Aquaculture Medium

https://journal.unram.ac.id/index.php/jmai/index. E-ISSN : 2798-0553

Volume 4, Number 2, May 2024

Embryogenesis Development of Pearl Catfish (*Clarias gariepinus*) The Fish Health and Environment Testing Laboratory of Muntilan

Perkembangan Embriogenesis Ikan Lele Mutiara (*Clarias Gariepinus*) di Laboratorium Pengujian Kesehatan Ikan Dan Lingkungan Muntilan

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ABSTRACT

Pearl catfish (*Clarias garipinus*) is one of the freshwater fish from Indonesia which has a very important economic value and is much loved by the community. There is still very little information about embryonic development (embryogenesis). The purpose of this study was to determine the development of the embryo. The study was conducted on March 27 to May 25, 2023 at the Muntilan Fish Health and Environmental Testing Laboratory, Central Java. Observations were made on the spawning eggs of 1 male Pearl catfish and 1 female pearl catfish. The material was analyzed descriptively with the help of images of the developmental phases of each egg and information on the hatching time of the eggs. The results showed that the embryonic development of Pearl catfish includes the phase of Zygote cell division (clevage), 27 minutes from 22:22 to 22:45, morula Stage 2 hours from 22:45 to 00:45, blastula stage 1 hour and 21 minutes. 00:45 - 01:06, the gastrula stage lasts 4 hours 10 minutes 01:06 - 04: 00, The organogenesis stage to larval hatching 1,225 minutes or 20 hours 25 minutes from the initial stage. Hatchability of eggs in this study was 83.47%.

ABSTRAK

Ikan lele mutiara (*Clarias garipinus*) merupakan salah satu ikan air tawar asal Indonesia yang memiliki nilai ekonomis sangat penting dan banyak digemari oleh masyarakat. Masih sangat sedikit informasi tentang perkembangan embrionik (embriogenesis). Tujuan dari penelitian ini adalah untuk mengetahui perkembangan embrio. Penelitian dilakukan pada 27 Maret hingga 25 Mei 2023 di Laboratorium Uji Kesehatan Ikan dan Lingkungan Muntilan, Jawa Tengah. Pengamatan dilakukan terhadap telur pemijahan 1 induk lele mutiara jantan dan 1 induk lele mutiara betina. Materi dianalisis secara deskriptif dengan bantuan gambar fase perkembangan setiap telur dan informasi waktu penetasan telur. Hasil penelitian menunjukkan bahwa perkembangan embrio lele mutiara meliputi fase pembelahan sel zigot (clevage), 27 menit dari 22:22 sampai 22:45, tahap morula 2 jam dari 22:45 sampai 00:45, tahap blastula 1 jam dan 21 menit. 00:45 – 01:06, tahap gastrula berlangsung 4 jam 10 menit 01:06 – 04:00, tahap organogenesis hingga penetasan larva 1.225 menit atau 20 jam 25 menit dari tahap awal. Daya tetas telur pada penelitian ini adalah 83,47%.

Kata Kunci	Embriogenesis, Daya Tetas, Ikan Lele Mutiara, Pemijahan		
Keywords	Embryogenesis, Hatching Rate, Pearl Catfish, Spawning		
Tracebility	Submission: 7/4/2024. Published : 25/5/2024		
Panduan Kutipan (APPA 7 th)	Sinuhaji, E. E. D., Usman, Z., & Valentine, R. Y. (2024). Embryogenesis Development of Pearl Catfish (<i>Clarias gariepinus</i>) The Fish Health and Environment Testing Laboratory of Muntilan. <i>Indonesian Journal of</i> <i>Aquaculture Medium</i> , 4(1), 1-8. http://doi.org/10.29303/mediaakuakultur.v4i2.3945		

INTRODUCTION

The African catfish *Clarias gariepinus* Burchell, 1822 is a species of catfish that has been cultivated almost all over the world. Pearl catfish cultivation began in Indonesia in 1985 and has now become one of the most popular cultivation products. Pearl catfish used in cultivation in Indonesia are imported either directly from African countries or through other countries. At the beginning of its introduction, pearl catfish showed its superiority as a cultivation commodity, but during cultivation, its superior performance decreased, requiring breeding efforts (Sunarma, 2004; Iswanto *et al.*, 2016). According to Hafiludin (2022), cultivating pearl catfish (*Clarias gariepinus*) has the advantages of fast growth, efficient feed, uniform size and disease resistance. Pearl catfish also has the advantage of a high growth rate, which is 20-70% faster than other catfish seeds, and the growth period is short, around 45-50 days from 5-7 cm seeds or 7-9 cm. Pearl catfish is a new species, pearl catfish which was developed by the Sukamandi Aquaculture Research Agency (BPPI) and has passed the species/variety release test on October 27 2014. Pearl catfish has the advantage of relatively complete breeding, especially in terms of growth, nutritional efficiency, uniformity of size, resistance to disease, easy to adapt to the environment, not easy to stress, and high productivity. The advantages of pearl catfish have led to the rapid development of their cultivation, which requires large quantities of seeds. The quantity and quality of cultivated fish seeds depends on the reproductive ability of the offspring. Spawning of pearl catfish can be done semi-artificially or artificially. Spawning is carried out by injecting the fish with the hormone ovaprim to stimulate gonad maturation and sperm production. The reproductive process occurs naturally without human assistance in a 1:1 ratio, namely 1 male and 1 female. There are several important aspects of the reproductive phase, including embryogenesis and egg hatching.

Embryogenesis is the gradual formation and development of the organ structure of the embryo after fertilization, which goes through several stages, namely cell division, morula, blastrula, gastrula and organogenesis. Egg yolk is used as an energy source in the embryo formation process, and the absorption of egg yolk or unfertilized eggs can be influenced by environmental temperature (Effendi, 2000).

METHODS

Time and Place of Research

This research was carried out from 27 March–25 May 2023 at the Fish and Environmental Health Testing Laboratory in Muntilan, Central Java.

Tools and Materials

The tools used in this research were a rectangular pool measuring 2.90 m x 1.83m x 62 cm, digital scales, basins, dropper pipettes, microscopes, measuring cups, blowers, syringes, kakabab, petri dishes, sesers and warings. The materials used in this research

were 1 male and 1 female catfish broodstock, ovulation hormone with the brand name ovaprim and floating pellet feed with the brand name STP (SPLA 12 GROWER) with a protein content of 32%.

Parent Rearing

The fish used in this research were parent pearl catfish that were 1 year old and had a body size of 30-40 cm with a weight of 1 kg, complete body organs and no defects. Mains maintenance is carried out in a concrete tank measuring 2.4m x 1.5m x 90 cm with a water level of 40 cm. Feeding of parent fish is given twice a day in the morning at 08.00 WIB and in the afternoon at 16.00 WIB using STP floating pellet feed (SPLA 12 GROWER) with a protein content of 32%.

Parent Selection

Parent selection is usually carried out in the morning so that the parent fish do not experience stress due to high temperatures. The parent pearl catfish must have the following characteristics: the male parent has prominent genitals and a tapered shape and has a flat head bone and if the stripping technique is used it will release sperm fluid from the genitals. Meanwhile, the female parent has a brighter body color. Compared to males, they have reddish colored genitals and have a large body shape and if the stripping method is used they will produce yellow eggs. The selected pearl catfish broodstock were 4 male broodstock and 20 female broodstock.

Spawning

The spawning technique used in this research is semi-artificial spawning, where spawning is carried out by injecting the hormone ovaprim to stimulate gonad maturity and sperm production in catfish. The ovaprim dose used for injections in female parents is 0.3 ml, while the ovaprim dose for male parents is 0.15 ml. The injection is carried out on the back of the fish at an angle of 30°.

Embryogenesis Observations

After spawning the resulting eggs are put into a sample box in the form of a measuring cup. The process of checking embryogenesis is carried out by taking the eggs using a drop pipette and then placing them in a petri dish for observation using a Yazumi binocular microscope. The check starts at 22.22 WIB, in the zygote phase the check is carried out for 27 minutes, in the morula phase the check is carried out for 2 hours, in the blastula phase the check is carried out for 1 hour 21 minutes, in the grastula phase the check is carried out for 4 hours 10 minutes and the next check is Organogenesis is carried out until the egg hatches.

Result

RESULT AND DISCUSSION

Fertilized pearl catfish eggs will undergo embryonic development including several phases, namely: division of the egg or zygote cell (clevage), morula, blastrula, gastrula, and organogenesis. The development of an egg or embryo begins with cell division, this phase occurs from the 0th to the 27th minute. The second phase, namely the morula phase, occurs from the 27th to the 147th minute, the third phase, namely the blastrula phase, occurs from the 147th to the 288th minute. The fourth phase, namely the grastula phase, occurs from the 288th to the 28th minute. 538, phase five is the organogesis phase, where this phase is the final phase of embryo development which hatches into transparent pearl catfish larvae, occurring at the 1,225th minute or 20 hours 25 minutes from the initial phase.

After the spawning process, pearl catfish eggs are moved into hatching ponds, there are 4 ponds used. The total number of eggs that hatched was 400,842 out of a total of 480,223 eggs produced, so the egg hatchability value obtained was 83.47%.

Time	Phase	Figure	Note
22.22	Fase Cleavage	1 cell	From the results of this telolecital cell defense, 2 groups of cells will be formed. The first is a group of main cells (blastoderm) that will form the
22.26		2 cells	body of the embryo, called formatic cells or clumps of inner cells (inner mss cells). The second is a group of complementary cells (trophoblast, periblast, auxiliary cells) which function
22.31		4 cells	as a protective membrane and bridge between the embryo and the mother or the external environment.
22.36		8 cells	
22.40		16 cells	
22.45		32 cells	

Table.1 Development of the embryo of the mutira catfish (*Clarias gariepinus*)

00.30		Early morula	Cells form a shape like a ball (spherical) due to continuous cell defense where they are close to each other.
00.35			
00.40	-		
	Morula phase		
00.45			
01.06	Blastula phase		In this blastula there are areas that will differentiate to form certain organs such as digestive tract cells, notochord, nerves and epidermis, ectoderm, mesoderm and entoderm. The group of daughter cells resulting from division takes the form of a relatively round object in the middle of which there is an empty cavity
04.03	Grastula phase		Gastrulation in teleost fish will end when the egg yolk is completely encased. During this process some of the mesoderm tissue located along

04.06			both sides of the notochord is organized into segments called somites.
15.38	Organogenesis phase	6	The process of forming the body organs of a developing creature. The body's organ systems originate from 3
18.42			sprout leaves, namely ectoderm and mesoderm. From the ectoderm, the nervous system and epidermis of the skin are formed. From the endoderm, the digestive tract along with the digestive glands and respiratory organs are formed. Meanwhile, from the mesoderm the skeleton, muscles, blood vessels, excretion organs, reproductive organs and the skin corium will emerge.

Discussion

Embryogenesis is the process of cell division and cell differentiation of fish embryos that occurs during the early stages of fish development until egg hatching. The stages of embryogenesis consist of zygote, morula, blastula, grastula and organogenesis. The zygote will begin to divide by mitosis to produce a multicellular organism, the time required for the formation of this zygote is 15 minutes (Anonymous, 2009 in Permana, 2015). Based on the practical results, zygote formation takes around 27 minutes starting from 22.22 -22.45 WIB. The result of this process is called an embryo. Morula is a ball-like cell formation (spherical) due to continuous cell division where the presence of one cell to another is tight, the time required at this stage is 2 hours (Anonymous, 2009 in Permana, 2015). Based on the practical results, the time required from the zygote process to the morula process is around 127 minutes, namely from 22.45 – 00.45 WIB. The next stage, blastula is a continuation of the morula which continues to divide. The blastula stage is marked by the start of cell changes by forming irregular curves. Based on the practical results, the morula to blastula process takes around 1 hour 21 minutes, namely from 00.45 - 01.06 WIB. Furthermore, the gastrula is a further formation of the blastula whose body curves have become more pronounced and have layers of the embryo's body wall and body cavity. The time required at this stage is 4 hours (Anonymous, 2009 in Permana, 2015). Based on the practical results from the blastula process to the gristula process, it takes around 4 hours 10 minutes, namely from 01.06 – 04.0 WIB. The final stage of embryogenesis is organogenesis, namely the process of forming body organs in living creatures (Anonymous, 2009 in Permana, 2015).

The next process is organogenesis, namely the formation of the body's organs. Embryology includes the development process after fertilization to organogenesis before hatching or birth. Organogenesis is the stage in which the formation of body organs occurs from the three layers above, namely ectoderm, metoderm and endoderm. Each layer forms a different organ. Ectotherms form the epidermis layer on the teeth, eyes and auditory nerves. Mesoderm forms the respiratory, pericranial, peritoneal, liver and bone systems. Meanwhile, entotherms form sex cells and endocrine glands. Based on the practical results, the hatching process occurred the next day at 18.42 WIB.

According to Effendie (2000), the normal range for egg development is until the egg hatches (18-20 hours). This is different from the existing literature, the factors that influence the embryogenesis process include temperature, light intensity, and reduced oxygen pressure (Afandi, 2009 in Permana, 2015). The length of development of catfish eggs is due to the lack of light intensity and temperature. Apart from that, in the practicum the treatments experienced artificial spawning. External factors that influence the hatching of fish eggs are temperature, dissolved oxygen, pH, salinity and light intensity. The hatching process generally takes place more quickly at higher temperatures because at high temperatures the metabolic process runs faster so that embryo development will be faster which will result in more intensive movement of the embryo in the shell. However, temperatures that are too high or change suddenly can hinder the hatching process, causing embryo death and failure to hatch. A good temperature for fish hatching is 27 – 300C. Fertilized touro are bright brownish yellow, while unfertilized eggs are pale white. In the egg hatching process, sufficient oxygen supply is required. To meet the need for dissolved oxygen in water, aeration is installed in each hatching tank.

Fertilized	Not Fertilized

Sumantadinata (1983) said the factors that influence the hatchability of eggs are:

- 1. Egg quality. Egg quality is influenced by the quality of feed given to the parent and the level of egg maturity.
- 2. Environment, namely water quality consisting of temperature, oxygen, carbon dioxide, ammonia, etc.
- 3. The movement of the water is too strong which causes violent collisions between eggs or other objects, resulting in the egg breaking.

CONCLUSSION AND SUGGESTION

The development of an egg or embryo begins with cell division, this phase occurs from the 0th to the 27th minute. The second phase, namely the morula phase, occurs from the 27th to the 147th minute, the third phase, namely the blastrula phase, occurs from the 147th to the 288th minute. The fourth phase, namely the grastula phase, occurs from the 288th to the 28th minute. 538, phase five is the organogesis phase, where this phase is the final phase of embryo development which hatches into transparent pearl catfish larvae,

occurring at the 1,225th minute or 20 hours 25 minutes from the initial phase. The number of eggs that hatched was 400,842 with an egg hatchability value of 83.47%.

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