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Embryo and larvae development of Nilem Fish, *Osteochilus vittatus* reared in batik liquid waste

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Abstract. *Nugrahesthi GH, Wijayanti GE, Habibah AN. 2023. Embryo and larvae development of Nilem Fish,* Osteochilus vittatus *reared in batik liquid waste. Nusantara Bioscience 15: 105-112.* The embryonic and larval stages are critical phases of an organism's development. For aquatic organisms, development is affected by environmental factors such as liquid waste. A batik liquid waste is a waste product of the textile industry usually streamed directly into the aquatic environment. This study aimed to observe the effects of exposure to batik liquid waste effects on developing Nilem (*Osteochilus vittatus* Valenciennes, 1842) fish embryos and larvae. The research was conducted using a completely randomized design. One hundred two-celled embryos were kept in batik liquid waste with dilution concentrations of 0% (the control), 5%, 10%, 15%, and 20% of water until the age of 4 days after hatching with five replications. The time of embryo evaluation was 60th minutes, 120th minutes, and 180th minutes after fertilization; the larval stage evaluation time was the 24th, 48th, 72nd, and 96th hour after fertilization; five embryos were evaluated for each replication. The results showed that embryo exposure to batik liquid waste affected the height of the blastoderm embryo, accumulation of waste in the chorion of the embryo, deceleration of embryonic development, increased larval abnormalities, decreased the survival rate of larvae, and acceleration of yolk absorption of fish larvae. Embryos were successfully hatched and produced larvae only in the control and 5% batik liquid waste medium. Batik liquid waste interfered with *O. vittatus* embryo development and generated mortality above 5%.

Keywords: Batik waste, blastoderm, development, embryogenesis, Nilem Fish

INTRODUCTION

Batik was recognized as one of the cultural heritage of Indonesia by the United Nations, Educational, Scientific and Cultural Organization (UNESCO). Therefore, it enhanced batik production and increased economic growth (Ariyanti et al. 2021). Small and medium entrepreneurs carry out batik production; they conduct traditional techniques for producing batik (Muslimah et al. 2020). This technique generated wastewater released before treatment into the river or environment (Muslimah et al. 2020).

The wastewater problem hinders increasing batik industry production (Indrayani et al. 2020; Oedjijono et al. 2021). Batik liquid waste contains dyes and organic compounds that contaminate water bodies. Some techniques are required to separate and degrade the organic compounds from the dyes, requiring a continuous process (Ariyanti et al. 2021). Wirosoedarmo et al. (2019) stated that wastewater produced by the batik industry contains dark-colored organic waste, elevated temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Suspended Solid (TSS) content. The wastewater is due to chemical and coloring substances used during the batik production and coloration process. Batik production comprises techniques: canting, hand-drawn batik, block, printing, *tritik*, and tie-dye. Canting is widely used in the small batik industry; this is a traditional technique for making batik. There are six stages for making batik through the traditional technique: material preparation, waxing, dyeing, dye fixation, wax disposal, and product drying. These processes run as follows: the cloth was submerged into the desired color and toward the sun drying. Dye use during the preparation process was done if only necessary. The following step was dipping in starch solution; this process aimed to shed the glossy effect. Rinsing and sun drying are the next steps. Next, the waxing process uses a block design, dipping into the wax, then applying it to the cloth. The dyeing process applies the desired color of batik; The cloth is submerged into a sodium silicate solution for about 3-4 hours, then submerged in water. The following steps are rinsing and drying, also wax disposal. This process takes about 1-1.5 hours for submerging, boiling, and rinsing; 18 hours for submerging before the batik product's drying process. Washing and rinsing are carried out repeatedly to remove the oil, excessive wax, and dyes (Daud et al. 2022). These batik-making processes mostly utilize clean and not-so-clean water; the water utilization quantity for batik production is related to the wastewater (Handayani et al. 2021). The water used in batik production created an environmental problem (Widianarko et al. 2021). Batik production is part of the textile industry that is the major contributor to environmental water contamination. Therefore, water treatment before releasing wastewater from the textile industry, including batik production, is necessary. However, batik industries' wastewater is usually disposed of without treatment. This batik liquid water consists of several toxic components of heavy metals such as chromium, ferrum (Fe), lead (Pb),

silicon (Si), calcium (Ca), magnesium (Mg), cuprum (Cu), and zinc (Zn) (Daud et al. 2022).

Heavy metals were toxic to the fish embryo; for example, they caused subtraction of the eye size of the embryo (Zhang et al. 2015). Therefore, heavy metal is a major pollutant for freshwater fish species. Furthermore, several reports on heavy metal affected many fish species length and body weight (Häder et al. 2021), such as chromium, copper, and cadmium, affected the Pejjerey fish's embryo (*Odonthestes bonariensis* Valenciennes, 1835) (Miranda and Somoza 2022). Also, chromium significantly affected the development of the *Danio rerio* Hamilton, 1822 (zebrafish) embryo; it caused several abnormalities such as yolk sac edema, faint pigmentation, impairing skeleton, pericardial edema, lordosis, hatching overdue, tail shortening, notable subtraction of development of the embryonic eye, movements of the embryo, and heartbeats (Nisha et al. 2020). Furthermore, Xu et al. (2021) investigated chromium's effect on zebrafish larvae; it proved that chromium had a neurotoxicity effect on zebrafish embryos. In addition, Krzykwa et al. (2018) stated that neurological and cardiovascular systems are more reliable for measurable endpoints of toxicity tests.

Like heavy metals, dyes, especially synthetic dyes, were harmful to aquatic animals like fish. Several eco-toxicological effects from dyes for fish, such as alteration of enzyme activity, morphology, size, histopathological significances, and diminishing of carbohydrates, lipids, and protein in tissue, resulted in mortality (Al-Tohamy et al. 2022). Several dyes used for batik making usually depend on fabric material. For example, indigo and naphthol dyes were used for cotton and linen (Dailin et al. 2022). Indigo dye was insoluble in water, and naphthol contained more hazardous chemicals; this was harmful to fish (Dailin et al. 2022). Nasrin et al. (2022) examined Basic Red 18 dye to tilapia. Therefore, this dye was toxic for the treated fish; it showed causing abnormalities in several organs: gills, heart, stomach, intestine, and liver of *Tilapia mossambica* Peters, 1852.

Moreover, fish usually were used to asses water status (Braunbeck et al. 2014). The embryo phase is the critical phase of the organism. This phase is one of the most important stages for increasing the production in fish aquaculture (Olaniyi and Omitogun 2013). Moreover, Dahlke et al. (2020) stated that the embryo and larvae stages were the critical phase in determining their developmental stage further. Therefore, the embryo was a convenient model for testing some toxic material, and this stage was sensitive and suitable for the toxicology test (Wlodkowic and Campana 2021).

Furthermore, batik liquid waste damage the chorion of Nilem Fish embryo (Habibah et al. 2022). As the embryo protector, the chorion was totally damaged in the incubation of 20% batik liquid waste for 24 hours. Embryos of Nilem Fish were incubated in several batik liquid waste concentrations: 5%, 10%, 15%, 20%, and 0% as the control. Observation of the chorion showed a breakdown of the chorion in 20% batik liquid concentration. However, research about the effects of batik liquid waste on embryo and larva development of Nilem Fish were not conducted yet. Therefore, this research aimed to determine the effect of batik liquid waste exposure on embryos and larvae development of Nilem Fish (*Osteochilus vittatus* Valenciennes, 1842).

MATERIALS AND METHODS

Material

This study used Nilem Fish (*O. vittatus*), 10% Neutral Buffered Formalin (NBF) as a fixative solution, 0,9% NaCl solution for diluting fish milt, cavity slides, and eyepiece micrometer. The study used a Completely Randomized Design (CRD) model with five batik liquid-waste medium concentrations for embryo rearing medium. These five concentrations were 0% (control), 5%, 10%, 15%, and 20%. The evaluation was conducted at the embryonic development stage of 60^{th}, 120^{th}, and 180^{th} minutes after fertilization and the larval stage of 24^{th}, 48^{th}, 72^{nd}, and 96^{th} hours after fertilization. The treatment was replicated five times. Therefore, a total of 25 experimental units were maintained during the research, and 110 embryos per unit.

Procedures

Dilution of batik liquid waste

Batik liquid waste was obtained from the waste collection tank after the dyeing process of the batik industry in Sokaraja Sub-district, Banyumas District, Central Java, Indonesia. Batik liquid waste was diluted to fulfill percentage levels of 5%, 10%, 15%, and 20%. The dilution of batik waste was done by adding water obtained from the campus environment of the Faculty of Biology, Universitas Jenderal Soedirman, Banyumas District, Central Java, Indonesia. Wastewater from each percentage level was put into a basin with the addition of water until it reached ± 1,000 mL volume and was equipped with aeration.

Assisted spawning of Nilem Fish

Mature females and males of Nilem Fish were induced using GnRH analog (Ovaprim) at a dose of 0.5 mL/kg for females and 0.3 mL/kg for males (Simanjuntak and Wijayanti 2005). After 8 hours of induction, induced fish were then stripped. Eggs collected were accommodated in a container, a small concave basin with size 15 cm in diameter until assisted fertilization takes 5-10 minutes. Milt of male fish was taken using a syringe without a needle and then diluted with 0.9% NaCl solution with a ratio of 1:9. Assisted fertilization was done by putting milt into a container containing eggs and slowly adding water to activate spermatozoa and gently shaking the container. After the eggs reached the 2-cell stage, the eggs were put into each basin of the experimental unit equipped with aeration.

Sampling of embryo and larvae

Samples of 25 embryos were obtained from each treatment. In addition, five embryos were collected from each basin of 5 replication for each treatment. Embryo samples were taken at incubation times of 60^{th} minutes, 120^{th} minutes, and 180^{th} minutes. A sampling of embryos that had developed into larvae was continued at the 24^{th}, 48^{th}, 72^{nd}, and 96^{th} hours. All samples obtained were immediately fixed in a 10% NBF fixative solution.

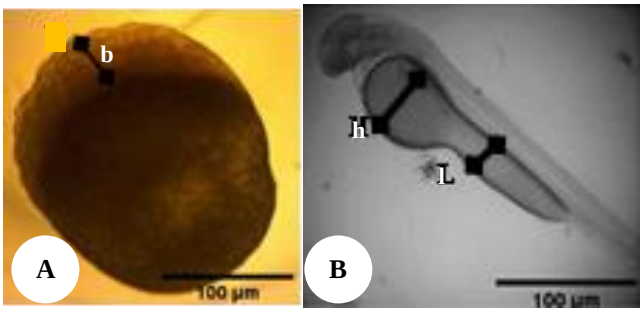

Figure 1. A. Embryo of Nilem Fish (*O. vittatus*) morula stage. Description: b. Height of blastoderm. B. Nilem larvae age 96 hours (Windari 2013). Description: l. Elongated yolk sac, h. Shortened yolk sac

Embryo and larvae observation

Embryos and larvae were observed under a light microscope at an objective magnification of 4 X. The blastoderm height was measured using an eyepiece micrometer previously calibrated. Measurement of blastoderm height is represented in the Figure 1A.

The proportion of an abnormal sample was observed and quantified. Abnormal embryos were categorized if there was the disintegration of the vitelline membrane, the blastomere size was not uniform, or a blastomere detached from the blastoderm and was found in the perivitelline space (Hallare et al. 2005). Abnormal larvae were categorized if there were one or more forms of abnormality, such as pericardial edema, bent or curved tail vertebrae, and bent or curved pectoral fins (Mukti 2005). The cumulative time required in the development process to reach a certain stage was calculated and recorded. Then the cumulative development time of treatment embryos was compared with the cumulative development time of the control embryos. Finally, the percentage of larval survival rate was calculated from the number of larvae that lived at the end of each evaluation time compared to the total larvae of initial rearing, which was 400 embryos.

The yolk absorption rate was calculated based on the volume of the yolk sac using an eyepiece micrometer with the following formula:

$$LPKT = \frac{V_0 - V_t}{T}$$

Where:
LPKT : Yolk sac absorption rate
V0 : Early yolk sac volume
Vt : End yolk sac volume
T : Time (hour)

V=0,1667 π 〖LH〗 ^2

V : Egg yolk volume
L : Elongated yolk sac diameter
H : Shortened yolk sac diameter

The measurement of the length of the yolk sac diameter is represented in the Figure 1B.

Data analysis

Height of embryo blastoderm, abnormal embryo, and larvae, cumulative development time, larval survival rate, and yolk absorption rate were analyzed using ANOVA with SPSS software version 16.0. The data were first tested for homogeneity using Levene and normality using Kolmogorov-Smirnov before being analyzed using ANOVA. Then, when data were normally distributed and homogenous, it was analyzed using ANOVA or Independent Sample T-Test (if only two data groups were being tested) with a significance of P<0.05. Finally, post hoc analysis was carried out to determine the time of embryo maintenance with the highest level of damage. The post hoc used was the Tukey HSD for data with ANOVA significance value <5%.

RESULTS AND DISCUSSION

Height of embryo blastoderm

Blastoderm formation indicates embryo development after fertilization (Figure 2). The average blastoderm height at the 60th and 180th minutes evaluation time showed subtle differences (p= 0.08 and p=0.232, respectively). Therefore, it can be interpreted that the batik liquid waste treatment has no significant effect (p>0.05) on the height of the embryo's blastoderm at 60th and 180th minutes after fertilization. Meanwhile, the results for 120th minutes evaluation time showed a significance value (p = 0.002), which means that the treatment of batik liquid waste has an effect (p<0.05) on the height size of the embryo's blastoderm. Therefore, Tukey HSD further tests were carried out to know the details of the concentration of batik liquid waste, which affects the size of the blastoderm height at the 120th minutes evaluation time. Based on Tukey's test, 20% batik liquid waste significantly affected the mean height of the blastoderm embryo, which was 271.72±19.68 µm.

Embryo abnormality-2

Batik liquid waste was not affected (p>0.05) on the level of abnormality of Nilem Fish embryos at all three evaluation times (60th minutes, 120th minutes, 180th minutes) (Figure 2). Abnormalities of the control and treatment embryos tend to show a constant abnormality rate. This was possible because the chorion function, which acts as a protector, could still protect the embryo in the range of immersion time of 60th minutes, 120th minutes, and 180th minutes. The chorion that surrounds the embryo functions as a means of respiration and excretion of the embryo. In addition, the chorion also acts as the first line of defense against foreign substances entering the embryos. Research on the development of zebrafish embryos by Henn and Braunbeck (2011) proved the role of the chorion as a protector. It revealed that embryos without chorion incubated in pronase increased mortality, which was not found in these control embryos. Furthermore, the number of coagulated embryos identified physical damage to dechorionized embryos.

Larvae abnormality

Embryos reared in batik liquid waste at concentrations of 10%, 15%, and 20% did not continue until the hatching

stage, so the treatment did not obtain larvae (Figure 3). The Independent Samples T-Test (between the control larvae and those exposed to 5% batik liquid waste) showed a significant value of ($p<0.001$) (Figure 4), which means that batik liquid waste had a highly affected ($p<0.01$) on the level of larval abnormalities resulted hatching failure at 24 hours post-fertilization (hpf). Larval abnormalities included pericardial edema, spinal curvature, and bent pectoral fins (Figure 5).

Larvae survival rate

Batik liquid waste affected the survival rate of Nilem Fish. The concentration of 5% batik liquid waste decreased the survival rate of Nilem and along with the evaluation time (Figure 6).

Larval yolk absorption rate

Larval yolk absorption was observed between control and treatment group of 5% batik liquid waste medium. The absorption of yolk was presented in Figure 7.

Time cumulative development

The time required for each stage to complete the stages of development is called the time cumulative development (Table 1).

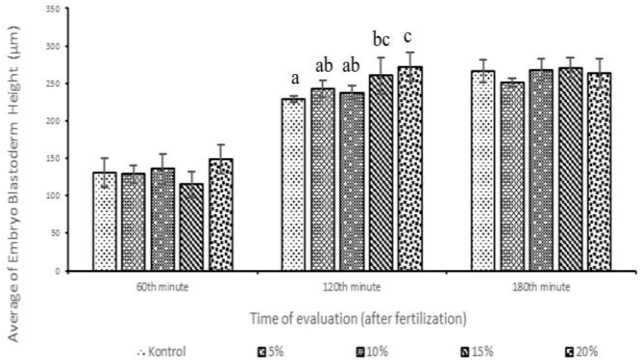

Figure 2. Average and the standard deviation of embryo blastoderm height of Nilem Fish (*Osteochilus vittatus*) in batik liquid waste medium and control at 3 evaluation times; 60th minute, 120th minute, and 180th minute after fertilization. Note: different letters were significant

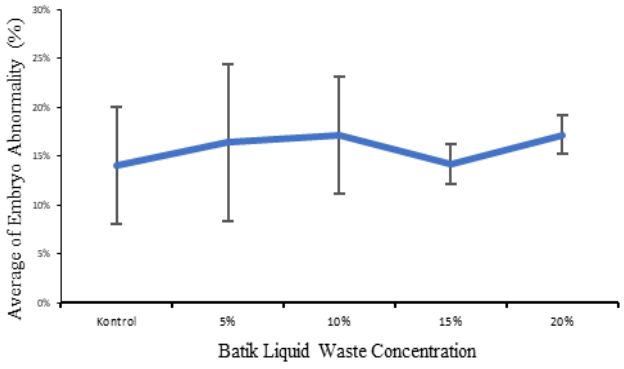

Figure 3. Average and standard of deviation of embryo abnormality of Nilem Fish (*O. vittatus)* control and in batik liquid waste medium

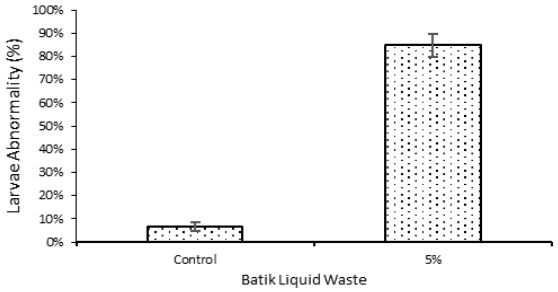

Figure 4. Abnormality of Nilem Fish larvae (*O. vittatus*) control and in 5% batik liquid waste medium

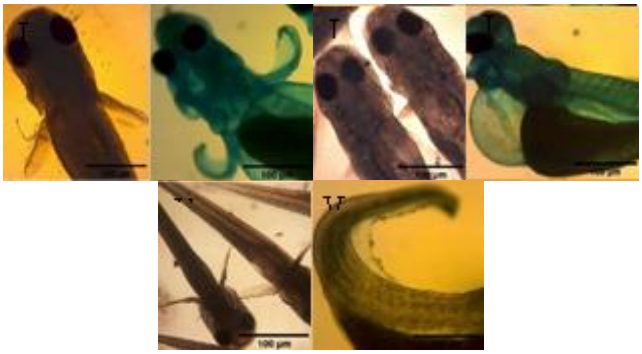

Figure 5. Morphology of pectoral fins, heart, and vertebrae of Nilem (*O. vittatus*) larvae 0% (Control) and 5% in batik liquid waste medium: I. Morphology of the control group larvae of pectoral fins, II. The morphology of the pectoral fins in the 5% batik

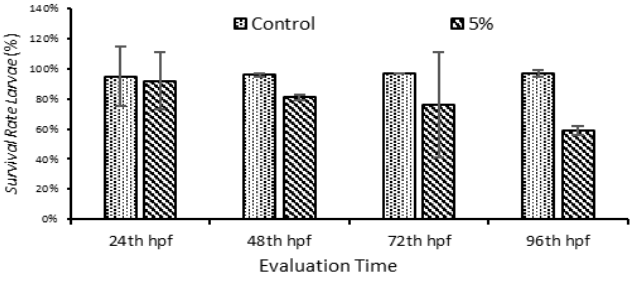

Figure 6. Larvae survival rate and standard of deviation of Nilem Fish (*O. vittatus*) in 5% batik liquid waste medium

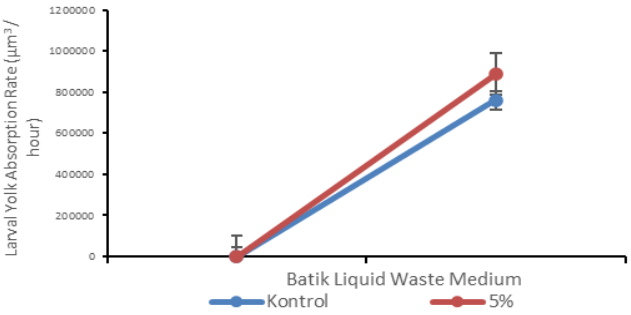

Figure 7. Absorption rate of yolk larvae Nilem Fish (*O. vittatus*) the control and in 5% batik liquid waste medium

Table 1. Cumulative developmental time (minutes) of Nilem Fish embryo (*O. vittatus*) control and in batik liquid waste for each embryonic stage

Developmental stage	Batik liquid waste concentration	Cumulative time of embryo development (minutes)				
		0% (Control)	5%	10%	15%	20%
2 cell–Morula		59±3[b]	40.33±3,51[a]	105.67±2,51[c]	113.67±2,08[d]	57.33±2,08[b]
Morula–Blastula		14.66±1.15[a]	30.33±4.50[b]	49±1.00[d]	42.66±3.51[cd]	40.33±1.52[c]
Blastula–Gastrula		66.66±3.78[b]	63±3.00[ab]	63.33±0.57[ab]	66±2.00[ab]	57±5.56[a]
Gastrula–Hatching		1390.3±2.51	1997.7±3.78	-	-	-

Discussion

The development of the embryos of the research subjects was affected by toxicants when the embryos entered the two-cell stage or 30th minutes after fertilization. The development of blastoderm embryos in the control and reared in batik liquid waste at 60th minutes tended to be normal due to the short exposure to batik liquid waste. Embryo development up to the 60th minute was included in the early stages of embryo development so that maternal factors largely regulated development. Furthermore, O'Farrell (2015) suggested that the development of eggs outside the mother's body was supported by maternal factors deposited into the oocyte cytoplasm during the oogenesis phase so that the oocyte has been prepared by the mother to start the cell division cycle.

Embryos in batik liquid waste at the time of evaluation of 120th minutes tend to show faster development than the controls; the higher average of height of the blastoderm proved it. The highest blastoderm size indicates the embryo is at the peak stage of the blastula. At 120th minutes, the control embryos were in the late morula stage until the early blastula, while the embryo in exposure to batik liquid waste currently entered the blastula stage. Toxicants accelerate blastula development in embryos exposed to batik liquid waste; it is assumed this stage possesses high sensitivity to toxicants. Häder and Erzinger (2018) stated that conditions of water temperature, water pH, and form of toxicants affect the level of toxicity and sensitivity of organisms to toxicants. O'Farrell (2015) suggested that embryonic development during the late blastula stage to early gastrula is a transitional cycle at the end of development involving maternal gene products toward zygotic transcriptional activation. This embryonic development is characterized by the appearance of cell shape and morphogenetic changes in a relatively short period. Changes in internal factors due to the activation of embryonic genes occur, especially in development at the 120th minute to the 180th minute. The development of the control embryos at the 180th minute reached the late gastrula stage. In contrast, the embryos reared in the waste showed an average development up to the late blastula or early gastrula stage. Furthermore, the development of embryos exposed to batik waste would be continued because of the possibility of embryos initiating adaptation mechanisms to tolerate toxicity due to the presence of the chorion as a protective blastomere (Braunbeck et al. 2005).

In this study, developmental deterioration was experienced by embryos in exposure to batik liquid waste treatment. The maintenance of treatment embryos at 180th minutes showed development to the late blastula or early gastrula stage, while the control embryos reached the late gastrula stage. Von Hellfeld et al. (2020) reported the developmental retardation of late blastula to early gastrula in zebrafish (*D. rerio*) embryos exposed to toxic mercury. Delayed embryo hatching evidenced the developmental delay in this research subjects in 5% batik liquid waste-treated embryos with a delay of about 60th minutes longer when compared to the control embryos. Meanwhile, embryos in batik liquid waste treatment above 5% did not hatch. Slow developments due to exposure to toxicants, as suggested by Küçükoğlu et al. (2013), on zebrafish (*D. rerio*) embryos on exposure to zinc chloride began to hatch on the 7th day after fertilization, compared to the control embryos hatched on the 4th day after fertilization. Moreover, Nisha et al. (2020) found that toxic material (chromium) in water caused abnormalities in zebrafish embryos, such as eye and tail development. Like zebrafish embryos, chromium that might be involved in the batik liquid waste becomes toxic to Nilem Fish embryos. The mechanism of heavy metal could cause abnormalities in fish embryos through oxidative stress, and free radicals evocation was an indication.

The accumulation of batik liquid waste in the chorion was characterized by color alteration of the chorion layer. The higher the concentration of batik liquid waste, the darker the color. The role of the chorion as a respiratory and excretory pathway was supported by a pore structure evenly distributed throughout the chorion layer. The small pore size has the potential as a route for toxicants to enter the embryo. Toxicants that successfully penetrated the chorion layer were able to interfere with the development of the embryo's blastomere. Habibah et al. (2022) investigated the chorion and oolemma of Nilem Fish embryos reared in batik liquid waste. It showed that a concentration of 20% batik liquid waste affected supreme damage of chorion and oolemma compared to other concentrations, 0%, 5%, 10%, and 15%.

The cumulative time required for embryos in batik liquid waste medium to reach certain stages of development is shown in Table 1. Batik liquid waste at a concentration of 10% to 20% interferes with embryonic development time, especially at the morula to blastula stages of development. The 10% and 15% concentrations of batik liquid waste tended to slow the development, while the 20% concentration tended to accelerate the development to the early gastrula stage. The formation of morula-stage blastoderm by 10% and 15% batik liquid waste embryos in long duration is due to the toxicant content that has

successfully penetrated the chorion, as reported by Feitosa et al. (2021) that accumulation of toxicants around the chorion causes a decrease in oxygen levels so that it affects embryonic development. The difference in development time can be caused by the quantity of the toxicant used as a medium for embryo maintenance. The high toxicant content of batik liquid waste, especially at a dilution concentration of 20%, may accelerate embryo development. However, the development will be stopped at a certain stage due to toxicants that damage the blastoderm. In line with the observations, embryos reared in waste concentrations above 5% stopped at the late gastrula stage, so they did not develop into larvae. The difference in hatching duration of 5% batik liquid waste embryos was about ±60 minutes compared to the control embryos. Batik liquid waste with high polarity and a small molecular weight is estimated to interfere with the normal development of embryos and cause embryos to experience hatching delays (Cao et al. 2009).

The anomaly observed in the test could be attributed to the inhibition of DNA synthesis, which causes protein and enzyme activity disruption due to excessive levels of toxicants. Research by Scholz et al. (2008) reported that the activity of certain transporter proteins, such as the ATP binding cassette-transporter, a transporter that can prevent the entry of certain molecules into cells, can be affected by environmental chemicals. Inhibition of the transporter activity can potentially increase the entry of toxicants through the chorion so that the number of toxicants in contact with the embryo increases. In addition, toxicant interactions at the molecular level could cause gene transcription or protein expression changes, which can disrupt embryonic metabolism. Researchers Wiegand et al. (1999) suggested that embryos exposed to toxicants will increase excretory metabolism by controlling stress due to exposure to foreign substances. One of the metabolisms observed is the increase in glutathione as a detoxifying enzyme. Increased activity of glutathione allows detoxification to be carried out through the physiological degradation of toxicants. However, the activity of detoxifying enzymes cannot tolerate high concentrations of toxicants.

Changes in the rearing environment in the form of toxicants resulted in abnormal larval development. Changes in the shape of the vertebrae can characterize the form of larval abnormalities. Researcher Küçükoğlu et al. (2013) informed that one of the mechanisms that cause vertebral damage is disruption during early vertebral development caused by inhibition of collagen synthesis. Toxic substances contained in batik liquid waste inhibited collagen synthesis during the development of vertebrae of Nilem Fish larvae during the study.

The survival rate of the control group larvae tends to be stable; there was no change, while 5% batik liquid waste larvae have decreased linearly (Figure 6). The respiratory disorders due to toxicants in the waste were assumed to cause the decreased survival rate of larvae reared in 5% batik liquid waste from time to time. In addition to the mouth, exposure to toxicants can enter through the gill surface, which would then be transported to the entire surface area of the fish's body (De Oliveira et al. 2016). The linear decreased curve was in line with the increase in larval yolk consumption. Yolk as an energy source was used by larvae until they reached the age of day 4 or 96 hours after fertilization. Research by Wulandari et al. (2020) reported that yolk shrinkage in 24 hours post-fertilization (hpf) larvae tended to be faster than yolk shrinkage in 36 hpf to 96 hpf larvae. Based on observing the 5% batik liquid waste larval survival rate reduction curve, the external feed can be given earlier at 50 hpf to replace the energy source previously provided by the yolk.

The larval yolk absorption rate showed a higher value of 5% batik liquid waste than the control (Figure 7). Rearing in batik liquid waste caused stress on Nilem Fish larvae. Under stressful conditions, larvae require more energy, so their metabolic rate is faster than the control larvae. A higher metabolic rate caused higher energy consumption. When yolk reserves were nearly depleted, the metabolic needs were fulfilled from tissue-level catabolism for energy production. Therefore, the external feed can be given earlier, shortly after the opening of the larvae's mouth, to replace the energy source used (Mariska et al. 2013). The increase in the absorption rate of 5% batik liquid waste larvae was due to the larval effort to maintain homeostasis to stay alive by hydrolysis of the yolk into the larval body tissue. Research by Wiegand et al. (1999) informed that the enzymatic process in embryos requires additional energy used for growth, but in embryos exposed to toxicants, more energy was needed for detoxification. Research by Long et al. (2011) reported that the detoxification of aquatic organisms was mediated by the ATP Binding-Protein Cassette (ABC) transporter. Gills, as organs of excretion and detoxification, were the main link between animals and their environment. Exposure to toxicants causes an increase in the expression of ABC transporter genes in the gills to limit the absorption of environmental toxicants.

Based on the results of the research that has been done, it can be concluded that the exposure of batik liquid waste to embryos in the third observation evaluation time (60^{th} minutes, 120^{th} minutes, and 180^{th} minutes) affected the height of the blastoderm of the embryo, deteriorate the development of the embryo, and accumulated batik liquid waste in the chorion characterized by changing the color of the embryo's chorion. Furthermore, exposure to batik liquid waste on larvae aged 24 to 96 hours after fertilization also increased larval abnormalities, decreased larval survival rates, and accelerated the absorption rate of larvae yolks.

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