Investigation of the Embryonic Developmental Stages in Zebrafish *Danio rerio* (Hamilton, 1822)

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Abstract

The embryonic stages of zebrafish *Danio rerio* (Hamilton, 1822) species were studied from January 2018 to January 2019. The zebrafish, *Danio rerio* (Hamilton), is one of the most important vertebrate model organisms for scientific researchers. It is very cheapest and breeds large amounts of eggs easily. The genetic structure is surprisingly close to that of *Homo sapiens.* They have their regenerative power for their fins, skin, heart etc. during their larval stages. In the present study, a totally 30 individuals of sexually matured zebrafish, *Danio rerio* (Hamilton) were examined in embryonic stage and larval development by placing with the ratio of 2 males: 1 female for their breeding. The water temperature at 25-26°C and pH at 6.8 were managed, respectively. At each time of the breeding process, about 100 eggs were obtained including fertilized and unfertilized eggs. Five replications of embryogenesis in Zebrafish *Danio* were studied. The development of zygote, cleavage, blastula, gastrula, segmentation, pharyngular and hatching and early larval development were recorded according to the changing of time after fertilization under the light microscope and stereomicroscope by placing the concaving slide. Indeed, the present embryonic study provides a detailed illustration of the development of zebrafish (*D. rerio*) in nature and the knowledge for future researchers.

Keywords: Danio rerio, Embryogenesis, Zygote, Cleavage, Blastula, Gastrula, Segmentation, Pharygula, hatching, Ornamental fish

Introduction

Danio rerio (Hamilton), commonly known as Zebrafish, is an ornamental fish belonging to the minnow family (Cyprinidae) of the order Cypriniformes as a freshwater popular aquarium fish. It is often called a "tropical fish" and found in private ponds and wild types. The zebrafish *Danio rerio* is one of the most important vertebrate model organisms in developmental biology in a popular aquarium species for many years (Creaser, 1934). A staging series is a tool that provides accuracy in developmental studies. Their eggs are optically translucent eggs, fertilization is external and hatching is rapid. Still, knowledge of early ontogeny is of critical importance in understanding the biology of a species and the functional trends and environmental preferences of the different developmental stages (Koumoundouros *et al.*, 2001; Borcato *et al.*, 2004). A detailed understanding of the ontogeny is therefore essential to identify species-specific adaptations and their ecological consequences (Verreth *et al.*, 1992).

In inland water bodies like lakes, ponds and river systems, the numerous fish are suitable and popular as freshwater aquarium fish. It is a freshwater popular aquarium fish or ornamental fish. The keeping of ornamental fish as pets is an ancient practice, as an alternative to outdoor recreation. It will positively affect human health by reducing stress, lowering heart rate and blood pressure. Most of the ornamental fish trade in the world market comes from Southeast Asian countries with rich biodiversity including freshwater or marine ornamental fish (Biffer, 1997). Therefore, the ornamental fish sector is an extensive and global component of international trade, fisheries, aquaculture and development. This has brought in great sources of wealth and foreign exchange earnings for countries, endowed with the right conditions (Weerakoon and) - fish species commercialized as aquarium ornamental pet fish (Cardoso *et al.*, 2019). Since 1985, the value of international trade in exports of ornamental fish has increased at an average growth rate of approximately 14 percent per year. Developing

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countries account for about two-thirds of the total export value (Bartley, 1999). Globally, ornamental fish culture and trade is a major activity with greater than 1 billion animals per year traded (Whittington and Chong, 2007). Aquarium ornamental pet fish constitute a major segment of the pet industry, with the United States, Europe, and Japan dominating the market (Noga, 2010). The highly demanded species are goldfish, dragonfish, koi carp, discuss, angel fish, flower horn, Oscar and including zebrafish. In Myanmar, there are many kinds of zebrafish such as *Leopard danio, Brachydanio rerio, Cyprinus chapalio, Cyprinus rerio, Danio frankei, Danio lineatus, Nuria rerio, Perilamopus striatus etc.* Therefore, freshwater ornamental fish industry has tremendous potential for the development of the country. Because they are mostly involved in trade from captive breeds. Tin Ei Khin (1996) studied some Myanmar aquarium fishes selected from wild catch for export.

However, lack of information about the zebrafish situation and investigations on the Developmental Biology of the fish. Moreover, the investigations were very few zebrafish in Myanmar. Because there are shortages of expertise, tedious laboratory work and a rather low focus on research.

As a matter of fact, the present study was carried out for the detection of Morphogenesis in zebrafish *Danio rerio*. Kimmel *et al.,* (1995) stated that the development of that species is rapid, with precursors to all major organs developing within 3 hrs and larvae displaying food-seeking and active avoidance behaviours within five days post fertilization, *i.e,* 2-3 days after hatching. And the developmental rates of domesticated strains in the laboratory have been different from wild fish (Eaton and Farley, 1974a). Their eggs are large relative to other fish (0.7 mm in diameter at fertilization) and optically transparent, the yolk being sequestered into a separate cell. And fertilization is external so live embryos are accessible to manipulation and can be monitored through all developmental stages under a dissecting microscope (Kimmel *et al.,* 1995).

In addition, the zebrafish is excellent and widely used animal laboratory model organism in scientific research not only in the educational sector, the subject of Embryology, Developmental Biology, Stem cell, Toxicology, Animal behaviour and Molecular biology especially but also in human diseases such as disorders of the nervous system, muscular dystrophy, human cancers, and controlling cell growth. It Is also a useful model for genetic studies, genome sequencing, mitochondrial DNA Pigmentation gene, Immune system, infectious disease, cardiovascular disease and drug discovery.

It has regenerative power such as fins, skin, heart, lateral line hair cells, and brain during larval stage. Its embryonic development is very rapid, and its embryos are relatively large, robust, and transparent, and able to develop outside their mother. Furthermore, well-characterized mutant strains are readily available. Therefore, zebrafish have also been modified by researchers to produce many transgenic strains.

Other advantages include the species' nearly constant size during early development, which enables simple staining techniques to be used, and the fact that R54t65dcits two-cell embryo can be fused into a single cell to create a homozygous embryo. It is native to freshwater habitats in South Asia: India, Pakistan, Bangladesh, Nepal and Bhutan while in the northern limit: South Himalayas and its range further south from the Western and Eastern Ghats regions. It has frequently been said to occur in Myanmar (Burma), but this is entirely based on pre-1930 records and likely refers to close relatives only described later, notably *Danio kyathit*. Zebrafish is also a popular aquarium fish striped danio as Ah MAE Zin in Myanmar. And other Danio species, usually popular as Kyantthit Danio, Leopard in the ornamental fish sector also. Nowadays zebrafish multicolor captive breed for sale aquarium fish from aquarium shops. To date, the information on embryonic development in the study of

zebrafish *Danio* species. As these points, the present study will focus on recording the zebrafish *Danio* species in embryonic developmental stages during the study period by the following objectives;

- to study the reproductive biology of zebrafish *Danio rerio*
- to examine the embryogenesis stages of zebrafish
- to determine the duration of developmental stages during the reproductive time

Materials and methods

Study area and study period

The zebrafish were purchased from the aquarium shop located in Tarmwe Township of Yangon Region. The laboratory experiments were carried out at Zoology Department, Yangon University during the study period from January 2018 to January 2019.

Materials

The sexually matured fish 20 individuals of female and 40 individuals of male with the size are around 1-1.5 inches (Plate 1) were collected in the plastic bag with oxygen from the aquarium shop and transferred into the aquarium glass tank at the Department of Zoology to examine the developmental stages under the High-resolution Microscopy and Camera Lab (Plate 2). Four plastic containers were used for their mating and breeding process.

Methods

For the mating setup, a total of 30 fish (10 Females and 20 Males) were chosen and placed in plastic containers for the breeding process waiting to stimulate courtship behavior and examined their continuous embryo development. At first, zebrafish Danio were placed in a separate tank which was fitted inside the larger tank overnight. The matured one female was placed into the small container which was upperside and the matured two males were placed into the larger tank at the underside. The water temperature was maintained at 26ºC and pH 6.8. Two hours later, the male Danio were removed into the small container with the female Danio. They display courtship behavior and start to deposit eggs and sperm into the water. In the present study the depositions were found at 3am very early. And then, the smaller container together with all the fishes were removed. The eggs were left at the bottom of the large container. All laid eggs were collected with the tube into the petridish for the examination of development stages under the light microscope at the High-Resolution Microscopy and Camera Lab in the Zoology Department, University of Yangon. The unfertilized eggs were removed by the tube to throw the other place.

Five replications for the breeding process were performed and examined embryogenesis developmental stages of studied fish species under the microscope according to their changing times. The images of embryonic developmental stages were recorded by the digital camera throughout the study period.

Danio Male Danio Female Female and Male

Plate 1. Morphology of zebrafish *Danio rerio* (Hamilton,1822)

Results

The breeding paired of zebrafish danio was with the ratio of 2 males: 1 female. In each time of breeding process, about 100 eggs were obtained including fertilized and unfertilized eggs.

Embryogenesis of zebrafish

Seven stages of embryogenesis were observed such as zygote, cleavage, blastula, gastrula, segmentation, pharyngula and hatching stages. The cell division of a major developmental process occurred during the first three days after fertilization. The embryonic stages were identified and named based on morphological features, generally by examination of the live embryo with the light microscope. The unfertilized eggs were enclosed with the transparent chorion, light pink color and spherical shape. Their animal pore and vegetal pore cannot be differentiated. They have granular cytoplasm which is uniformly distributed over the entire egg. The embryonic developmental stages lasted 72 hours. The hatching stages and three larval stages were shown in plate 3.

Zygote period (0-¾ hours post fertilization/ hrs) of fertilized eggs

The zygotic period was found a little minute at 3-3:45 am after fertilization. After the fertilization period, the first cleavage stage was about 45 minutes. This period included one developmental stage.

In the stage of one cell (0 hrs); the enlargement of perivitelline space occurred and the chorion swells and lifts away from the newly fertilized egg. The cytoplasm was streamed toward the animal pole to form one cell (blastodisic) after 10 minutes after fertilization. It formed a small dome-like structure on the top of the vegetal pole. The blastodisc was a so-called animal pole and the yolk mass was the so-called vegetal pole. They can be found clearly. During the early cleavage stages, this segregation was continued (Plate 3)

Cleavage period (3/4 hrs – 2¼ hrs) of a fertilized egg;

This cleavage period was taken about 15 minutes at the 3:45am-5:00am after the first cleavage of the cells or blastomeres. The cytoplasmic divisions were meroblastic. Cleavage occurred only in the blastodisc and only the cytoplasm of the blastodisc became the embryo.

They incompletely undercut the blastodisc. The blastodisc color was lighter than the yolk mass. The stages of six developments occurred during this cleavage period (Plate 3). The cell cycles of 2 cell stages through the 7 stages of embryonic development occur rapidly and synchronously as 2 cells, 4 cells, 8 cells, 16 cells, 32 cells and 64 cells.

Two cell stages were taken around (3/4 hrs). After fertilization, the first vertical cleavage appeared, dividing the blastodisc into two equal halves of blastomeres. The furrow arose near the animal pole and rapidly toward the vegetal pole. This type of cleavage was known as meroblastic cleavage (Plate 3).

Four cells stage (1 hrs): In one hour after fertilization, the two blastomeres were incompletely divided into four cell stages. The second division of blastodisc produced four equal sizes of blastomeres on the yolk (Plate 3).

Eight cells stage (1¼ hrs): The third cleavage occurred one hour and 15 minutes after fertilization, resulting in an embryo with eight cells. It was found as two rows of four cells over the yolk (Plate 3).

The stages of 16 cells stage (1.5 hrs): Sixteen globular blastomeres were observed after the appearance of the fourth cleavage. This embryonic stage was difficult to distinguish from

the eight cells stage, because they look similar in face view. But, blastomeres were smaller than those of the previous stage (Plate 3).

Thirty-two cells stage (1.75 hrs): about one hour and forty-five minutes, thirty-two small-sized blastomeres were observed on the yolk. The numbers of blastomeres were difficult to count as they were increasing and changing in size and shape. The plane of the blastodisc was curved, marginal cells were more vegetal, and they lie partly in front of the nonmarginal ones positioned closer to the animal pole (Plate 3).

The embryonic stages of sixty-four cells (2 hrs): about two hours after fertilization sixty-four small globular blastomeres were observed which were difficult to count. The shape of the blastodisc was observed as the mound looks distinctly higher in the side view. For the first time, some of the blastomeres were completely covered by another one. The cleavage period ended at the sixty-four cell's stage (Plate 3).

Blastula period (2¼ hrs -5¼ hrs); of a fertilized egg;

The blastula period started at 5:00am-7:40 am. The blastodisc begins to form a ball-like structure termed as the blastula period, within 128 cell stages or eight zygotic cells to the time of onset of gastrulation. Important processes occur during this blastula period; the embryo enters mid-blastula transition, the yolk syncytial layer forms and epiboly begins. The five embryonic developmental stages were recorded in the blastula period. They were 128-cell, 256-cell, 512-cell, 1k-cell, High, Oblong, Sphere, Dome-like structures and 30%-epiboly. Start with 128 cells and duplicate reaching about 1000 cells in 3 hours post fertilization. Two areas were markedly differentiated, the animal-vegetal axis. At 4 hours the egg is spheric and a dome shape formation can be seen inside. The embryo enters the mid-blastula transition, the yolk syncytial layer forms and epiboly begins.

Before the high blastula stage (2¼-3 hrs): a total of 128 blastomeres were subdivided three times at about 15 minutes intervals to arrange as a high mound of cells, a solid half ball perched on the yolk cell (Plate 4).

In the high blastula stage (3¼ hrs): Blastoderm was eventually changed to form the high mound by the accumulation of proliferating blastomeres. Bulging blastoderm consists of small bubble-shaped cells over the yolk which has translucent and granular cytoplasm (Plate 4).

At the oblong blastula stage (3¼ hrs): Cells of the blastoderm gradually become smaller and thicker than in the previous stage. The blastodisc was compressed and down to the yolk cell. The construction of the blastodisc margin was likely due to the elevation of non-yolky cytoplasm during the one-cell stage. Blastomere was extended slightly over the yolk (plate 4).

This sphere stage (4 hrs): The blastoderm was gradually flattened. The surface of the blastoderm became uniformly smooth and approximately spherical in shape. One distinguishing characteristic of this stage was a very deep plane of focus of the face between the yolk cell and the upper part of the yolk cell (Plate 4). The embryo enters the mid-blastula transition, the yolk syncytial layer forms and epiboly begins.

Dome stage (4.3 hrs): Internal yolk syncytial layer was begun deep to the blastodisc to dome toward the animal pole. This prominent and rapidly occurring change in the interface between the yolk cell and the blastodisc was the beginning of epiboly (Plate 4).

Gastrula period (5¼ hrs -10 hrs) of a fertilized egg

It was recorded at the time of 7:40am-1:00pm. Epiboly describes cell movements. The primary germ layer and embryonic axis were also produced; three developmental stages were recorded within this period. 50% epiboly, Germ-ring, Shield, 80% epiboly, 90% epiboly and tail bud. The shape of the embryo stretches along the animal-vegetal axis. The neural plate representing a primordial brain is formed. The cells that will form the notochord, axial somite derived from muscles, and specific neurons of the hindbrain were present. At the end of the epiboly, the yolk plug was closed and gives rise to the tail bud.

The germ-ring stage (5.45 hrs): Gastrulation started at five hours and forty-five minutes after fertilization. The blastoderm extended outward over the surface of the yolk. The periphery of the blastoderm thickened to form the marginal ring or germ ring (Plate 4).

The shield stage (6-9 hrs): An embryonic shield occurred in the animal pole as well as the germ ring at six hours after fertilization. Both epiblast and hypoblast were locally thickened at the shield. Both layers undergo convergence and extension. Within about three hours after the beginning of the shield, epiboly formation was reached until 90 % between these conditions, the blastoderm eventually descended and covered almost the yolk cell. The remaining uncovered yolk cell protruded from the vegetal pole to form a yolk plug (Plate 4).

The bud stage (10 hrs): Epiboly was continued until 100% as the blastoderm completely covered the yolk plug. The shape of the embryo stretches along the animal-vegetal axis. The neural plate representing a primordial brain is formed. Convergence and extension movements established an embryonic axis on the dorsal region of the embryo. A distinct swelling of the tail bud developed at the posterior or caudal end of the embryonic axis. Some cells of the mid-line of the embryonic shield were distinctly seen to form the segmentation (Plate 4).

Cells that will form the notochord, axial somite derived from muscles, and specific neurons of the hindbrain were present. At the end of the epiboly, the yolk plug was closed and gave rise to the tail bud.

Segmentation period (10-24 hrs) of fertilization of egg

There were 3-somite, 6-somite, 10-somite, 14-somite, 18-somite, 21-somite, and 26-somite. Between ten-to-twenty-four-hour post fertilization occurred the varieties of morphogenetic movements. The somites were developed, the rudiments of the primary organs became visible, the tail bud became more prominent and the embryo elongated (Plate 4). First body movement appears and the first cells differentiate morphologically. The body length of the embryo increases quickly. The muscle segments and the vertebral cartilage are arranged along the somites. Pronephric kidneys and ducts develop below the third somite pair. All the sensory tissues such as optic, olfactory and ear tissues start to form at this stage.

Five somites stage (11.7 hrs): eleven hours and forty-minute post-fertilization, five to six somites occurred at the middle portion of the embryo and the primitive streak appeared. Its study time was 1:00 pm-1:00 am. And it is also called the tail bud period. The antero-posterior axis was a slightly thick line and encircles the two thirds of the yolk. The anterior portion of the primitive streak was seen to lift to form the region of the head. The embryo was indicated with the head and tail ends. Its maximum length was about 0.8 mm long. The brain primodium was distinctly thickened to form the neural keel in the anterior trunk between the six and ten somites stage (Plate 4).

The tail of the embryo was elongated by the extension of early rudiment tail buds in the seventeen somites stage (16 hrs). This stage was formed at a rate of about two per hour until

the end of the segmentation period. The V-shape appearances of somites. The notochord was more prominent. At the tail end, the posterior region of the yolk had many changes into kidney bean shape and rapidly became constriction into the thinner and cylindrically shaped posterior elongation of the yolk sac (yolk extension). The tail bud was protruding away from the body of the embryo (Plate 4).

Twenty somites stage (19 hrs): The length of the yolk extension was more than half the diameter of the yolk ball. The brain has become a hollow structure. In the 17-somites state (Plate 4), all the trunk myotomes weak contraction nearly occurred.

Twenty-six somites stage (22 hrs): the posterior trunk straightening was nearly complete, but the elongated tail was still curved ventrally. The olfactory placodes were thickening anterior and dorsal to the forebrain. The somite was formed more slowly than others. The rudiment of the median fin fold appeared along the length of the tail in the dorsal midline. The spontaneous myotomal contractions produce a lashing from side to side and their frequency increases transiently upon dechorionation (Plate 4).

Paryngular period (24-48 hrs) of the fertilized egg

In this embryonic stage were observed at the 1:00 am- 9:00 pm. In the pharyngula period were found the Prim-6, Prim-16, Prim-22, High-pec and occurred during the second day of the development (Plate 5). The formation of the pharyngeal arches gives rise to the mandibula and gills. The lengthening of the embryo slows down and the fins form and cells start pigmentation. The embryo was most evidently a bilaterally organized creature with a well-developed notochord, and a newly completed set of somites. The nervous system was hollow and expanded anteriorly. The lengthening of the embryo slows down and the fins form and cells start pigmentation. The embryo was most evidently a bilaterally organized creature with a well-developed notochord, and a newly completed set of somites. The nervous system was hollow and expanded anteriorly.

The embryo was a newly completed set of somites that extend to the end of a long post-anal tail. The nervous system is hollow and expanded anteriorly. During the first few hours of the pharyngula period, the head also straightens out. The morphogenesis of head straightening shortened the head, it was moncompact along the anterior-posterior axis. The rudiments of the eye and the ear approach one another. The media fin fold was present, prominent and formed fin rays. The pair pectoral fins began their morphogenesis Mesenchymal cells were gathering together to form fin buds. The melanophores were arranged at a well-defined set of longitudinal body stripes. The heart began to beat and formed well-delineated chambers. Blood began to circulate through a closed set of channels (Plate 5).

Hatching period (48-72 hrs) of the fertilized egg

In this stage at the 9:00 pm - 3:00 am, there were Long-pec, Pec-fin, Protruding-mouth. During the hatching period, it was continuously grown at about the same rate as earlier. The pectoral fins were developed during the early part of this stage. At the same time a circulatory channel appeared through the formation of a tubular heart at the place below the head. The head was elevated from the yolk optic cups and the lobes of the brain had markedly enlarged. Distributions of the melanophores appeared on the trunk and tail. Up to about ten melanophores occupied each lateral stripe. Eyes were more prominent and the pigmentation of the retina was dense. Morphogenesis of many of the organ rudiments is now rather complete and slows down considerably. The mouth was wide open and pectoral fins, the jaws and the gills continued to develop. At the early part of the hatching period the small open mouth was located between the eyes and at the end of this period the mouth protrudes beyond the eye. It was almost ready to hatch (Plate 5).

Early larva period (72 hrs)

The hatched larva had completed most of its morphogenesis in three days post-fertilization, and it continued to grow rapidly (Table 1). During the hatching period, the embryo was at rest, the early larva gradually began to swim about actively. Inflation of the swim bladder and protrusion of the mouth can be seen. Swift escape responses, herald respiration and the seeking of prey and feeding (Plate 5).

Table 1. Periods of early development

Period	Duration (hrs)	Time	Day
Zygote Period	0-3/4	3-3:45 AM	1st day
Cleavage Period	¾-2 ¼	3:45 AM-5:00AM	1st day
Blastula Period	2 ¼ -5 ¼	5:00 AM-7:40 AM	1st day
Gastrula Period	5 ¼ -10	7:40 AM-1:00 PM	1st day
Segmentation Period	10- 24	1:00 PM-1:00AM	2nd day
Pharyngula Period	24-48	1:00 AM- 9:00 PM	2nd day
Hatching Period	48-72	9:00 PM -3:00 AM	3rd day

Discussion

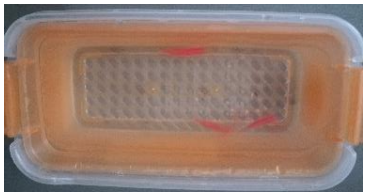
Based on the present study noted that the female zebrafish will be able to spawn at the interval of four to seven days, laying 100 eggs in each clutch. Developmental stage of a living embryo can be prevented at pH 7 stated by Westerfield, (1994). In the present study, the water temperature was maintained at 26 ºC and pH 6.8. The cleavage of one cell stage of fertilized eggs was performed within 30 min after fertilization. This result was agreed with Frocse *et al*. 2007.

Kimmel (1995) also stated that one cell stage was performed 10 min after fertilization. After fertilization, the first cleavage also ends in completion after a 15 min interval. In the present study, two cell stages were observed within 45 min (3/4 hrs) and four cell stages were found one hour after fertilization. This also agreed with Kimmel *et.al,* 1995, who described that duration of cleavage for two cells stage (3/4 hrs) after the first zygote cell cycle was vertically orientated as usual until the 32 cells stage. Sixteen cell stages (1 ½ hrs), thirteen two-cell stages (1 ¾ hrs) and sixteen four stages (2 hrs) that occurred after the zygote period. The Blastula period (2 ¼ hrs), gastrula period (5 ¼ hrs) segmentation (10 1/3 hrs), and pharyngula period (24 hrs) were also the same as the recent study. Completion of rapid morphogenesis of primary organ systems of the hatching stage occurred within 48 hrs and swim bladder inflates food-seeking and active avoidance behaviors of the early larva stage were observed within 72 hrs were also relevant to Kimmel *et.al,* (1995) who has stated.

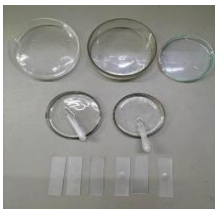
Kar and Subbiah (2013) stated that the zebrafish (*Danio rerio*) is a powerful model organism for the study of vertebrate biology, being well suited to both developmental and genetic analysis. The present study noted that the early development of zebrafish embryos was external, rapid, and visually accessible. Suppose to zebrafish only important biomedical organism's popularity has grown. One reason that zebrafish embryos are transparent and they develop outside of the uterus that could be manipulated at all developmental stages. Developmental process allows studying the details starting from fertilization throughout development.

Conclusion

In conclusion, the present finding supports becoming familiar with the stages of the embryonic development of the Zebrafish and to know the behavioral pattern and important facts about notice of fish during the reproductive time. The finding of this research will also provide information on the educational sector and further research as outlined.



Plastic container with Danio Net

Concave slide Petridish Pipette and slide High Resolution Lab

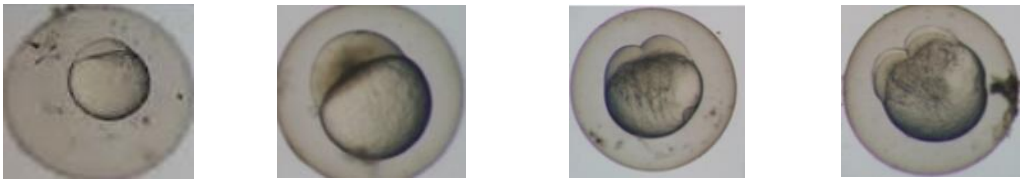


Plastic container with Danio



Aquarium tank at the Zoology department

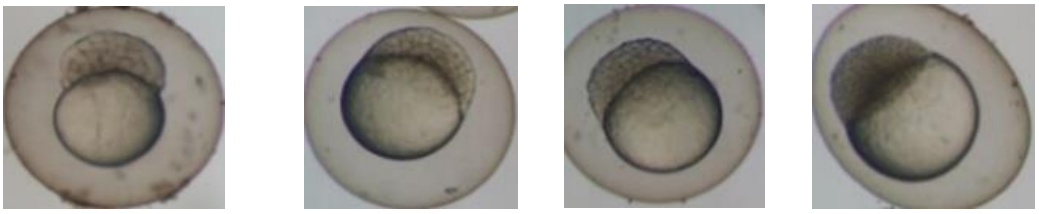
Plate 2. Materials used for breeding process



Zygote 1 cell 2 cell 4 cell

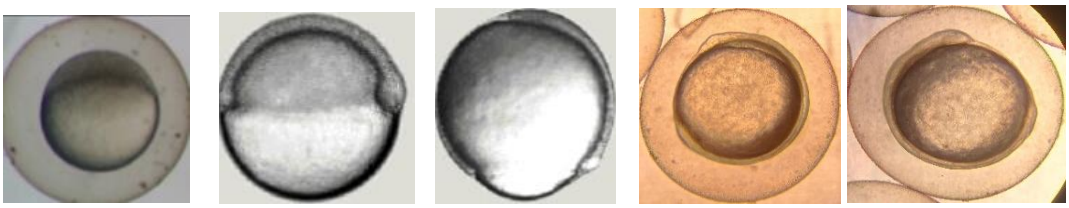
8 cell 16 cell 32 cell 64 cell

Plate 3. Embryos development during the cleavage



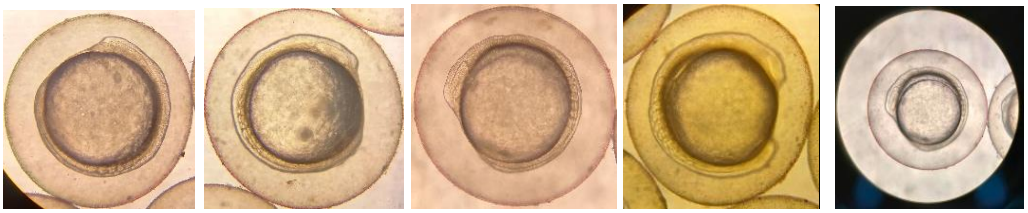
128 cell 256 cell stage 512 cell High stage

(A) Blastrula period

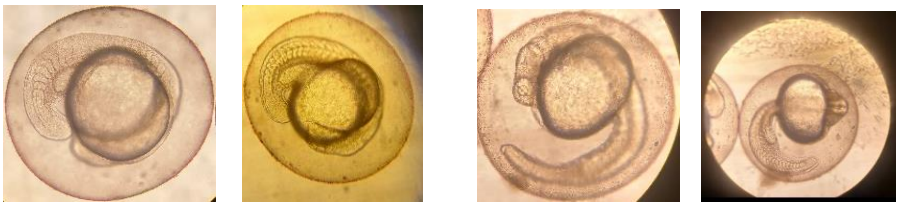


Oblong stage 50% epiboly 80% epiboly 90% epiboly Tail Bud

(B) Gastrula period



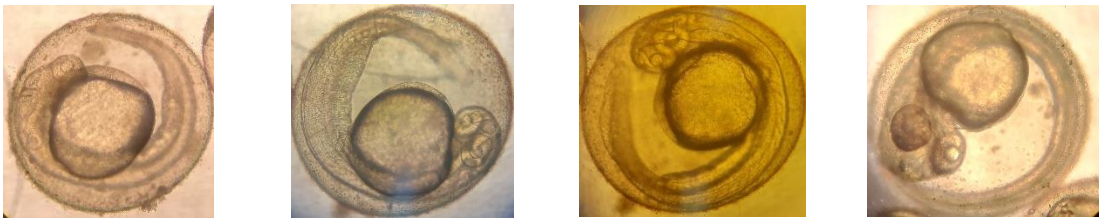
3 somites 6 somites 10 somites 14 somites



18 somites 21 somites 26 somites

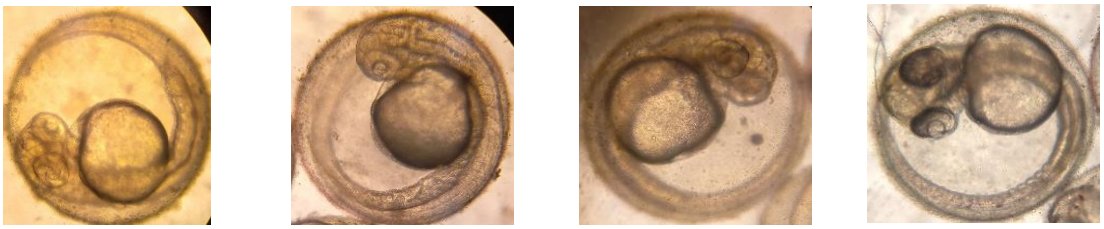
(C) Segmentation period

Plate 4. Embryos development during the cleavage



Prime 6 Prime 16 Prime 22 High pec

(A) Pharyngula period



Long-pec Pec-fin Protruding-mouth

(B) Hatching period 48 to 72 hrs

(C) Early larval stage

Plate 5. Embryos development during the cleavage

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