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Analysis of Reproductive Performance of the Nile Tilapia (*Oreochromis niloticus*) in the Application of Biofloc and Non-Biofloc Technology

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ABSTRACT
This study evaluated the reproductive performance of the Nile tilapia (*Oreochromis niloticus*) in biofloc and non-biofloc systems. An experimental approach was used, with fish cultured under controlled conditions and reproductive parameters—fecundity, egg diameter, and gonadal maturity—analyzed at 3, 4, and 5 months of age. Data were statistically assessed using an Independent Sample T-Test. The results showed no significant differences between the two systems in fecundity, egg diameter, gonadal maturity levels, or gonadal maturity index ($P > 0.05$). However, the highest fecundity was observed in the non-biofloc system, while the largest egg diameter was recorded in the biofloc system. The histological analysis of gonadal maturity levels indicated variations in developmental stages between the two systems, though the gonadal maturity index did not significantly differ. These findings suggest that both culture methods effectively support reproductive performance. Beyond protein, macronutrients such as lipids and vitamins should be considered, as deficiencies may hinder reproductive productivity. Optimizing culture conditions and nutrient balance is crucial for sustainable and efficient tilapia aquaculture. Further research is needed to refine biofloc applications and to enhance long-term reproductive outcomes.

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is one of the most widespread and popular freshwater fish commodities in aquaculture due to its high economic value **(Putra *et al.*, 2022)**. The advantages of tilapia include relatively fast growth, good environmental tolerance, large body size, as well as high survival ability and easy maintenance **(Rahman *et al*., 2021)**. Tilapia has a reproductive type known as partial spawner or batch spawner that allows various tilapia species to spawn several times a year and to produce different numbers of eggs in each spawning cycle **(Saroni *et al.*, 2024)**. Factors that influence reproductive quality and the number of larvae include environmental conditions and the quality of feed provided **(Sefianingsih *et al.*, 2023)**. In addition, the growth and reproductive performance of tilapia is highly dependent on external factors such as feed quality and the environment in which the fish are cultivated. For example, feed that has a high protein content during the resting period can significantly improve fish reproductive performance **(Izquierdo *et al.*, 2022)**. The reproductive quality of individuals classified as batch spawners shows ovaries with asynchronous patterns that contain oocytes at all stages of maturity without the dominance of certain populations **(Salamah & Zulpikar, 2020)**. Therefore, optimal feed selection is very important to improve the reproductive quality of fish **(Izquierdo *et al.*, 2022)**.

Tilapia culture methods are also faced with challenges, one of which is the problem of pollution due to the use of waste water containing ammonia and nitrogen from the remains of feed and fish feces **(Sunaryo & Kristianto, 2022)**. As a solution, the use of biofloc technology has been proposed to address this issue. Biofloc technology serves to convert toxic nitrogenous waste into bacterial proteins that fish can utilize as a feed source. The heterotrophic bacteria involved in this process can grow in the presence of sufficient carbon, which increases the carbon to nitrogen ratio (C/N ratio) in the culture system **(Saha *et al*., 2022)**. The application of bioflocs not only reduces ammonia levels in water but also provides a source of single-cell protein to support fish growth **(Astarini *et al.*, 2024)**. Protein is an important component in fish nutrition that supports growth, reproduction, and other physiological functions **(Munaeni *et al.*, 2024)**. In the process of vitelogenesis, oocyte development is highly dependent on the intake of protein and energy obtained from food, which in turn affects ovarian maturation **(Munaeni *et al.*, 2022; Putra *et al.*, 2022)**. Research shows that reproductive performance can be improved by the contribution of bioflocs that function as "native protein" **(Saroni *et al.*, 2024)**. In addition to micronutrients, other chemical compounds in bioflocs also play an indirect role in reproduction, one of which is polyhydroxybutyrate (PHB). This compound is believed to be utilized as an alternative energy source for tissue synthesis and cellular repair **(Suehs *et al*., 2025)**. As a result, protein absorption can be optimized, allowing fish to allocate more nutrients for growth and reproduction.

Research on the development of reproductive organs in the Nile tilapia (*Oreochromis niloticus*) under two different aquaculture systems, namely non-biofloc and biofloc, is crucial to supporting the sustainability of aquaculture systems and enhancing productivity from upstream to downstream. The biofloc system has been proven to improve water quality, provide additional nutritional sources, and optimize fish growth and health, including reproductive aspects **(Astarini *et al*., 2024)**. Environmental factors and nutrient availability in the rearing medium play a significant role in fish reproduction, particularly in gonadal maturation and oocyte development **(Purbomartono *et al*., 2023)**. Furthermore, previous studies have shown that reproductive success is highly dependent on the interaction between feed quality, gut microbiota, and protein utilization efficiency in fish **(Munaeni *et al*., 2024)**. Understanding how biofloc and non-biofloc systems affect the reproductive organs of the Nile tilapia will allow for the development of more efficient, environmentally friendly, and sustainable aquaculture strategies to support food security and the aquaculture sector as a whole.

MATERIALS AND METHODS

Experimental design

This study employed a stratified random sampling design. Stratified random sampling involves dividing the population into homogeneous groups, where each group consists of subjects with similar characteristics. Samples are then randomly selected from each group. The purpose of this design is to ensure that the sample proportion remains aligned with the population proportion **(Suriani *et al*., 2023)**. This design is commonly used when there is significant variation within the population **(Firmansyah & Dede, 2022)**. The samples used in this study consist of the Nile tilapia (*Oreochromis niloticus*) cultivated under biofloc and non-biofloc systems. Each culture system includes three age groups (strata): 3, 4, and 5 months.

Stratified random sampling biofloc system and non-biofloc system

The experiment was conducted to compare biofloc and non-biofloc culture systems across three Nile tilapia age groups: 3, 4, and 5 months. The experimental procedures were as follows:

Three round tarp tanks, each with a diameter of 3 meters and a height of 1.2 meters, were prepared, resulting in a total water volume of approximately 11.3 m^3 (11,300 liters) per tank. Nile tilapia were stocked at a density adhering to the recommended standard for biofloc and non-biofloc systems, ranging from 100 to 150 fish per cubic meter (100–150 fish/m^3). This stocking density yielded approximately 1,130–1,695 fish per tank, adjusted according to optimal capacity and management practices.

Following stocking, 10% of the total fish population from each tank (approximately 113–170 fish per age group) were randomly sampled and transferred to separate observation tanks. These fish were monitored closely to assess growth performance and overall health.

Throughout the one-month experimental period, water quality parameters—including temperature, pH, dissolved oxygen (DO), and floc density—were recorded weekly. Additionally, in the final week, at least 11 fish from each age group were randomly sampled for histological analysis to evaluate gonadal development.

These procedures enabled a comprehensive assessment of the effectiveness of biofloc versus non-biofloc systems in supporting the growth, health, and reproductive development of Nile tilapia at different developmental stages.

Fish sampling criteria

Fish samples were selected based on specific criteria to ensure uniformity and reliability in the experimental results. The jJuvenile Nile tilapia (*Oreochromis niloticus*) were sourced from the same hatchery to maintain genetic consistency. The selection process involved measuring body weight and total length, ensuring that only individuals within a predetermined size range were included to minimize variability in growth and reproductive development. 3 and 4 months old: Approximately 15–20cm in length and 100–150 grams in weight. 5 months old: Approximately 20–25cm in length and 200–250 grams in weight. Fish were visually inspected for any external deformities, lesions, or abnormal swimming behavior, and only healthy individuals without signs of disease or stress were chosen. Additionally, a brief acclimatization period was conducted before the experiment to allow the fish to adapt to the experimental conditions, reducing potential stress-related factors that could affect reproductive performance.

Reproductive parameters, including fecundity, egg diameter, and gonadal maturity, were analyzed at 3, 4, and 5 months of age. Data were statistically assessed using an Independent Sample T-Test to determine significant differences between the two culture systems, providing insights into the impact of biofloc or non biofloc technology on reproductive traits.

Fecundity

The female broodstock was dissected to obtain the gonads. The gonads were divided into 3 parts, namely anterior, middle, and posterior. The measurement results were recorded to be processed data using the formula for calculating egg fecundity **(Parveen *et al.*, 2022)** as follows: Fecundity = weight of gonads (W)/weight of some gonads (w)*egg sample (n).

Egg diameter

Egg diameter was measured using a binocular microscope (Olympus DP22) whose results came out directly on the Olympus CellSens application connected to the computer in the form of microns. The diameter of the egg on the microscope showed the development of the oocyte and was then measured horizontally and vertically for having an imperfect round shape (1). The diameter was measured 5 times in each field of view (2). Each age of fish in the culture system used 3 fields of view.

Histological observation of gonadal maturity levels

Observation of the level of gonad maturity through histology was done using haematoxylin-eosin staining. The first stage of fish gonad samples were immersed in 10% NBF (Neutral Buffered Formaline) solution for at least 24 hours. The next stage was dehydration (70% alcohol I, 70% alcohol II, 90% alcohol I, 90% alcohol II, absolute alcohol I, absolute alcohol II) for 45 minutes each. After the dehydration stage, then the clearing stage (Xylol I, Xylol II) was carried out for 45 minutes each, next was embedding and blocking (liquid paraffin), slicing (sectioning) using a microtome with a thickness of 4-6 microns and placing it on an object glass, and the last stage was staining using Hematoxylin-Eosin (HE) and closing **(Rahmawati, *et al*., 2022).**

Gonadal maturity index observation

Gonad maturity was assessed by initially weighing each female broodstock to obtain the total body weight. Subsequently, fish were dissected to extract the gonads, which were weighed separately. The gonad maturity index (GMI) was calculated using the method described by **Putra *et al.* (2022)**, where the weight of the gonads was expressed as a percentage of the total body weight of the fish, using the following formula:

$$\textbf{GMI (\%)=Gonad Weight (g) / Body Weight * 100}$$

Water quality

During the study, water quality parameters measured included temperature, pH and dissolved oxygen (DO) levels. Measurements of temperature, pH, TDS (Total Dissolved Solid) and dissolved oxygen (DO) levels were conducted weekly. Tools used for measurement included a thermometer for temperature, pH meter for pH, TDS meter to measure TDS and DO meter for dissolved oxygen level. Observations on water quality were made over a period of 30 days or throughout the duration of the study. Observations of flocs were made by measuring the volume of flocs formed at various densities. A total of 20mL of water sample was deposited in a measuring cup for 30 minutes, then the volume of floc that settled was recorded and calculated.

Data analysis

Data were analyzed using an Independent Sample T-Test to assess the reproductive performance of tilapia in biofloc and non-biofloc systems at 3, 4, and 5 months of age. If $P < 0.05$, there was a significant difference in male gonad development in a given age group; otherwise, the difference was not significant.

RESULTS

Fecundity

Tilapia fecundity data in biofloc and non-biofloc systems were analyzed using an unpaired t-test in SPSS, showing insignificant differences at each age observed. The highest fecundity value for 3-month-old fish in the non-biofloc culture system was 578 with an average body weight of 51.66 grams and gonad weight of 1.22 grams. Fish aged 4 months obtained the highest fecundity value in non-biofloc culture system fish of 804 having an average body weight of 75 grams and gonad weight of 1.67 grams. The highest fecundity value obtained at the age of 5 months is the non-biofloc culture system fish of 1350, with an average body weight of 86.66 grams and gonad weight of 3.13 grams. The fecundity value obtained in tilapia reared in biofloc and non-biofloc culture systems, the results are shown in the following graph (Fig. 1).

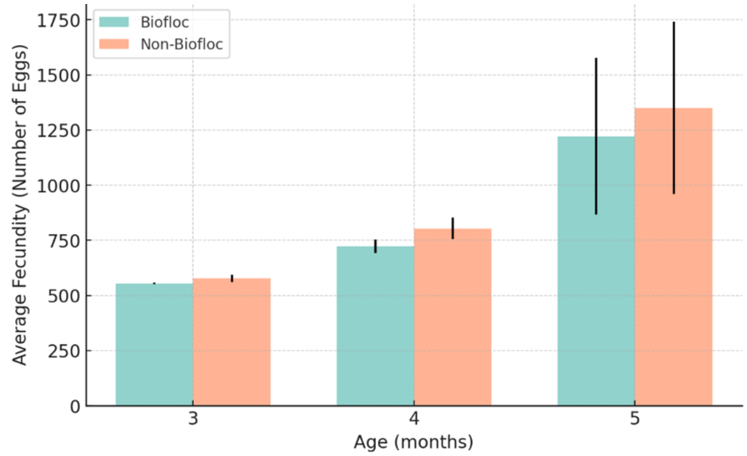


Fig. 1. Average of fecundity

Egg diameter

Tilapia egg diameter data in biofloc and non-biofloc systems were analyzed using an unpaired t-test in SPSS, showing unsignificant differences at each age observed. Based on the graph in Fig. (2), the largest egg diameters in tilapia aged 3, 4, and 5 months in the biofloc system were 260.21, 304.178, and 1093.752µm, respectively. Calculation of the average egg diameter from three fields of view showed similar results between biofloc and non-biofloc systems, as shown in the following graph (Fig. 2).

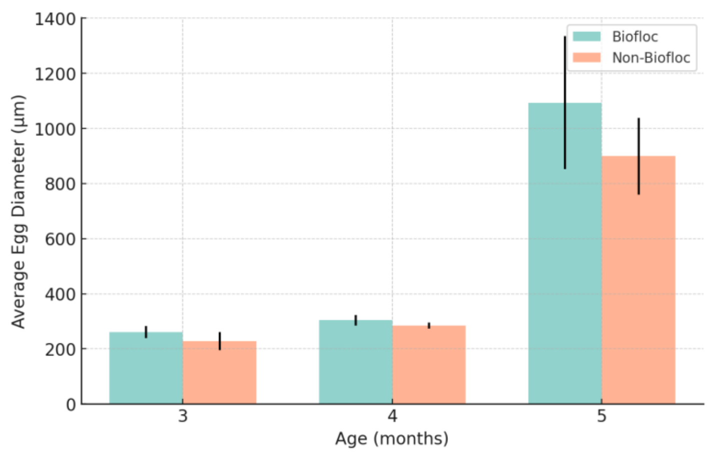
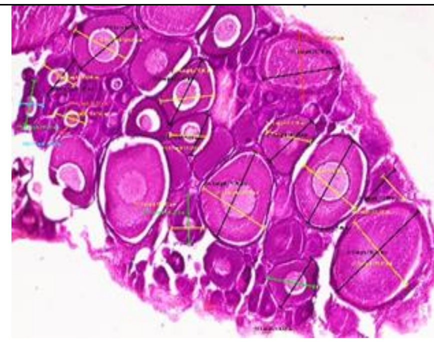
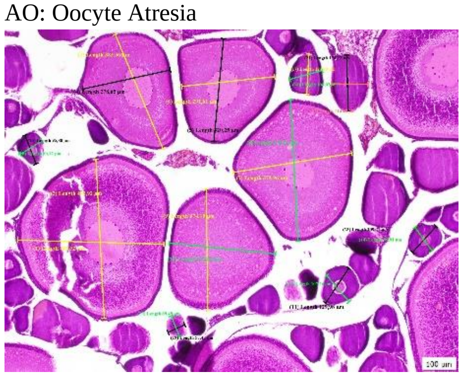
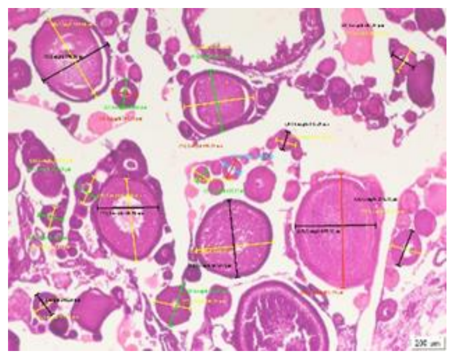


Fig. 2. Average of egg diameter

Histology of gonadal maturity level

Observation of the level of gonadal maturity through the histology method was carried out to determine the dominance of the level of oocyte maturity in detail. This method provides detailed information about egg development. The stages of female gonadal oocyte development according to **Nibamureke and Wagenar (2021)** include OI (Early oocyte), OII (Perinucleus), OIII (Cortical Alveolus), OIV (Vitelogenic oocyte), and OV (Mature oocyte). Histology of the gonads of tilapia aged 3, 4, and 5 months was observed using a binocular microscope with a magnification of 40x-100x. The results of the analysis in Table (1) show that the stage of oocyte development in tilapia in biofloc and non-biofloc systems is different at each age.

Table 1. Histology results of female gonads of tilapia biofloc system

Age	Biofloc	Non Biofloc
3 months	 OI: Early oocyte OII: Perinucleus OIII: Cortical Alveolus OIV: Vitellogenic oocyte AO: Oocyte Atresia	 OII: Perinucleus OIII: Cortical Alveolus
4 months	 OI: Early oocyte OII: Perinucleus OIV: Vitellogenic oocyte	 OI: Early oocyte OII: Perinucleus OIII: Cortical Alveolus OIV: Vitellogenic Oocyte AO: Oocyte Atresia
5 months	 OV: Mature oocyte	 OIV: Vitellogenic Oocytes OV: Mature Oocyte

Gonad maturity index

The average gonadal maturity index of the Nile tilapia in the biofloc system at 3 months of age was 0.36%, at 4 months was 0.62%, and at 5 months was 2.42%. Meanwhile, in the non-biofloc system, the values were 1.58, 2.12, and 3.46%, respectively. Based on the measurement results of the gonadal maturity index, it was found that each age group had a non-significant index range, as indicated by the t-test results with significance values (2-tailed) > 0.05, namely 0.179, 0.105, and 0.082. Overall, the gonadal maturity index at each age did not differ significantly between the biofloc and non-biofloc systems.

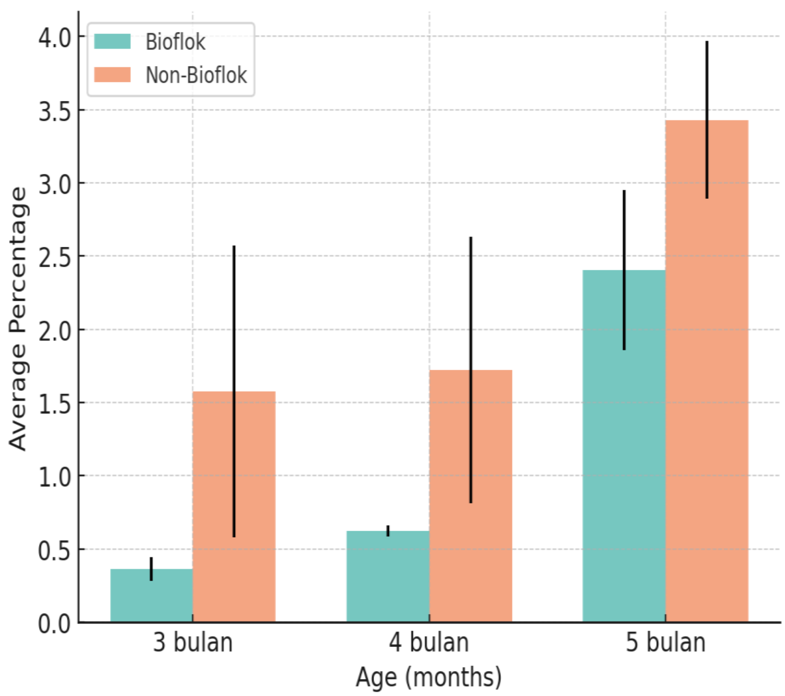


Fig. 3. Average of gonad maturity index

Fish gonad oocyte size

The size of oocytes in female fish gonads can be known through histology preparations of fish egg diameter. The size of oocyte development was analyzed mophologically according to **Arianti *et al*. (2017)**. The results obtained are shown in Table (2).

Table 2. Diameter size of oocytes

Biofloc diameter range (µm)			
Oocyte stage	**Age (month)**		
	3	**4**	**5**
OI	78,875±53,39	87,96±62,305	
OII	91,895	118,62±91,255	
OIII	344,62±148,565	204,395±156,155	
OIV	603,635±308,425	442,72±218,35	433,9±414,505
OV			1586,02±785,085
Oocyte Dominance	OII&OIII	OIII&OIV	OV
Non Biofloc Diameter Range (µm)			
Oocyte Stage	**Age (month)**		
	3	**4**	**5**
OI			146,81±106,23
OII	240,845±100,45	275,22±129,24	
OIII	367,23±250,94	393,65±310,185	
OIV	541,935	932,6±516,955	407,745
OV			1377,375±587,165
Oocyte Dominance	OII&OIII	OIV	OIV & OV

Water Quality

Water quality measurements on biofloc and non-biofloc rearing media are exhibited in Table (3).

Table 3. Water quality of biofloc cultivation system

Parameter	Unit	Biofloc	Non Biofloc	Literature
pH	-	6-6,8	8,3-9,1	6,5 - 8,5 (SNI-2009)
Suhu	°C	26,2-29,9	29,5-31,2	25 – 32 (SNI-2009)
DO	ppm	5,11-7,11	8,25-10,67	>3 (SNI-2009)
TDS	ppm	273-720	166-185	1000 ppm (PP No 82 tahun 2001)
Volume Flok	%	3-6%	-	<8% (Sumitro *et al*., 2021)

DISCUSSION

Based on the analysis of reproductive performance in Nile tilapia (*Oreochromis niloticus*) cultured under biofloc and non-biofloc systems, fecundity results showed no significant difference between the two systems. Statistical analysis using the independent t-test for all three age groups (3, 4, and 5 months) yielded significance (sig.) values greater than 0.05 ($P > 0.05$), indicating that the cultivation system (biofloc vs. non-biofloc) had no statistically significant impact on fecundity. Analysis of egg diameter in Nile tilapia (*Oreochromis niloticus*) across biofloc and non-biofloc cultivation systems indicated no significant differences. Statistical testing using independent t-tests for all three age groups resulted in significance (sig.) values (2-tailed) greater than 0.05 ($P > 0.05$). Despite the lack of statistically significant differences, larger average egg diameters were consistently observed in fish reared under the biofloc cultivation system across all age groups. This outcome suggests that fecundity and egg size may not be solely influenced by parental nutritional supply and water quality conditions, but could also be affected by other factors associated with the biofloc environment. Fish that have the same genetics can produce different numbers of eggs because the nutritional supply received by each female parent is different. Fish at the age of 3-4 months (young age) have a smaller fecundity value than fish at the age of 5, this is because the utilization of feed in young fish is focused on growth **(Rohma *et al*., 2012)**. In addition, fecundity is closely related to diameter size; if the fecundity value is low, then the size of the egg diameter is large and vice versa. Microorganisms that make up biofloc can be utilized as an additional feed for fish; in some studies biofloc contains essential amino acids, antioxidants, and vitamins **(Ekasari *et al*., 2016)**.

The nutrients received by the mother will increase the yolk content, resulting in an increase in diameter. The biofloc culture system contributes as an additional feed that supports survival, development, and reproduction. In some studies, biofloc has the protein and HUFA content needed in the development of female fish gonads. Research by **Cardona *et al.* (2016)** has similar results, namely biofloc has fatty acid nutrients that can support gonadal development, especially an increase in ovarian size, but the differences in each study are due to differences in the composition of microorganisms that make up biofloc. Diameter size correlates with fecundity value, the acquisition of high fecundity value, the size of the egg diameter is small and vice versa. Additionally, the diameter of the egg affects the survival of the resulting larvae. The size of the large egg diameter according to **Muhardisah (2020)** has a greater quantity of food reserves for embryos and larvae when hatching.

The variation in oocyte size is thought to be due to tilapia having an asynchronus breeding type which can be recognized from the diverse oocyte development groups (two to three stages). Fish with asynchronus breeding type have a long spawning cycle if environmental conditions are favorable. Oocyte development according to **Soeprijanto *et al.* (2022)** is divided into 5 stages, which are grouped into primary growth and secondary growth. Primary growth itself is oocyte stage 1 to stage 2, while secondary growth is oocyte stage 3 to stage 6. Stage 1 (chromatin nucleus) oocytes have a size of 5-25µm, stage 2 (perinucleus stage) oocyte size ranges from 50-205µm. Stage 3 (endogenous stage) has an oocyte size of 100-340µm, stage 4 oocytes range from 172-683µm. Stage 5 (exogenous vitellogenesis) and 6 (maturation) oocyte sizes are 214-970µm and 422-1965µm. Atresia is found at the 5th and 6th stage of gonadal maturity, in trout 30% of the total oocytes occur atresia. Follicular atresia is influenced by the reproductive cycle, temperature, and nutrient supply. The lack of cultivation media conditions causes the eggs to degenerate so that the atresia follicle layer will be reabsorbed. Ovarian development is influenced by nutrients consumed by the mother during the gonadal development phase. Floc aggregates according to **Cardona *et al.* (2016)** has a fat content that can be assimilated by the female parent during the growth period. Fat reserves owned by the mother in the spawning phase will be channeled to the ovaries as a raw material for the vitelogensis process. In addition, in the vitelogensis process according to **Santo *et al.* (2014)**, protein is needed as a raw material in the process of synthesizing yolk protein (vitellogenin) and choriogenin. High protein levels can increase the proportion of oocytes and the diameter of fish eggs.

Morphological classification of oocyte development stages followed the criteria outlined by **Nibamureke and Wagenar (2021)**, who categorized oocyte sizes into five distinct developmental stages. The early aocyte stage (OI) is the first stage to signal that meiotic division of prophase oogonia begins. The oocyte has a nucleus enveloped in a thin layer of cytoplasm (nucleus) which will be visible in the ovarian lumen followed by squamous follicular cells. The perinucleus stage (OII) is characterized by the development of the oocyte accompanied by the growth of nucleoli in the nucleus which have different numbers in each oocyte. A white ring will begin to appear around the nucleus. The final phase of stage (OII) will see the formation of yolk and fat granules in the cytoplasm, as a sign toward stage (OIII). Cytoplasmic development occurs in the cortical alveolus phase (OIII) as a result of the formation of fat and yolk granules around the nucleus. The position of the nucleus is in the center (core), in some nucleoli are attached to the membrane. This stage has an irregular shape and varies in size in some fish species. The zona radiata can be clearly seen. An increase in size, number of yolk granules and fat in the cytoplasm occurs in the vitelogenic oocyte (OIV) stage. The nucleus disappears at the mature oocyte (OV) stage, and the nucleolus exits into the cytoplasm, making the oocyte difficult to identify. Yolk granules fall off and spread to the ovarian lumen, thinning of the zona radiata occurs due to an increase in cell size, resulting in rupture of the follicular epithelium.

The pH value in the biofloc system is still at the optimal level, while the non-biofloc system is slightly higher. Fluctuations in pH value are related to the process of breaking down organic matter and respiration of fish that produce CO2. According to **Arifin (2016)**, the ideal pH value is 4-9, but for optimal growth in tilapia the ideal pH ranges from 6-8. The temperature in biofloc and non-biofloc systems is at an optimal level. This is in accordance with the statement of **Habibah *et al.* (2017)**, the optimal temperature range for tilapia is 28-32°C, while at a temperature of 37°C causes the ovaries of female tilapia to shrink. Water temperature according to **Soeprijanto *et al*. (2022)** plays a role in the performance of hormones in the reproductive system in species; in goldfish, temperatures below 14°C cause the vitelogenesis process to be inhibited.

Dissolved oxygen content in biofloc and non-biofloc systems is in the optimal range. Microorganisms that make up biofloc according to **Zaidy (2022)** require sufficient oxygen to convert organic matter, feed residues and fish metabolism. Total dissolved solids (TDS) in non-biofloc systems have low values, while in biofloc media, the TDS value is higher. Total dissolved solids (TDS) affect the level of water brightness in the media. When total dissolved solids (TDS) values approach 1000ppm, water transparency significantly decreases. Reduced transparency adversely affects photosynthesis, disturbs fish visibility, and leads to decreased fish appetite, potentially impacting growth performance and overall health **(Gusrina, 2008)**.

CONCLUSION

Based on the results of this study, it can be concluded that the reproductive performance of tilapia in biofloc and non-biofloc systems, assessed through fecundity, egg diameter, histological analysis of gonadal maturity level, gonad maturity index, and oocyte size, showed no significant differences based on the t-test at all age levels. These findings indicate that both culture systems can support tilapia reproduction with similar effectiveness. However, to optimize reproductive performance, it is crucial to reassess the chemical compounds present in biofloc that may influence fish reproduction. Additionally, for fish farmers aiming to focus on hatchery-based cultivation, enhancing macronutrients such as protein and fat, as well as micronutrients like vitamins, in the biofloc system is essential. This study serves as a valuable reference for aquaculture practitioners in developing more effective strategies to improve fish productivity and ensure the sustainability of the aquaculture industry.

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