Effect of Active Detergent Ingredients on Successful Fertilization and Embryo Development of Sea urchins *Tripneustes gratilla* (Linnaeus, 1758)

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Abstract

The success of fertilization and development of sea urchin embryos *Tripneustes gratilla* can be used as a bioindicator of the water quality against the accumulation of pollutants. One of the contaminants that are often used is detergent with an active ingredient in the form of LAS (Linear Alkylbenzene Sulphonate). The purpose of this study was to analyze the effect of LAS (Linear Alkylbenzene Sulphonate) on the success of fertilization and development of sea urchin embryos. This research was conducted in September 2021 at the Zoology Laboratory, Pattimura University, Ambon. *Tripneustes gratilla* were treated with exposure to the active ingredient LAS (Linear Alkylbenzene Sulphonate) with a concentration of 0.0; 0.5; 1, and 2 $mg.L^{-1}$. The parameter used to assess the success of fertilization is the formation of membrane fertilization. Parameters of embryo development are the division of 2 cells, 4 cells, 8 cells, 16 cells, and 32 cells to form a morula, blastula, and hatching blastula. The results showed that the active ingredient of detergent LAS with a concentration of 0.5 $mg.L^{-1}$ caused delays in the process of fertilization and embryo development. Meanwhile, the active ingredient LAS with concentrations of 1 and 2 $mg.L^{-1}$ caused failure in the fertilization process and the embryonic development process of *Tripneustes gratilla* (Linnaeus, 1758).

Keywords: Detergent, embryo, fertilization, *Tripneustes gratilla.*

INTRODUCTION

Detergent is one of the main components of household waste and, in a certain amount, can pollute the aquatic environment because it can cause a lot of foam on the surface of the water [1]. The main component of detergents is surfactants (Surface Active Agents), where surfactants are a type of active ingredient that causes a decrease in the surface tension of the liquid [1]. The most frequently used detergent surfactant is LAS (Linear Alkylbenzene Sulphonate) [2]. One of the aquatic organisms that can be used as a bioindicator of water quality is the sea urchin. It is due to the biological condition of sea urchins that are responsive to changes in environmental conditions [3]. One type of sea urchin that can be used as a bioindicator is *T. gratilla*.

The accumulation of detergent in the sea will cause a lot of foam that can cover the surface of the water, thus disrupting the diffusion of oxygen (O_2) from the air into the water [1]. When the oxygen supply in the water is disturbed, the respiration process in aquatic organisms will also be disrupted [4].

Dissolved oxygen comes from the diffusion process and the photosynthesis process of phytoplankton and is used for respiration by aquatic organisms and for the decomposition of organic substances by microorganisms [5]. The dissolved oxygen level in waters, according to the Minister of Environment, is <5 $mg.L^{-1}$ [6]. In addition, the concentration of detergent in water also affects the pH, namely, the higher the concentration of detergent in a water, the higher the pH of the water [7]. Detergents have a pH that ranges from 10-12, while the ideal pH for water ranges from 7-8.5 [6]. The degree of acidity (pH) is a description of the concentration of hydrogen ions that accumulate in a liquid [5]. The existence of variations in pH in water affects the life of organisms that live in these waters considerably. For example, the presence of phytoplankton which supported by the availability of nutrients in these waters [8]. Available environmental conditions such as temperature, salinity, dissolved oxygen, pH, and strong currents affect the survival and reproduction of *T. gratilla* [9], such as successful fertilization and embryo development because the environment is a fertilization medium for sea urchins.

Surfactants are molecules with a polar hydrophilic group and a non-polar hydrophobic group so that the hydrophobic component can damage the egg cell membrane and enter the cell while the hydrophilic will dissolve egg protein and fat in water. Damage to the membrane due to the surfactant will cause fluid to enter the egg cell so that the egg cell does not develop, and after that, cell death will occur [10]. In addition,

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surfactants also suppress the work of respiration, namely inhibition of respiratory activity, which results in the disruption of the oxygen uptake process by the eggs so that the eggs are deprived of oxygen and will die [11]. Damage to eggs is indicated by the opening of the egg cell membrane, the color of the egg changes to pale white, and the gelatin capsule that encloses the egg will turn yellow-brown and then harden [10].

Embryos exposed to pollutants will experience disturbances at their developmental stage. It can result in abnormalities in the form of developmental delays, developmental abnormalities and also decreased differentiation in the embryonic layer [12]. Thus, evaluation of the effect of pollutant toxicity can be carried out by identifying abnormalities in sea urchin embryos [13]. Research on the effect of LAS detergent on water quality has been carried out. However, research using eggs and embryos of *T. gratilla* sea urchins as a bioindicator of contaminants of active detergent ingredients in the coastal area of Ambon City has never been carried out. Therefore, it is necessary to conduct a more specific analysis regarding the effect of the active ingredient of LAS detergent on the success of fertilization and development of sea urchin embryos because this understanding can be used as the basis for developing water quality bioindicators. With the research using *T. gratilla* sea urchins from the waters off the coast of Ambon Island as a model animal in the development of water quality bioindicators, it is hoped that the population of *T. gratilla* sea urchins in nature can be monitored in the future.

METHODS

This research was conducted in September 2021 at the Zoology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon. The treatment design in this study consisted of several concentrations of LAS (Linear Alkylbenzene Sulphonate) surfactant with three replication on each treatment (Table 1).

Table 1. Treatment Group

Code	Treatment
P1	Seawater + no detergent (Control)
P2	Seawater + LAS 0.5 mg.L^{-1}
P3	Seawater + LAS 1.0 mg.L^{-1}
P4	Seawater + LAS 2.0 mg.L^{-1}

Fertilization and Embryo Culture

Sea urchins were obtained from the coastal waters of Latulalat, Ambon-Maluku. The water are far from residential areas. It also classified as free of industrial activities. Thus, they are not contaminated by pollutants, which are the main factors underlying the selection of these locations.

Sea urchins taken from nature with a body diameter of 75-80 mm [14] were cleaned and injected with 0.5 M KCl solution as much as 1-3 mL through the peristomial membrane to stimulate spawning. After that, the female sea urchins were placed on a beaker containing seawater, while the male sea urchins were placed on an empty beaker for 30 minutes with the oral position facing down. Eggs and sperm that come out are accommodated in a beaker filled with sea water. The aboral side view of male and female sea urchins shortly after spawning is shown in Figure 1.

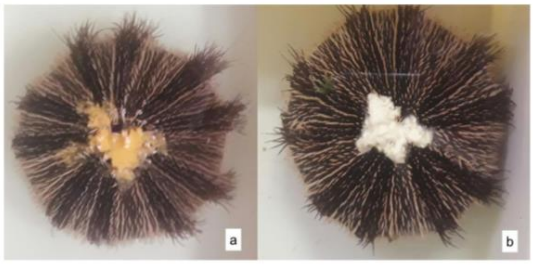

Figure 1. Sea urchin Spawning Aboral View; Oocyte Release (A) and Spermatozoa Release (B) [15]

To analyze the effect of LAS on the success of fertilization, the fertilization process was carried out by mixing 0.1 mL of eggs and 0.1 mL of sperm which had been diluted (0.1 mL of concentrated sperm in 1 mL of seawater). The density of sperm was 1 individual.mL^{-1} in a petri dish containing 20 mL of seawater and left until the egg is fertilized (embryo phase). Fertilization was carried out in seawater with several different concentrations of LAS (Table 1). Observation of the success of fertilization was carried out 15 minutes after the eggs and sperm were mixed. Eggs were placed in counting columns (Sedgewick Rafter Chambers) to be counted and observed under a microscope with a magnification of 40x.

Furthermore, to analyze the effect of LAS on embryonic development, fertilization was carried out by mixing 0.1 mL of egg and 0.1 mL of sperm which had been diluted (0.1 mL of concentrated sperm in 1 mL of seawater) to reach the embryonic stage in 1000 ml of seawater. After that, the embryos were cultured at a density of 1 individual.mL^{-1} at different concentrations of LAS (Table 1) in 20 mL of seawater.

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The parameters used in this study were the percentage of successful fertilization. The parameters used in this study were the percentage of successful fertilization. It is the number of successfully fertilized eggs indicated by the formation of a fertilization membrane and the percentage of normal embryos. The number of embryos that observed were underwent division starting from 2 cells, 4 cells, 8 cells, 16 cells, 32 cells to form morula, blastula and hatching blastula. Then the embryos were placed in a counting column (Sedgewick Rafter Chambers) and observed under a microscope with a magnification of 40x.

Water Quality Measurement

Water quality was measured for each treatment on each parameter, namely temperature, pH, DO, and salinity. The tools used in this study are a thermometer to measure temperature, a pH meter to measure pH, a DO meter to measure DO, and a refractometer to measure salinity.

Data Analysis

Data in the form of a description of the success of fertilization and embryo development were analyzed descriptively. Data in the form of the percentage of successful fertilization and embryo development were processed using Microsoft Excel 2013.

RESULT AND DISCUSSION

The Effect of LAS on Fertilization Success

The success of fertilization can be observed based on indications of the formation of a fertilization membrane in the form of a transparent layer that covers the entire outer surface of the egg (Figure 2). The percentage of eggs that were successfully fertilized in the control treatment; 0.5 mg.L^{-1}; 1 mg.L^{-1} and 2 mg.L^{-1} were 100%, 7%, 0% and 0%, respectively (Figure 3). It indicates that in the 1 mg.L^{-1} treatment and 2 mg.L^{-1} treatment, the eggs were not fertilized, which was characterized by the absence of a fertilization membrane. The environmental conditions available in the control treatment (0.0 mg.L^{-1}) allowed the fertilization process to take place without any disturbance so that the eggs were completely fertilized. The fusion of spermatozoa and egg takes 15 minutes. During the fertilization process, the movement of the cytoplasm increases so that the cell surface becomes irregular. Shortly before the first division begins, the membrane will stop vibration so that the cell surface becomes regular and the hyaline layer thickens [16].

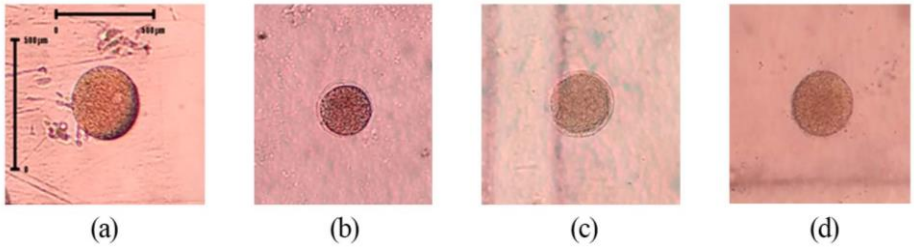

Figure 2. Sea urchin eggs *Tripneustes gratilla* in control treatment, treatment 0.5 mg.L^{-1} LAS (P2), treatment 1 mg.L^{-1} LAS (P3) and treatment 2 mg.L^{-1} LAS (P4). Eggs before fertilization (a); eggs with immature fertilization membranes (b); eggs with complete fertilization membranes (c), and unfertilized eggs (d).

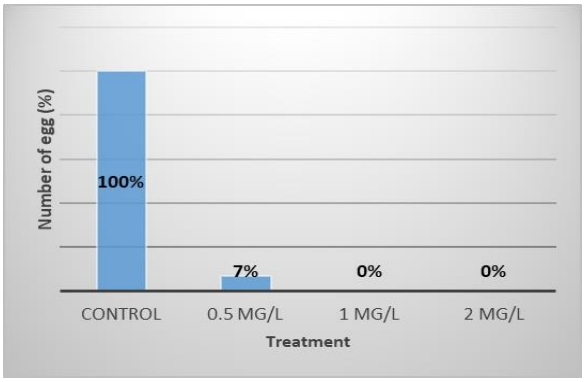

Figure 3. The results of observations of fertilization of *Tripneustes gratilla* sea urchins in the control treatment, 0.5 mg.L^{-1} LAS treatment, 1 mg.L^{-1} LAS treatment, and 2 mg.L^{-1} LAS treatment.

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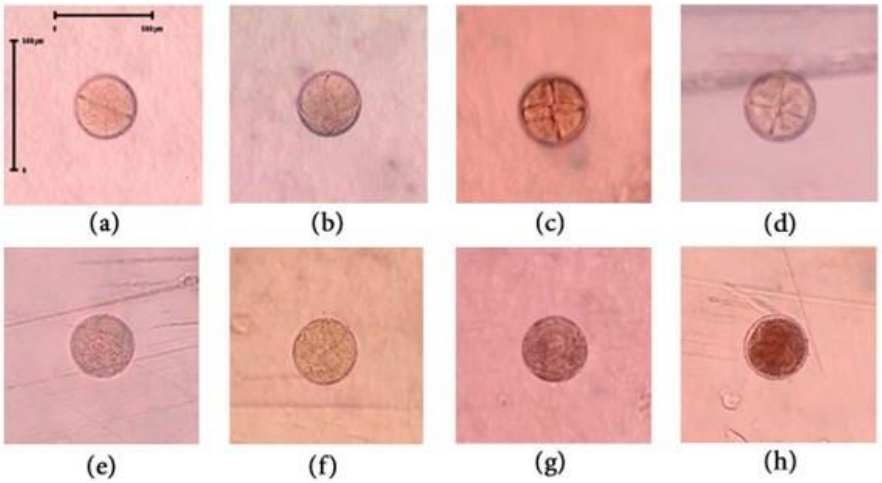

Figure 5. Embryos that failed to develop and embryos that died after being treated with 0.5 mg/L LAS (P2), 1 mg/L LAS (P3) and 2 mg/L LAS (P4): embryos that failed to divide 2 cells (a); embryos that fail to divide 4 cells (b); embryos that fail to divide 8 cells (c); embryos that fail to divide 16 cells (d); 32 cell (e) failed embryos; embryos that fail to morula (f); dead morula (g); dead blastula (h).

Furthermore, at a concentration of 0.5 mg.L^{-1} the percentage of successful fertilization was 7%, which means that most of the eggs were not fertilized. Unfertilized eggs are eggs that experience a delay in the fertilization process in the form of incomplete membrane formation (Figure 4). LAS at a concentration of mg.L^{-1} has started to have an effect on egg mortality [11]. LAS quality standard in a waters is 0.5 mg.L^{-1} [17]. LAS with a concentration of 1 mg.L^{-1} and 2 mg.L^{-1} caused damage to the cell membrane due to the interaction between the cell membrane and the LAS hydroxyl group so that the egg cells were penetrated by the LAS. The hydrophobic component of the surfactant damages the egg cell membrane and enters the cell, while the hydrophilic component of the surfactant dissolves egg protein and fat in water [10]. Damage to the membrane will cause fluid to enter the egg so that the egg is not fertilized. Fertilization occurs when the spermatozoa move closer to the jelly layer of the egg cell then the acrosome membrane will fuse with the spermatozoa plasma membrane and exocytosis occurs resulting in the release of enzymes from the acrosome granules. This enzyme allows spermatozoa to penetrate the jelly layer of the egg.

Effect of LAS on Embryo Development

The process of embryonic development begins after the egg is successfully fertilized. Under normal conditions, embryogenesis takes place over 8 hours 45 minutes, starting with the cell division process until it reaches the morula stage and ends at the blastula stage (Table 2).

Table 2. Embryonic Development Stage

Time Duration	Embryo Stage	Treatment	%
1 h, 12 min	2-cells	control	97
		0.5 mg.L^{-1}	8
		1.0 mg.L^{-1}	0
		2.0 mg.L^{-1}	0
2 h, 3 min	4-cells	control	95
		0.5 mg.L^{-1}	13
		1.0 mg.L^{-1}	0
		2.0 mg.L^{-1}	0
2 h, 28 min	8-cells	control	95
		0.5 mg.L^{-1}	10
		1.0 mg.L^{-1}	0
		2.0 mg.L^{-1}	0
2 h, 51 min	16-cells	control	95
		0.5 mg.L^{-1}	0
		1.0 mg.L^{-1}	0
		2.0 mg.L^{-1}	0
3 h, 12 min	32-cells	control	95
		0.5 mg.L^{-1}	0
		1.0 mg.L^{-1}	0
		2.0 mg.L^{-1}	0
3 h, 32 min	morula	control	95
		0.5 mg.L^{-1}	0
		1.0 mg.L^{-1}	0
		2.0 mg.L^{-1}	0
8 h, 45 min	blastula	control	94
		0.5 mg.L^{-1}	0
		1.0 mg.L^{-1}	0
		2.0 mg.L^{-1}	0

Note: Time duration after fertilization; h=hour, min=minutes. At the blastula stage, the embryo hatches from the fertilization membrane that encloses it during embryogenesis.

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In the control treatment, the embryos developed successfully under normal conditions. However, in the 0.5 mg.L^{-1} treatment, the embryos experienced developmental delays at the 2-cells, 4-cells, 8-cells stages, and died at the 16-cells, 32-cells, morula, and blastula stages. Accumulated LAS concentrations exceeding 0.5 mg.L^{-1} are toxic to various aquatic organisms [17]. It is in line with the results proposed by a previous study where at a concentration of 0.5 mg.L^{-1}, there were abnormalities in eggs caused by the entry of surfactants through the egg membrane resulting in chromosomal abnormalities and gene damage. Certain concentration of surfactants could denature proteins. It will damage several enzymes and hormone systems involved in embryonic development [11]. LAS threshold standard in a water is 0.5 mg.L^{-1} [17].

Meanwhile, in the 1 mg.L^{-1} treatment and 2 mg.L^{-1} treatment, the embryo failed to develop and died at each stage of its development. An increase in the concentration of LAS 1 mg.L^{-1} and 2 mg.L^{-1} causes the activation of hatching enzymes such as chorionase enzymes which are inhibited so that embryos fail to hatch, besides that the embryos also fail to divide, are disabled, and die [3]. The egg mortality rate increased along with the increase in LAS concentration as reported in previous studies, namely at a concentration of 1.5 mg.L^{-1} and 3 mg.L^{-1}, eggs died which was marked by a change in egg color which was initially bright or transparent then turned white or brown cloudy [11].

Culture Media Water Quality

The quality of the culture media was measured during the culture process, which included several parameters, namely temperature, pH, DO, and salinity. The results of water quality measurements in culture media showed that the temperature of the culture media increased with the increase in the concentration of the active ingredient of LAS detergent, which resulted in a greater percentage of embryo mortality. Temperature greatly affects the development of the short planktonic period of sea urchins [18]. When the ambient temperature becomes higher, then the hatchability will also increase. Otherwise, if the ambient temperature becomes lower, then the hatchability will also decrease [19]. An increase in temperature will increase the rate of respiration so that the need for oxygen will also increase. Respiration results in an increase in carbon dioxide in seawater due to reacting with acidic carbonic acid, so that the pH value will also decrease. A decrease in the pH value affects sperm movement, which becomes slower [20].

Table 2. Culture media water quality

LAS (mg.L^{-1})	Temperature (°C)	pH	DO (mg.L^{-1})	Salinity (ppt)
0*	26.6	7.62	3.0	35.0
0.5	26.7	7.59	3.1	34.4
1	26.8	7.57	3.2	34.2
2	26.9	7.56	3.2	34.0

Note: *control (seawater, no LAS).

Furthermore, dissolved oxygen (DO) levels in the culture media increased in line with the increase in the concentration of the active ingredient in LAS detergent. The increase in DO levels was due to an increase in the percentage of embryonic death, so that the embryo's need for oxygen was also lower. Then the salinity in the culture media decreased when the concentration of the active ingredient in LAS detergent increased. Echinoderms are generally not resistant to low salinity except for species that live in tidal areas, such as sea urchins [21]. The salinity of marine waters ranges from 30-40 ppt [22].

CONCLUSION

The active ingredient of detergent LAS (Linear Alkylbenzene Sulphonate) with a concentration of 0.5 mg.L^{-1} caused delays in the process of fertilization and embryo development. Meanwhile, the active ingredient LAS (Linear Alkylbenzene Sulphonate) with concentrations of 1 mg.L^{-1} and 2 mg.L^{-1} caused failure in the fertilization process and the embryonic development process of *Tripneustes gratilla*.

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