Histological analysis of ovarium development of *Hampala macrolepidota* Kuhl & Van Haselt, 1823 from Jatigede Reservoir, West Java

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Abstract. This research was conducted from May to September 2018 at the Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, University of Padjadjaran. Fish samples were taken from Jatigede Reservoir, Sumedang, West Java. The hampala fish consisted of female hampala barb with gonadal maturity level (GML) IV, GML V, and GML VI. Ovarian histological preparations were made at the Animal Microtechnical Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. This research aimed to determine the stages of ovarian development of hampala barb, and determine the types of spawning. The hampala barb in the Jatigede Reservoir had five stages of oocyte development, namely chromatin nucleolus stage, perinucleolus stage, cortical alveolus stage, vitellogenesis, and oocyte maturation with multi-spawner group synchronous type of ovarian development.

Keyword: *Hampala macrolepidota*, multiple spawner, oocyte development, synchronous group, Jatigede Reservoir.

1. Introduction

Jatigede Reservoir area is ± 4122 ha and is located in Sumedang, West Java. This reservoir was built by damming the Cimanuk River and is a multi-function reservoir. The functions of this reservoir are as a power plant, irrigation, fishing area, and flood control [1]. Fish communities that inhabit reservoirs were originally fish from dammed river waters [2]. One of the indigenous fish of Cimanuk river that could be found in Jatigede is hampala barb (*Hampala macrolepidota*). Morphological characteristics of this species are that adult fish had black spots between the dorsal pin and the abdominal pin, then they would be vague on large sizes. an elongated body and flat. The head part between the eyes is rather prominent. The dorsalis pin is full of scales and the edges are dark in color. The other part of the caudalis pin is dark red. Dorsal pin, pin pectoralis, abdominal pin, and yellowish red anal pin [3].

Hampala barb is an excellent fish for anglers, preferred for consumption, and had economic value making it one of the target fishes [4]. Catching hampala barb continuously and not selective about size accelerate the decline in the population of wild fish in nature. Maintenance and conservation of wild fish required a rational management effort [5]. To support the efforts of conservation of endangered fish, information related to the biology of fish reproduction is needed. Until now, information related
to gonadal histology and the development of vascular fish eggs which are one of the endangered fish species included in the IUCN Red List are very limited.

The stages of egg cell development histologically are the basis for reproduction studies related to reproductive strategies. Fish reproduction strategies are very diverse [7]. Reproduction is efforts to produce new individuals through the spawning process. The reproductive cycle of each fish varies, seen from oocyte development and spawning season [6]. The development of oocytes consists of several stages; namely the initial development stage (characterized by the formation of chromatin and perinuclear nucleus), the cortical alveoli stage, the stage of vitellogenesis, and the stage of maturation (initial maturation and final maturation) [6, 8]. Furthermore, studies on fish reproduction could be used to support fish management and conservation programs designed to maintain or increase fish stocks. Therefore, research related to the development of histological gonads of hampala barb is very important to know about the reproductive strategies of hampala barb to support a fish conservation program as an effort to maintain or increase fish stocks.

2. Materials and Methods
Fish samples were taken in May 2018 from Jatigede Reservoir, Sumedang Regency, West Java Province. Hampala barb were caught using gill nets with mesh sizes between 4-7 inches. The collected fish was then dissected and gonads with gonadal maturity level (GML) IV, V and VI were taken and preserved using Bouin’s fixative solution. Morphological determination of the gonad maturity level of morphological fish was carried out.

Histological gonad preparations for hampala barb were stained by haematoxylin-eosin (Eurlich, Mayer) at the Animal Microtechnical Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. The development of oocytes was referred to the criteria of [7, 10, 11].

3. Results and Discussion
3.1 Oocyte development phases
The ovaries of hampala barb showed five phases of oocyte development as follows.
Phase I. Chromatin nucleoli (C)
This phase is the initial stage of oocyte development. Oocytes appeared around the lumen of the ovary. Oocytes had a large nucleus surrounded by cytoplasm. The diameter of the oocyte ranged from 20-100 μm.

Phase II. Perinucleolar (PN)
This phase is characterized by the appearance of nucleoli (n) in the nucleus and cytoplasm appearing darker than the nucleus (N) in hematoxylin staining. Nucleoli had varying sizes and generally located in the peripheral nucleus. The diameter of the oocyte ranged from 100-150 μm.

Phase III. Cortical alveoli (CA)
This phase is characterized by the formation of cortical alveoli (CA) in the cytoplasm. In Hematoxylin-Eosin staining, cortical alveoli looked like an empty structure. Chorion (C) started to be seen in this phase. Nucleoli were numerous and in the peripheral part of the nucleus. The diameter of the oocyte ranged from 150-250 μm.

Phase IV. Vitellogenesis
This phase is characterized by the presence of egg yolk (Y) around the nucleus. The nucleus had an irregular shape. Fatty granules (O) were seen in the cytoplasm. The diameter of the oocyte ranged from 250 – 700 μm.

Phase V. Mature
In this phase the nucleus disappeared. Cytoplasm is filled with egg yolk granules (Y). The diameter of the oocyte ranged from 700 – 1.000 μm.
The phase of oocyte development in fish varied. In teleost fish, the oocyte development phase is usually divided into four to five phases depending on the species and criteria used by each author [7,12,13,14,15,16]. The oocyte stage could be characterized by cytoplasmic volume [12,17], the appearance of the nucleus and nucleoli, and the presence of egg yolk granules. Based on observations, five stages of oocyte development were found in hampala barb; (1) chromatin nucleoli stage, (2) perinucleolar stage, (3) cortical alveoli, stage (4) vitellogenesis stage, and (5) mature stage. The chromatin nucleolus and perinuclear phase are the initial phases of oocyte development (previtellogenesis). The chromatin nucleolus phase is characterized by the presence of a large nucleus surrounded by cytoplasm. In Haematoxylin-Eosin (H&E) staining, the oocyte nucleus in this phase looked darker than the cytoplasm. The second phase in the oocytes development of hampala barb is the perinuclear phase. This phase is characterized by the appearance of nucleoli in the nucleus in varying shapes and amounts. In cytoplasmic H&E staining it looked darker than the nucleus. In the perinuclear phase, the oocyte had a large nucleus and contained several nucleoli in the peripheral part of the nucleus [18].

The third phase of the development of hampala barb oocytes is the alveoli cortical phase. This phase is characterized by the appearance of cortical alveoli around the nucleus. Cortical alveoli were oval-shaped structures that look like empty structures in H&E staining [11]. This phase is an important phase for oocytes to go to the vitellogenesis phase [6]. Histochemical analysis showed that cortical alveoli contain carbohydrates and protein [19,20,21]. Furthermore, cortical alveoli contain polysaccharides in the form of glycoproteins and glycoconjugates [21]. Chemical content in the cortical alveoli played an important role in the process of fertilization and early embryonic development [21,22].

Entering the Phase-IV or vitellogenesis, there were visible yolk and fat beads around the nucleus and cytoplasm. Moreover, in this phase the nucleus became irregular. Vitellogenesis is an important phase for oocytes because in this phase oocytes accumulate phospholipid-rich yolk protein (YP) precursors called vitellogenin (Vtgs). Vitellogenin as a dimeric protein that had two identical subunits with the main components being phosphate, lipids, carbohydrates, and proteins. Vitellogenin is also an important ion carrier such as calcium, magnesium, iron, zinc, copper, and other minerals and also vitamins such as retinoids and carotenoids [23]. The presence of carotenoid pigments caused the yolk to appear orange [6]. Vitellogenin is synthesized by the liver and transported through the bloodstream to the ovary to be absorbed by oocytes and processed into yolk protein (YP) derivatives and stored as yolk granules, yolk globules or yolk globules or platelets. When vitellogenesis ended, the ovary would be filled with perfect oocytes filled with egg yolks which would then undergo maturation and ovulation [23]. In phase V or mature oocytes, the cytoplasm of the oocyte is filled with egg yolk granules. The more mature the oocyte, the oocyte diameter would increase. This is caused by the increasing number of egg yolk that meet the oocyte. During oogenesis, oocyte size increased significantly due to the accumulation of lipids and egg yolks in the cytoplasm continuously [16].

3.2 Type of ovarian development

Based on observation of the cross section histology of the ovary of hampala barb with stage of maturity IV and V, it was known that in one cross section of ovary there were seen two different groups of eggs (oocytes). In the ovary, most oocytes had a large size and were in phase V or mature. However oocytes of small size (phases I and II) were also seen around oocytes of phase V. This showed that the fish (Hampala macrolepidota) had a type of ovarian synchronous development or developed simultaneously in group.
In fish with a synchronous ovarian type, there are at least two oocyte groups that could be identified at the same time during the spawning period [24,25,26]. In the ovary with synchronous group type there are an oocyte population that are the same or dominates and would be removed in the next spawning season while in ovaries with asynchronous oocyte types from all stages of development were seen without any dominant population [27,28]. Furthermore, fish ovaries had three types based on the form of oocyte development [6,29], they are the type of developing simultaneously (synchronous), developing simultaneously in groups (synchronous group), and developing not simultaneously (asynchronous). Fish with synchronous ovarian development type are fish that have at least two oocyte populations and there is one more dominant oocyte population. Large oocytes are released in the first spawning season and then small oocytes will be released during the next spawning season [10, 27, 30].

Types of ovarian synchronous group development also found in the *Hampala bimaculata* species originating from Betung Kerihun National Park, West Kalimantan. In the mature ovary phase, two groups of eggs are found, oocyte phase IV and oocytes in the previtellogenic and vitellogenic phases (phase I, II, and III) where the ovaries were dominated by oocytes in phase IV. The presence of these two oocyte groups in the ovary indicated that *Hampala bimaculata* had a synchronous ovarian developmental type [26].

3.3 Types of hampala barb spawning based on ovarian histology
Spawning type is a mechanism by which female fish emitted mature oocytes during the spawning period [26]. Based on the results of histological observations on hampala barb ovaries with GML VI, the number of oocytes in phase I and II and also the presence of post ovulatory follicles (POFs) were seen. This indicated that hampala barb (*Hampala macrolepidota*) has a type of partial spawner or multiple spawner, which means that these fish had a spawning period several times a year. The presence of immature eggs showed that the fish spawned partially [31]. The type of partial spawner spawning is a type of spawning that lasts a long time because the fish spawned their eggs part by part [32, 33]. This result was in accordance with Musrin’s research which stated that *Hampala macrolepidota* is a partial spawner.
In addition to oocytes in phases I and II, many oocytes have been found to had ovulated (ovulated eggs). Ovulation is the release of mature oocytes from the follicle into the ovarian cavity [34] or the release of oocytes into the ovarian lumen when the oocytes are in metaphase during the second meiotic division [35, 36]. The ovulated oocytes would remain in the lumen of the ovary until the emergence of spawning stimuli from the environment or through artificial techniques [36, 37]. Oocytes that had been ovulated but not immediately removed or spawned and remain in the ovary would be over-ripening or overripe. This over-ripening condition occurs because of changes in morphology, physiology, histology, biochemistry, cellular, and molecular [36, 37, 38, 39, 40, 41]).

Oocytes that have been ovulated generally have diameters between 800 – 1,000 μm, but there were also oocytes which had a diameter between 400 - 600 μm that have ovulated. This is presumably because the oocyte had degenerated and reabsorbed because the fish were late spawning so that the oocyte shape became irregular and the oocyte diameter shrinks. The atretic oocytes decreases in size as the oocytes is broken down and reabsorbed [28]. Ooocytes that had been ovulated would degenerated and reabsorbed if there was no stimulation of spawning from the environment or stripping manually [36,37]. In nature, fish spawning could be delayed because fish could not find a suitable environment for spawning or due to changes in environmental conditions such as dam construction [36, 42].

3.4 Types of hampala barb spawning based on the distribution of egg diameter

The distribution of the diameter of hampala barb eggs in GML IV (figure 3) showed the presence of two peaks. The first mode of 25% was in the interval 140 - 257 μm which is a group of young oocytes (previtellogenic phase) and the second mode of 19% was found at intervals of 730 - 847 μm (mature phase). In this GML IV, overall, the highest percentage was found in the previtellogenic phase oocytes at 39%, followed by oocytes in the mature phase by 34%, and oocytes in the vitellogenin phase at 27%. The existence of the two peaks of the mode indicated that the hampala barb was a partial spawner or multiple spawner.
The distribution of egg diameters at GML V (Figure 4) is not much different from the distribution of egg diameters in GML IV. The highest mode is found in mature phase oocytes at 26% and 17%. The third highest percentage is in the previtellogenic phase oocyte, which is 16%. Overall, in this GML V the mature phase oocytes have increased than before. Mature oocyte phase in GML V is 53%, oocyte in the vitellogenic phase is 18% and oocyte in the previtellogenic phase is 28%. There are several peaks on the graph reinforcing that hampala fish are multiple spawners.

Based on the figure 5, it is known that the distribution of egg diameters in the ovaries of hampala barb at GML VI has one peak at intervals of 21-139 μm. Whereas in GML IV and GML V which are mature phase ovaries, the distribution of egg diameters varied from small to large diameter. In the graph distribution of egg diameters with GML IV and V also seen the existence of the two to three highest modes which were large eggs and small eggs. This reinforces the fact that hampala barb (*Hampala macrolepidota*) was a partial spawner or multiple spawner. This was in line with the results of Fikriyah *et al* [43] who observed morphologically the density of oocyte fish that the diameter of
hampala barb eggs in GML III, GML IV, and GML V had several size classes with different frequencies indicating that the hampala barb (*Hampala macrolepidota*) in Jatigede Reservoir has a partial spawner spawning type. In fish and invertebrates often found bimodal egg diameter distribution [32,44]. The first mode consisted of immature eggs and the second mode consisted of ripe eggs. This spawning model is called partial spawning. The same thing also occurred in *Hampala bimaculata* species where during the spawning season the distribution of egg diameter in mature ovaries showed a polymodal (multiple modes) which means that *Hampala bimaculata* is a batch of spawner or multiple spawner [26].

4. Conclusions
Based on the results, it could be concluded that:
1. *Hampala macrolepidota* from the Jatigede Reservoir has five stages of oocyte development, they consist of chromatin nucleolus phase, perinucleolar phase, cortical alveoli phase, vitellogenesis phase, and mature phase.
2. The type of development of oocytes in ovaries is a synchronous group
3. Type of spawning is batch spawner or multiple spawner.

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