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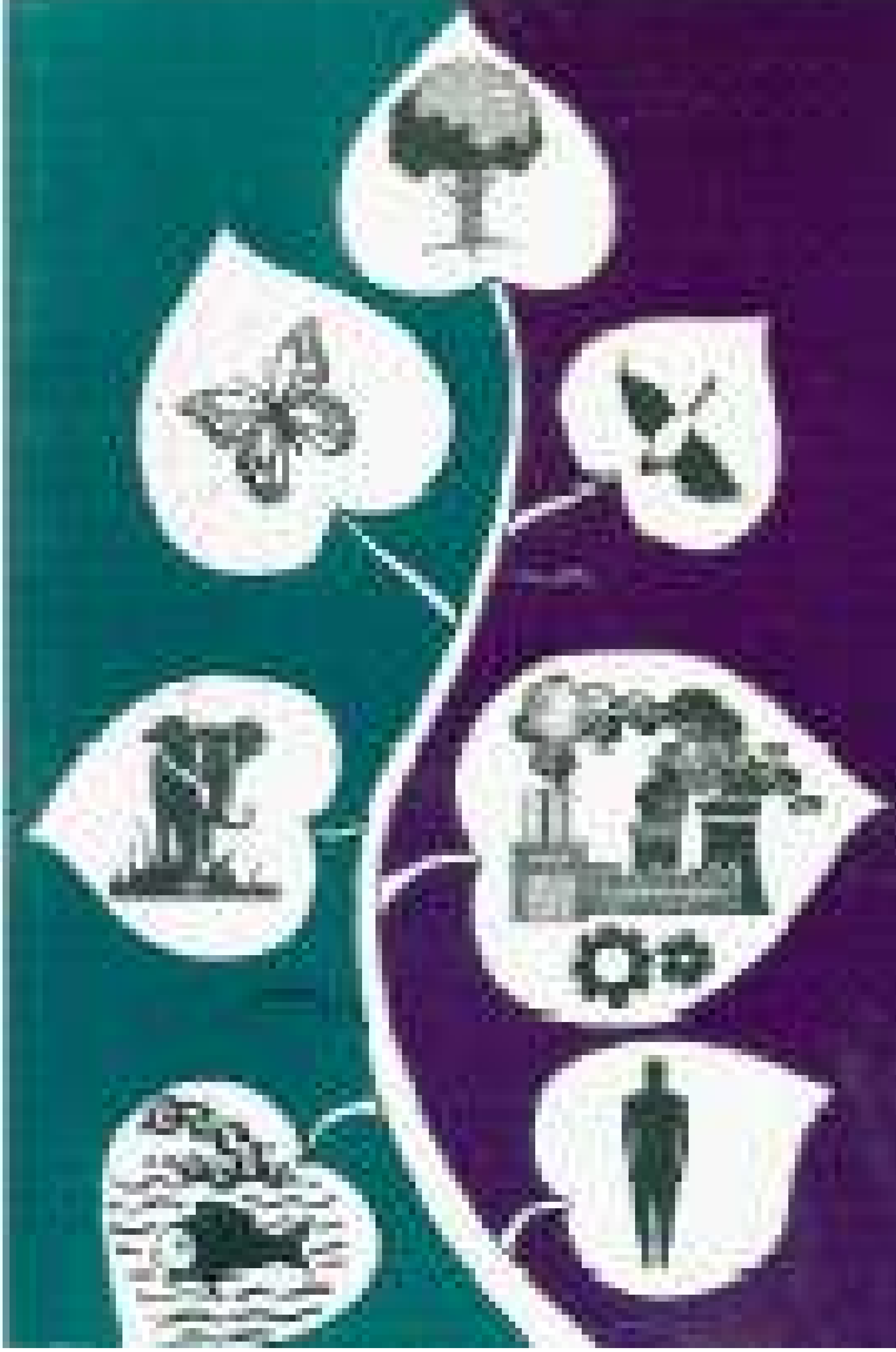

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Enhancement salinity inhibits toxicity of heavy mercury (Hg) metals to development of *Oreochromis niloticus* L. Embryos

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ABSTRACT

Tilapia (*Oreochromis niloticus* L.) is one of the freshwater fish that lives in freshwater and has valuable commodities in Indonesia. Contamination of heavy metals into the environment has become a problem that needs world attention. Mercury (Hg) is one of the metal elements that has high toxicity and has the potential to accumulate in the body of the organism. The concentration of heavy metals can increase with decreasing salinity. Exposure to Hg at low salinity is feared to cause abnormalities and deaths in the embryo of *Oreochromis niloticus* L. This study aims to determine the effect of exposure to Hg on different salinity on embryo development *Oreochromis niloticus* L. and find out the optimal salinity for embryo development exposed to Hg. Parameters of observation include hatchability, embryo development time span and percentage of larval abnormalities that have been exposed to Hg at doses of 0.005 mg/L at 0 ppt salinity, 10 ppt and 20 ppt. Calculations are carried out in a Completely Randomized Design (CRD). The results show that the use of salinity can reduce the effect of exposure to Hg at salinity of 20 ppt with the highest hatchability of 76.80b ± 4.56. There is an effect of exposure to Hg on different salinity to embryo development *Oreochromis niloticus* L. Toxicity Hg is inversely proportional to salinity, the higher the salinity, the lower Hg toxicity.

Key words: *Embryo, Hg, Oreochromis niloticus L., Contamination, Salinity.*

Introduction

Oreochromis niloticus is one of economic commodities in Indonesia (Saifulloh *et al.*, 2019). Environmental contamination is a change of environmental condition (land, air and water) that is not profitable such as damaging and harming humans, animals and plants due to the entry of living things, substances, energy and other components into the environment (Sastrawijaya, 2009). One of the heavy metal contamination is mercury (Hg) is an invaluable metal that contains very high toxicity and accumulated accumulated by the organization (Green Ruiz, 2009).

The Hg metals in both organic and inorganic forms that enter the waters are toxic and can accumulate in the body of organisms that live in waters (rivers, lakes and seas) through metabolic processes. The Hg levels of sea water ranges from <10-30 µ / L, whereas in freshwater ranges from 10-100 µ / L. The toxicity of mercury in waters differs between fresh, brackish and sea waters because salinity is one of the factors that can affect the accumulation of heavy metals in living things. The concentration of metals will increase with decreasing salinity (Miller and Connel, 1995).

Contamination of heavy metal Mercury (Hg) will be a threat to fish farming activities that are actively

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developed by Indonesian people, including the early stages of tilapia (*Oreochromis niloticus* L.) which is very influential in the success of fish hatchery activities. *Oreochromis niloticus* L. was recommended by the *US Environmental Protection Agency* (USEPA) as a toxicological test animal, because it has a wide distribution, has high environmental tolerance capabilities and is easily maintained on a laboratory scale (Yuniar, 2009).

Efforts to find out how much the influence of heavy metal Hg with salinity on the development of embryos of *Oreochromis niloticus* L., it is necessary to do research that is by exposure to heavy metals Hg at different salinity. In this study, *Oreochromis niloticus* L. Stari Jatimbulan was used as a superior product. This research is expected to be able to know the effect of heavy metal Hg with different salinity and optimal salinity level for the development of the embryo of *Oreochromis niloticus* L. strain Jatimulan so that it can help increase the fish commodity numbers in Indonesia.

Materials and Method

This research was conducted from January to February 2017 at the Laboratory of Pengembangan Budidaya Air Tawar (PBAT) Umbulan, Pasuruan, East Java, Indonesia. This study was an experimental study with variables in the form of embryo development of *Oreochromis niloticus* L. Jatimbulan strain with exposure to heavy metals Hg and different salinity.

Instruments and Materials

The instrument of this research is a tool for spawning in the form of a container for holding tilapia fish, digital scales, bowls, petri disc, seser and sieves. The tools for hatching are aquariums measuring 40x60x50cm^3, spawning ponds of 2x6x2m^3 size tilapia, hatching glasses, aerated faucets, paralon pipes, straws, and waterpams. Tools for observation are binocular microscopes, glass preparations, pipettes, rulers, pH pens, DO meters, thermometers, refractometers, cameras, and tissue.

The materials used in this study include egrgs used in this study derived from the natural spawning of the parent *Oreochromis niloticus* L. strain Jatimulan which is naturally spawned with a number of female 9 tails (600 gram / head) and male 3 tails (800 gram / head). The purity of the parent strain *Oreochromis niloticus* L. strain Jatimulan has been verified by PBAT Umbulan.

Work Procedures

The preparation stage of the container used in this study is the aquarium that is used measuring 40x60x50cm^3. Then making a salinity stock solution is made by means of fresh water added with salt. Salinity dose of 0 ppt as control, 10 ppt and 20 ppt was measured using a refractometer to reach the salinity used. Mercury chloride (HgCl2) is diluted into distilled water as a stock solution, then the solution is taken using a pipette in accordance with the required dose of 0.005 mg/L.

In this study there were four groups, including groups A, B, C and D. Group A was the control group with salinity of 0 ppt and without exposure to heavy metals. Group B is a group with a salinity treatment of 0 ppt and exposure to heavy metals Hg 0.005 mg/L. Group C is a group with a salinity treatment of 10 ppt and exposure to heavy metals Hg 0.005 mg/L. Group D is a group with a salinity treatment of 20 ppt and exposure to heavy metals Hg 0.005 mg/L. Fish spawning is done by pairing the male and female parent in the spawning pond with a ratio of 1: 3 male and female fish. The eggs were incubated and hatched on incubation media by exposure to heavy metal Hg in different salinity according to the treatment group. The development of embryos in eggs is observed at the 2nd, 4th, 45th, 75th, 85th, 100th and 120th hours after fertilization. Observation of embryos in eggs using a light microscope with 400x magnification. Egg observation was carried out to determine changes in embryo shape and the zygote stage, cleavage, blastula, gastrula, segmentation, pharyngula and post hatching. The time of observation is based on periods of embryonic development of *Oreochromis niloticus* L. strain of East Java.

Data Analysis

Fertilization Rate calculation uses the formula below:

$$FR = \frac{\text{Number of fertilized eggs}}{\text{The total number of eggs}} \times 100\%$$

Calculation of hatching power (Hatching Rate (HR) of eggs using the formula below:

$$HR = \frac{\text{Number of eggs hatched}}{\text{Number of eggs sampled}} \times 100\%$$

The eggs that will be observed are taken randomly using the RAL method using 5 and 10 mL pipettes. Data on embryo development obtained will be described descriptively. Fertilization Rate (FR) and Hatching Rate (HR) of eggs are presented in the form of SPSS data ANAVA test and Duncan's advanced test (Amalia, Rahardja and Triastuti, 2019). This research has fulfilled the ethical principle requirements, namely respecting animal life forms, analyzing benefits and losses, and fulfilling a sense of justice.

Results

The results of the development of *Oreochromis niloticus* L. Jatimbulan strain incubated by exposure to heavy metal Hg 0.005 mg / L at 0 ppt salinity, 10 ppt and 20 ppt experienced difference time and normal stage. The speed of development stage of *zygote*, *cleavage*, blastula, gastrula, segmentation, pharyngula and hatching can be seen in Table 1. The development of the *Oreochromis niloticus* L. embryo in the Jatimbulan strain at 0 to 2 hours after fertilization of all treatments simultaneously entered the zygote phase. Treatment A as control (salinity 0 ppt without heavy metal Hg) enters the cleavage phase at the 2nd to 4th hour after fertilization, as is the case with treatment B, C and D. At 4th to 22nd hours after fertilization, treatments A, B, C, and D enter the blastula phase. Treatment C (salinity of 10 ppt + heavy metal Hg 0.005 mg/L) is known to begin to experience tardiness in embryo development at 26 - 48 hours after fertilization, namely the gastrula phase while treatment A, B and D have entered the segmentation phase at that hour.

Treatment B (salinity 0 ppt + heavy metal Hg 0.005 mg/L) is known to experience a pharyngular phase longer than the treatment of controls A, B, and D at 48 hours to 120 hours. Treatment D (20 ppt salinity + heavy metal Hg 0.005 mg/L) is known to reach the hatching phase earlier at 90 hours equal to treatment A (salinity 0 ppt without heavy metal Hg). The results showed the treatment that entered the hatching phase at the latest was treatment B (salinity 0 ppt + heavy metal Hg 0.005 mg/L) and treatment C (salinity 10 ppt + heavy metal Hg 0.005 mg / L) when compared to treatment A (control).

The development time of *Oreochromis niloticus* L. Jatimbulan strains exposed to Hg heavy metals in different salinity for more details is shown in Table 2.In treatment C (Salinity of 10 ppt + exposure to heavy metals Hg 0.005 mg / L) and treatment D (Salinity of 20 ppt + exposure to metals heavy Hg 0.005 mg / L) completing the cleavage phase was faster than treatment A and B. with exposure to heavy metals Hg 0.005 mg / L has the fastest development time span, which is 2 hours. Treatment A (salinity 0 ppt without heavy metal Hg) and treatment B (salinity 10 ppt + heavy metal Hg 0.005 mg/L) has the same development time span, which is 3 hours. Salinity treatment of 0 ppt, 10 ppt and 20 ppt with each exposure to heavy metal Hg 0.005 mg/L entering the development stage of the blastula simulta-

Table 1. The speed of development stage of *Oreochromis niloticus* L embryo Jatimbulan strain with exposure to heavy metal mercury (Hg) 0.005 mg/L at different salinity.

Time span observation time (hours) (Fujimura and Okada, 2007)	Treatment			
	A	B	C	D
0 – 2	*Zygote*	*Zygote*	*Zygote*	*Zygote*
2 – 4	*Cleavage*	*Cleavage*	*Cleavage*	*Cleavage*
4 – 22	Blastula	Blastula	Blastula	Blastula
22 – 26	Gastrula	Gastrula	Gastrula	Gastrula
26 – 48	Segmentation	Segmentation	Gastrula	Segmentation
48 – 90	*Pharyngula*	*Pharyngula*	Segmentation	*Pharyngula*
90 – 120	Hatching	*Pharyngula*	*Pharyngula*	Hatching
120 – 124	Early larvae	Hatching	Hatching	Early larvae
>124	Early larvae	Hatching	Early larvae	Early larvae

Explanation: Treatment A. control (salinity 0 ppt + nonmetal), B. salinity 0 ppt + mercury 0.005 mg / L, C. salinity 10 ppt + mercury 0.005 mg / L, D. salinity 20 ppt + mercury 0.005 mg / L. hpf = hour post fertilization (hours after fertilization), ppt = parts per thousand.

neously ie at the 3rd hour simultaneously after fertilization.

However, treatment D (salinity of 20 ppt + heavy metal Hg 0.005 mg/L) has a faster development time span, i.e. for 19 hours. The segmentation stage is known in treatment B (salinity 0 ppt without heavy metal Hg 0.005 mg/L) has the longest time span, ie at 30 to 58 hours for 28 hours. The A and D treatments are known to reach the segmentation phase first compared to treatment B and C. Treatment D (salinity 20 ppt + heavy metals Hg 0.005 mg / L) is known to enter the pharyngula phase with the fastest time span compared to treatments A, B, and C which are during 41 hours. Treatment A (control) has time span of 42 hours then followed by treatment C for 45 hours.

Treatment B (salinity of 10 ppt + heavy metal Hg 0.005 mg / L) is known to enter the hatching phase at the latest compared to other treatments ie at the 105th hour with time span of 27 hours. D treatment (salinity of 20 ppt + heavy metal Hg 0.005 mg/L) is known to enter the earliest hatching phase compared to treatment A (control) which is at the 89th hour after fertilization with time span of 30 hours. The duration of embryo development results of the study showed the fastest shown by treatment D (salinity of 20 ppt + heavy metals Hg 0.005 mg/L) when compared to treatments A, B and C.

The development of the Jatimbulan tilapia embryo cannot be separated from the abnormalities resulting from exposure to heavy metal mercury in different salinity treatments. Abnormalities that are caused are divided into two, namely abnormalities of embryo development and developmental abnormalities of larvae. The developmental abnormalities of the larvae were detected in the form of hemorrhage, yolk sac abnormalities, jaw abnormalities, abnormal bone and abnormal caudal. These abnormalities can be seen in Figure 1.

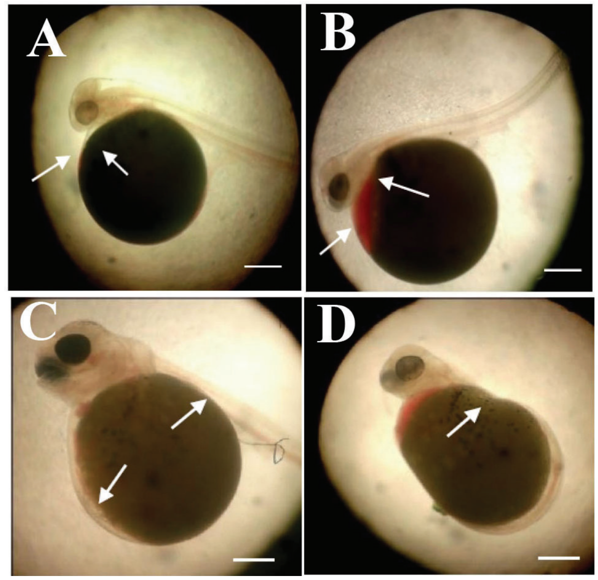

Fig. 1. Developmental abnormalities of *Oreochromis niloticus* L. larvae of the Jatimulan strain incubated in exposure to heavy metal Hg with different salinity at the hatching phase. a, normal embryo. b, hemorrhage (bleeding) in the embryo. c, normal yolk sac. d, yolk sac is defective. Arrows = where the abnormality occurs, Scale line = 1 mm.

The most incomplete morphology of embryo development was found in the salinity treatment of 0 ppt with the addition of heavy metal Hg 0.005 mg / L. Abnormal embryos cause abnormalities in the larval phase and can result in death. Changes that are seen are haemoragi and yolk morphological abnormalities. The embryo with this disorder changes during the next stage and not a few suffer death. Jaw, bone and caudal abnormalities are also seen in the larval phase shown in Figure 2.

Table 2. Time of development of *Oreochromis niloticus* L. embryo Jatimbulan strain exposed to heavy metal Hg at different salinity.

Treatment	Time Span Development (Hour)						
	Zygote	*Cleavage*	Blastula	Gastrula	Segmentation	*Pharyngula*	Hatchery
A	0-1	1-4	4-22	22-26	26-48	48-90	90-120
B	0-1	1-4	3-24	24-30	30-58	58-105	105-138
C	0-1	1-3	3-24	24-27	27-50	50-95	95-128
D	0-1	1-3	3-22	22-26	26-46	46-87	87-117

Explanation: Treatment A. control (salinity 0 ppt + nonmetal), B. salinity 0 ppt + Hg 0.005 mg / L, C. salinity 10 ppt + Hg 0.005 mg / L, D. salinity 20 ppt + Hg 0.005 mg / L. hpf = hour post fertilization (hours after fertilization), ppt = parts per thousand.

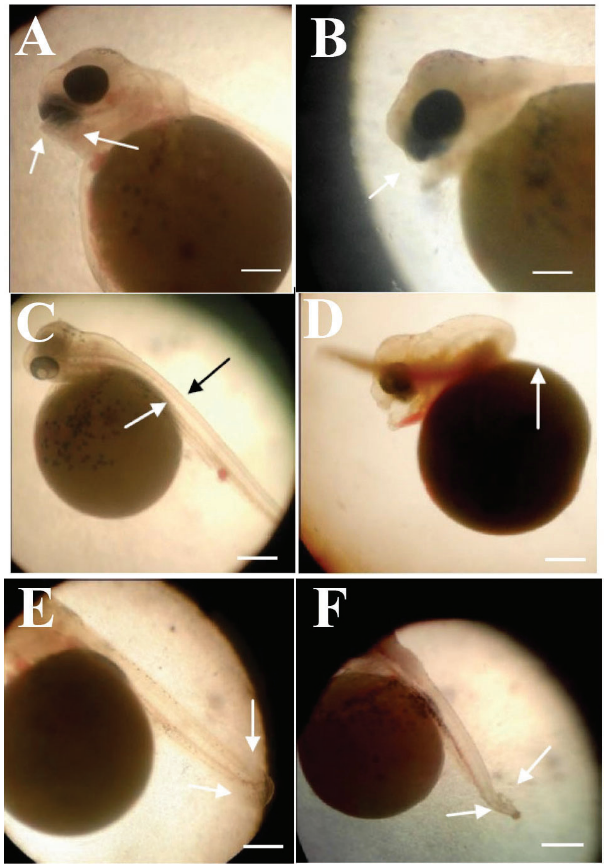

Fig. 2. Developmental abnormalities of *Oreochromis niloticus* L. larvae of the Jatimulan strain incubated in exposure to heavy metal Hg with different salinity. a, normal jaw. b, Deformed jaw. c, normal bone. d, Bone defects. e, normal Caudal. f, Caudal defect. Arrows = place of occurrence of abnormality, scale line = 1 mm.

The results showed that the larval abnormalities of *Oreochromis niloticus* L. Jatimbulan strain exposed to Hg heavy metals at different salinity were varied. The results of the statistical tests on each treatment can be seen in Table 3. The results of observation of the number of abnormalities in the development of early larvae exposed to heavy metals Hg 0,005 mg / L obtained different variations. Treatment of B salinity of 10 ppt with metal exposure had the highest concentration of 80.41% ± 5.39. The second highest embryo abnormality is in treatment B (salinity 0 ppt + heavy metal Hg 0.005 mg / L) which is equal to 40% ± 54.77. The lowest number of abnormalities is indicated by treatment D (salinity of 20 ppt + heavy metals Hg 0.005 mg / L) which is equal to 32% ± 4.90 compared to treatment A (control). The control treatment in the form of incubation media with salinity 0 ppt without exposure to heavy metals has a percentage of abnormalities of 12.8% ± 3.35.

The results study of the effect of heavy metal Hg on different salinity to the development of the *Oreochromis niloticus* L. embryo strain of Jatimbulan obtained a FR value of 97.6%. The value of hatchability of eggs in each can be seen in Table 4.ANAVA test results showed that the dose of the addition heavy metal Hg to salinity had a significant effect ($P <0.05$) on the averages hatchability of *Oreochromis niloticus* L. eggs of Jatimbulan strain. Duncan's Multiple Range Test results show the highest hatchability is in treatment A, namely the salinity control treatment 0 ppt without heavy metal treatment that is 83.20a ± 3.34.

The second highest hatchability is treatment D (20 ppt + Hg 0.005 mg / L) with the treatment of heavy metals Hg 0.005 mg / L that is 76.80b ± 4.56. D treatment (20 ppt + Hg 0.005 mg / L) showed that the results were significantly different from treatment B (0 ppt + Hg 0.005 mg / L) which was 4.80d ± 5.21. The third highest average hatchability of Jatimbulan tilapia eggs was followed by C treatment (10 ppt + Hg 0.005 mg / L) which was 49.60c ± 4.56. C treatment when compared with the control treatment showed significantly different results ($p <0.05$).

Table 3. Percentage of number abnormalities of *Oreochromis niloticus* L. larval Jatimbulan strain exposed to heavy metals Hg 0.005 mg/L at different salinity.

Treatments	Σ Larva	Abnormalities
A Control (salinity 0 ppt + non logam)	104	12,8 % ± 3,35
B (salinity 0 ppt + 0.005 mg/L Hg)	6	40% ± 54,77
C (salinity 10 ppt + 0.005 mg/L Hg)	62	80,41% ± 5,39
D (salinity 20 ppt + 0.005 mg/L Hg)	96	32% ± 4,90

Explanation: Treatment A. control (salinity 0 ppt + nonmetal), B. salinity 0 ppt + Hg 0.005 mg/L, C. salinity 10 ppt + Hg 0.005 mg/L, D. salinity 20 ppt + Hg 0.005 mg/L.

Table 4. Statistical test results of hatchability of *Oreochromis niloticus* L. eggs of Jatimbulan strains exposed to heavy metals Hg 0.005 mg/ L at different salinity.

Treatment	Hacthability ± SD
A (Control 0 ppt + non logam)	$83.20^{a}\pm 3.34$
B (0 ppt + 0,005 mg/L Hg)	$4.80^{d}\pm 5.21$
C (10 ppt + 0,005 mg/L Hg)	$49.60^{c}\pm 4.56$
D (20 ppt + 0,005 mg/L Hg)	$76.80^{b}\pm 4.56$

*Different superscript notations in the same column shows the comparison between treatments has a significant difference (P <0.05). Treatment of A. control (0 ppt + nonmetal salinity), B. salinity 0 ppt + Hg 0.005 mg / L, C. salinity 10 ppt + Hg 0.005 mg/L, D. salinity 20 ppt + Hg 0.005 mg/L.

In this study, measurements of water quality parameters were carried out to maintain the stability of the research environment. The stable scope of the media, is expected that media water quality does not affect the results of the study. Observations of water quality showed that the egg incubation media was stable and did not undergo fluctuating changes between treatments. The water quality parameters of the incubation media show the treatment temperature between 27 - 28°C and Dissolved Oxygen (DO) 6-8 mg / L.

Discussion

Observation of development *Oreochromis niloticus* L. embryo of Jatimulan strain during the study incubated by exposure to heavy metal Hg in different salinity experienced differences in the development time of each treatment. The longest range time is at treatment B salinity 0 ppt with exposure to heavy metals Hg 0.005 mg / L. Comparison of treatment A (control) 0 ppt without heavy metals with treatment B formation of the gastrula phase 4 hours later than normal development. The range of treatment time D (salinity 20 ppt with heavy metal Hg 0.005 mg / L) accelerated embryo development 2 hours earlier in the segmentation phase with a span of 20 hours compared to treatment B, C and A (control).

The acceleration of embryo development at salinity of 20 ppt is caused by the content of chloride cells found in the *Oreochromis niloticus* L. eggs Jatimbulan strains increase with increasing saline (Maetz and Bornancin, 1975). Chloride cells appear on the yolk sac membrane in the early phase of the embryo and then on the skin during the last stage of embryo development. Chloride cells contained in the membrane of the yolk pouch and turn out to be complex in response to changes in salinity (Kaneko *et al.*, 2002). Chloride cells play a role in controlling osmoregulation, can increase Na +, K + - ATPase activity in salt exchange to increase tolerance ability and play an important role in the process of salt secretion (Foskett and Scheffey, 1982; Zainuddin *et al.*, 2017). The role of chloride cells causes the liquid in fish eggs to become thicker and closer to the concentration of liquid in the hatching media, so the energy used for osmoregulation activities and other processes that occur in the egg decreases and the remaining energy can be used for growth (Cioni *et al.*, 1991). At higher salinity freshwater fish shows higher development and growth (Boeuf and Payan, 2001).

The phenomenon of tardiness in the development of *Oreochromis niloticus* L. eggs of Jatimulan strain incubated by exposure to heavy metal Hg 0.005 mg / L at different salinity has the potential to cause abnormal embryo development. Development of abnormal embryos can produce defective larvae and potentially cause death. The deformed larvae observed in this study had morphological abnormalities such as abnormal yolk sac shape, abnormal hemorrhage, jaw shape, and defective caudal shape. The morphology of the *Oreochromis niloticus* L. embryo of Jatimbulan was thought to be due to the influence of heavy metal Hg 0.005 mg / L on incubation media.

In fresh water and water with low salinity, Hg will form uncharged complexhydroxide and chloride ions, whereas in seawater with high salinity, Hg will form a negative complex with chloride ions. Molecules that do not undergo change are more easily delivered to membrane biology, bio-availability of mercury to methyl forms increases with decreasing salinity levels (Ullrich, Tanton and Abdrashitova, 2001). The available mercury in the water media enters through the pores of ZRI and ZRE by damaging the cell membrane and carrying out active transport to the mitochondria (the place of energy formation). The toxicity of mercury is not able to be suppressed by low salinity and causes the slow development of the embryo which causes abnormalities in the development of the embryo of *Oreochromis niloticus* L. strain of East Java. Research on larvae after the mummichog or killfish embryos

(*Fundulus heteroclitus*) exposed to Hg 5 and 10 µg / L resulted in anatomic abnormalities in this species (Weis and Weis, 1995). Other studies of zebrafish embryos (*Danio rerio*) exposed to Hg have weak heart defects, edema and spinal abnormalities. Most zebrafish embryos die after 6 days after fertilization (Samson *et al.*, 2001).

The average hatchability of *Oreochromis niloticus* L. eggs Jatimulan exposed to the highest heavy metals Hg in the highest salinity during the study was found in treatment A (control) ie 83.20a ± 3.34, the lowest in treatment B (0 salinity + heavy metal Hg) 0.005 mg / L which is 4.80d ± 5.21. Low hatchability is caused by several factors, which are well-fertilized eggs and abnormalities from the embryonic phase. Salinity incubation media with exposure to heavy metals Hg 0.005 mg / L directly influence embryo development. *Oreochromis niloticus* L. has pores in the internal radia zone (ZRI) and external radia zone (ZRE). Fish eggs consist of chorion which has elastic pores, and varies in thickness and strength.

The egg layer after chorion, the yolk membrane is the protoplasmic layer that surrounds the yolk. The yolk membrane is not like a chorion layer that has a pore. The chorion layer and yolk membrane are separated by a chamber containing previteline fluid. The eggs are fertilized and placed in water so the previteline chamber will be filled with colloidal liquid from yolk mass. So that it can draw water from outside the egg surface to enter and the hardening process of the egg occurs through the pores in the chorion. The pores will pass water and electrolytes from the outside to enter inside, but colloidal fluid cannot pass through the pore because it is very fine (Leitritz and Lewis, 1976). Hg 0.005 mg/L heavy metal exposure in different salinity was indicated to interact directly with the embryo at all stages and reduce the hatchability of *Oreochromis niloticus* L. eggs of Jatimbulan strain.

Observations of water quality during the study was also observed as a supporting variable. Higher temperatures than optimal temperatures may result in faster embryo development (Lin *et al.*, 2006). Water temperature is known to be an environmental factor that is very important in influencing the development of fish embryos (Blaxter, 1991). The average temperature between salinity treatments is 0 ppt, 10 ppt and 20 ppt evenly ranges between 27 - 28^{o}C. There were no significant differences in water quality parameters.

Suggestions that can be given based on this research are *Oreochromis niloticus* L. Jatimbulan strains can be used as bio indicators in freshwater which detected environmental contamination of heavy metals Hg by knowing the development of these fish.

Conclusion

Based on the results and data processing, it can be concluded that the toxicity of heavy metals Hg is inversely proportional to salinity, the higher the salinity, the lower the toxicity of heavy metals Hg. The optimal salinity level for the development of the *Oreochromis niloticus* L. embryo of Jatimbulan strain exposed to Hg 0.005 mg/L heavy metals ie 20 ppt with the highest hatchability, relatively faster time span and the lowest number of abnormalities.

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