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OOSITE DEVELOPMENT SEREN FISH (*DIPLOCHEILICHTHYS PLEURTAENIA*, BLEEKER 1855) FROM JATIGEDE RESERVOIRS AT A DIFFERENT LEVEL OF GONAD MATURITY

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KeyWords
Diplocheilichthys pleurotaenia, gonadal maturity levels, oocyte development, spawning types, Jatigede Reservoir, synchronous group, partial spawner.

ABSTRACT

This research aims to determine the development of oocytes in each gonad development and reproduction type of seren fish. This research was conducted from May to December 2018. Seren fish (Diplocheilichthys pleurotaenia) originated from Jatigede Reservoir. Preparation was made at the Animal Microtechnical Laboratory, Faculty of Mathematics and Natural Sciences (MIPA) Padjadjaran University, analysis of sample preparations was carried out at the Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University. Seren fish used consisted of gonadal maturity levels III, IV, and V. Analysis of the development phase of fish oocytes based on criteria made by Wallace and Selman (1981), West (1990), and Erkmen and Kirankaya (2016). The results showed that the development of oocytes at all different levels of gonadal maturity were found in 5 stages of oocyte development, namely chromatin nucleoli, perinuclear, cortical alveoli, vitellogenesis, and maturity. Synchronous ovarian developmental type and partial spawner spawning types.

INTRODUCTION

Seren fish is a native fish of Cimanuk River which can live and breed in Jatigede reservoir. This fish is used by the local community as a consumption fish. This reservoir was built by damming the Cimanuk River and is a multi-function reservoir. The results of communication with local fishermen seren fish in the jatigede reservoir from the start of inundation until June 2018 seren fish in Jatigede Reservoir are very easy to catch and very many catches while in mid-September 2018 seren fish are rarely caught even though fishermen use gill nets the size of gills standard. This is thought to be due to the inability of the seren to compete with other introduced fish in the Jatigede reservoir so that it results in a decline in fish populations and threatens the preservation of fish resources.

To meet animal needs, fishermen in Jatigede Reservoir utilize catches from nature and carry out rearing continuously, this can damage the sustainability of fish (Yurisman et al. 2010). Although fish are considered biological resources which have the characteristics of being able to recover (Renewable), but if catching is done continuously will result in population decline. The growth of fish populations in nature is very dependent on their reproductive abilities and responses to environmental changes (Wootton and Potts, 1984).

One of the things we can learn from fish reproduction is spawning patterns and oocyte cell development. Spawning patterns can be suspected by observing the distribution pattern of egg diameter in fish ovaries (Ernawati, 2009). There are 4 types of reproduction of freshwater fish in tropical waters, namely: bing bang spawner, total spawner, partial spawner, and small brood spawner (Lowe, 1975). By knowing the types of development of reproductive organs and fish spawning patterns, we can apply them in fish management systems to be better.

Marques et al. (2000) explain that studies on fish reproduction can be used to support fish conservation management and programs to maintain or increase fish stocks. Herawati et al. (2018) states that seren fish have negative allometric growth patterns. The condition factor ranges from 0.973-1.105. The sex ratio of male and female seren is 1: 1. Maturity Index of male and female serene gonads is relatively similar. Seren fish measuring 225 mm have an average fecundity of 10,032 items, while fish sizes are 260 mm with an average fecundity of 23,471 items. Seren fish are better caught using a 5-inch mesh for the preservation of Seren fish in the Jatigede Reservoir. At present no one has examined the development of reproductive organs and seren fish spawning patterns in the Jatigede Reservoir, therefore research on oocyte development at different Gonad Maturity levels is needed as a reference in aquaculture, especially seren fish spawning.

MATERIALS AND METHODS

This research was conducted from May 2018 to December 2018. Fish samples were taken from the Jatigede Reservoir in May 2018. Preparation was carried out at the Animal Microtechnical Laboratory, Faculty of Mathematics and Natural Sciences, Padjadjaran University while for analysis of sample preparations were carried out at the Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences Padjadjaran University.

The tools used in the research are: surgical instruments, cool boxes, petri disks, tweezers, small jars, millimeter blocks, ovens, microscopes equipped with PCs that have zen software, microtomes.

The materials that will be used in this research are: seren fish taken from Jatigede reservoir and consisting of three Maturity Levels Gonad III, IV, and V, Bouin solution, 70% alcohol, 80% alcohol, 90% alcohol, and 100% alcohol, paraffin blocks, hetatoxylin and eosin dyes, physiological NaCl, and xylol solutions.

This research uses exploratory methods, fish are caught using gill nets 80-100m long, 6-8m wide, with 3-6inch mesh size, fish are taken to the animal microtechnical laboratory to be made gonad preparations using the paraffin method.

PROCEDURE

This research was conducted from March to December 2018. Preparation was carried out at the Animal Microtechnical Laboratory, Faculty of Mathematics and Natural Sciences, Padjadjaran University while for analysis of sample preparations were carried out at the Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University. This research includes several stages consisting of the seren fish sampling process, seren fish surgery process, determination of Gonad Maturity Level (GML), Gonad Maturity Indeks (GMI) calculation, Fecundity calculation, preparation of seren fish gonad preparations, and analysis of seren fish gonads.

The parameters of observations made in this research are: The development phase of fish oocytes was analyzed based on the literature made by Wallace and Selman (1981), West (1990), and Erkmen and Kirankaya (2016).

The data obtained is presented in tabular and figure form. Data related to Gonad Maturity Level and oocyte development phase were analyzed descriptively qualitatively while fecundity data, Gonad Maturity Index, and oocyte diameter were analyzed descriptively quantitatively.diameter were analyzed descriptively quantitative.

RESULT AND DISCUSSION

Determination of fish GML seen from the gonad morphological characteristics which include the shape, color, and proportion of filling the body cavity. Seren fish with GML III (Figure 1A) have yellowish gonad characteristics and fill 1/3 of the body of the fish, fish body weight of 287 gr, with gonad weight of 48.35 gr, have a fecundity value 36,746 grains and have a GMI value 16.84%.

The GML IV phase or the mature phase in seren fish (Figure 1B) can be seen with the characteristic of a yellowish-white egg with a larger oocyte size compared to GML III and nearly filling ½ the body of the fish. The body weight of the fish is 229 gr, with a gonad weight of 53.6 gr, has a fecundity value 48.508 grains, and has a GMI value of 23.41%.

While in the GML V or mature phase (Figure 1C) we can see the difference in color and size of oocytes in fish. In GML V, eggs appear greenish yellow and fill in 2/3 of the fish's body. Fish body weight of 296 gr, with gonad weight of 56.4gr, has a fecundity value 60,686 grains, and has a GMI value of 19.05%. The average fecundity in fish according to environmental conditions according to Moyle (2004) in Bakhris (2008).

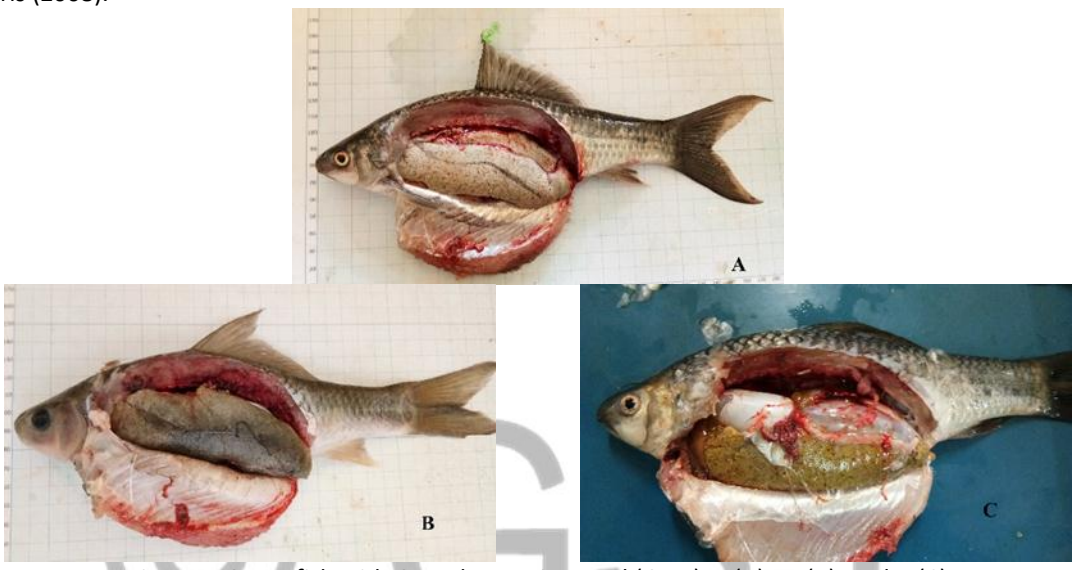
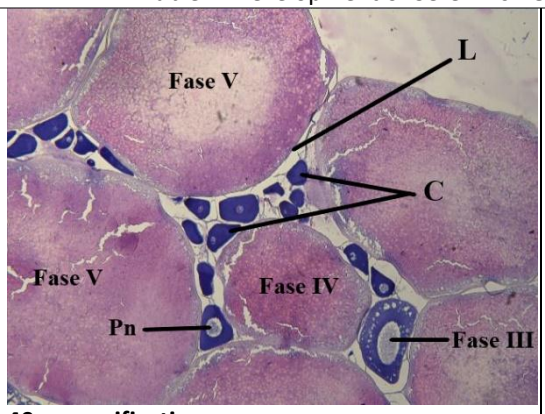


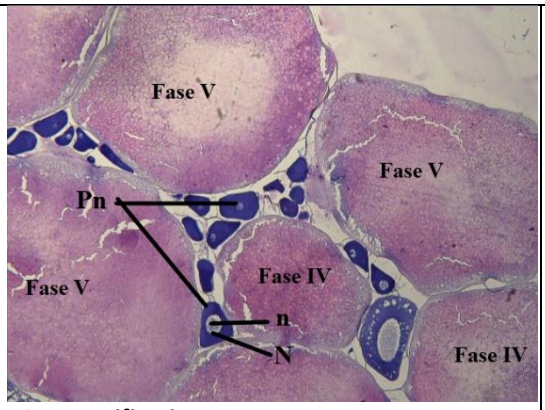
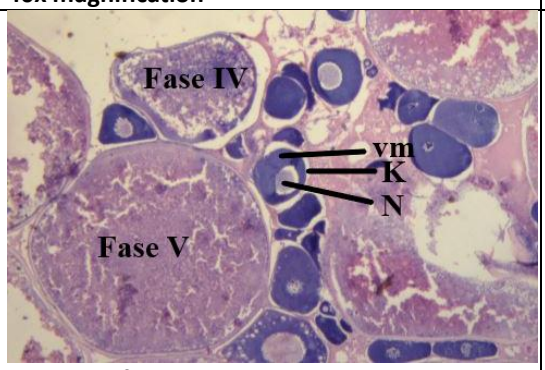
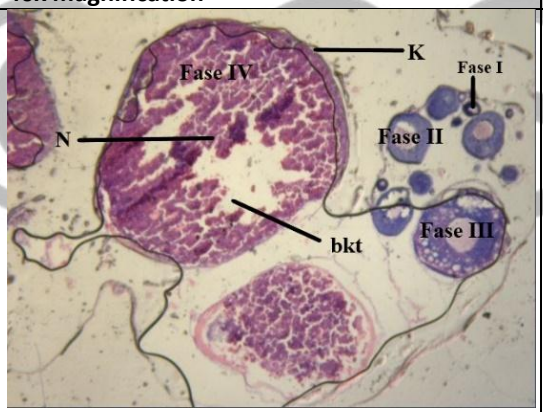
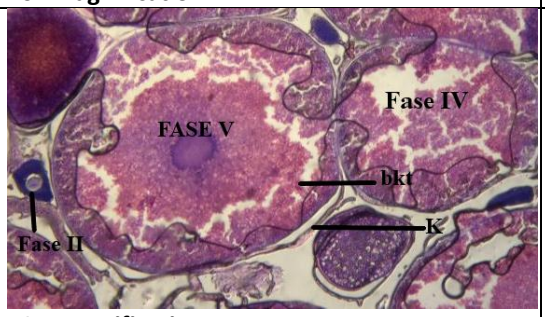
Figure 1. Seren fish with Gonad Maturity Level (GML) III (A), IV (B), and V (C)

Oocyte Development

Based on observations on seron gonads, five stages of oocyte development were found, namely chromatin nucleolus (Kn), perinuclear (Pn), cortical alveoli (Ka), vitellogenesis, and maturity. This is in accordance with the references made by Wallace and Selman (1981), West (1990), and Erkmen and Kirankaya (2016) who divide the stages of fish oocyte development into 5 stages.

Table 1. Development of Seren Fish Oocytes

Phase I. Kromatin nukleus(Kn) stage	 **40x magnification**	This phase is the initial stage of oocyte development, namely the chromatin nucleus (C) phase. Oocytes appear around the lumen of the ovary (L). Oocytes have a large nucleus surrounded by cytoplasm. Oocyte diameter ranges from 20-100 µm.

Phase II. Perinukleolar (Pn) stage	 **40x magnification**	This phase is characterized by the appearance of nucleoli (n) in the nucleus (C) appearing darker than the nucleus (C) in hematoxylin staining. Oocyte diameter ranges from 100-150 µm.
Phase III. Kortikal Alveoli (Ka) stage	 **40x magnification**	This phase is characterized by Chorion (K) starting to be seen clearly in this phase. vitelline membrane (vm) is clearly located inside Chorion (K). The nucleus (N) is in the peripheral part of the nucleus. Oocyte diameter ranges from 150 - 250 µm.
Phase VI. Vitellogenesis stage	 **40x magnification**	This phase is characterized by the presence of egg yolk (bkt) around the nucleus (N). The nucleus has an irregular shape. Egg yolk is seen in the cytoplasm. Oocyte diameter ranges from 250 - 700 µm.
Phase V. Mature stage	 **40x magnification**	In this phase the nucleoli are clearly visible. Chorion (K) is still visible and cytoplasm is filled with egg yolk (bkt). Oocyte diameter ranges from 700 - 1,000 µm.

Note: K = chorion, bkt = egg yolk granules, bl = fat granules, N = nucleus, n = nucleoli, C = chromatin nucleolus stage, PN = perinuclear stage, vm = vitelline membrane.

The development of oocytes in fish consists of several stages, namely the initial stage (characterized by the formation of the nucleus chromatin and perinuclear), the cortical alveoli stage, the stage of vitelogenesis, and the stage of maturation (McMillan 2007). But the development of fish oocytes also do not have to follow the stages that already exist, can be adjusted to the development period of the fish oocytes. According to Nagahama (1983) oocyte stage can be characterized based on cytoplasmic volume, appearance of the nucleus and nucleoli, and the presence of egg yolk granules.

Chromatin nucleus phase

The chromatin nucleus phase of the Oocyte appears around the lumen of the ovary. Oocytes have a large nucleus surrounded by cytoplasm and have oocyte diameters ranging from 20-100 µm.

Perinuclear phase

Perinucleolar phase, the nucleoli appear darker than the nucleus with hematoxylin staining. In this phase oocytes have diameters ranging from 100-150 µm.

Alveoli cortical phase

The cortical phase of the alveoli which is marked by the chorion begins to be clearly seen and the vitelline membrane is clearly visible inside the chorion. The nucleus is in the peripheral part of the nucleus. In this phase the diameter of the oocyte ranges from 150 - 250 µm. Cortical alveoli have an important role in the stage of vitelogenesis because an increase in the size of cortical alveoli (egg yolk and fat) will shift to the periphery of the cytoplasm and strengthen the cytoplasmic wall (Arianti et al. 2017). According to Ravaglia & Maggese (2002) revealed that cortical alveoli movement to the periphery of the cytoplasm is caused by increased accumulation of egg yolk.

Vitellogenesis phase

Vitelogenesis phase where in this phase is characterized by the presence of egg yolk around the nucleus. Egg yolk are seen in the cytoplasm and oocytes in this phase with diameters ranging from 250 - 700 µm. At this stage some fish have a mixture of egg yolk and fat that is difficult to distinguish during ripening and oocytes become difficult to find at the time of dissolution of the nucleus. This is caused by shrinkage and distortion of oocytes. Yon et al. (2008) revealed that the mature stage, the nucleus could not be observed because egg yolk and fat granules filled the entire cytoplasm homogeneously with a larger size.

Mature Phase

Mature phase where in this phase the nucleoli are clearly visible. The chorion is still visible and the cytoplasm is filled with egg yolk. In this phase the diameter of oocytes ranges from 700 - 1,000 µm. Montchowui et al. (2012) state that during oogenesis, oocyte size increases significantly due to the accumulation of lipids and egg yolks in the cytoplasm continuously. The more mature the oocyte, the oocyte diameter will increase. This is caused by the increasing number of egg yolk that meets the oocyte.

Spawning Type

The type of fish spawning can be determined by analyzing the development of oocytes in the ovaries of female fish. Stages of development of oocytes can also be related to the level of fish gonad maturation. Observation of the cross section of seren ovaries of Gonad Maturity Levels (GML) III, IV, and V, it can be seen that in one cross section there are two different groups of egg cells (oocytes). The results of observations on the preparation of seren fish Gonad Maturity Levels (GML) III, IV, and V (figure 2) show small oocytes ranging from 50-250 µm and large oocytes ranging from 300-1000 µm which are oocellogenesis phase oocytes and mature phase. Also seen are small oocytes (phase I and II) in GML IV and V although not as many as large oocytes.Castillo and Gatlin, 2015). In the utilization of enzymes in aquatic feed, a major concern is their stability at various processing conditions including the heat, pressure and moisture. Extrusion is a process that exposes the feed ingredient mixture to high temperature, high pressure and strong shear force over a short period of time, which can inactivate some antinutritional factors and increase the feed safety by destroying pathogenic microorganisms. Compared to a pelleted diet, an extruded diet can improve feed utilization by improving digestibility of starch (Glencross et al., 2012; Krogdahl et al., 2005; Venou et al., 2009), energy (Glencross et al., 2011), and protein (Gaylord et al., 2008; Glencross et al., 2008). However, the high temperature and high pressure during the extruding would cause damage to some heat-sensitive nutrients, such as vitamins and dietary enzymes.

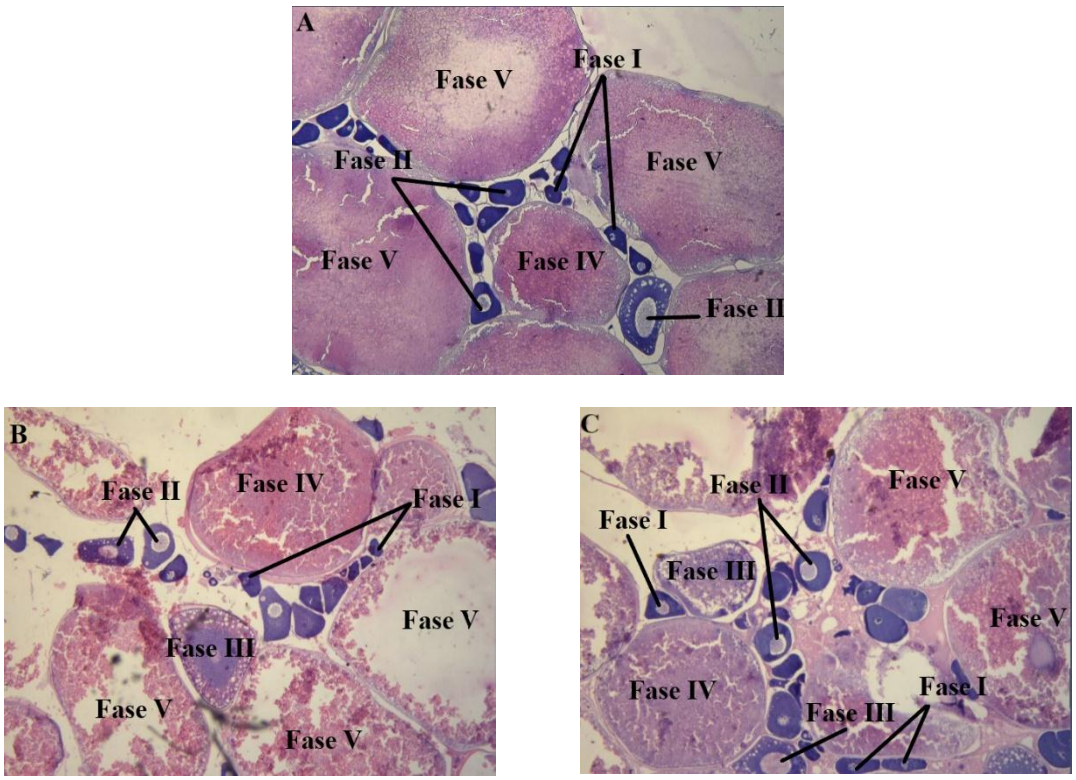


Figure 2. Cross section of seren ovaries of Gonad Maturity Levels GML III (A), GML IV (B), and GML V (C) with 40x magnification

This shows that seren fish (*Diplocheilichthys pleurotaenia*) is a fish that has a type of oocyte developmental development in groups (group synchronic). According to Selman & Wallace (1989) fish ovaries can be classified into three types based on the form of oocyte development, namely the type of developing simultaneously (synchronic), developing together in groups (group synchronic), and developing not together (asynchronic). Fish that issue eggs in groups (group synchronic) have two stages of development in the cross section of the ovary, namely large oocytes (homogeneous) and small oocytes (heterogeneous) (Ganias et al. 2004). Selman & Wallace (1981) in Sjafei et al. (2008) stated that fish with a synchronous ovarian developmental type are fish that have at least two oocyte populations and there is one more dominant oocyte population. Large oocytes are removed in the first spawning season and then small oocytes will be removed during the next spawning season.

Ovarian Histology

The type of spawning or spawning patterns in fish can be estimated from the distribution of egg diameters. According to Prabhu (1956) and Kagwade (1968) in Warjono (1990), the type of fish spawning is related to the development of egg diameter in the ovary. Measurement of egg diameter in mature gonads is useful for estimating spawning frequency by looking at the mode of spreading (Prabhu 1956 in Susilawati 2000). Spawning time can be estimated from the frequency of fish egg size. The type of fish spawning seen from the distribution of the diameter of the egg size is divided into two, namely partial spawner and total spawner. Partial spawner is a type of gradual spawning where fish release their eggs little by little twice the spawning season. The first peak on the distribution of diameter is the first time issued when spawning and then followed by a second spawning on eggs that are on the second peak. Total spawner is a type of spawning that is not gradual where the fish release their eggs thoroughly (Sulistiono et al. 2001).

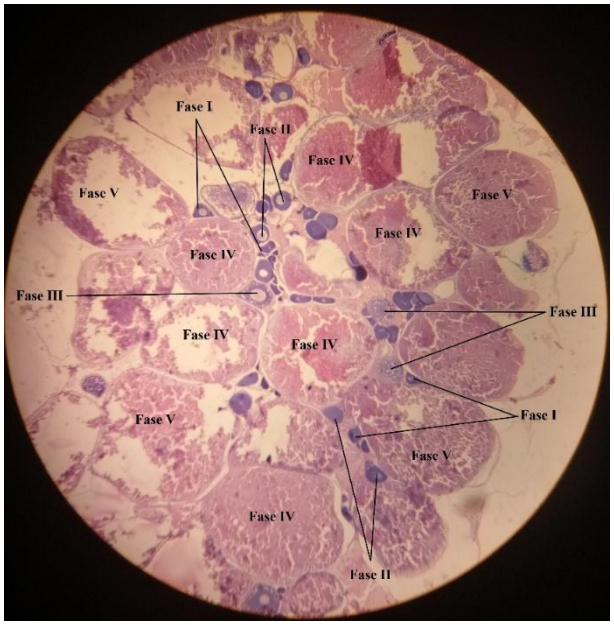


Figure 3. Cross section of seren fish ovaries in GML V

Based on histologi observations on seren ovaries, it can be seen in GML IV and V that there are many oocytes in phases I and II (Figure 3). This shows that the existence of groups of eggs that are not yet ripe, this makes that the seren fish spawn partially spawn. Type of partial spawner spawning is a type of spawning that lasts for a long time because the fish spawned their eggs partially.

Egg Diameter

Based on the measurement results of serene fish oocyte diameter in GML III, GML IV, and GML V, the following data were obtained.

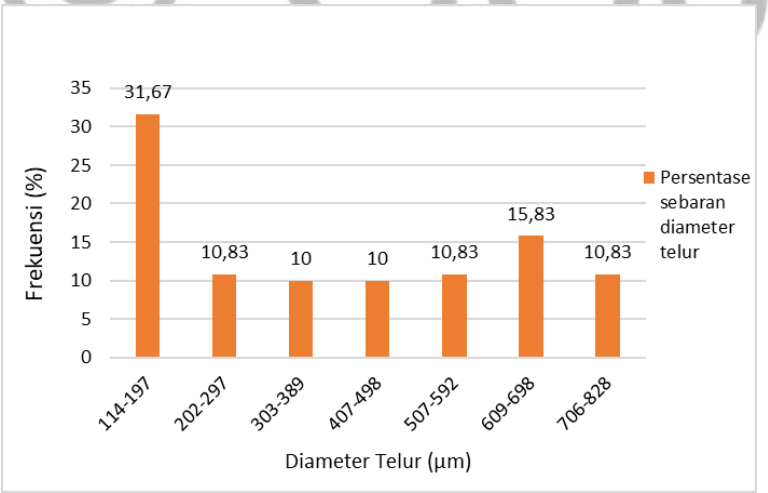


Figure 4. Distribution of seren fish egg diameter in GML III

Distribution of seren fish egg diameter in GML III (figure 4) shows the most egg diameter size, namely eggs with a diameter of 114-197 µm which are eggs in the previtellogenic phase of 31.67%. While other egg sizes have a percentage that is not so far that ranges between 10% -15% of the total eggs observed. In GML III, the most eggs are eggs in the previtellogenic phase with a total overall percentage of 42.5%. While egg size with vitellogenic phase has a total percentage of 20% and egg size with mature phase has a total percentage of 37.49%. It can be seen that there are groups of eggs in GML III that indicate seren fish are partial spawner fish.

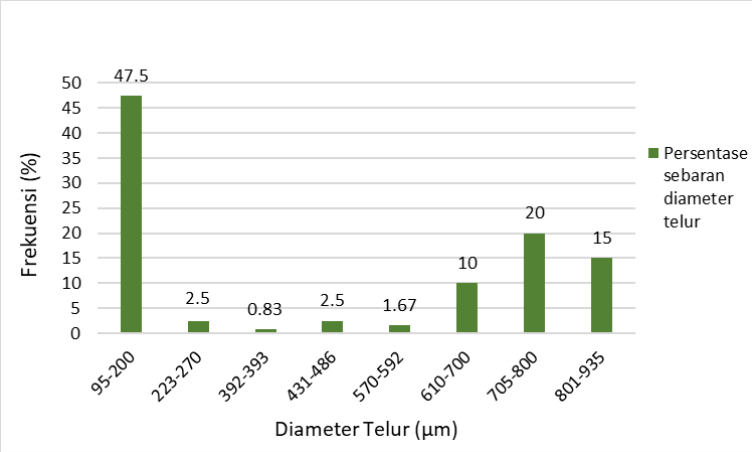


Figure 5. Distribution of seren fish egg diameter in GML IV

Distribution of seren fish egg diameter in GML IV (figure 5), it is clear that there are two different groups of eggs, namely small oocyte groups (phase I&II) and large oocytes (phase IV&V). In the distribution of GML IV seren fish eggs not much different from the distribution of egg diameters in GML III. The highest mode remains in the previtellogenic phase with a total percentage of 50% while the total percentage in the vitellogenic phase of 5% can be said that in GML IV it is clear that there are different groups of eggs. In the mature phase in GML IV by 45%.

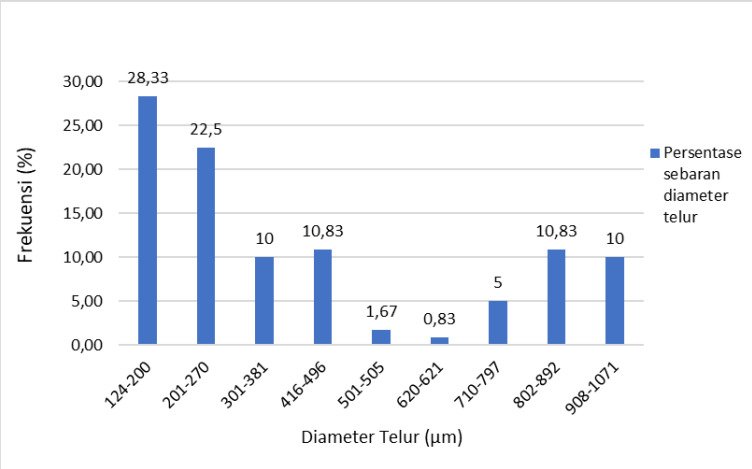


Figure 6. Distribution of seren fish egg diameter in GML V

From the data distribution diameter of GML V seren fish eggs (figure 6) there are still two oocyte groups present in GML V seren fish namely small oocyte groups (phase I & II) and large oocyte groups (mature phase). Seen from the data above the highest mode in the distribution of GML V seren fish eggs is in the previtellogenic phase with a total percentage of 60.3%, then the total percentage of the next phase is the vitellogenic phase of 13.33% and the mature phase which has a total percentage value of 25 , 83%. This reinforces that seren fish are fish that have a partial spawner spawning type. Effendie (1997) in Unus and Omar (2010) states that in fish and invertebrates, there is often a bimodal egg diameter distribution or two modes, namely the first mode consists of immature eggs and the second mode consists of ripe eggs. This spawning model is called partial spawning.

Seren Male Testicles

Samples of male seren fish obtained from Jatigede reservoir amounted to three samples and have similarities in the development of the gonad that is mature / ready to spawn. Can be seen how the gonad cross section of male seren fish that has been cature in Figure 7.

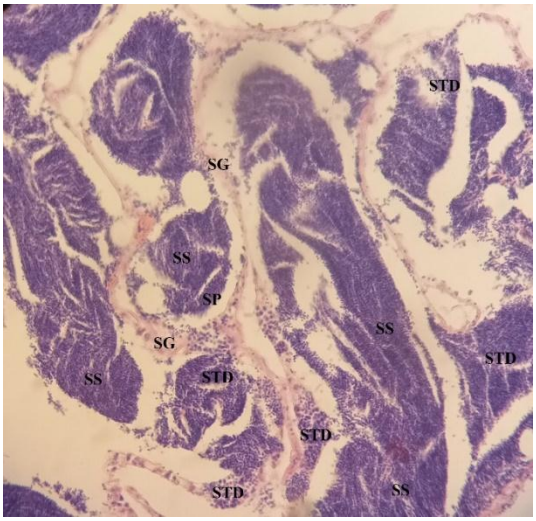


Figure 7. Testicular cross section of male seren fish
Note: SP = Primary Spermatocytes, SS = Secondary Spermatocytes, STD = Spermatids, SG = Spermatogonia.

Based from the observation of seren fish testes, there are many secondary spermatocytes in the seren fish testicles. This shows that male seren fish are in a mature / ready to spawn condition. In accordance with research conducted by Rahmawati (2014) on stingray waders (*R. lateristriata*), gonadal testicles have more spermatogonium cysts in their testes, whereas testes in gonad's mature cells will have many secondary spermatocytes and spermatid cells in the testes while the composition of spermatogonia cysts is less.

Conclusion

Based on the results obtained, it can be concluded that seren fish from Jatigede reservoir have five stages of oocyte development consisting of chromatin nucleolus phase, perinuclear phase, alveoli cortical phase, vitellogenesis phase, and mature phase. Seren ovarian development types are synchronous groups and partial spawner spawning types.

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