Embryogenesis of climbing perch fish *Anabas testudineus* Bloch 1792 at incubation temperature of 28°C

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Abstract. Information on the embryonic development of fish is important to understand the early life history of fish, this information is useful for fish breeding, especially in crossbreeding and genetic engineering. Therefore, this study aimed to observe the process of embryonic development and the incubation time of climbing perch eggs which were incubated at a temperature of 28 °C. The results showed that the embryonic development of betok fish was divided into six phases, namely the cleavage phase (3 hours and 5 minutes), morula (4 hours and 30 minutes), blastula (5 hours, and 40 minutes), gastrula (8 hours, and 47 minutes), organogenesis (15 hours, and 39 minutes) and hatched. At an incubation temperature of 28 °C, the climbing perch fish eggs hatched 18 hours and 38 minutes after fertilization.

1. Introduction
Climbing perch (*Anabas testudineus*) or locally name betok is a freshwater fish that has economic value and has the potential to be developed as a target for commercial aquaculture [1-3]. In Indonesia this fish is distributed Sumatra, Kalimantan, Java, Sulawesi [4, 5]. Betok fish is omnivorous that tend to be carnivorous [6]. According to Fitrani *et al.* [7] betok fish habitat is swamps, small rivers, irrigation channels, and paddy fields [8].

Studies on the climbing perch fish have been carried out by several researchers, for instance; Fitrani, Muslim and Jubaedah [7] studied the bioecological aspects of climbing perch and concluded that climbing perch is omnivorous, they spawn more than once a year at the beginning of the rainy season. Turyati *et al.* [9] have also investigated the biological aspects of this fish and concluded that climbing perch fish first matured at the size of 9.5 cm in male, and 9.2 cm in female, the fish had fecundity ranging from 336 - 21,616 eggs with an average size egg diameter of 0.046-0.052 cm. Wibowo and Helmizuryani. [10] studied the cultivation aspects of this fish and concluded that stocking density affects the growth and survival of climbing perch. In addition, Putra *et al.* [11] studied the application of Artemia enriched with several ingredients and concluded that climbing perch fish fed Artemia enriched with soybean meals resulted in better growth and survival. Jamsari *et al.* [12] studied the genetic diversity of climbing perch in Sumatra, Indonesia dan Peninsular Malaysia.

According to Muslim [12], the success of spawning is influenced by at least two factors, namely internal and external factors; the internal factors include; fish health, hormone secretion, and gonadal maturity, while the external factors include environmental and feeding factors. Temperature, light
intensity, and water pH are the important factors that influenced the spawning of the fish. Changing in temperature affects the biological and physiological processes of fish [13-15], when the temperature passed the ambient limit can cause damage to gills and blood cell abnormalities.

Embryogenesis study has been carried out on several species of fish, for instance in fighting fish Betta imbellis where the embryos are hatched 35 hours after fertilization, and embryonic development was divided into five phases, namely: division phases, morula, blastula, gastrula, and organogenesis [16]; rainbow Melanotaenia spp. where the eggs were hatched for 142 hours 59 minutes after fertilization at an incubation water temperature of 22.7-28.3 °C [17], the baung fish Hemibagrus nemurus hatched for 24 hours after fertilization [18]. Studies of embryogenesis in A. testudineus have also been carried out by several researchers, such as: incubation in different media and pH [19], embryogenesis of diploid and triploid [20], and performance and developmental stages in the laboratory [21]. However, embryonic development of climbing perch A. testudineus at incubation temperature of 28 °C has never been carried out. This study is important to understand the process of embryonic development in climbing perch fish at incubation temperature of 28 °C which can be useful for crossbreeding purposes in laboratory, especially gynogenesis and triploidization. Therefore, this study aims to observe the process of embryogenesis in climbing perch A. testudineus at incubation temperature of 28 °C.

2. Material and Methods

2.1. Site, time, and broodstock

The study was conducted at the Fish Hatchery Laboratory, Faculty of Marine and Fisheries, Syiah Kuala University from March to April 2021. A total of 10 males and 15 females of broodstock fish (A. testudineus) were collected from the wild then acclimatized for two weeks prior used for spawning. The broodstock was injected with a half dosage of Ovaprim (0.5 ml per kg body weight) two days intervals to induce the gonad maturation. The broodstock was fed on a commercial diet two times a day (at 8 AM and 4 PM). After two weeks, the late maturity broodstock was selected for spawning.

2.2. Spawning process

Before spawned, male and female brood fish are reared separately for two days, and during this process, the broodfish was not fed. Then the broodfish was intramuscularly injected with a single dosage of ovaprim 1 ml kg of body weight, then male and female fish were placed in the same container with a sex ratio male: female ratio is 3: 1. Approximately 8 hours after hormonal injection, sperm and eggs were taken by the abdominal gentle pressure of the broodstock. The sperm and eggs were put in different plastic jars and kept on an icebox (4 °C).

Then the sperm was diluted with a physiological solution (0.9% NaCl solution, [22]). Fertilization was conducted by mixing 0.6 ml of eggs with 0.2 ml of sperm (egg to sperm ratio, 3:1), then several drops of tap water were added, and then stirred evenly using chicken feathers and kept for 10 min approximately to allow the sperm to fertilize the egg. Then the fertilized eggs were rinsed with tap water, and then 100 eggs were taken randomly and then incubated at 28 °C in an aquarium equipped with aeration. The development of the zygote was observed 15 minutes intervals until hatching.

2.3. Embryogenesis observation and data analysis

The embryogenesis process was observed by randomly taken several eggs from the incubation aquarium every 15 min, and then placing the eggs in the object container and observing them using a Zeiss Primo Star microscope. During the observation of embryogenesis, the picture of embryonic development and the duration of every phase of embryonic development were recorded. Data on the phase and time of embryonic development for each phase were presented in plate/figure and then analyzed descriptively.
3. Results and Discussion
The embryogenesis of the climbing perch fish is divided into 6 phases, namely: the cleavage phase, morula, blastula, gastrula, organogenesis, and hatching. The cleavage phase is divided into 5 cleavage stages; the first division produces one blastomere with a duration of 34 minutes after fertilization. However, [23] reported 30 min, and [24] was 1 h for first cleavage in *A. testudineus*. The subsequent division produces two blastomeres or 2 cells in equal size. This division occurs 59 minutes after the fertilization process and lasts for 25 minutes. Subsequent cleavage to produce four blastomeres occurred 100 minutes after fertilization, this process lasted for 41 minutes. In this division, the two blastomeres divide to produce four blastomeres equal in size. Furthermore, each blastomere was re-divided to produce eight blastomeres which occurred at 125 minutes after fertilization, or this division process lasted for 25 minutes. This blastomere continues to undergo cell division to produce 16 blastomeres at 140 minutes after fertilization, this phase lasts for 15 minutes. Furthermore, each blastomere re-divided to produce 32 blastomeres occurred 185 minutes after the fertilization process or lasted for 45 minutes (Figure 1a-g). While [21] report, 4-cell, 8-cell, 16-cell, 32-cell stages were visualized at 1 h and 5 min, 1 h and 25 min, 1 h and 50 min, 2 h and 15 min respectively after fertilization.

After the cells have 32 blastomeres, the embryonic cells continue to develop into smaller parts and enter the Morula phase, a phase where the blastomeres condense become blastodisk at the anima pole which forms two cell layers. This Morula phase occurs 270 minutes after the fertilization process, and this process lasts for 85 minutes. According [21] stage morula varies from 2 h 40 min – 4 h. Furthermore, the cells at the anima pole condense into a small blastodisk to form two layers of cells of the same size; Then enter the blastula phase, in this phase two layers of cells are formed, namely the ectoderm layer or outer layer of the embryo and the endoderm layer or inner layer of the embryo. This phase also formed a collection of cells in the form of a cavity filled with fluid (blastocoel) called blastoderm. The Blastula phase occurs 340 minutes after fertilization, and the cell division process in this phase lasts for 70 minutes, while [21] stage blastula from 4 h - 5 h and 30 min. After the blastula phase is completed, the embryonic development enters the Gastrula phase which is marked by the formation of the head and tail of the embryo. The gastrula phase occurs 527 minutes after the fertilization process, or this process lasts for 187 minutes. According [21] gastrula stage completed after 5 h and 30 min to 7 h and 30 min after fertilization. In this phase, the eye buds and somites are formed, which are mesoderm blocks located on both sides of the neural tube of embryos that develop from anterior to posterior parts.

Furthermore, the development of the embryo enters the organogenesis phase, which is the last phase where the organs of the embryo begin to develop. The organogenesis phase in climbing perch occurred 939 minutes after the fertilization process, and this process lasted for 412 minutes. In this phase, the organs such as the tail/caudal part, somites, heart, eyes, head, body, and others begin to be clearly seen. After the organogenesis phase is complete, the embryo will hatch 1118 minutes after fertilization, or 179 minutes after the organogenesis phase.

The results showed that the cleavage phase in climbing perch was occurred for 3 hours 5 minutes after fertilization and the first cleavage has occurred 59 minutes after fertilization. Whereas in tawes fish *Osteochilus hasselti* this phase lasts more quickly, namely for 1 hour 26 minutes after fertilization with the first division occurring at 26 minutes after fertilization. In addition, the embryonic stage of climbing perch occurs longer than tawes fish. According to Djuwita et al. [25] that the speed of embryonic cell division depends on the volume and distribution of egg yolks contained in the zygote, this condition is species depending. Therefore, it is suspected that the volume and distribution of egg yolks in climbing perch is less than tawes fish. This is related to the size of the eggs cells where the egg size of the climbing perch ranges from 0.23 – 1.20 mm while the egg size of the tawes fish ranges from 0.8 – 1.42 mm.

In addition to the internal factors mentioned above, the speed of embryogenesis is also influenced by external factors, including temperature, light intensity, and pH. However, this study was not examined these factors, because this is a basic study to understand the process of embryonic cell division in climbing perch at ambient temperatures without treatment.

In general, a lower water temperature causes the cell division process to occur slowly and even causes cell death due to failure to divide. This is because at low temperatures metabolic activity runs more
slowly so that embryonic development also slows down [26]. On contrary, at higher temperatures, the hatching process will occur more quickly due to increase cell metabolism, but the number of eggs hatched will decrease because at higher temperatures some embryos cannot survive and cause cell death, or resulted in abnormal larvae. [27] stated that at high temperatures, the metabolic rate of the embryo increases so that the energy source in the form of egg yolks will be quickly depleted before all embryonic development processes are completed so that it will produce abnormal larvae or even fail to hatch.

Figure 1. The embryonic development of climbing perch A. testudineus incubated 28 °C. (a-g) Cleavage stage, (h) Morula stage, (i) Blastula stage, (j) Gastrula stage, (k) Organogenesis stage, (l) larva stage
In addition, the light intensity and dissolved oxygen also affect the incubation time of eggs, in dark conditions or less light intensity, the embryos will hatch longer [28]. The developing embryo in the egg requires sufficient oxygen, dissolved oxygen in the water enters the egg by diffusion through the surface layer of the eggshell [29]. The lower dissolved oxygen will slow down the development of the embryo and can cause abnormal development. The average dissolved oxygen during this study was 7.65 mg/l, this is still within the optimum limit for embryonic development.

Apart from the factors described above, the pH of the egg incubation medium (water) is also playing an important role in the process of embryogenesis. The pH of the incubation media affects the activity of the chorionase enzyme by reducing the hardness chorion to become softer [12] so that facilitated embryo to break the egg wall to get out of the eggshell (hatch). This enzyme will work optimally in the pH range of 7.1 - 9.6 [30] while the average pH in this study was 7.40, and therefore still in the optimal range.

In terms of incubation time, the eggs of climbing perch (A. testudineus) which were incubated at 28 °C hatched within 18 hours and 38 minutes after the fertilization. At the same incubation temperature, tawes (O. hasselti) eggs hatch after 18 hours and 19 minutes of fertilization [31]. Therefore, although the process of cell division of climbing perch is slower than tawes fish, especially in the cleavage phase, however, the hatching time is relatively almost the same. This may be due to in the subsequent phases the process has been going faster.

4. Conclusion
The embryogenesis of climbing perch A. testudineus at an incubation temperature of 28 °C is divided into six phases, namely; cleavage which lasts for 3 hours 5 minutes, morula lasts 4 hours 30 minutes, blastula for 5 hours 40 minutes, gastrula for 8 hours 47 minutes, organogenesis for 15 hours 39 minutes, and hatching 18 hours 38 minutes after fertilization.

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