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The Effect of Different Salinity Concentrations on the Embryogenesis Development of the Freshwater African Catfish, *Clarias gariepinus,* Burchell 1822

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ABSTRACT

The African catfish *Clarias gariepinus*(Burchell, 1822) is a freshwater fish, massively cultivated, and highly demanded in Southeast Asia countries, particularly Indonesia. The market demand for the commodity is annually increasing, hence, it can provide better profits in the future. To anticipate a decrease in seed production, it is necessary to carry out a seed procurement process to ensure that production levels continue to increase in terms of quantity and quality. Therefore, this study aimed to determine the effect of different salinities on the embryogenesis phases of catfish *C. gariepinus* and the quality of the larvae produced. The experimental method used was a completely randomized design with five treatment groups and four replications. The salinity levels used included D0- D4, with concentrations of 0 (control), 2, 4, 6, and 8ppt, respectively. The results showed that the different treatments had significant effects on the speed of embryonic development and the quality of the larvae produced. The highest average percentage of abnormality was found in D4 (2.0%), followed by D3 and D2 with values of 1.25% and 0.5%, respectively. Meanwhile, no abnormal larvae were detected in D0 and D1. It was concluded that the increase in salinity is inversely proportional to the period of embryogenesis. Meanwhile, an increase in salinity is directly proportional to larval abnormalities. Further study on the effect of different salinity on larval physiological adaptability is recommended.

INTRODUCTION

Freshwater African catfish, *Clarias gariepinus*, Burchell 1822 is a phenomenal catfish that is still highly demanded by the Indonesian people **(Suratno & Putra, 2022)**. The species is wildly cultivated by people in Southeast Asia countries, such as Indonesia, Thailand, Malaysia and Vietnam **(Muchlisin *et al.*, 2014)**. Since its introduction in the 1980s, the catfish culture of *C. gariepinus*, Burchell 1822, is still the most produced species compared to other local catfish species such as *C. batrachus* and *meladerma* **(Iswanto, 2004)**. This is because it has faster growth, tolerance to low environmental quality, and disease resistance compared to other local species, which have low growth and are intolerant to pathogens **(Weerd, 1995; Lenormand *et al.*, 1999)**. From 2012-2018, the production of Indonesian catfish increased annually by 38% starting from 2012

with 441,217 tons. In 2013, 2014, 2015, 2016, 2017, and 2018, the rate increased by 543,774, 679,379, 719,619, 764,797, 1,280,0009, and 1,771,867 tons, respectively. This indicates that the Indonesian catfish production from 2012 to 2018 increased by 88.4%; while from 2019 to 2020, an increase was recorded of 1.49 million tons (16.8%) **(MMF, 2020)**. To anticipate a decline in seed production in the future, it is necessary to design a strategy from scientists to ensure the continuity of production increase in terms of quantity and quality every year.

A world significant problem facing catfish hatcheries, especially in Indonesia is the inadequate supply of catfish seeds. This is due to the high mortality of eggs during the embryonic and larval stages **(Marimuthu *et al.*, 2019)**. The success of fish hatchery is closely related to the process of embryogenesis, which is influenced by internal and external factors. The internal factors include genetics, hormones, and egg yolk volume, while the external are pH, temperature and salinity **(Kamler, 2012)**. The hatching process occurs faster when the embryo in the shell is actively moving, and one of the influential factors is salinity **(Borode & Oyintoke, 2005; Su *et al*, 2013)**. Furthermore, salinity can affect the hatchability of fish eggs due to an osmoregulation process in fish eggs. Furthermore, the insertion of eggs from freshwater fish into high-salinity media can cause swelling. This happens due to a hyperosmotic state where the fluid outside the egg flows in, thereby leading to swelling and breaking **(Gracia *et al.*, 2004)**.

Salinity is one of the important factors affecting the metabolism, distribution, and survival rate of fish. Several studies about *C. gariepinus* focused on efforts to improve the performance of the parental gonads and the types of hormones that are effective for improving the reproductive performance of catfish **(Olaniyi & Akinbola, 2013; El-hawarry *et al.*, 2016; Natea *et al.*, 2017; Mosha, 2018; Zidan *et al.*, 2020; Abdel-latif *et al.*, 2021)**. Previous studies were also carried out on artificial insemination using cryopreserved sperm **(Muchlisin *et al.*, 2015; Müller *et al.*, 2019; Eka *et al.*, 2020)**. There are also several studies on the use of herbal ingredients to improve the reproductive performance of eggs and broodstock, such as African walnut (*Tetracarpidium conophorum*) **(Dada & Aguda, 2015),** *Telfairia occidentalis* leaf extract powder **(Dada & Ejete-Iroh, 2016)**, garcinia kola seed meal **(Dada, 2012)**, adding to curcumin and thyroxine hormone **(Rawung *et al.*, 2020)**. Some studies focused on materials that can be toxic to catfish embryos and larvae, such as agricultural pesticides **(Agbogessi *et al.*, 2013)**, buprofezin **(Marimuthu *et al.*, 2013)**, formalin (**Yisa *et al*, 2014)** and gold nanoparticles **(Marimuthu *et al.*, 2020)**.

In addition, several studies have been carried out on the influence of environmental parameters. **Marimuthu *et al.* (2019)** reported that differences in pH have a significant effect on the hatching and survival rate of *C. gariepinus* with a recommended pH level of 6.7- 7.5 for an optimal hatching. On the other hand, **Robert *et al.* (2019)** revealed the effect of temperature and fertilizer on catfish eggs and embryos. Although research on the impact of salinity on *C. gariepinus* has been previously reported by **Borode *et al.* (2002)**, comprehensive and visually embryogenesis development studies on the effect of salt concentration in culture media on egg performance and catfish embryo have not been reported. Therefore, this comprehensive study is very important because it can provide scientific contributions and information to the aquaculture community regarding the description of embryogenesis, the level of abnormality in the larvae of African catfish, *C. gariepinus*. The purpose of the study was

to elucidate the effect of different salinities on the embryogenesis phases of catfish *C. gariepinus* and the quality of the larvae produced. The results are expected to support the improvement of sustainable hatchery business performance in the African catfish *C. gariepinus*, Burchell 1822, aquaculture environment.

MATERIALS AND METHODS

Experimental design

The catfish broodstock *C. gariepinus* was obtained from certified local hatcheries, and the samples were selected based on the previous standard, namely broodstock with matured gonads **(Marimuthu *et al.*, 2019)**. Spawning was carried out artificially using the synthetic hormone ovaprim, a synthetic analog of the gonadotropin-releasing agent (Syndel Laboratories, Canada) at a dose of 0.3mL/ kg and 0.2mL/ kg for females and males, respectively. The weight of the sample ranged from 0.9 – 1kg. The hormone was injected intramuscularly with a depth of 2- 2.5cm and a slope of 30- 45 degrees. Subsequently, the broodstock was incubated for 8 hours, and the male and female brood were separated to prevent the parents from spawning themselves. The sperm was then collected after 8 hours of injection, followed by rinsing and dilution in a physiological solution. To collect the egg, the female brood was stripped, and the abdomen was cleaned using dry tissue to avoid water contamination. They were then placed in a bowl, mixed with the sperm, and stirred using chicken feathers carefully to avoid breaking. To facilitate fertilization, clean water was added to the eggs, and they were rinsed 3 times to remove the remaining sperm.

The experimental design consists of five treatments and four replications with a total of 20 experimental units. Furthermore, the treatments were D0 with 0ppt (control), D1 with 2ppt, D2 with 4ppt, D3 with 6ppt, and D4 with 8ppt. The amount of salinity was determined using the method proposed by previous studies with few modifications **(Zhang *et al.*, 2010)**. The working procedures included preparation of hatchery containers and weighing of salt for salinity determination, spreading salt to egg hatching media, performing artificial spawning, stocking eggs, water quality management, identification of embryogenesis development, abnormalities, and data recap. The containers used were 20 mini aquariums, each of which was filled with 2 liters of water. The salt was then weighed, based on the salinity in the treatment, followed by the installation of aeration to supply dissolved oxygen in the hatching container.

The density of stocking was 50 eggs/ L of water, hence, the number of eggs in one hatching container was 100 to obtain a total of 2000. Before stocking, the aeration was removed to ease the samples immediately after they were settled to the bottom of the container. During the incubation period, the water in the hatchery was easily contaminated by the remaining sperm at fertilization. The high population of sperm can reduce water quality and interfere with the hatchability of catfish eggs, hence, siphoning was carried out. After water siphoning was refilled, water was taken from the prepared reservoir, based on the salinity of each treatment. The parameters of water quality included salinity, temperature, and pH, as reported in previous studies **(Salsabilla *et al.*, 2021)**.

Identification of embryogenesis development

The development of embryogenesis started from the cleavage phase (cell division), followed by morula, blastulation (blastoderm formation), gastrulation (closure of the yolk sac), and organogenesis until the embryo hatching stage. Observation of embryo development was carried out by taking random egg samples from the five incubation media for each treatment using a dropper and placing them on an object glass. It was then observed under a microscope integrated with a computer (Olympus Microscope CX23).

The observation of egg development was carried out 30 minutes after laying, then every 1 hour interval until they hatched **(Andriyanto *et al.*, 2013)**. The total incubation period was calculated from the time of first incubation until the eggs hatch completely. The classification of embryonic development was performed based on the method proposed by a previous study **(Okomoda *et al.*, 2018)**. Observation data on the development of catfish embryos *C. gariepinus* included descriptions of hourly embryonic development images covering each phase until hatching. Research data on the embryogenesis of the sample were descriptively recorded. Observations of abnormalities were carried out 5 days after the eggs hatched into larvae **(Marsela *et al.*, 2018)**. The level of larval abnormalities was calculated using the equation proposed by **Effendie (1979)**, as follows:

$$\text{Level of larval abnormalities (\%)} = \frac{\text{Number of abnormal larvae}}{\text{Number of total larvae}} \times 100\ \%$$

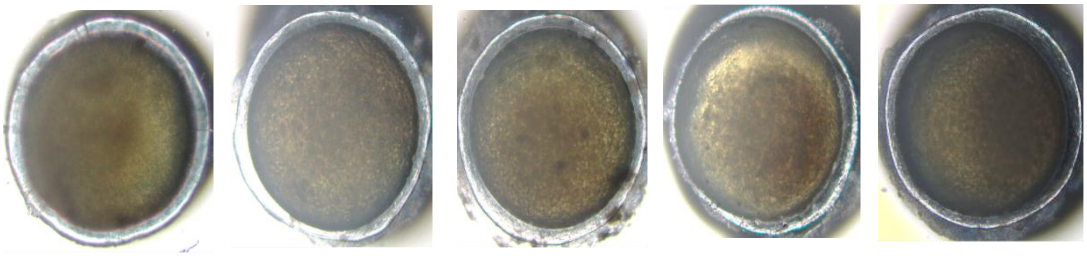
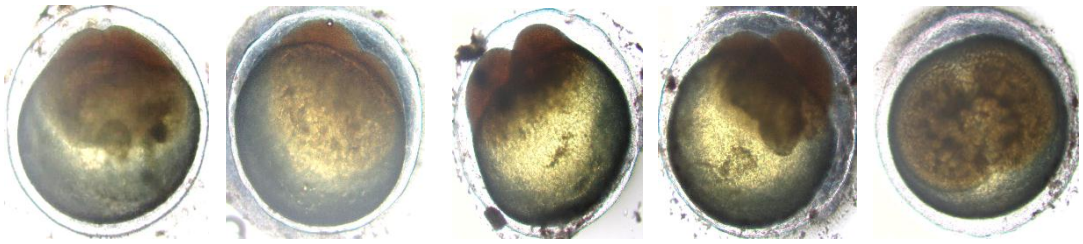
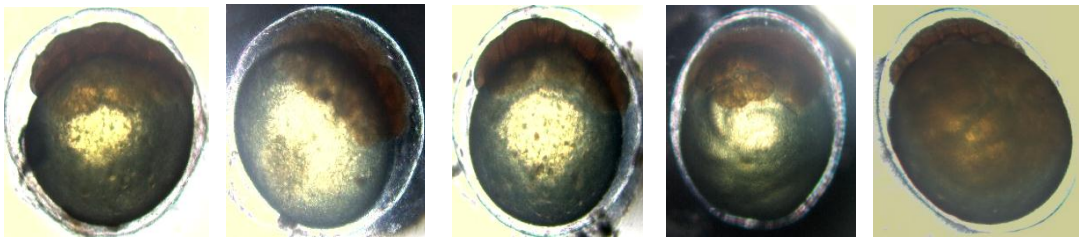
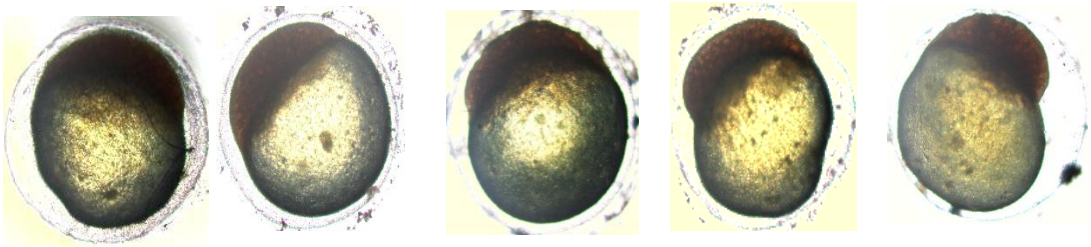
RESULTS

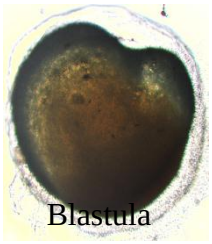
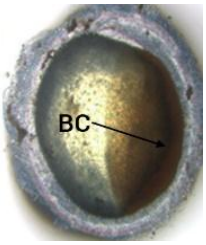
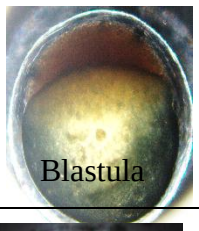
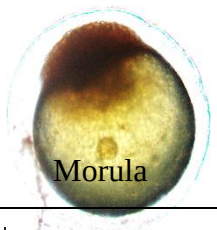
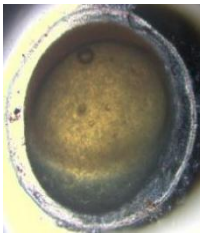
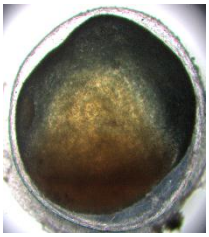
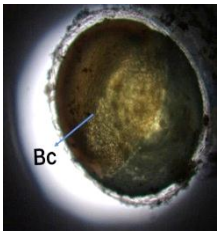
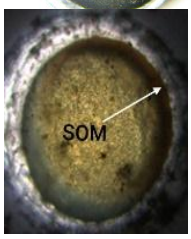
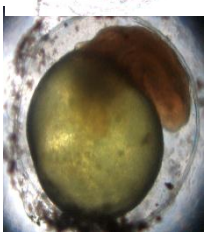
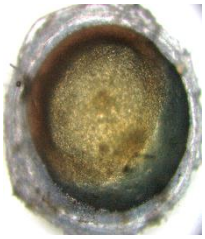
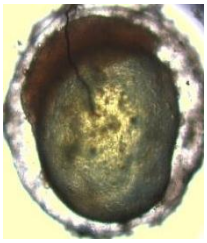
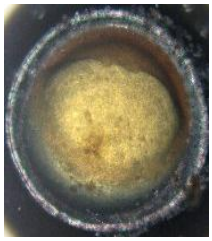
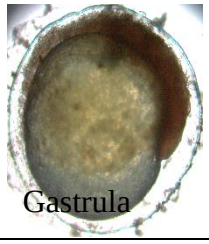
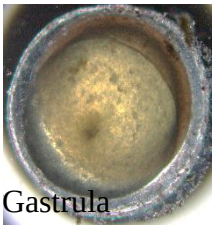
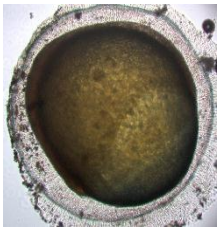
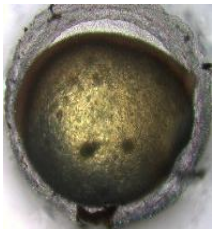
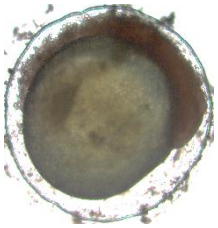
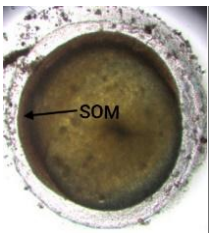
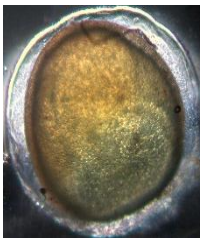
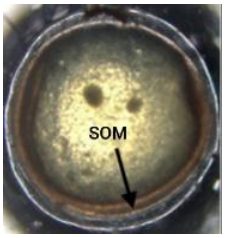
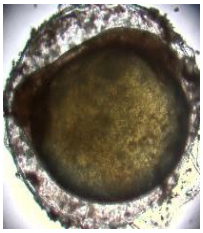
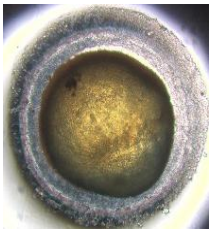
Embryo Development and hatching time at different salinities

Fig. (1) shows the results of observations on the embryogenesis development and hatching duration of *C. gariepinus* at different salinities, namely D0 (control), D1 (2 ppt), D2 (4 ppt), D3 (6 ppt) and D4 (8 ppt). The treatment with the fastest hatching time of 970 minutes was D3, followed by D4 at 1,150 minutes. Observation of embryo development began when the eggs were inserted into a container based on the treatment at 9.40 pm. At the 30th minute after stocking or 10.10 pm, the eggs underwent the first division phase from several levels, namely 2 cells in D0, D1, D2 and D4. Meanwhile, in the D3 treatment, the eggs entered the 4 cell division phase. The division into 16 cells occurred in D0, D1, D2, and D4; while for D3 (6 ppt), the 32 division phase was observed at 130 minutes or 11.10 pm.

At 00.10 am or 190 minutes after fertilization, the eggs in all treatment groups entered the morula phase. Furthermore, at 250 minutes or 01.10am, the samples in D0, D1, D2, and D3 entered the blastula phase, while D4 was still in the morula stage. The eggs in D0 and D3 entered the gastrula phase at 02.10 am or 310 minutes. At 08.10 am or 670 minutes after fertilization, the samples in D3 were in the organogenesis stage with the appearance of the tail and head, while D0, D1, D2, and D4 were still in the gastrula phase. At 11.10 am or 850 minutes, D2 and D3 treatments were in the organogenesis stage. For the D3 treatment, the embryo showed movement in the tail, while the shape of the eyes and tail appeared in D2. At 01.41 pm, the eggs in D3 hatched into larvae with a hatching period of 16 hours and 11 minutes. Meanwhile, in D0, D1, D2, and D4, the samples were still in the organogenesis phase. At 4.37 pm with the difference in hatching time from the previous treatment, namely 3 hours 18 minutes, the eggs in D4 hatched into

larvae after 19 hours & 7 minutes. The D0, D1, and D2 eggs were still in the organogenesis phase. At 5.26 pm after fertilization with a period of 1 hour & 3 minutes from the previous treatment, 4 samples in D2 hatched into larvae, with a hatching period of 19 hours 56 minutes. The eggs in D1 hatched after 20 hours & 42 minutes at 6.12 pm, with a time difference of 1 hour and 38 minutes from the previous treatment. At 8.45 pm, the samples in D0 hatched into larvae after 4 hours.

Time	Treatment					Duration (Minute)
	0 ppt	2 ppt	4 ppt	6 ppt	8 ppt	
21:40						40
22:10						70
	Fertilized egg 2 cells	Fertilized egg 2 cells	Fertilized egg 2 cells	Fertilized egg 4 cells	Fertilized egg 2 cells	
23:10						130
	16 cells	16 cells	16 cells	32 cells	16 cells	
00:10						190

	Morula	Morula	Morula	Morula	Morula	
01:10	 Blastula		 Blastula	 Blastula	 Morula	250
02:10	 Gastrula	 Blastula	 Blastula	 Gastrula	 Blastula	310
03:10	 Gastrula	 Gastrula	 Gastrula	 Gastrula	 Gastrula	370
04:10		 Gastrula	 Gastrula	 Gastrula	 Gastrula	430
05:10	 Gastrula	 Gastrula	 Gastrula	 Gastrula	 Gastrula	490
06:10						550

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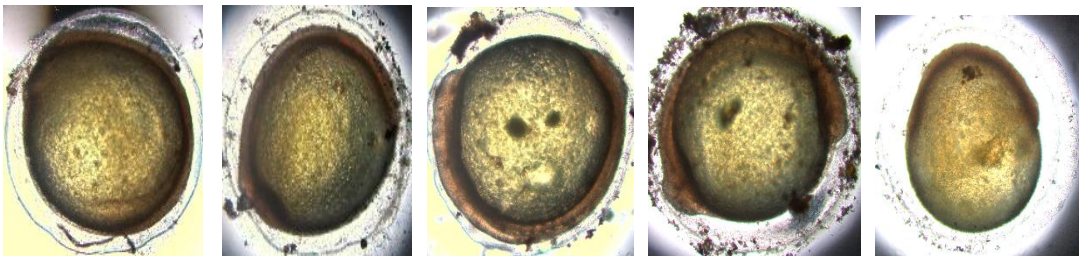
07:10						610
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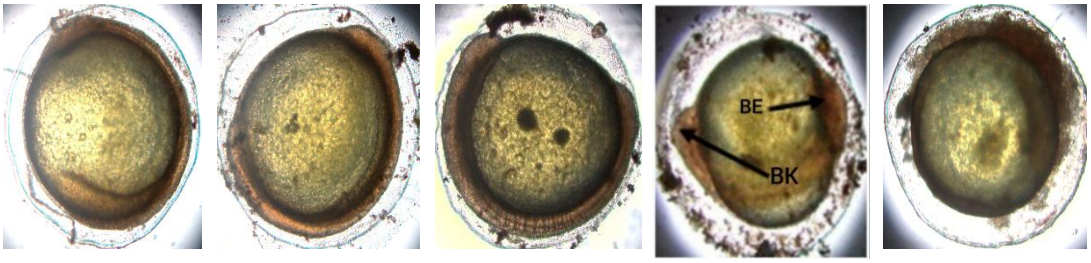
08:10						670
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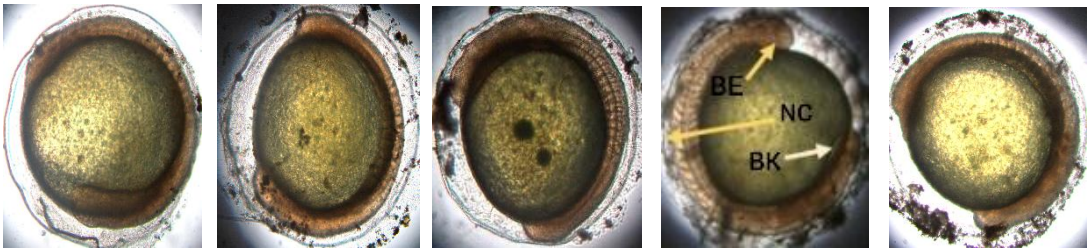
09:10						730
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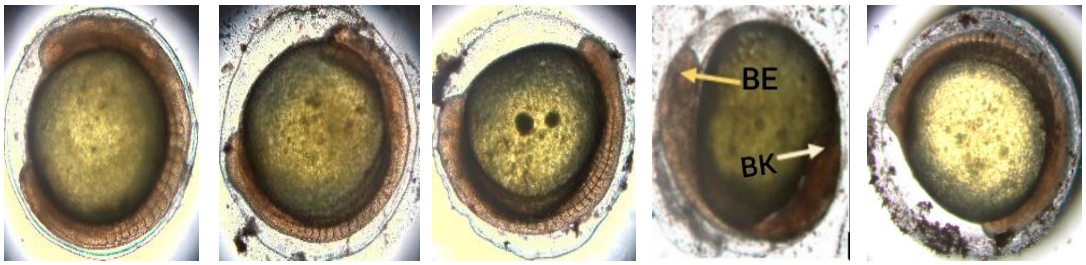
10:10						790
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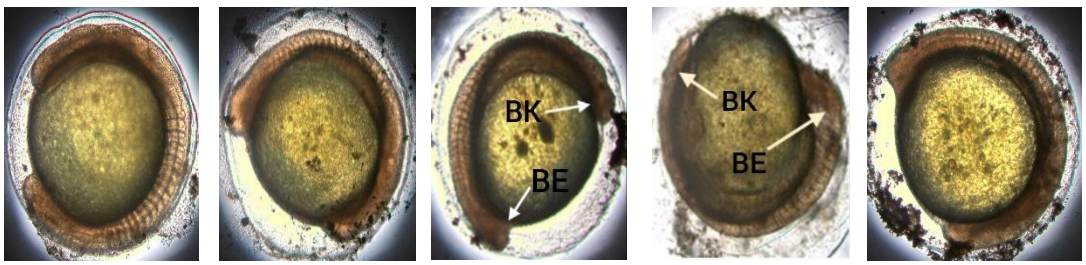
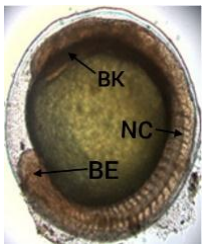
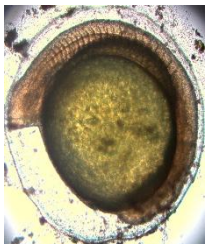
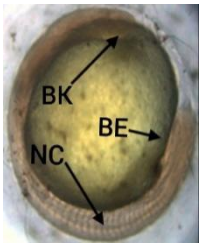
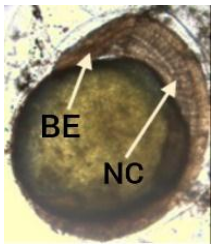
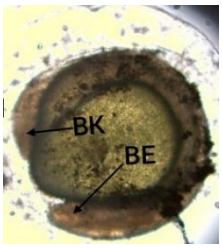
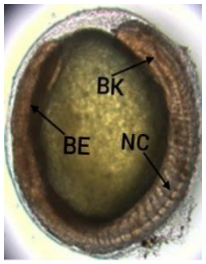
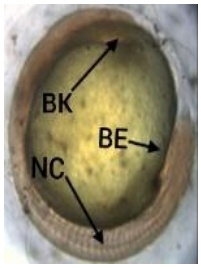
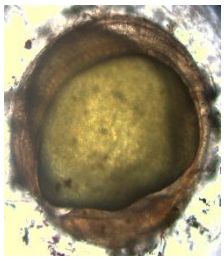
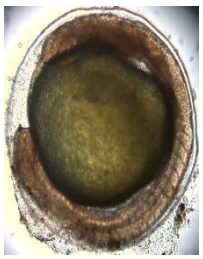
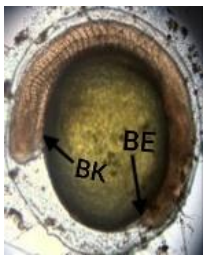
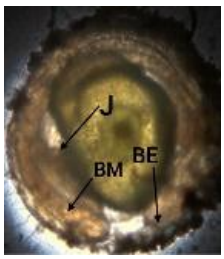
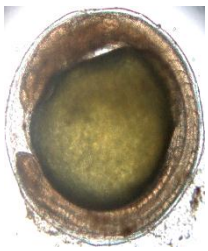
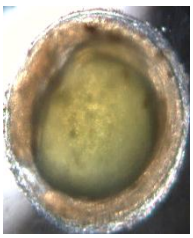
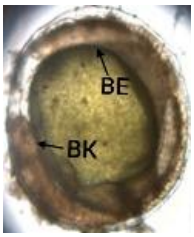
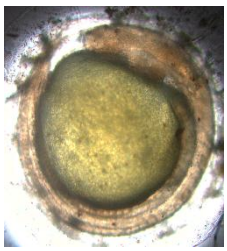
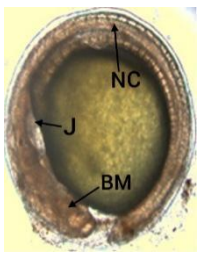
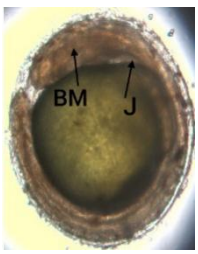
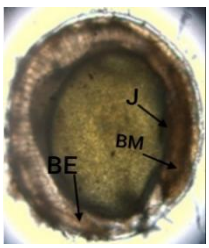
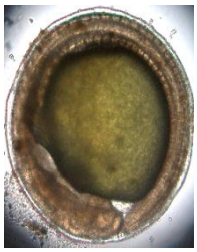
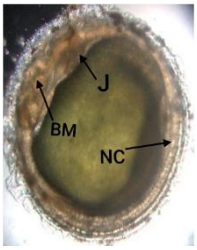
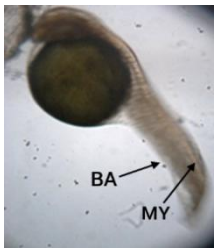
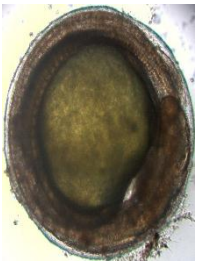
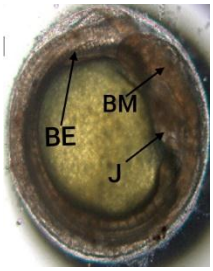
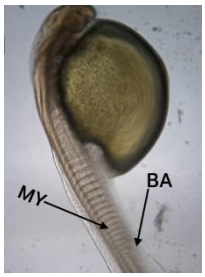
11:10						850
	Gastrula	Gastrula	Organogenesis	Organogenesis	Gastrula	

12:10						910
	Organogenesis	Organogenesis	Organogenesis	Organogenesis	Organogenesis	
13:10						970
	Organogenesis	Organogenesis	Organogenesis	Larvae hatch	Organogenesis	
14:10						1.030
	Organogenesis	Organogenesis	Organogenesis		Organogenesis	
15:10						1.090
16:10						1.150
	Organogenesis	Organogenesis	Organogenesis		Larvae hatch	

17:10	 Organogenesis	 Organogenesis	 Larvae hatch	1.210
18:10	 Organogenesis	 Larvae hatch		1.270
19:10	 Organogenesis			1.330
20:10	 Larvae hatch			1.390

Explanation: Eyes (BM), Tails (BE), Somit (SOM), Blastocoel (BC), Notochord (NC), Anus (BA), Myomere (MY), Heart (J),

Fig. 1. The stages of embryogenesis of *C. gariepinus* eggs at different salinities

Larval abnormality

Fig. (2) shows that there were no abnormal larvae in D0 and D1. This indicates that they were the appropriate salinity levels for the development of embryos and larvae of catfish *C. gariepinus.* In D0, the concentration of liquid in eggs and hatching media was the same, hence, the energy available was only used for embryo development.

Furthermore, the highest larval abnormality rate of 10.75% was obtained in D4, while a value of 7.25%, was recorded in D3.

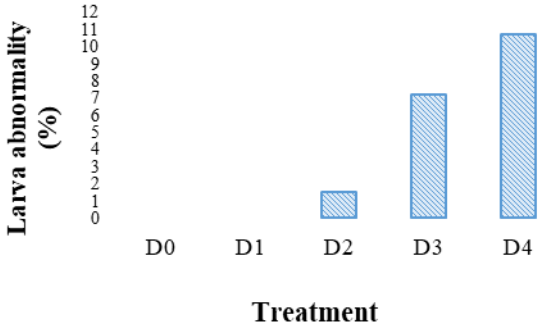


Fig. 2. The percentage of larval abnormality

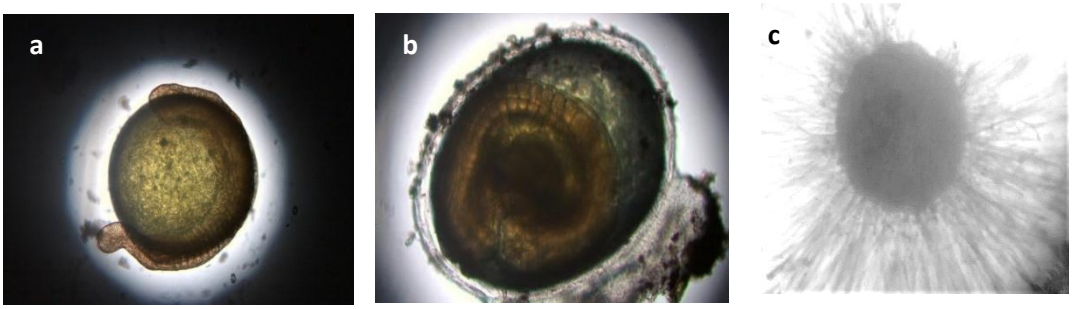


Fig. 3. The defective embryo (a), dead embryo (b) and fungus attack embryo (c)

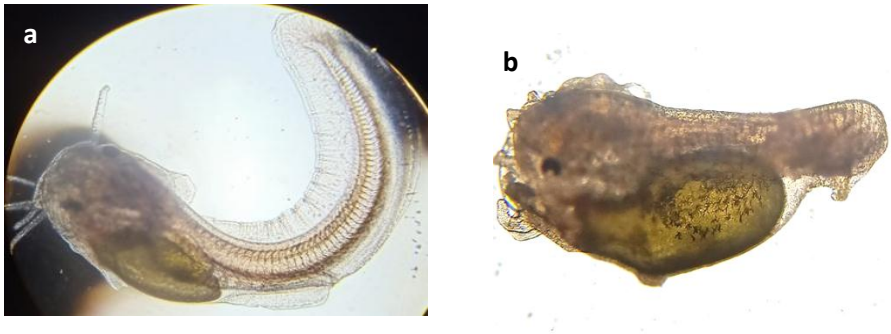


Fig. 4. The appearance of normal larval (a) and the abnormal larva (b)

DISCUSSION

Embryo Development and hatching time at different salinity levels

This study's results showed that salinity affected the egg development, hatching time, and larval deformation of *C. gariepinus*. Moreover, the larval development still has normal stages from cleavage, morula, blastulation, gastrulation, and organogenesis to

embryo hatching and exiting the eggshell. The fastest hatching time was obtained at a salinity of 6ppt (D3), followed by 8ppt (D4) and 4ppt (D3). The highest larval abnormalities was observed at high treatment levels of 6 and 8 ppt. The higher the salinity, the faster the hatching rate and the higher the level of abnormalities in *C. gariepinus* larvae. This finding coincides with previous studies, using other species, such as marble goby *Oxyeleotris marmoratus* **(Nguang *et al.*, 2012)**, lesser sandeel *Ammodytes tobianus* (Linnaeus, 1758) **(Bonisławska *et al.*, 2014)**, climbing perch *Anabas testudinus* **(Amornsakun *et al.*, 2019)**, pabda *Ompok pabda* **(Alam *et al.*, 2020)**, and the common carp *Cyprinus carpio* (Linnaeus, 1758) **(Iffar *et al.*, 2020)**. **Amornsakun *et al.* (2017)** reported that, the hatching time of snakehead *Channa striatus* fish eggs was between 1.192 and 1.442 minutes at a salinity of 5 ppt. In catfish eggs, mortality increased significantly at 8 and 10ppt, but the rate decreased **(Hossain *et al.*, 2021)**. The embryos of some stains of the Nile tilapia can tolerate up to 20ppt **(Fridman *et al.*, 2012)**, which exceeds the tolerance level of the fish used in this study.

Snakehead fish *Channa striata* were also reported to have a longer hatching rate of 1,192 minutes at 5ppt salinity, while it became slower at >5 ppt **(Amornsakun *et al.*, 2016)**. Several studies revealed that the hatching time for siamese gourami, green catfish, and climbing perch was 1,330, 1,080, and 1,255 min, respectively **(Amornsakun *et al.*, 1997; Amornsakun *et al.*, 2004; Amornsakun *et al.*, 2019)**. **Hadid *et al.*, (2014)** stated that treatment with hypoosmotic media concentrations can cause slower embryo formation, leading to a longer hatching time. These findings are consistent with this current study, where the samples in treatments D0, D1, D2, D3, and D4 hatched at 1,390, 1,270, 1,210, 970, and 1,150 minutes, respectively.

Observations showed that the salinity treatments had different effects on the speed of embryo development. The higher the level, the slower the development rate, and the lower the quality of the larvae. **Alderdice (1988)** stated that high salinity in the hatching media beyond the tolerance range can lead to the use up of energy for maximum osmoregulation. This condition then causes an inadequate amount of energy for embryo development, thereby affecting hatching time. From the observation, the morula phase can be clearly seen with the cells in the egg dividing, followed by the 32-cell division and formation of a second layer. The cells look less clear or vague at the poles. **Rizzo and Bazzoli (2020)** stated that, the morula phase was characterized by transverse division as well as the formation of a second layer that is faintly visible at the poles. This phase ends when all cells produce blastomeres of the same size. Subsequently, they condense into small blastodiscs and form 2 cell layers. In the D4 treatment, it was suspected that the concentration of the hatching medium was hyperosmotic, which led to slower embryo formation because the samples had to perform maximum osmoregulation. This finding is in line with that of **Hart (1990)** who reported that, when the concentration of fluid in the egg and the media (salinity) is not ideal, the osmotic hatching media become a burden. The condition then leads to an increase in the energy needed to maintain osmosis in the samples. The blastula phase is characterized by the formation of the second layer of eggs, which is denser when compared to the morula stage. **Hempel (1979)** reported that, the blastula phase occurs when the embryo undergoes cell division, which is marked by the formation of an empty cavity.

In this study, the concentration of liquid in the hatching medium (salinity) affected the enzyme performance and metabolic rate in the eggs, leading to the formation

of embryos in the shell more intensively. **Bonislawska *et al.* (2013)** revealed that salinity in the hatching medium can affect the activity of the embryo in the shell. This occurred in D0 since the eggs did not need to adapt to the salt level, which can reduce their energy for embryo development. **Fridman *et al.* (2012)** stated that, in hatching media without salinity, embryos and larvae do not need to adapt to the environment. Their adaptation can accelerate osmoregulation and metabolism as well as reduce egg yolk energy for larval growth. The samples in D1, D2, and D4 were still in the blastula phase, but one hour later or at 370 minutes, the eggs in D0, D1, D2 and D4 entered the gastrula phase. This stage was characterized by the blastoderm almost covering the entire yolk. **Holliday (1969)** revealed that, the gastrula phase begins with thickening on the outer edge of the blastodisc, and a ring-like circle is formed which is called the sprout ring. Changes from the characteristic gastrula stage is characterized by the movement of cells around.

In the D2 treatment, the concentration of fluid in the eggs was close to that of the liquid in the hatching medium, hence, the energy for osmoregulation decreased and the remaining was used to accelerate the development of the embryo. When the fluids concentration approaches each other and the egg can tolerate a given salinity change, the metabolic energy for osmoregulation becomes less, which makes the remaining sufficient for embryonic development **(Holliday, 1969; Hossain *et al.*, 2021)**. In D0, the hatching media was not affected by salinity, hence, the development of the eggs into larvae occurred within the normal hatching time. Treatments D1 and D2 showed good development but slower hatching time. This was because the egg used some of its energy for osmoregulatory activities, such as balancing its osmotic pressure with that of the hatching medium. In D2, it was suspected that, the samples were hyperosmotic to the media, thereby prolonging the hatching time and embryo formation. **Hadid *et al.*, (2014)** reported that, the absence of salinity in the media causes slower embryo formation and longer hatching time. This is due to the increased activity of enzymes, which leads to reduced metabolic rate.

In the D3 treatment, cell division into the blastula phase occurred quickly, and it disrupted the next development process. The most sensitive phase in embryonic development is the pre-embryonic stage, namely before reaching the blastula phase **(Arantes *et al.*, 2012).** This finding is consistent with **Holliday (1969)** that the occurrence of quick cell division phase can disrupt the next formation stage because the egg cannot pass its critical phase. **Muslim and Yonarta (2017)** revealed that, eggs can hatch even when the larval development does not have the ability to adapt to the environment. The results showed that the hatching rate can affect the quality of the larvae produced. Abnormal larvae were also found in D3 and D4. In D4, there were many rotten eggs and dead embryos, as shown in Fig. (3b). It was suspected that the development of embryos to larvae cannot tolerate changes in salinity. The hypertonic concentration of the media causes the outflow of liquid from the egg into the media **(Rizzo & Bazzoli, 2020)**. This was because there is a wide difference between the concentration of fluid in the hypotonic fish eggs and that of the hypertonic medium. Consequently, the eggs can no longer tolerate the changes in salinity, and this causes turgor/increased pressure. This condition can also cause plasmolysis or shrinkage due to the outflow of fluid from the egg, which then leads to the death when the change in salinity exceeds the tolerance limit. **Hadid *et al.* (2014)** attribued failure in egg development to the disruption of the

osmolarity between the osmotic medium and the samples, which is not ideal. The medium is likely to become a burden, and only some of the embryos can hatch with the occurrence of defective morphological conditions, as shown in Fig. (3a).

In D0, the hatchability of *C. gariepinus* catfish eggs was low since the hatching media did not influence the salinity and other anti-bacterial agent. Consequently, the eggs were attacked by a fungus; namely, *Saprolegnia* sp., as shown in Fig. (3c). **Heltonika (2014)** stated that high salinity levels can cause the death of microorganisms that are unable to adapt with the environment. The majority of freshwater microorganisms cannot survive at high salinity concentrations. In the D1 treatment, the eggs were still attacked by fungus due to the low concentration in the medium, which allowed fungal growth. In the D2 treatment on the media, the fungal attack was very low. Meanwhile, the fungal growth activity stopped but affected the quality of the larvae produced in D3 and D4. The use of high salinity in hatching media can affect the eggs and larvae. This finding concurs with that of **Kadarini *et al*. (2018)** who reported that, the condition can lead to egg rot and the death of many embryos due to the state of the hypertonic media.

Larval abnormality

Mubarokah *et al*. (2014) stated that when the concentration of water in the intracellular and extracellular fluids is the same, and the solute cannot enter or leave the cell, this condition is called isotonic. In the D1 treatment, the concentration between hatching media and eggs was close to isoosmotic; hence, a lot of energy was used for the development of embryos and larvae. **Alderdice (1988)** reported that, when the concentration of fluid in the egg is close to the concentration of fluid in the media, and the egg can tolerate changes in salinity given, the metabolic energy is used for osmoregulation, and the remaining energy is for embryonic development. Treatments D0 and D1 provided suitable salinity for the development of embryos and larvae as evidenced by the absence of abnormal larvae in the hatching media (Fig. 4a). For treatment D2, the level of abnormality was 1.5%, which was low for the slight difference between D0 and D1.

In the D3 treatment, the larval abnormality rate was 7.25%, followed by the highest of 10.75% at D4. The 6 ppt salinity treatment the fastest cell division and hatching, which affected the quality of the resulting larvae, namely the emergence of abnormal larvae, as shown in Figure 4b. This occured due to impaired cell division, which led to the inability of the egg to pass through its critical phase. In the D4 treatment, the embryos to larvae were not able to tolerate the given salinity, and this led to an increase in the abnormality rate. **Hadid *et al.* (2014)** revealed that, the concentration of fluid in the media and eggs is not the same; hence, the eggs use energy to carry out higher osmoregulation, inhibiting optimal development. The current observations showed that in the D4 treated with 8ppt, the catfish larvae *C. gariepinus* could not swim normally. The larvae were only at the bottom of the treatment container due to an imbalance in the larval body. Even at salinity concentrations of 6ppt, *C. gariepinus* catfish eggs hatched with the occurrence of abnormal larvae. This shows that the increase in salinity is directly proportional to the abnormality of the larvae, where the higher the increase in salinity, the higher the abnormality. **Putra *et al*. (2020)** postulated that, the level of abnormality can also be influenced by several factors including environmental pressures as well as errors

in maintenance and spawning efforts. **Kadarini *et al*. (2018)** showed that, genetic abnormalities found in larvae can cause a decrease in the total body length of fish due to mutations in the gene locus. To avoid the abnormalities, a suitable environment and highly nutritious fish feed are required **(Putra *et al*., 2019)**.

CONCLUSION

It can be concluded that, salinity has an effect on the stages of embryogenesis development of catfish *C. gariepinus*. The higher the salinity, the slower the development of the embryo. At higher levels, many of the eggs break quickly before the embryo is completely ready to hatch. Rotten eggs were also found and the embryos died in the middle of the development. Salinity affects the quality of the larvae produced, where the increase in the levels is directly proportional to the abnormality of the larvae. Therefore, the higher salinity, the higher the abnormality of the larvae obtained. Future study on the fish larval physiological metabolism and adaptability to different water salinity is highly recommended.

Data Availability Statement

The data supporting the findings of this study are available within the article and its supplementary material.

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Ethical statement

The use of animals during the work followed the institutional guideline of animal welfare.

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