CHRONIC AFFECTS OF DETERGENT SURFACTANT (LINEAR ALKYLBENZENE SULFONATE / LAS) ON THE GROWTH AND SURVIVAL RATE OF SEA BASS (*Lates calcalifer* Bloch) LARVAE

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ABSTRACT

Chronic Effects of Surfactant Detergent Linear Alkyl-Benzene Sulfonate (LAS) On the Growth and Survival Rate of Sea Bass (*Lates Calcalifer* Bloch) Larvae

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Sea bass (*Lates calcalifer* Bloch) is one of the economically important marine fishes, which is getting more important for marine-culture. This fish is categorized as a euryhaline species, i.e. has a wide salinity tolerance range. This species can live in the sea with salinity between 32 – 35 ppt, and in the river, estuarine and mangrove areas with the salinity between 0 – 25 ppt. The adult sea bass spawn in marine waters and the larvae and juvenile are mostly found in the estuarine. Estuarine is known as a good nursery and feeding ground, however it is also known as a pollutant trap. Therefore, the larvae of seabass and other euryhaline species are very susceptible to this condition.

Surfactant detergent Linear Alkyl-benzene Sulfonate (LAS) is a non-ionic soft detergent, which has a long straight carbon chain and a powerful cleaning capability. It is toxic to aquatic organisms, and however it is biodegradable. Therefore, it is widely used for cosmetic and household purposes. Some research found that the toxicity (LC_{50}-96 hours) of LAS detergent on invertebrate *Daphnia magna* was 2.7 mg/l, on gastropods was 19.4 mg/l and on shrimp (*Panaeus japonicus*) was 4.5 mg/l. The acute and chronic effect of this detergent on tropical marine fish is not yet known.

This research was done to find out the chronic effect (LC_{50}-96 hours) and acute effects, of detergent LAS on the larvae of sea bass (*Lates calcaliver* Bloch). A Bioassay method was applied to find out the acute toxicity, and Probit Analyses was used to find out the LC_{50}-96 hours of detergent LAS on sea bass larvae. Randomized Design was used to observe the chronic effects on the growth, survival rate of the sea bass larvae. There were six treatments applied, i.e.: treatment A (0% of LC_{50}-96 hours); B (5% of LC_{50}-96 hours); C (10% of LC_{50}-96 hours); D (15% of LC_{50}-96 hours); E (20% of LC_{50}-96 hours); F (25% of LC_{50}-96 hours). Analyses of variance were used to find out if there was a significant different in the treatment, followed by Multiple Range Duncan Test to find out the different among treatments. The histology of the gill and liver of the sea bass exposed to different concentration of detergent LAS was also observed.

The results showed that the LC_{50}-96 hours of detergent LAS on sea bass larvae was 1.18 mg/l and considered as moderately high toxicity. The absolute biomass growth of sea bass larvae was not affected by sub-lethal concentrations of detergent ALS, however, chronic concentrations of detergent LAS affected the daily growth rate of sea bass larvae significantly ($p<0.01$).

As a conclusion, the acute toxicity of LAS detergent on sea bass (*Lates calcaliver* Bloch) larvae was 1.18 mg/l. The sub-lethal concentrations of detergent LAS on the sea bass larvae did not influence the biomass growth and survival rate but affected the daily growth rate of sea bass larvae significantly. The sea bass larvae exposed to the sub lethal concentrations of LAS detergent for 30 days resulted in the gill damage, i.e.: hypertrophy, hyperplasia, telengeastases and melanization of the gill. The congestion and vacuolar degeneration of the liver were also observed.

Key Words: Detergent Linear Alkyl-benzene Sulfonate (LAS), Sea bass larvae, LC_{50}-96 hours; Chronic Effects; Growth; Survival Rate

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INTRODUCTION

Background

Sea bass (*Lates calcaliver* Bloch) is one of the high economic value marine fishes from Indonesian waters and categorized as a euryhaline and a catadromous species. The fish spawns in the open sea with water salinity between 32 – 35 ppt, and the eggs drifted to the shore: estuary and river to hatch. Furthermore, he mentioned that the larvae abundantly found in this area at the salinity between 0 – 25 ppt. Therefore, sea bass is chosen as cultivated species in marine, brackish and fresh waters(Soetomo, 1997).

Aquatic environment, especially estuarine is vulnerable from effluent discharged from terrestrial activities, is therefore, susceptible to the aquatic pollution (Supriharyono, 2000). According to Katz (1971) **in** Owen (1975), the waste can be divided in to four categories, i.e.: domestic, agriculture, industry and radioactive wastes.

One of the most common domestic wastes that commonly enter the aquatic ecosystem is detergent. The use of detergent as the washing substance is widely known in Indonesia. At first the most common active substance of detergent used is Alkyl Benzene Sulfonate (ABS) that is a non-biodegradable chemical substance (Chiao *et al*, 1992). Linear Alkyl-benzene Sulfonate (LAS) isan anionic surfactant then replaced the usage of ABS. According to Heath (2000), LAS was four times more toxic than ABS, however it is biodegradable. Effluent of LAS was found in the marine, brackish and fresh water ecosystems (Dugan, 1972 **in** Supriharyono, 1998).

Problems that occurred due to detergent pollution in the aquatic ecosystems are mostly the water quality degradation due to the low diffusion rate of oxygen from the air in to the water, which resulted in the oxygen intake failure of the aquatic organisms. In short term, the accumulation of detergent in the water may disturb the vision (eyes) of the fish as well as create gill damage (Sitorus, 1997).

Detergent may also affects the liver of aquatic organisms indirectly through absorption of certain tissue. As liver acts as detoxicant of any toxic substances enters the body (Yatim, 1990). It was mentioned further that the first liver damage found was congestion, i.e. the increase of the blood volume in the blood capillaries. The failure of oxygen intake by the fish and liver damage result in the growth retardation (Himawan, 1988, Yatim, 1990).

Sea bass is one of the aquatic organisms, which is vulnerable to the detergent pollution. As an economic valuable species, sea bass is chosen as fish for aquaculture development. The constrain for culturing this species is its insensitivity on aquatic pollution, such as detergent. The fish growth retardation was observed in the lower stage of detergent pollution, and further stage of the detergent pollution was the increase mortality of the fish especially during its larval stage. Research on the toxicity of detergent, especially LAS on sea bass larvae has not been done. The

increase use of LAS for domestic and industrial purposes and the development sea bass culture promote the investigation on the acute toxicity of LAS detergent on sea bass larvae.

Objectives

The objectives of this investigation were: to find out the acute toxicity (LC_{50}-96 hours) of Linear Alkyl-benzene Sulfonate (LAS) detergent, the chronic (sub lethal) effects on larvae growth and survival rate and the histological damage of the gill and liver of sea bass (*Lates calcaliver* Bloch) larvae

MATERIALS AND METHODS

Materials

Tested fish.-- Sea bass (*lates calcalifer* Bloch) at the size of 0.5 – 1 cm in length and average initial body weight of 1.26 – 1.33 g were used. The fish were kept in the experiment unit for one week for aclimation. During adaptation period the survival rate of the larvae should be ≥ 90%. The stocking density of the larvae was 10 fish/10 litres of water (Departement Pertanian, 1983; Rand and Petrocelli, 1985).

Tested media.-- The water used as the media during the investigation was sea water obtained from Central Brackish Water Research Centre, Jepara at the salinity of 25 ppt.

Tested substance.-- The tested substance in this research was active substance of Linear Alkyl -benzene Sulfonate (LAS). A stock solution (100 mg/l) of LAS was prepared prior investigation. For the application on each treatment the following formula (CEA, 1993) was used:

$$V_1 \times M_1 = V_2 \times M_2$$

V_1 = volume that will be applied (ml)

M_1 = Concentration of stock solution (100 mg/l)

V_2 = Volume of tested media (10.000 ml)

M_2 = Concentration of each treatment (mg/l)

Buffered Formalin Solution.-- This solution was used for tested animals preservation prior histological investigation.

Methods

The research was done in two steps, i.e. first step was preliminary finding investigation and the second step was the chronics toxicity experiment

Preliminary Finding Investigation.-- A bioassay method was applied to find out the LC_{50}-96 hours of LAS on sea bass larvae, which consisted of two steps.

Firstly to investigate the LC_{100}-24 hours, i.e. the highest concentration of LAS (N) that caused 100% mortality of tested larvae during 24 hours exposure and LC_0-48 hours, the lowest concentration of LAS (n) that resulted in 100% survival rate of the tested fish larvae after 48 hours exposure. Ten (10) tested fish larvae were kept in the 1 liter of water and exposed to the LAS. The LAS concentrations range for preliminary finding investigation were: A= 0,001 mg/l, B= 0,01mg/l, C= 0,1 mg/l, D= 1 mg/l, E= 10 mg/l, F= 100 mg/l, G= 1000 mg/l dan K= 0 mg/l.

The results of the preliminary finding investigation showed that the LC_{100}-24 hours, (N) of LAS was 10 mg/liter and LC_0-48 hours (n) was 1 mg/liter.

Since those concentrations range were too wide, second step was applied by using those results to narow down the concentrations range of LAS following the formula of Komisi Pestisida (Pesticide Committee) (1983).

$$(1) \quad \log \frac{N}{n} = k\left(\log \frac{a}{n} \right)$$

Remarks

N : The highest limit concentration (mg/l)

n : The lowest limit concentration (mg/l)

a : The lowest concentration in the concentration range)mg/l)

k : Number of tested concentrations (a, b, c, d, e, f and g)

To find out the LC_{50}-96 hours the following formula was applied.

$$(2) \quad \frac{a}{n} = \frac{b}{a} = \frac{c}{b} = \frac{d}{c} = \frac{e}{d} = \frac{f}{e} = \frac{g}{f}$$

It was found that 100% (N) mortality was found at concentration 1.38 mg/l. The results of the second step was meant to narrow down the concentrations range to find out the LC_{50}-96 hours (lays between 1–1.38 m/l) following the formula of Komisi Pestisida (Pesticide Committee) (1983). Therefore, the concentrations range to find LC50-96 hours were: A= 1.05 mg/l, B= 1.09 mg/l, C= 1.15 mg/l, D= 1.20 mg/l, E= 1.25 mg/l, F= 1.31 mg/l, G = 1.38 mg/l and K = 0 mg/l. Ten (10) sea bass larvae were kept in 1 liter container contaminated with those LAS concentrations. The mortality data were analysed using Probit Analyses. The result showed that the LC_{50}-96 hours of LAS on sea bass larvae was 1.18 mg/l

Chronics Toxicity Experiment.-- After the LC_{50}-96 hours of LAS on sea bass larvae was obtained, the chronics toxicity experiment was run to investigate the sub-lethal impact of ALS on the growth, survival rate, gill and liver histological changes on se bass larvae. Completely Randomized Design was applied. There were six treatments used, each treatment replicated three times. The treatments applied following (Hubert, 1980) were:

1. A = LAS Concentration 0 % of LC_{50}-96 hours (0 mg/l)
2. B = LAS Concentration 5 % of LC_{50}-96 hours (0.094 mg/l)
3. C = LAS Concentration 10 % of LC_{50}-96 hours (0.188 mg/l)
4. D = LAS Concentration 15 % of LC_{50}-96 hours (0.283 mg/l)
5. E = LAS Concentration 20 % of LC_{50}-96 hours (0.377 mg/l)
6. F = LAS Concentration 25 % of LC_{50}-96 hours (0.472 mg/l)

Data Collection.

The data collected during this study were: absolute growth, specific growth rate and the survival of the fish larvae. The histological changes of the fish gill and lives were also observed.

Growth.—The growth data parameter observed in this study were the absolute biomass growth of the larvae was recorded following Stickney (1979) and the Specific Growth Rate following Effendi (1979) :

$$\mathbf{W} = Wt - Wo$$

W = Absolute biomass growth (g)

Wt = Initial biomass weight (g)

Wo = The biomass Weight at the end of investigation (g)

The specific growth rate (%/day) of the larvae was recorded following Effendi (1979) :

$$\mathbf{SGR} = \frac{\mathbf{LnWt - LnWo}}{\mathbf{t_1 - t_2}}$$

SGR = Specific Growth Rate (%/day)

$\mathbf{t_2}$ = Duration (end) of the investigation (day)

$\mathbf{t_1}$ = Time of the investigation started (day)

Survival Rate.-- The survival rate of the sea bass larvae was observed and calculated followinf Effendi (1979) :

$$\textbf{Survival rate} = \frac{\textbf{Total number of live fish at the end of investigation}}{\textbf{Total initial number of fish}} \textbf{ X 100 \%}$$

Data analyses

Probit Data Analyses was used to find out the LC_0-24 hours, LC_{100}-28 hours and LC_{50}-96 hours of LAS on sea bass larvae. While to find out the chronic effects of sub-lethal concentrations of LAS on sea bass growth and survival rate, Analyses of Variance was applied. If there was significant effect of the treatment, the data were further analyzed using Multiple Range Duncen Test to find out the significant different between treatments.

RESULTS AND DISCUSSION

Results

Preliminary Finding Investigation.

The preliminary finding investigation showed that the LC_{50}-96 hours of LAS on sea bass larvae was 1.18 mg/l.

According to Indonesian Pesticide Commission the LC50-96 hours of LAS on sea bass was moderately high (1 mg/l $< LC_{50}$-96 hours $<$ 10 mg/l). Therefore, the present of ALS in the aquatic environment should be taken in to consideration. Even though LAS is considered biodegradable, however, effluent contains this substance should be controlled and monitored to avoid aquatic pollution.

Chronics Toxicity Experiment.

The chronic toxicity experiment was carried out for 30 days to find out the sub-lethal effects of LAS on the growth, survival rate, gill and liver histological changes of the tested fish larvae. The concentrations used in this study were: 0%; 5%; 10%; 15%; 20% and 25% of LC_{50}-96 hours (i.e. 1.886 mg/l) of LAS on sea bass larvae (Hubert, 1980): A (0 %); B (0.059 mg/l); C (0.199 mg/l); D (0.178 mg/l); E (0.238 mg/l); F (0.297 mg/l).

1. Survival Rate of Sea-bass Larvae.

There was no mortality found during 30 days investigation on the chronic effects of LAS on sea bass larvae.. This result showed that the sea-bass larvae was still being able to survive (100% survival rate) during 30 days exposure to the sub lethal concentration of LAS, however, the chonic effects of LAS sub-lethal concentration on growh and gill damage were found . This result may be due to the characteristic of LAS, i.e. easily degradable. According to Vives-Rego *at al* **In** IPCS (1996), almost 70% of LAS composition at the concentration of 20 mg/l in the sea water at 22^{o} C would be degraded during 10 days. Furthermore, Von Bock & Man (1971) **In** IPCS (1996) mentioned that in the sea water 97 % approximately of 10 mg/l LAS composition was degraded in two weeks.

2. Growth.

2.1. The Absolute Biomass Growth of Sea-bass Larvae

The biomass growth was observed weekly, and it showed a steady increase. The data biomass growth is presented at Table 1

Table 1. The Absolut Biomass Growth (gram) of Sea-bass Larvae after 30 days exposed to sub-lethal concentrations of LAS in Each Treatments and Replications

Treatments	Replicates	Wo	Wt	Δw
A (0.000 mg/l)	1	1.29	4.10	2.81
	2	1.31	4.05	2.74
	3	1.40	4.54	3.14
	Total	4.00	12.69	8.69
	Average ± sd	1.33 ± 0.059	4.23 ± 0.270	2.90 ± 0.214
B (0.094 mg/l)	1	1.24	4.28	3.04
	2	1.28	4.15	2.87
	3	1.30	4.42	3.12
	Total	3.82	12.85	9.03
	Average ± sd	1.27 ± 0.031	4.28 ± 0.135	3.01 ± 0.128
C (0.188 mg/l)	1	1.25	4.3	3.05
	2	1.24	4.27	3.03
	3	1.27	4.35	3.08
	Total	3.76	12.92	9.16
	Average ± sd	1.25 ± 0.015	4.31 ± 0.040	3.05 ± 0.025
D (0.283 mg/l)	1	1.26	4.33	3.07
	2	1.28	4.38	3.10
	3	1.25	4.34	3.09
	Total	3.79	13.05	9.26
	Average ± sd	1.26 ± 0.015	4.35 ± 0.026	3.09 ± 0.015
E (0.377 mg/l)	1	1.40	4.51	3.11
	2	1.32	4.46	3.14
	3	1.32	4.44	3.12
	Total	4.04	13.41	9.37
	Average ± sd	1.35 ± 0.046	4.47 ± 0.036	3.12 ± 0.015
F (0.472 mg/l)	1	1.30	4.49	3.19
	2	1.23	4.40	3.17
	3	1.26	4.42	3.16
	Total	3.79	13.31	9.52
	Average ± sd	1.26 ± 0.035	4.44 ± 0.047	3.17 ± 0.015

The Analyses of Variance of the absolute biomass growth of sea-bass larvae is shown in Table 2. The results of the analyses showed that there was no significant different in the treatment. It mean that the absolute biomass growth of sea-bass larvae was not affected by the sub-lethal concentration of LAS

Tabel 2. Analisyses of Variance of the Absolute Biomass Growth of Sea-bass larvae

Source of Variance	Df	TSqr	MSqr	F_{calc}	F_{table}	
					0.05	0.01
Treatment	5	0.140	0.028	2.660	3.110	5.060
Eror	12	0.127	0.011			
Total	17	0.267				

2.2. The Specific Growth Rate of Sea-bass Larvae

The specific growth rate of sea-bass larvae is shown as a daily growth rate in %. It is shown in Table 3. The results showed that the specific growth rate of the sea-bass larvae exposed to the sub-lethal

concentrations of LAS were higher compared to the daily growth rate of the larvae exposed to the media without any LAS

Table 3. The daily growth rate of sea-bass larvae exposed to sub-lethal concentrations of LAS and the media without any LAS after 30 days investigation

Treatments	Replicates	Wo	Wt	SGR(%)
A (0.000 mg/l)	1	1.29	4.10	3.854
	2	1.31	4.05	3.762
	3	1.40	4.54	3.922
	Total	4.00	12.69	11.54
	Average ± sd	1.33 ± 0.059	4.23 ± 0.270	3.85 ± 0.080
B (0.094 mg/l)	1	1.24	4.28	4.129
	2	1.28	4.15	3.921
	3	1.30	4.42	4.079
	Total	3.82	12.85	12.13
	Average ± sd	1.27 ± 0.031	4.28 ± 0.135	4.04 ± 0.109
C (0.188 mg/l)	1	1.25	4.3	4.118
	2	1.24	4.27	4.122
	3	1.27	4.35	4.104
	Total	3.76	12.92	12.34
	Average ± sd	1.25 ± 0.015	4.31 ± 0.040	4.11 ± 0.009
D (0.283 mg/l)	1	1.26	4.330	4.115
	2	1.28	4.38	4.101
	3	1.25	4.34	4.149
	Total	3.79	13.05	12.36
	Average ± sd	1.26 ± 0.015	4.35 ± 0.026	4.12 ± 0.025
E (0.377 mg/l)	1	1.40	4.51	3.899
	2	1.32	4.46	4.058
	3	1.32	4.44	4.043
	Total	4.04	13.41	12.00
	Average ± sd	1.35 ± 0.046	4.47 ± 0.036	4.00±0.088
F (0.472 mg/l)	1	1.30	4.49	4.132
	2	1.23	4.40	4.249
	3	1.26	4.420	4.183
	Total	3.79	13.31	12.56
	Average ± sd	1.26 ± 0.035	4.44 ± 0.047	4.19 ± 0.059

In general, the average growth rate of sea-bass larvae icreased when the sub-lethal concentrations were raised. The average specific growth rate of sea-bass larvae were: treatment A 3,85%/day, treatments B, C, D, E and F were 4,04%/dayi; 4,11%/day; 4,12%/day; 4%/day and 4,19%/day. Those data were transformed to Arcsin transformation. The Analyses of variance of the specific growth rate of the sea-bass larvae shown in Table 4

Table 4. Analyses of Variance of specific growth rate of sea-bass larvae

Source of Variance	Df	TSqr	MSqr	F_{calc}	F_{table}	
					0.05	0.01
Treatment	5	0.473	0.095	8.626**	3.110	5.060
Eror	12	0.132	0.011			
Total	17	0.605				

** : Highly Significat Different

Those analyses of variance shows that the treatments resulted in a highly significant different to the daily growth rate of sea-bass larvae during 30 days investigation (p > 001)

To finde out the dirretence between the treatments, a Multiple range Duncen Test was carried out, and it is shown in Table 5.

Table 5. Result of a Multiple Range Duncen Test on the daily growth rate of sea-bass larvae after 30 days experiment

Treatment	Mean	Difference				
F	11.7966	F				
C	11.7370	0.05963	C			
D	11.7155	0.08111	0.021472	D		
B	11.5990	0.19761*	0.13798	0.11651	B	
E	11.5365	0.26009*	0.20045*	0.17898	0.06247	E
A	11.3093	0.48729**	0.42766**	0.40618**	0.28968**	0.22720*

* = Significantly different
** = Highly Significantly different

2. 3. Histopathology

The histopathology of the gill and liver of sea-bass larvae exposed to the sub-lethal concentrations of LAS were analyzed to find out the effects of the sub-lethal concentrations of LAS on the gill and liver tissue of the tested fish larvae after 30 days exposed to the sub-lethal concentrations of LAS..

2.3.1. Histopathological analyses of the gill

The results showed that macroscopically, the gills were in a normal conditions. i.e. fresh red coloration, the gill lamella were arrange normally and the mucus were transparent. However, after the histology of gills were analyzed further microscopically, the results shown in Figure 1, 2, 3, 4 and 6

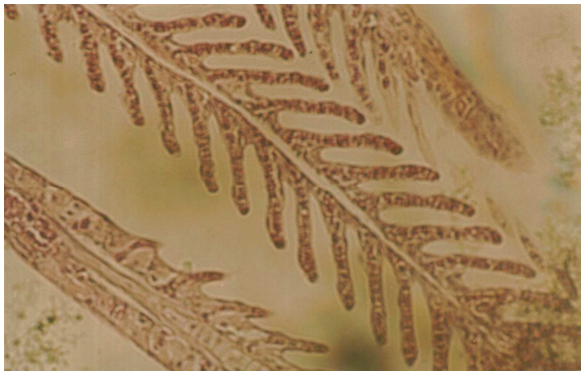

Figure 1. Histopathology of the gill in treatment A (0,0 mg/l LAS). Magnified 400x. It showed that there was no gill damage observed. The gill was still in normal condition

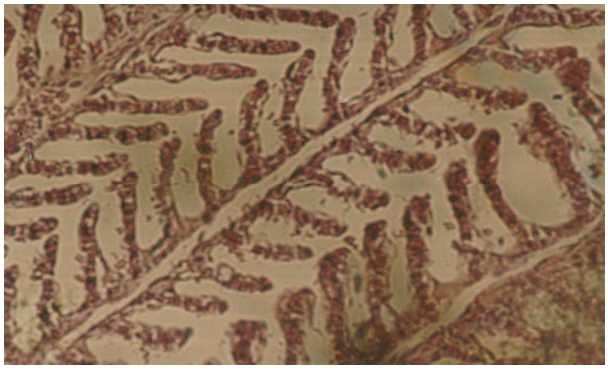

Figure 2. Histopathology of the gill in treatment B (0,094 mg/l LAS). Magnified 400x. It showed a hipertrophy. Hipertrophy was happened because of the increase size of the cell. The gill lamellae was swollen but the cell number were not change

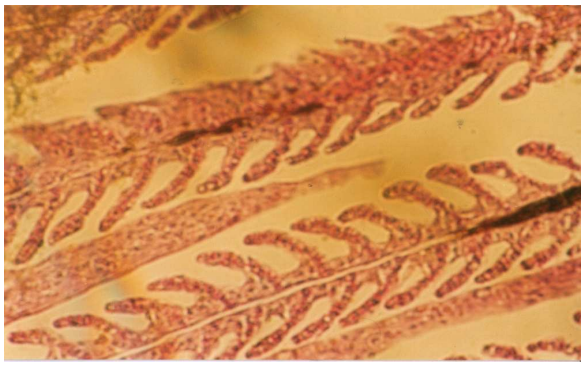

Figure 3. Histopathology of the gill in treatment C (0,188 mg/l LAS). Magnified 400x. It showed a pathological conditions: hypertrophy: the gill cells were swollen and melanization: black coloration of the of the blood capillaries

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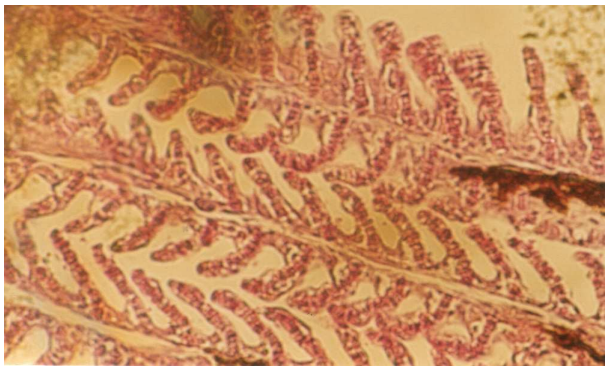

Figure 4. Histopathology of the gill in treatment D (0,283 mg/l LAS). Magnified 400x.It showed hypertrophy of the gill lamellae and melanization. The hyperthrophy in this treatment was almost allover the secondary gill lamellae. The gill cells were obviously swollen

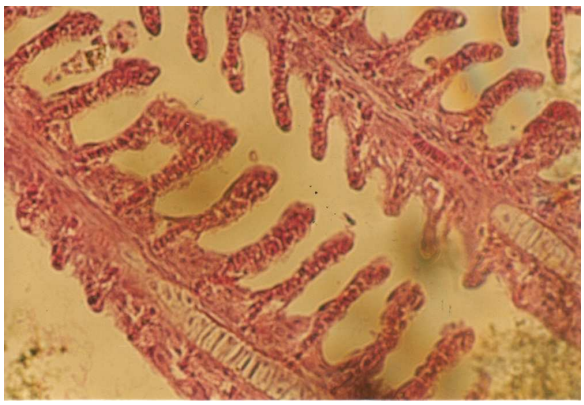

Figure 5. Histopathology of the gill in treatment E (0,377 mg/l LAS). Magnified 400x.It showed hypertrophy and hyperplasia of the gill lamellae. It shown that the swollen of the gill lamellae due to the increased number of the cell, i.e. the cell was in a normal size but the gill lamellae was swollen/bigger

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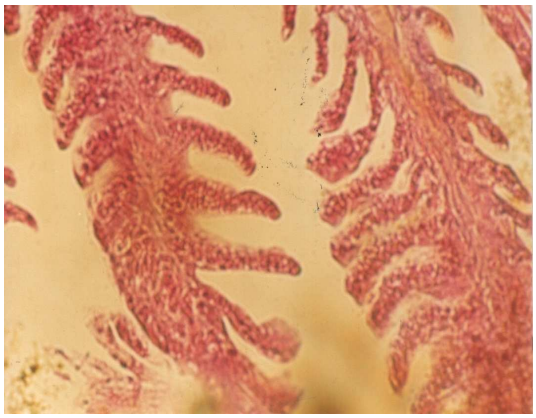
(a)



(b)

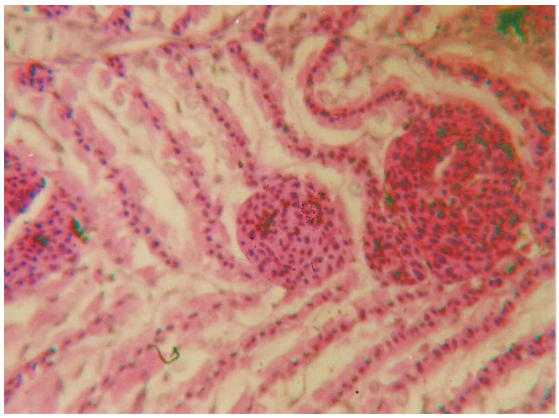


Figure 6. **(a)** Histopathology of the gill in treatment F (0,472 mg/l LAS). Magnified 400x. It showed hypertrophy and hyperplasia of the gill lamellae. The number of hyperplasia lamellae was dominant compared to the hypertrophy lamellae. This was due to the increase number of the cells so that the lamellae overlapped and sticked each other. **(6b)**The gill lamellae cell damage (telengeastacist) was also found Telengeastacist was showed by the swollen and showed an accumulation of local the blood capillaries

2.3.2. Histopathological analyses of the liver

The macroscopic observation of the sae-bass larvae liver was not abled to carry out since the whole tested fish were immersed in the fixation solution. Therefore, only microscopic histopathological analyzed was done. This was observed to find out thehistopathological damage of the liver of the tested fish after 30 days exposed to the sub-lethal concentrations of LAS.

In general, congestion tissue and degeneration vacuolar of the liver tissue of treatment F was observed. The results of histopathology of the liver were shown in Figure 7, 8, 9, 10, 11, 12.

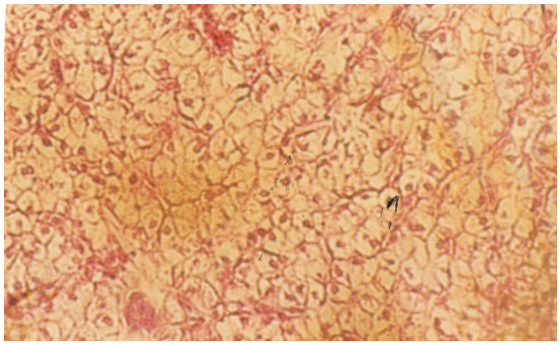


Figure 7. Histology of liver of Treatment A (0.0 mg/l LAS). Magnified 400x. No histological damage was found, the hepatocyt of the liver was clearly shown.

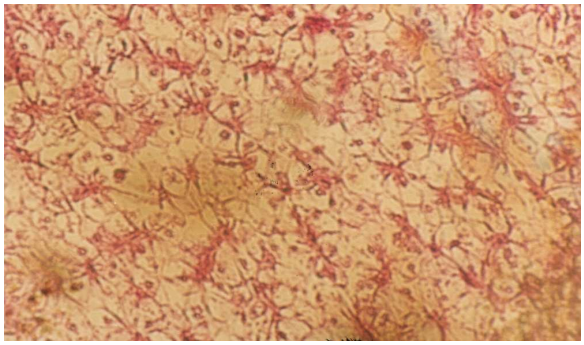


Figure 8. Histology of liver of Treatment B (0.094 mg/l LAS). Magnified 400x. Congestion of some of the tissue shown by red colorization of the blood capillaries because of the swollen and increase number of the red blood cell.

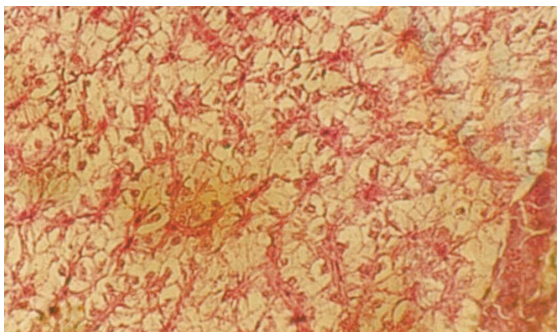


Figure 9. Histology of liver of Treatment C (0.188 mg/l LAS). Magnified 400x. More Congestion of the tissue in treatment C was observed compared to treatment B

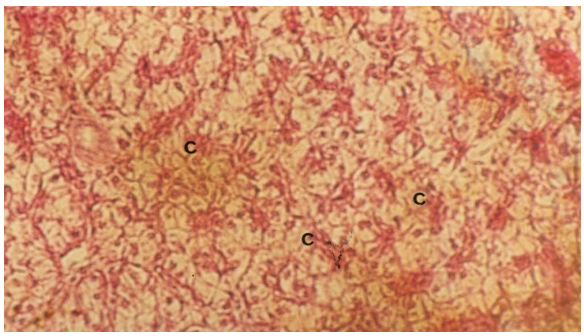
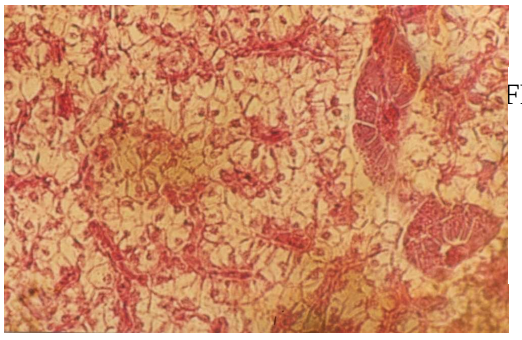


Figure 10. Histology of liver of Treatment D (0.283 mg/l LAS). Magnified 400x. More Congestion of the tissue in treatment D was observed compared to treatment C



FFigure 11. Histology of liver of Treatment E (0.377 mg/l LAS). Magnified 400x. More Congestion of the tissue in treatment E was observed compared to treatment D. The congestion were spread allover the liver tissue

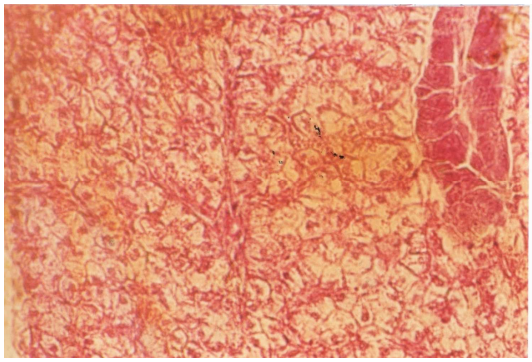
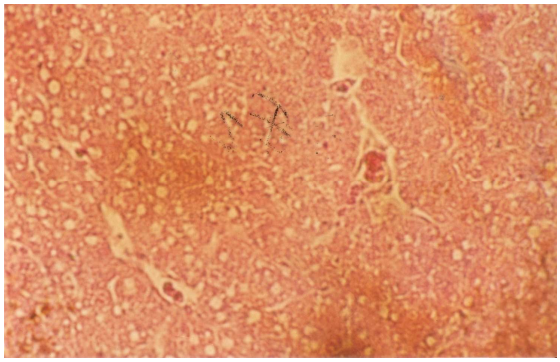
(a)  **(b)** 

Figure 12. **(a)** Histology of liver of Treatment F (0.377 mg/l LAS). Magnified 400x. More congestion were spread allover the liver tissue.

(b) Tissue damage: a vacuolar degeneration showed by empty vacuoles/holes around nucleus caused by swollen of the vacuolar.

2.3.4. Water Quality Controll and Management.

The water quality (pH, DO, salinity, alkalinity, hardness, ammonia) were controlled and managed daily to give a good living conditions of the media.

Discussion

1. Growth.

The average absolute biomass growth and specific growth rate of sea-bass larvae increased as the increase of the LAS concentration in the media (Table 1 and 3). Statistically the average absolute biomass growth of sea-bass larvae was not significantly affected by the LAS concentrations in the media (Table 2). However, of sea-bass larvae exposed to different concentrations of LAS significantly affected their specific growth rate ($p<0.01$) (Table 4).

The increase growth of sea-bass larvae exposed to the LAS may be due to the ability of the LAS to stimulate the growth of the sea-bass larvae. This assumption is support by Plumb (1964) who mentioned that the present of pollutant in the water media could give a positive response to the aquatic organisms included fish due to the abnormality, i.e. the increased number as well as the increase size of the cells tissue. Heath (2000) mentioned further that LAS as a pollutant had an ability to increase the thyroid hormone to promote the fish larvae growth. These results were also supported by Nugraha (2001) and Saijah (2003) whos found that the growth of common carp (*Cyprinus carpio*) juvenile showed a positive response after being exposed to 0.2 – 6.0 mg/l LAS for 35 days exposure.

At the beginning of the treatment, the average biomass of growth of the sea-bass were almost similar, however, there were observed a weekly growth variations until the end of the investigation. This variations resulted in the daily growth or specific growth differences. This condition most probably because of the different levels of homeostasis of the sea-bass larvae during the research. The fish larvae which had high homeostasis level would have the ability to utilize their metabolic energy for growth. Oppositely, the fish which had a low homeostasis level would only utilize their metabolic energy for live not for their growth. As mentioned by Warren (1971) that aquatic organisms had their own adaptation or homeostasis ability to withstand their internal conditions for their live and growth.

2. Survival Rate

There was no mortality of sea-bass larvae found during the research. This most probably because of the ability of sea-bass larvae to withstand the LAS in the media and also due to the natural condition of LAS, i.e. degradable. Von Bock & Man (1971) **In** IPCS (1996) showed that 10 mg/l ALS concentration will be 97% degraded after two weeks. Other studied by Vives-Ringo *et al* **In** IPCS (1996) found that 20 mg/l LAS concentration in the seawater at temperature of 22^{o} more than 70% would be degraded after 10 days.

3. Histology of the gill and liver

The histological analyses on the gill and liver of the sea-bass larvae showed a gradual damage in every treatment. The gill damage found was hypertrophy and hyperplasia of the gill lamellae (Figure 1,2,3,4,5 and 6).

Hypertrophy of the gill were found in every treatments except treatment A (0.0 mg/l LAS). The gill lamellae increase in size due to the increase size of the cell. This most probably because of the accumulation of LAS detergent in the gill which then resulted in abnormality cell formation and therefore, the gill lamellae became swollen.

While hyperplasia was found mostly in treatment F (0.472 mg/l LAS). It was found that The fish the larvae exposed to 0.422 mg/l LAS had a large size of gill lamellae and even much bigger than its normal size. This may be due to the wider of the blood capillaries locally (telengeastase) and its look like a pocket hole. Macroscopically, telengeastacist was shown by red colorization or red spot of the affected organ, whereas microscopically, it is shown by the widened of the wall of blood capillaries

Gill is the most soft of the fish organs, and it is the main organ for fish respiration. Gill is also the first organ affected by the pollutant in the media (Lagler *et al*, 1977). The present of LAS detergent in the water media resulted in the reduction of oxygen diffusion from the air in to the water, and caused respiration failure and thus mortality.

The liver histological damage was caused by respiration failure of the fish exposed to LAS detergent resulted in the congestion and vacuolar degeneration. Congestion is a blood circulation disturbance due to the increase volume of the blood in the blood capillary (Saleh, 1973). Macroscopically, the congestion of the fish liver showed dark red colorization, and microscopically, the blood capillary was widened and full of erythrocyte (Figure 7, 8, 9, 10, 11, 12).

Vacuolar degeneration is known as an acute swelling of the organ (Kurniasih, 1999). Microscopically, it was shown by the present of vacuoles in the cytoplasm and it is usually closed and/or around the nucleus. The vacuolar degeneration cannot be seen macroscopically, and it is usually shown by the swollen of the organ with pale colorization.

The liver damage of the fish exposed to LAS detergent may be due to the accumulation of the detergent in the liver tissue. Liver is known as the filter or detoxification of any toxic substances enter the body. The ability of the liver to detoxified any pollutant is limited, therefore, the accumulation of the pollutant in the liver would result in liver damage. In this research, the liver damage got worst as the

increase concentrations of the LAS in the media. The LAS detergent accumulation in the liver tissue resulted in necrosis, cirrhosis, and increase of fat in the surrounding tissue (Lu, 1995).

Based on the pathology observation of sea-bass larvae, it was found that the concentrations of LAS in the water media resulted in the increase the larvae growth abnormally due to the abnormal increase of the cell and tissue number.

CONCLUSION

Based on the results of the research, it could be concluded:

1. Lethal concentration ($LC_{50\text{-}96\ hours}$) of LAS detergent on sea-bass larvae (*Lates calcalifer* Bloch) was 1.18 mg/l. LAS detergent is considered as a moderately high aquatic pollutant
2. Sub-lethal concentrations of LAS detergen (0.094 mg/l; 0.188 mg/l; 0.283 mg/l; 0.377 mg/l and 0.472 mg/l) did not affect the absolute biomass growth of the sea-bass larvae,
3. Sub-lethal concentrations of LAS detergen (0.094 mg/l; 0.188 mg/l; 0.283 mg/l; 0.377 mg/l and 0.472 mg/l) significantly affected the specific growth rate of the sea-bass larvae,
4. Sub-lethal concentrations of LAS detergen (0.094 mg/l; 0.188 mg/l; 0.283 mg/l; 0.377 mg/l and 0.472 mg/l) resulted the histological damage of the gill and liver of sea-bass larvae. The gill damage was: hyperplasia, hypertrophy and telengeastasis. The liver of the fish larvae damage was congestion and vacuoler degeneration.

It is also recommended that LAS detergent concentration in the marine environment should be less than 0.094 mg/l to avoid gill and liver damage of the marine fish.

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Prof. Dr. dr. Ign. Riwanto, Sp.BD
Editor In Chief
Journal of Coastal Development
Research Institute Diponegoro University

Semarang, 16 February 2006

Dear Prof. Dr. dr. Ign. Riwanto, Sp.BD,

I enclose my revised manuscript '***Chronic Affect of Detergent Surfactant Linear Alkylbenzene Sulfonate (LAS) on the Growth and Survival Rate of Sea Bass (Lates calcalifer Bloch) Larvae'*** **(CD 011205)**

I am really sorry for the delay on submitting this correction, because this manuscript was addressed to different person and I have just received it few weeks ago. I hope that my manuscript can be published in your journal.

My great gratitude for your cooperation

Yours truly,

Ir. Sri Rejeki, M.Sc.
Fisheries Department
Faculty of Fisheries and Marine Sciences
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Cc:
Executive Secretary: Dr. Ocky Karna Radjasa