or if you can't find Instant ocean make a 1% solution of NaCl made from non-iodized salt (add 1 tsp salt to 1 pint of water, or 5 mL salt to 500 mL water).
- To determine the amount of brine shrimp eggs to use you must first determine the size of container you will be using for your culture.
  - Pint jar; up to ½ tsp. of eggs
  - 1 gallon, up to 1 tsp. of eggs
  - 3 gallon; up to 1 tbsp. of eggs
- Aerate vigorously. After 48 hr at 28.5° C
- To collect brine shrimp follow directions below
- Filter the shrimp through a cloth, wash with **fresh water** and **dilute into dH2O** at a ratio of 1 volume shrimp to 3 volumes water. This will remove the salt so you do not expose a hypertonic environment to your zebra fish when feeding.
- Feed 1 pasteur pipette of diluted shrimp per 8 adult fish.

- Other possible foods are daphnia, Drosophila and Drosophila larvae. Beware of tubifex worms, which may carry diseases.
- If you do not want to go through the hassle of feeding brine shrimp to them you can feed those Tetra flakes twice a day.

Feeding your Live Brine Shrimp:

- You will need to feed your live brine shrimp daily by adding some brewers yeast to dH_2O, forming a milky solution.
- Drop a few drops, using a pipette, per day so that you do not overfeed and cause contamination.

Collecting Brine shrimp

To collect brine shrimp, choose the tank with the oldest shrimp and turn off the air-lines that are used to aerate the shrimp culture. Place a lamp in front of the tank and turn it on. The brine shrimp will **move toward the light** and in five to ten minutes, they can be siphoned out.

To siphon the brine shrimp for a morning feeding:

- Place a 2-liter flask with a funnel and a handkerchief on the floor in front of the tank.
- Remove the lid from the tank and slowly lower the siphon into the water. (A layer of un-hatched eggs always covers the surface of the water and a small puff of air as the end of the siphon passes through this layer can clear the un-hatched eggs so they do not go into the siphon.)
- Position the end of the siphon directly in the thickest concentration of brine shrimp and start the siphon.

- The shrimp will collect on the handkerchief and the salt water will collect in the flask. **A clean empty beaker should be standing by to receive the brine shrimp**. A 600 ml beaker works nicely; however, if there are a lot of brine shrimp, a 1-liter beaker is required.
- The brine shrimp should be rinsed with fish water to remove any excess salt water before feeding. Keep a squirt bottle near the clean water tank for this purpose.
- To transfer the brine shrimp to the beaker, place one hand in the shape of a cup under the handkerchief and lift the shrimp out of the funnel. With the other hand, use the squirt bottle to rinse them off the cloth and into the beaker.
- If the concentration in the beaker is too thick, add more clean system water to thin it.
- Take the flask of salt water and pour it back into the brine shrimp tank.
- Locate the air-lines and turn them on so the water is bubbling again.

Feeding Brine Shrimp to baby zebra fish in Nursery

- Use a pipette (5 3/4" disposable Pasteur pipette) with a bulb to feed the baby fish in the Nursery.
- Fish that are 9-11 days old need only a couple of drops of the brine shrimp.
- Fish that are 12-17 days old can eat at least one full pipette
- Fish that are 17 and 21 days old, they will eat 1.5 to 2 pipettes full.

In addition to the fish in mouse cages in the Nursery, babies in regular tanks (in the regular, non-nursery facility) also need to be fed brine shrimp. All the babies in the main facility should be labeled (e.g. with pink pieces of tape on their tanks that have the word "babies" written on them). Simply start at one end of the main room and walk down each aisle looking for tanks with "baby stickers" on them. **A general rule for feeding is one pipette full per 10 fish, so a tank with 50 fish will get 5 pipettes of brine shrimp.**

Afternoon feedings

To collect brine shrimp for the afternoon feeding

- obtain a clean, empty mouse cage and place the flask that will hold the salt water in the mouse cage.
- Put the mouse cage and flask on the floor and siphon almost all the brine shrimp from the tank using the procedures outlined for the morning feeding.

Use two 1-liter beakers to hold the brine shrimp and pour the collected salt water back into the tank. A larger amount of brine shrimp is required because the afternoon feeding includes a supplement of baby brine shrimp for all the fish in the facility. **A general rule for feeding is one pipette full per 10 fish, so a tank with 50 fish will get 5 pipettes of brine shrimp** be used as a guideline for feeding the adult supplement in the afternoon. After all the fish have been fed, the beakers and pipette are cleaned as in the morning.

Feeding Baby Zebra Fish Embryos

Paramecia for Baby Fish (Seed Cultures):

1. To each bowl add 10-15 grains of of boiled wheat to 175 mL of system water (water that is de-chlorinated).
2. Add 20 mL of paramecia seed culture.

3. Grow for 7-12 days before using.
4. Store at 28.5°C on well-lit shelves.
5. Cultures remain useable and healthy for a month or more.

Paramecia for Baby Fish (Standard Procedure):

1. Fill plastic mouse cage or glass bowls with 2 liters of system water.
2. Add a large pinch (about 30 grains) of boiled wheat, ½ tablet of brewer's yeast, and 1/6 of a petri dish of paramecia culture to each mouse cage.
3. Cover and store in a warm, well-lit place. The covered mouse cages may be stacked three layers high with the highest layer closest to a warm light. It will be ready to feed first.
4. **Filtering:** When the culture is ready for feeding, you need to strain it by pouring slowly through a cotton handkerchief into a funnel. The paramecia will pass through, but the filter catches the thick stuff that has grown around the wheat. Feed up to 5 mL for each beaker of fish fry. Change the water a few hours after feeding.
5. The cultures will be ready to use in 4 days and will keep for about 3 weeks.

Paramecia for Baby Fish (small batch)

1. Use small container filled 2/3 full (about 200 mL) with system water.
2. Add 8-9 grains of boiled wheat, 1/5 of one 7.5 grain brewer's yeast tablet, and 8 mL of the paramecia seed culture.
3. Stack the containers 6-8 high, cover and store at 28.5 degrees Celsius on well lit shelves.
4. After 4-6 days, the paramecia are ready to feed to the fish larvae. Cultures will be good for about 3 weeks or more.
5. Filter as described above.

Velvet disease

Zebrafish are highly susceptible to velvet disease, Oodinium pillularis, a parasitic dinoflagellate algae. Most fish from dealers or pet stores carry velvet disease. This oval-shaped parasite attaches to the fish near the fins, especially the dorsal fin, and around the gills. You can see it under a dissecting microscope. When the parasite is mature, it drops off the fish and multiplies 60 times on the bottom of the tank. These new parasites then re-infect the fish. Fish with velvet disease have the characteristic behavior of rubbing their sides and flipping around in the corners of the tank. As the disease progresses, fish become lethargic, the fins (particularly the dorsal fin) are held close to the body, and the fish stop producing eggs. Most fish from pet stores or dealers carry velvet disease.

Symptoms of velvet disease:

- rubbing behavior
- lethargy
- fins held close to the body
- parasites near fins and gills

Although velvet disease is extremely contagious, it can be cured with minimal damage to the fish using a 3-day treatment with Atabrine (Quinacrine hydrochloride).

Treatment for velvet disease:

- **Day 1**
  - Turn off incoming water.
  - Slowly drip 2 liters of sea salts into an infected 10-gallon tank. (20 tablespoons of Instant Ocean in 2 quarts of system or tank water.)
  - Add 3.3 ml of the Atabrine stock solution (10 mg/mL H_20)

- **Day 2**

  - Add 3.3 ml Atabrine stock.

- **Day 3**
  - Add another 3.3 ml Atabrine stock for a total of 9.9 ml.
  - At the end of the 3-day period, clean the bottom of the tank thoroughly and slowly dilute out the salt and the Atabrine with fresh water.
  - Continue cleaning the bottom of the tank daily for several days.
- **Solutions:**
  - Atabrine Stock:10 mg/ml dH2O. Store in light tight bottle.
  - Salt Stock: 20 tablespoons (280 g) Instant Ocean Sea Salts (Aquarium Systems, Inc.) dissolved in 2 liters distilled water.

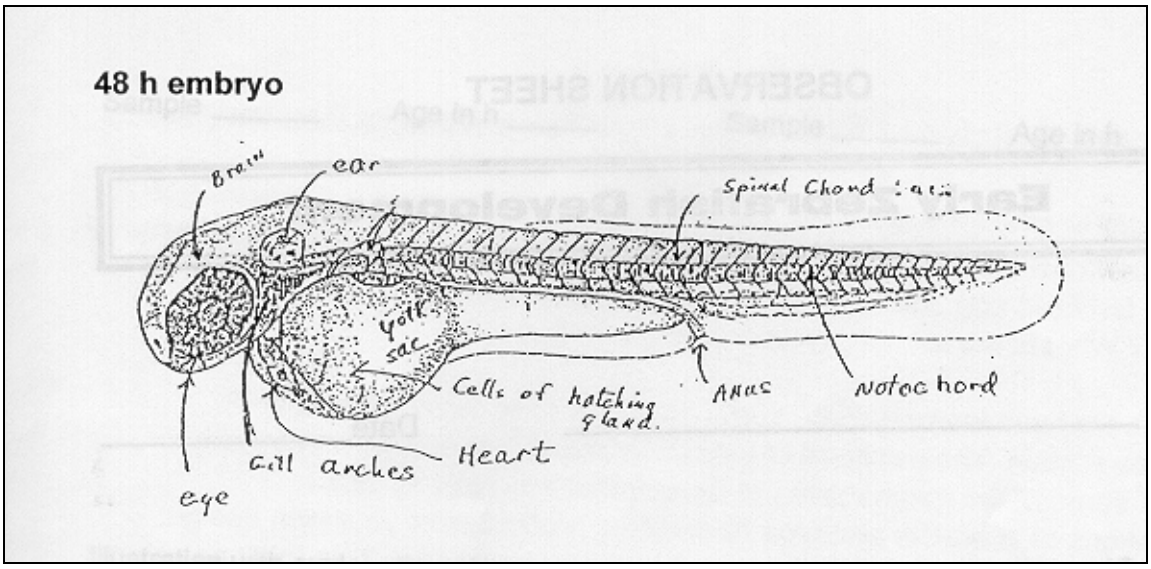
Classroom observation in embryology!

	Students work in pairs
	Each group of two students need to get one small petri dish (60 mm diameter X 15 mm)
	Label your small petri dish with your name and date, using masking tape or a wax pencil. This will be your baby zebra fish's home.
	Fill petri dish with egg water, you made from the recipe above.
	Place lid on dish.
	Place in incubator or environment to maintain a 28.5° Celsius temperature. If you do not have an incubator you can place the eggs in a small petri dish and then place the small dish into a larger one. You then float them in your fish tank to obtain the same temperature.

Observing Zebra Fish Embryology

The following table gives the approximate times after fertilization of key developmental stages.

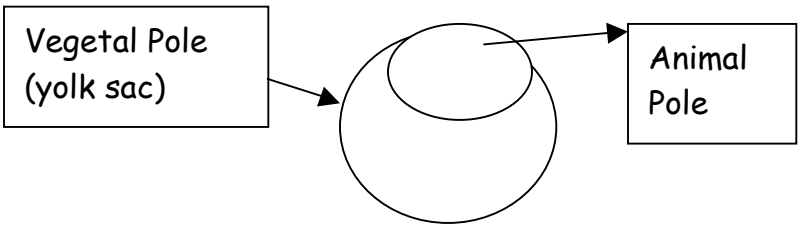
Stage	Time (28.5° C)
1st Cleavage	.7 hours
10th Cleavage	3 hours (Midblastula)
Epiboly	4.3 hours
Gastrulation	5 hours
First movements	18 hours
Heartbeat and Pigmentation	24 hours
Swimming	48 hours
Hatching and Feeding	72 hours



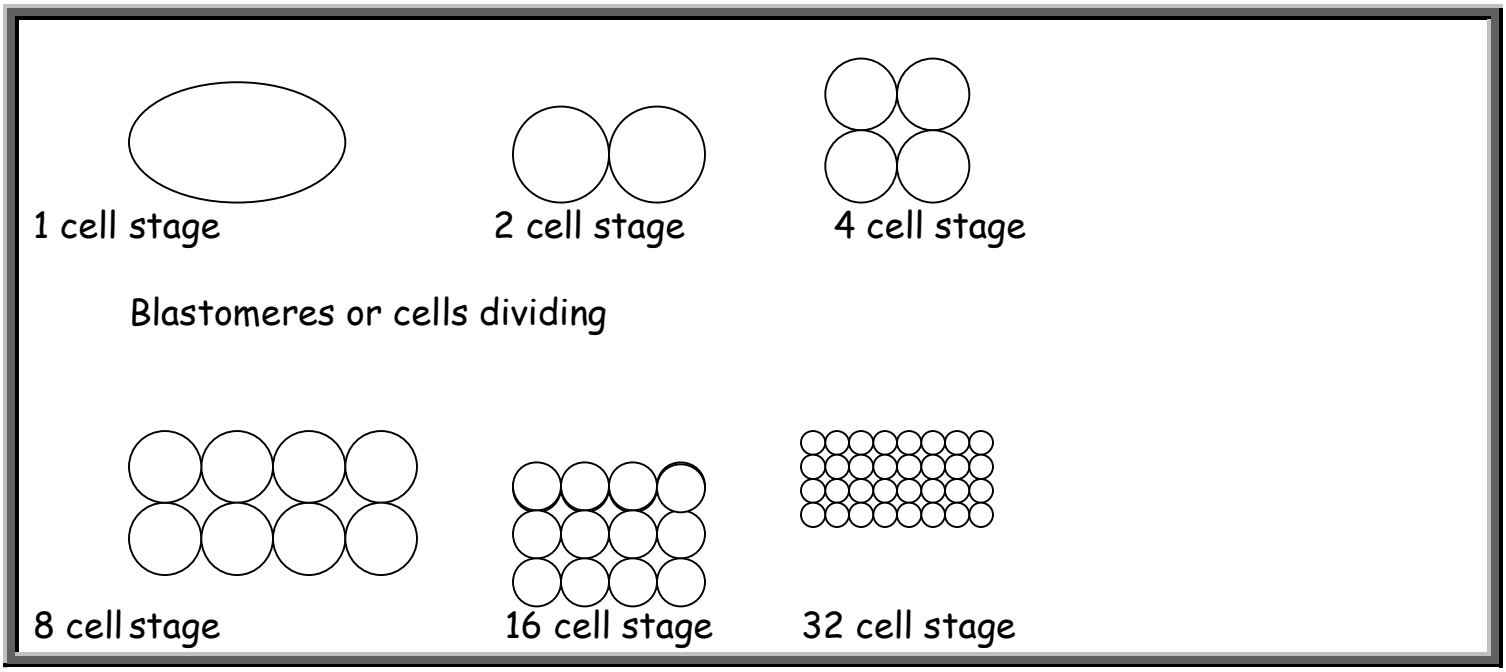
Part I: Observations of the Cleavage Period

During this period the first 6 cleavages occur. The cells or **blastomeres** divide synchronously at about 15 minute intervals. You can observe these early cleavages with a dissecting microscope, using transmitted light.

You will observe that the cleavages are partial; the region of the egg containing most of the yolk is not cleaved, only the yolk free cytoplasm of the animal pole will show cleavage.



Usually the first cleavages are all vertical and occur at right angles to one another as shown below:



This view is from the animal pole. Irregular cleavages may occur but generally don't produce abnormal development.

Observation of nuclei:

- Carefully adjust your lighting to focus on the nuclei. They are present during the first half of each cycle and their shapes change systematically. The nuclei are long and threadlike and globular in early Interphase and become spherical by late Interphase. As cells enter mitosis, the nuclei are elliptical and then take on the shape of an oval shortly before they disappear during prophase. The mitotic chromosomes are more difficult to observe.

Approximately 5 minutes after the nuclei disappear, you should see cleavage-taking place.

Note: It may be necessary to change to a light microscope with greater magnification to see the nuclei.

- **To do this you need to mount the embryo on a slide in a drop of water.**

Part 2: Recognition of later embryonic developmental stages: http://zfish.uoregon.edu/zf_info/zfbook/stages/stages.html

- Students will be given several embryo samples at different stages of development.
- They will need to arrange the samples in the proper order from youngest to oldest and then determine the age, in hours of each sample. Use the dissecting microscope for these observations.
- Using note-cards, draw and label, making sure you emphasize the features you noticed that supports your age determination. Use the example given for labeling and drawing. Indicate the animal pole, vegetal pole, sample, estimated age, stage and period. Use your book to describe the stage of development. What is actually happening at this time?
- During observations of later stages of zebrafish development, ask students to identify the embryonic layers present within the embryo and the structures that will evolve from each of the layers.

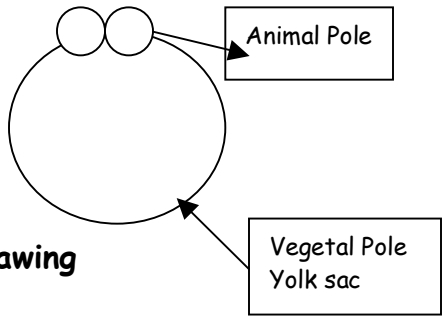
Example: 1

Estimated age: .75 hours

Stage: 2- cell stage

Period: Zygote

Drawing



Description:

_____

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_____

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Periods of Early Development

Period -h - Description

Zygote – 0 hour - The newly fertilized egg through the completion of the first zygotic cell cycle. Fertilization occurs when the sperm penetrates the plasma membrane of the egg.

Fertilization occurs together with the following steps:

- **Recognition** occurs next when the sperm secretes a protein that binds with special receptor molecules that reside on a glycoprotein layer surrounding the plasma membrane. This vitelline layer (or zona pellucida in humans) insures that fertilization occurs only between egg and sperm of the same species.
- **Penetration** occurs when the plasma membrane of the sperm and egg fuse and the sperm nucleus enters the egg.
- **Formation of the fertilization membrane** occurs when the vitelline layer forms a fertilization membrane, which blocks the entrance of any other sperm.
- **Completion of meiosis II in the egg occurs in humans**, sperm penetration triggers meiosis II in the egg, producing a polar body, which is discharged through the plasma membrane.
- **Fusion of nuclei and replication of DNA** occurs when sperm and the egg fuse, forming a zygote nucleus consisting of 23 pairs of chromosomes (in humans). Each chromosome replicates so that it consist of 2 identical chromatids.

Cleavage - $\frac{3}{4}$ hour – is a succession of rapid cell division that follows fertilization. The cells undergo the S (DNA synthesis) and M (mitosis) phases of the cell cycle, but often skip the G1 and G2 phases. Gene transcription is shut down during cleavage probably because of fast DNA replication and the embryo does not grow during this period of development. Cleavage partitions the cytoplasm of one large cell, the zygote into many smaller cells called Blastomeres, each with its own nucleus. The Blastomeres contain less cytoplasm than the original zygote. Embryo polarity is seen in this stage. This is where the egg has an upper, animal pole, and a lower vegetal pole (illustrated on page 12). Cells that are formed at the vegetal pole have more yolk, or stored food, because the yolk material, denser than the surrounding cytoplasm, settles to the bottom of the egg. Early cleavages are polar dividing the egg into segments that stretch from pole to pole. Other cleavages are parallel with the equator.

Blastula - 2 1/4 - Rapid, metasynchronous cell cycles (8, 9) give way to lengthened, asynchronous ones at the midblastula transition; epiboly begins

Gastrula - 5 1/4 - Morphogenetic movements of involution, convergence and extension form the epiblast, hypoblast and embryonic axis; through the end of epiboly. This occurs when a group of cells invaginate (move inward) into the blastula forming a 2-layered embryo with an opening from the outside into a center cavity. 3 germ layers form between the outer and inner layers of the invaginated embryo. These layers are the ectoderm (outside layer), mesoderm (middle layer), and endoderm (inside layer).

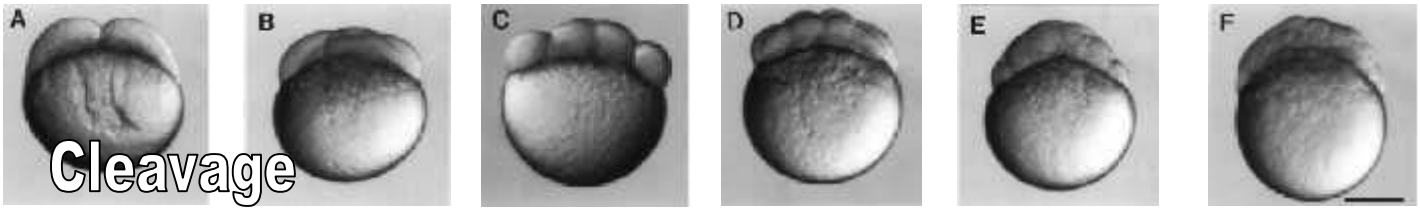
Segmentation - 10- Somites, pharyngeal arch primordia, and neuromeres develop; primary **organogenesis (the development of organs);** earliest movements; the tail appears. **Organogenesis** is when the cells continue to divide after gastrulation and start to differentiate. This is the point at which the cells have a determined fate. They will take on characteristics of specific tissues and organs. The formations of the following organs are characteristics of chordates. The notochord, are cells along the dorsal surface of the mesoderm germ layer. The notochord is a stiff rod that provides support in lower chordates. The neural tube is in the ectoderm layer directly above the notochord, a layer of cells forms the neural plate. The plate indents forming the neural groove, and then rolls up into a cylinder the neural tube. The neural tube develops into the central nervous system. Additional cells roll off the top of the developing neural tube and form the neural crest. These cells form various tissues, including teeth, bones, and muscles of the skull, pigment cells in the skin, and other tissues. Somites are seen and are arranged serially on both sides along the length of the notochord. Cells from the somites not only give rise to the vertebrae of the backbone they also form the muscles associated with the axial skeleton.

Pharyngula - 24 - Phylotypic-stage embryo; body axis straightens from its the early curvature about the yolk sac; circulation, pigmentation, and fins begin development

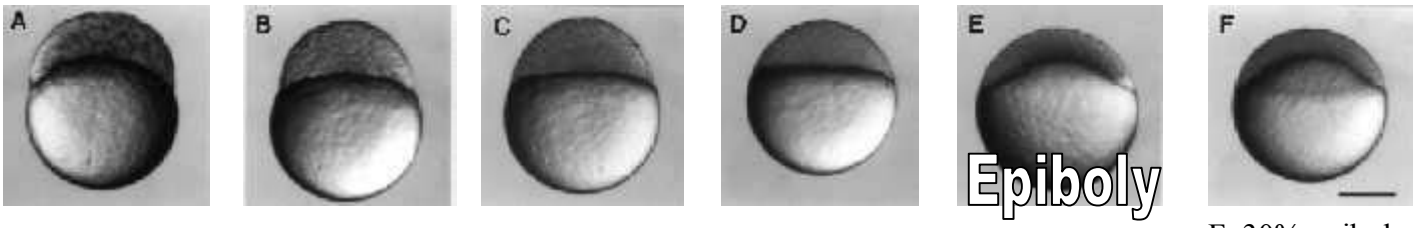
Hatching - 48 - Completion of rapid morphogenesis of primary organ systems; cartilage development in head and pectoral fin; hatching occurs asynchronously.

Early larva - 72 - Swim bladder inflates; food-seeking and active avoidance behaviors

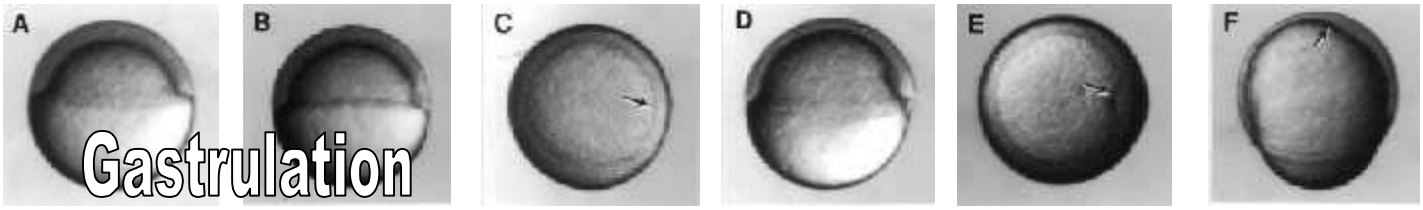
Zebra Fish Developmental Stages



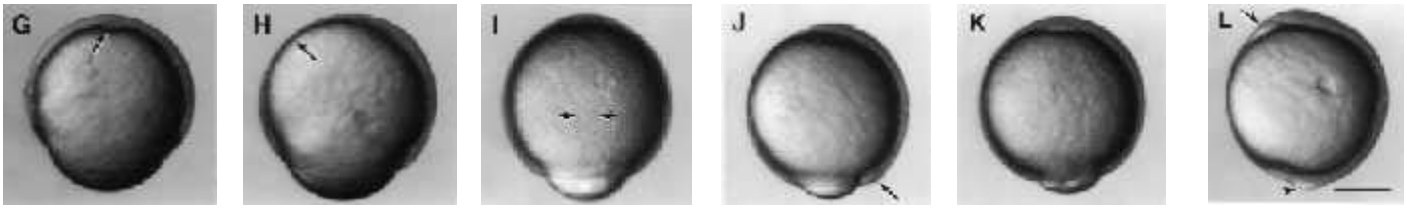
A: 2-cell stage (0.75 h).
B: 4-cell stage (1 h).
C. 8-cell stage (1.25h).
D: 16-cell stage (1.5 h).
E: 32-cell stage (1.75 h).
F. 64-cell stage (2 h).



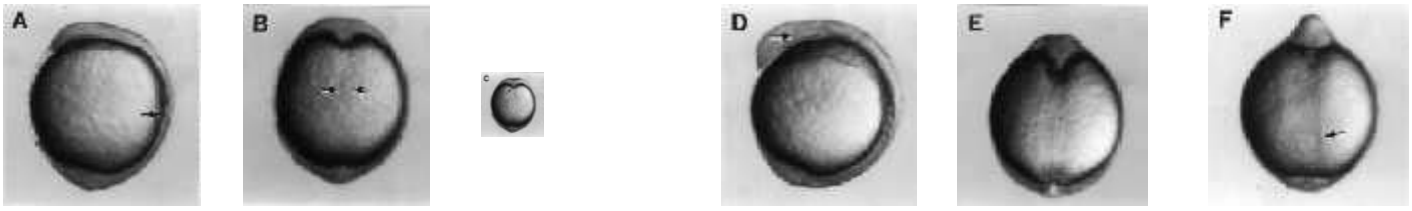
A: 256-cell stage (2.5 h).
B: high stage (3.3 h).
C. oblong stage (3.5 h).
D. sphere stage (3.8 h).
E: dome stage (4.3 h).
F. 30%-epiboly stage (4.7 h).



A: 50%-epiboly stage (5.25 h).
B. Germ ring stage (5.7 h).
C. Animal pole view
germ ring stage
D: Shield stage (6 h).
E: Animal pole view
shield stage
F: 70%-epiboly stage (7.7 h).

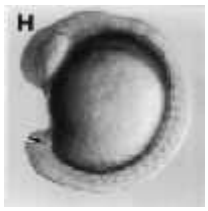


G: 70%-epiboly stage
H: 75%-epiboly stage (8 h).
I: 80%-epiboly stage (8.4 h).
J: 90%-epiboly stage (9 h).
K: 90%-epiboly stage
L: Bud stage (10 h)

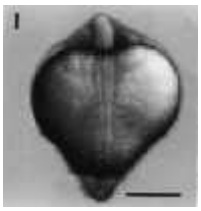


A: 2-somite stage (10.7 h).
B: 2-somite stage dorsal view.
C: 2-somite stage ventral view
D: 4-somite stage (11.3 h).
E: 4-somite stage dorsal view
F: 5-somite stage (11.7 h)

G: 8-somite stage (13 h).

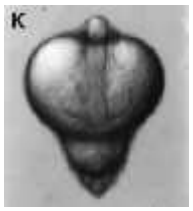


H: 13-somite stage (15.5 h).

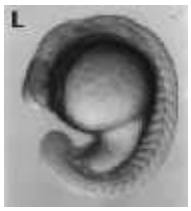


I: 14-somite stage (16 h).

J: 15-somite stage (16.5 h).



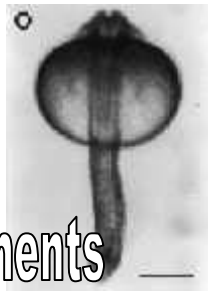
K: 15-somite stage dorsal-view



L: 17-somite stage (17.5 h).

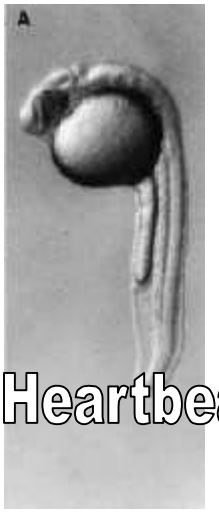
M: 20-somite stage (19 h).

N: 25-somite stage (21.5 h).

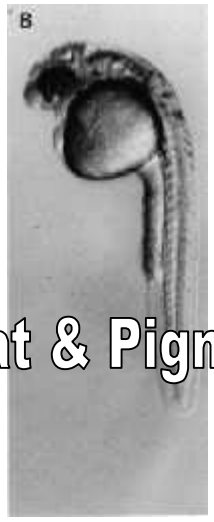


O: 25-somite stage dorsal view

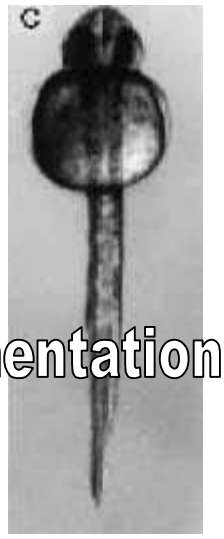
18 Hours-First Movements



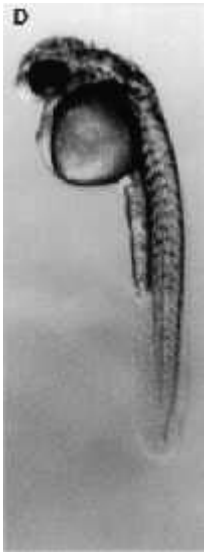
A:prim-5 stage (24 h).



B: prim-12 stage

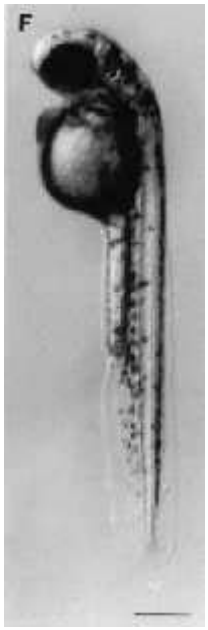


C: prim-12 stage (28 h).



D:prim-20 stage

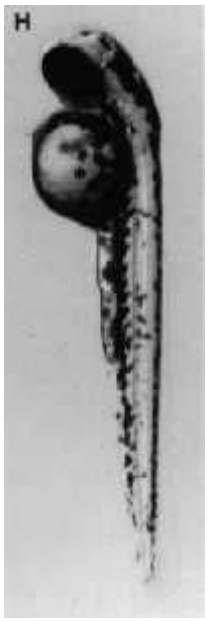
E:prim-20 stage (33 h).



F:prim-25 stage

Heartbeat & Pigmentation

G: prim-25 stage (36 h).



H high pec stage

I: high-pec stage (42 h).

A: Long-pec stage

B: Long-pec stage (48 h).

Swimming

C: Pec-fin stage

D: Pec-fin stage (60 h).

E: Protruding-mouth stage

F:Protruding-mouth stage (72 h).

Hatching & Feeding 72 hrs.

G: Early larva

H: Early larva (120 h).