Journal of Coastal Development
Volume 8, Number 3, June 2005 : 207-226

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

Original paper

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

Sri Rejeki, Desrina, Andi Rahmat Mulyana*

Aquaculture Study Program, Fisheries Department, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Indonesia

Received: December, 7, 2004 ; Accepted: May, 14, 2005

ABSTRACT

Sea bass (Lates calcalifer Bloch), one of the economically important marine fish, is getting more important for marine-culture. This fish is categorized as a euryhaline species, i.e. with a wide salinity tolerance range 32 – 35 ppt, and in the river, estuarine and mangrove areas with the salinity between 0 – 25 ppt. The adult of sea bass is spawn in marine waters while the larvae and juvenile are is spawn mostly found in the estuarine. Estuarine water 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, with a long straight carbon chain, has 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.

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 is used to find out the LC_{50}-96 hours of detergent LAS on sea bass larvae. Randomized design was carried out to observe the chronic effects on the growth and survival rate of the sea bass larvae. There were six treatments applied, i.e.: 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).

The results showed that the treatment of LC_{50}-96 hours of detergent LAS on sea bass larvae, 1.18 mg/l, was 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

*** Correspondence**: Phone 024-7470281, Fax. 024-8311525, E-mail: srirejeki7356@yahoo.com

Journal of Coastal Development
Volume 8, Number 3, June 2005 : 207-226

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

INTRODUCTION

Sea bass (*Lates calcaliver* Bloch) is one of the high economic value marine fish for plural form still fish, no es and so water from Indonesian water. Sea bass is categorized as a euryhaline and a catadromous species. The fish is spawn in the open sea with water salinity between 32 – 35 ppt, and the eggs drifted to the shore: estuary and river to hatch Furthermore, larvae was abundantly found in this area at the salinity between 0 - 25 ppt (Soetomo, 1997). Therefore, sea bass is chosen as cultivated species in marine, brackish and fresh waters.

Aquatic environment, especially estuarine, 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) as a non-biodegradable chemical substance. Further, Linear Alkyl -benzene Sulfonate (LAS), an anionic surfactant then replaced the usage of ABS (*Chiao et al, 1992*). 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 result 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 affect 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 was not 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.

The objectives of this investigation were:

1. To find out the acute toxicity (LC_{50}-96 hours) of Linear Alkyl -benzene Sulfonate (LAS) detergent on sea bass (*Lates calcaliver* Bloch) larvae.
2. To find out the chronic (sub lethal) effects of Linear Alkyl -benzene Sulfonate (LAS) detergent on sea bass (*Lates calcaliver* Bloch) larvae growth and survival rate.

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

3. To find out the histological damage of the gill and liver of sea bass (*Lates calcaliver* Bloch) larvae

MATERIALS AND METHODS

2.1. Materials

The materials used in this investigation were tested fish, tested substance, water media, and equipments.

Tested fish. Sea bass (*lates calcalifer* Bloch) at the size of 0.5 – 1 cm length and average initial body weight of 1.26 – 1.33 g was used. The fish were kept in the experiment unit for one week for adaptation. 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 (Departemen Pertanian, 1983; Rand and Petrocelli, 1985).

Tested media. The water used as the media during the investigation was sea water from Central Brackish Water Research Centre, Jepara, Central Java 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 to 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 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 to histological investigation.

Equipments. Eighteen (18) plastic containers in 12 liters capacity were used for holding the tested fish during the research. Each container was aerated individually using an air stone.

2.2. Methods

The research was done in two steps; the first step was preliminary finding investigation and the second step was the chronic 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 the range of those concentrations was too wide, the 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.

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

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

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

Following the formula (1) the concentrations range obtained were:

$$\log\frac{10}{1} = 7\left(\log\frac{a}{1}\right)$$

log 10 = 7 log a – 7 log 1
log 10 – 7 log a = 7 log1
1 = 7 log a – 0
log a = 1/7 = 0.14287
a = antilog 0.14287
a = 1.38 mg/l

$b = a^2/n$	$d = c^2/b$	$f = e^2/d$
$b = (1.38)^2/1$	$d = (2.62)^2/1.90$	$f = (4.97)^2/3.61$
b = 1.90 mg/l	d = 3.61 mg/l	f = 6.84 mg/l
$c = b^2/a$	$e = d^2/c$	$g = f^2/e$
$c = (1.90)^2/1.38$	$e = (3.61)^2/2.62$	$g = (6.84)^2/4.97$
c = 2.62 mg/l	e = 4.97 mg/l	g = 9.41 mg/l

It was found that 100% (N) mortality was at concentration 1.38 mg/l. The result of the second step was meant to narrow down the concentration 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.

$$\log\frac{1.38}{1} = 7\left(\log\frac{a}{1}\right)$$

log 1.38 = 7 log a – 7 log 1
log 1.38 – 7 log a = 7 log1
0.139 = 7 log a – 0
log a = 0.139 / 7
a = antilog 0.019
a = 1.05 mg/l

$b = a^2/n$	$d = c^2/b$	$f = e^2/d$
$b = (1.05)^2/1$	$d = (1.15)^2/1.10$	$f = (1.25)^2/1.20$
b = 1.10 mg/l	d = 1.20 mg/l	f = 1.31 mg/l
$c = b^2/a$	$e = d^2/c$	$g = f^2/e$
$c = (1.10)^2/1.05$	$e = (1.20)^2/1.15$	$g = 1.31^2/1.25$
c = 1.15 mg/l	e = 1.25 mg/l	g = 1.38 mg/l

Therefore, the concentration 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

Chronic 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

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

histological changes on sea bass larvae. Completely Randomized Design was applied. There were six treatments used, each treatment replicated three times. The treatments applied were the following (Hubert, 1980)

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)

2.2.1. Data Collection.

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

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

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) :

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

SGR = Specific Growth Rate (%/day)
t_2 = Duration (end) of the investigation (day)
t_1 = Time of the investigation started (day)

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

$$\text{Survival rate} = \frac{\text{Total number of living fisn at the end of investigation}}{\text{Total initial number of fish}} \times 100\ \%$$

2.2.2. 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 difference between treatments.

RESULTS AND DISCUSSION

3.1. Results

3.1.1. 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

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

should be taken in to consideration. Even though LAS is considered biodegradable, however, effluent which contains this substance should be controlled and monitored to avoid aquatic pollution.

3.1.2. 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).

3.1.2.1. Survival Rate of Sea-bass Larvae

There was no mortality found during 30 days of investigation on the chronic effects of LAS on sea bass larvae. This result showed that the sea-bass larvae was 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° 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.

3.1.2.2. Growth

3.1.2.2.1. 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.**

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

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

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 Analysis of Variance of the absolute biomass growth of sea-bass larvae is shown in **Table 2**. The results of the analysis showed that there was no significant difference in the treatment. It means that the absolute biomass growth of sea-bass larvae was not affected by the sub-lethal concentration of LAS.

3.1.2.2.2. 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 was higher compared to the daily growth rate of the larvae exposed to the media without any LAS.

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

Tabel 2. Analisysis 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				

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 increased when the sub-lethal concentrations were raised. The average specific growth rates of sea-bass larvae were: treatment A 3,85%/day, treatments B, C, D, E and F were 4,04%/dayi;

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

4,11%/day; 4,12%/day; 4%/day and 4,19%/day. These data were transformed to Arcsin transformation. The Analysis of variance of the specific growth rate of the sea-bass larvae is 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

This analysis of variance se analysis of variance shows the treatments resulted in a highly significant difference to the daily growth rate of sea-bass larvae during 30 days investigation (p > 001)

To find 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*

Keterangan : * = Significantly different
** = Highly Significantly difference

3.1.2.3. 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.

3.1.2.3.1. Histopathological analysis of the gill

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

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

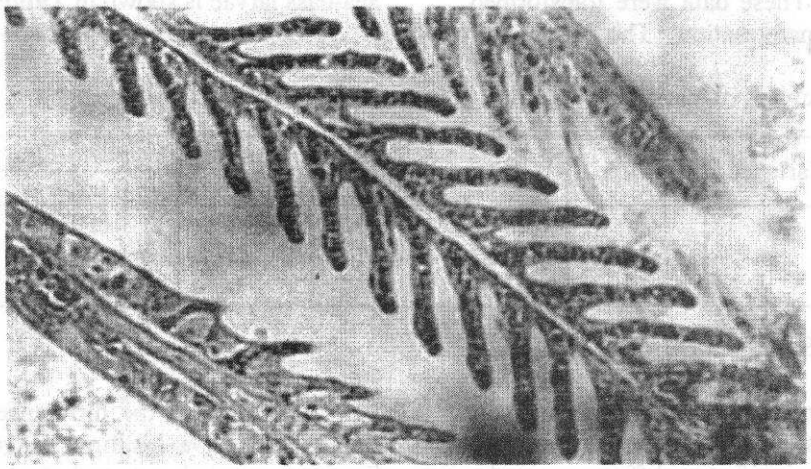

Fig. 1. Histopathology of the gill in treatment A (0,0 mg/l LAS). magnified 400x. It shows that there was no gill damage observed. The gill was still in normal condition.

Fig. 2. Histopathology of the gill in treatment B (0,094 mg/l LAS). magnified 400x. It shows a hipertrophy. Hipertrophy happened because of the increasing size of the cell. The gill lamellae was swollen but the cell number did not change

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

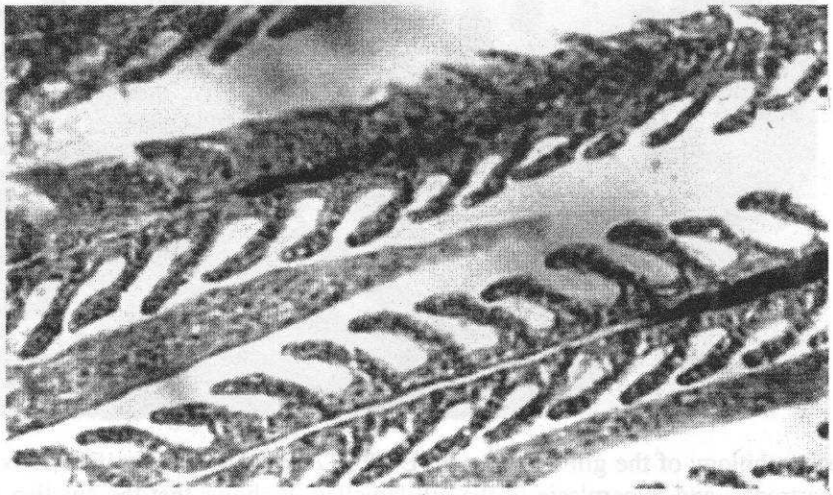

Fig. 3. Histopathology of the gill in treatment C (0,188 mg/l LAS), magnified 400x. It shows a pathological conditions: hypertrophy: the gill cells were swollen and melanization: black coloration of the of the blood capillaries

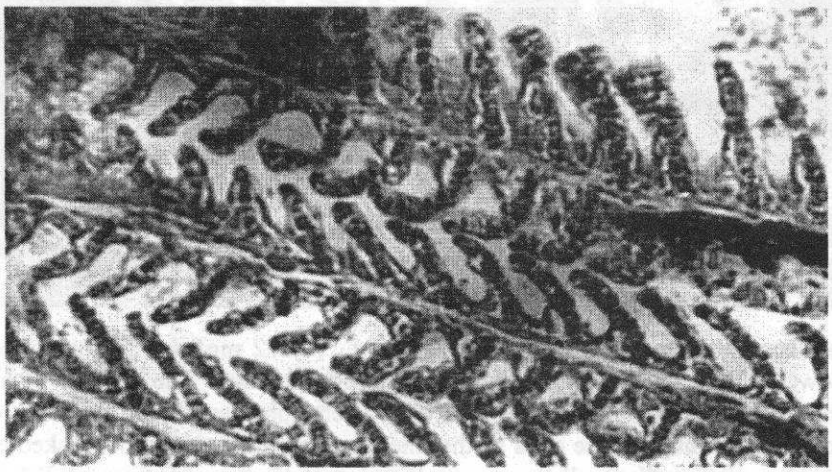

Fig. 4. Histopathology of the gill in treatment D (0,283 mg/l LAS), magnified 400x. It shows hypertrophy of the gill lamellae and melanization. The hyperthrophy in this treatment was almost all over the secondary gill lamellae. The gill cells were obviously swollen.

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

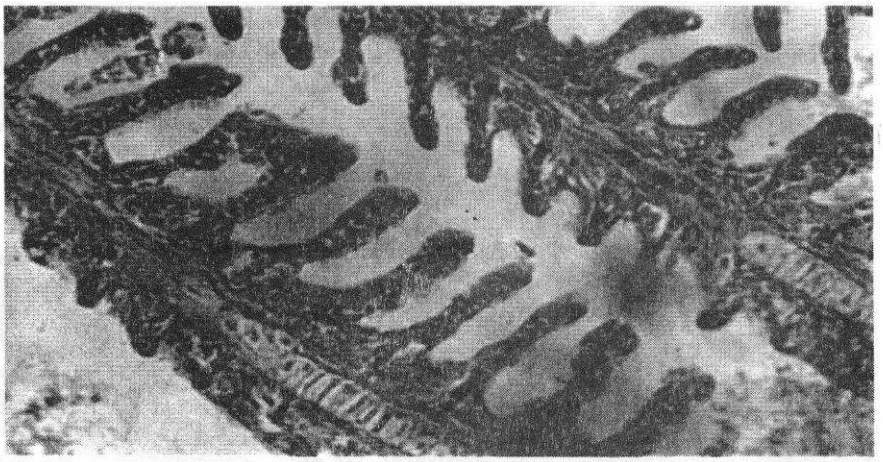

Fig. 5. Histopathology of the gill in treatment E (0,377 mg/l LAS) magnified 400x. It shows hypertrophy and hyperplasia of the gill lamellae. It shows that the swollen of the gill lamellae due to the increased number of the cell, i.e. the cell was in a normal size but the gill lamellae was swollen/bigger.

(a) **(b)**

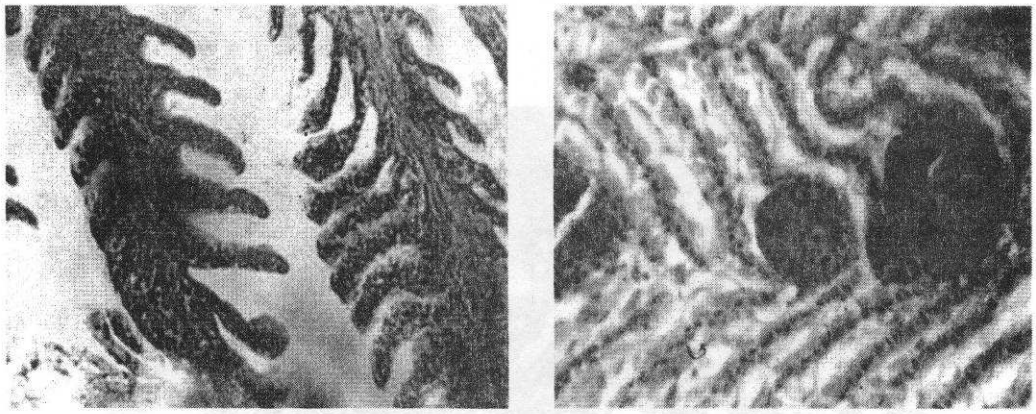

Fig. 6. **(a)** Histopathology of the gill in treatment F (0,472 mg/l LAS), magnified 400x. It shows hypertrophy and hyperplasia of the gill lamellae. The number of hyperplasia lamellae was dominant compared to the hypertrophy lamellae. This was due to the increasing number of the cells so that the lamellae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

3.1.2.3.2. Histopathological analysis of the liver

The macroscopic observation of the sae-bass larvae liver was not carried out since the whole tested fish were immersed in the fixation solution. Therefore, only microscopic histopathological analysis was done. This was observed to find out the histopathological damage of the liver of

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

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 were observed. The results of histopathology of the liver were shown in **Figure 7, 8, 9, 10, 11, 12.**

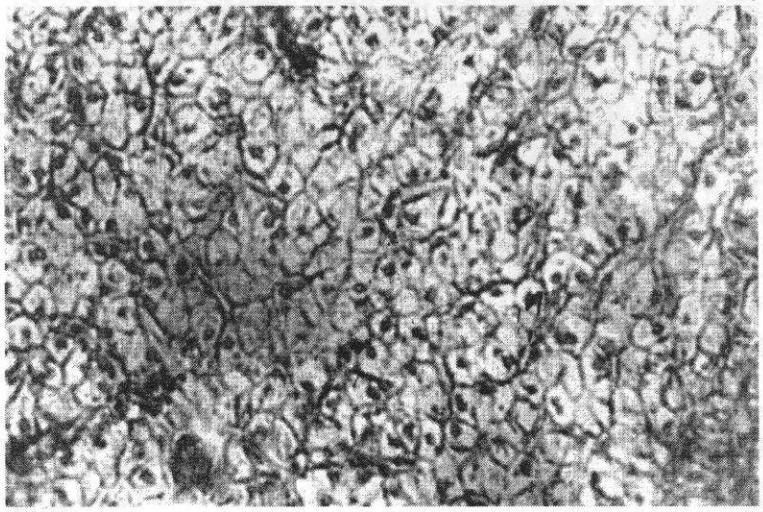

Fig. 7. Histology of liver of Treatment A (0.0 mg/l LAS), magnified 400x. No histological damage was found, the hepatocyt of the liver was clearly shown.

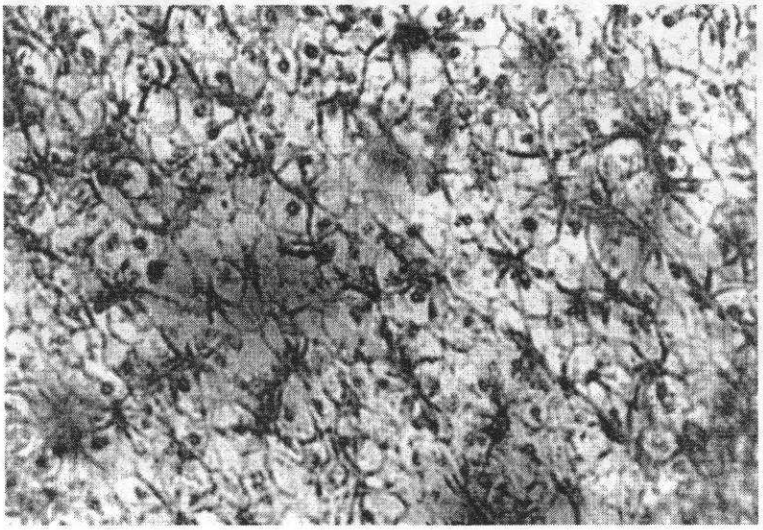

Fig. 8. Histology of liver of Treatment B (0.094 mg/l LAS), magnified 400x. Congestion of some tissue is shown by red colorization of the blood capillaries because of the swollen and increasing number of the red blood cell.

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

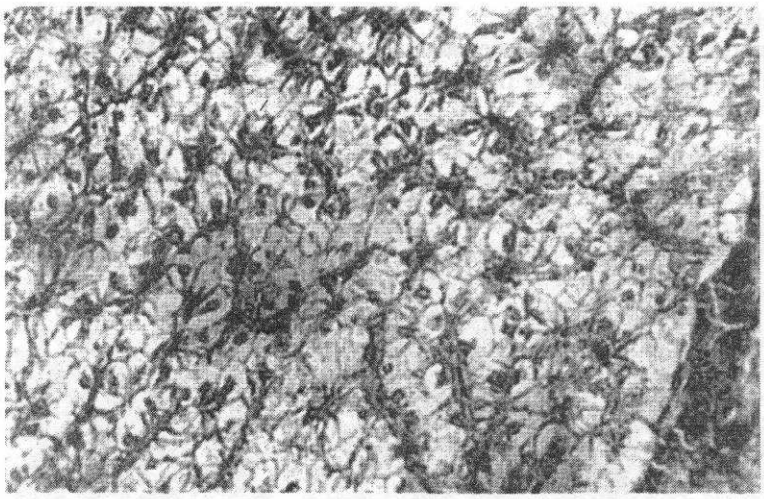

Fig. 9. Histology of liver of Treatment C (0.188 mg/l LAS), magnified 400x. More Congestion of the tissue in treatment C was observed compared to treatment B.

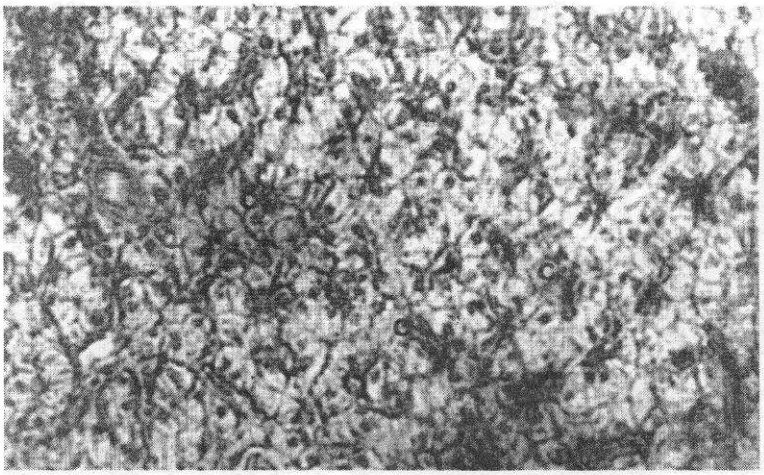

Fig. 10. Histology of liver of Treatment D (0.283 mg/l LAS), magnified 400x. More Congestion of the tissue in treatment D was observed compared to treatment C.

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

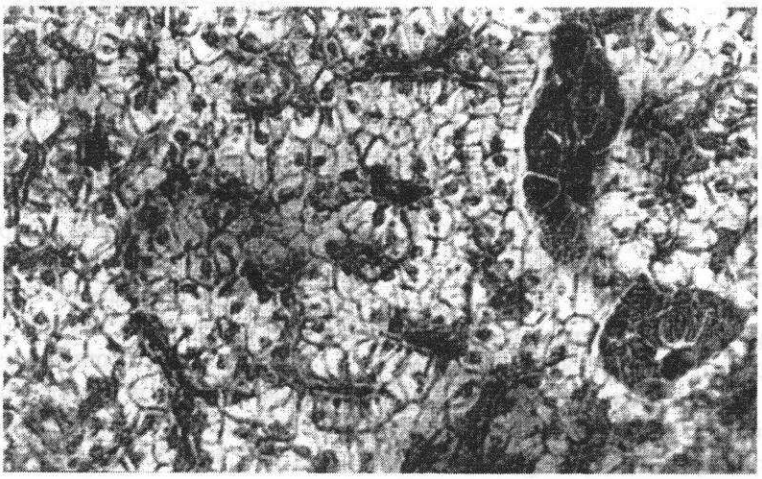

Fig. 11. Histology of liver of Treatment E (0.377 mg/l LAS), magnified 400x. More Congestion of the tissue in treatment E was observed compared to treatment D. The congestion was spread all over the liver tissue.

(a) **(b)**

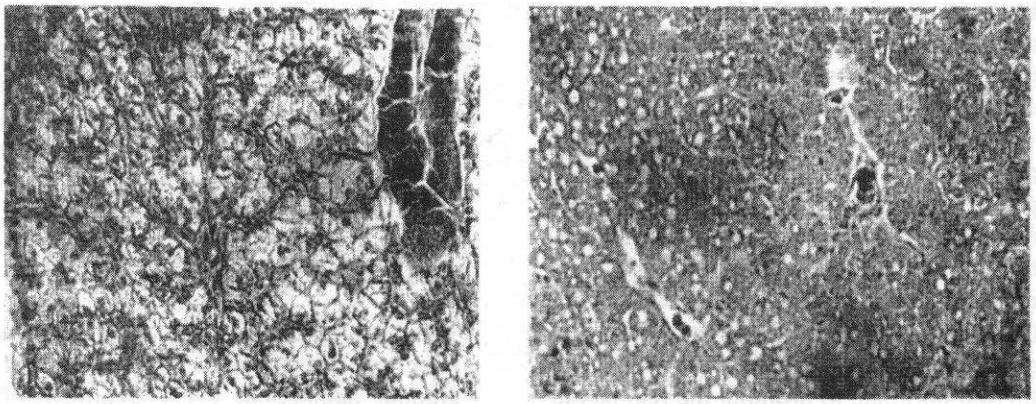

Fig. 12. **(a)** Histology of liver of Treatment F (0.377 mg/l LAS), magnified 400x. More congestion was spread all over the liver tissue.
(b) Tissue damage: a vacuolar degeneration showed by empty vacuoles/holes around nucleus caused by swollen of the vacuolar.

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

3.1.2.3.4. Water Quality Controll and Management

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

3.2. Discussion

3.2.1. 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, sea-bass larvae exposed to different concentrations of LAS significantly affected their specific growth rate ($p<0.01$) (**Table 4**).

The increasing 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 supported by Plumb (1964) who mentioned that the presence of pollutant in the water media could give a positive response to the aquatic organisms including fish due to the abnormality, i.e. the increasing number as well as the increasing 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) who 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 day exposure.

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

3.2.2. Survival Rate

There was no mortality of sea-bass larvae found during the research. This most probably due to 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. Another study by Vives-Ringo *et al* In IPCS (1996) found that 20 mg/l LAS concentration in the seawater at temperature of 22° more than 70% would be degraded after 10 days.

3.2.3. Histology of the Gill and Liver

The histological analysis 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 was found in every treatment except treatment A (0.0 mg/l LAS). The gill lamellae increases in size due to the increasing size of the cell. This was most probably due to the fish larvae exposed to 0,422 mg/l LAS had a large size of gill lamellae, even much

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

bigger than its normal size. This may be due to the increasing width of the blood capillaries locally (telengeastase) which look like a pocket hole. Macrospically, telengeastacist was shown by red colorization or red spot of the affected organ, whereas microscopically, it is shown by the widening of the wall of blood capillaries.

Gill is the softest 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 presence 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 increasing 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 presence 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 which enters the body. The ability of the liver to detoxify 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 increasing 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 of the larvae growth abnormally due to the abnormal increase of the cell and tissue number.

CONCLUSION

1. 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 in the histological damage of the gill and liver of sea-bass larvae. The gill damage includes: hyperplasia, hypertrophy and telengeastasis. The liver of the

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

fish larvae damage was congestion and vacuoler de-generation.

2. Recommendation

The 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.

REFERENCES

CEA (Canadian Executing Agency), 1993. *A Generic Protocol for conducting Tropical Acute With Fish and Invertebrate.* ASEAN–Canada Cooperative Programe on Marine Science – Phase II, Penang. 27 pp.

Chio, J.T., F.M. Dayrit., P.P. Ocampo, and V.R. Ocampo. 1992. A Study on the Environmental Impact of Detergent, III: Toxicity of Alkylbenzene Sulfonates and Coconut Fatty Alcohol Sulfates to Wards Fish and Rats. *Phil. J. Sci.* 1–39 pp.

Connel, D. W., dan Miller, G. J. 1995. *Kimia dan ekotoksikologi Pencemaran*. Penerbit Universitas Indonesia. Jakarta. (Diterjemahkan oleh Yanti Koestoer). 520 hlm. (Chemistry and Pollution Ecotoxicolog. Translated byYanti Koestoer. Indonesian University Publication. 520 pages)

Departemen Pertanian. 1983. *Pedoman umum Pengujian Laboratorium Toksisitas Lethal Pestisida pada Ikan untuk Keparluan Pendaftaran*. Komisi Pestisida. Departemen Pertanian. Jakarta. (General Guidance for Laboratory Test of Pesticide Lethal toxicity on Fish. For Registration Purposes. Pesticide Commission of Agriculture Department of Indonesia)

Effendi, H. 2000. Telaah Kualitas Air Bagi Pengelolaan Sumberdaya dan Lingkungan Perairan. Fakultas Perikanan dan Ilmu Kelautan. IPB. Bogor. 258 hlm. (Study on The Water Quality for Environmental Aquatic Resources Management. Faculty of Fisheries Institute of Agriculture Bogor. 268 pages)

Effendie, M. I. 1979. Metode Biologi Perikanan. Gramedia Pustaka Utama. Jakarta. (Fisheries Biology Method. Gramedia Jakarta)

Heath, A.G. 2000. *Water Polution and Fish Physiology*. Department of Biology. Virginia Polytechnic Institute and State University. Florida. CRC Press, Inc. 244 pp.

Himawan, S. 1998. *Patologi.* Universitas Indonesia. Jakarta. 448 hlm. (Pathology. Indonesia University 448 pages)

Hubert, J.J. 1980. *Bioassay*. Toronto Kendall/Hunt Publishing Company. 151 pp

IPCS (International Programe on Chemichal Safety), 1996. Linear Alkylbenzene Sulfonate and Related Compuonds: Environmental Health criteri 169 *in* www. Google. Com

Kurniasih. 1994. *Patologi Umum.* Laboratorium Patologi Fakultas Kedokteran Hewan. Yogyakarta. Universitas Gajah Mada. 51 hlm.

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

Kurniasih. 1999. *Deskripsi Histopatologi Dari Beberapa Penyakit Ikan.* Pusat Karantina Pertanian. Departemen Pertanian Jakarta. 55 hlm. (Histopathology Discription from Several fish Diseases. Centre of Fish Quarantine. Department of Agriculture, Jakarta 55 pages)

Lagler, K. F., J. E. Bardach, R. R. Miller and D. R. M. Passino. 1977. *Ichtyology.* John Willey and Sons Inc. New York. 197–217 pp.

Lu, C. F. 1995. Toksikologi Dasar: Asas, Organ Sasaran, dan Penilaian Resiko. Jakarta: Penerbit Universitas Indonesia. (Diterjemahkan oleh Edi Nugroho) (Basic Toxicology. Rules, Organ Affected and Risk Analyses. Indonesia University Publication. Translated by Edi Nugroho)

Nugraha, D. M. 2001. *Pengaruh Surfaktan Alkyl Sulfate (AS) Terhadap Larva–juvenil Ikan Mas (Cyprinus carpio).* Skripsi. Bogor: Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor. (Effects of Surfactan Alkyl Benzene (AS) on the Juvenile of Common Carp (*Cyprinus carpio*). Final Project faculty of Fisheries and Marine Sciences, Institute of agriculture Bogor)

Rand, G.M dan S.R. Petricolli. 1985. *Fundamental of Aquatic Toxicology.* Washington: Hemisphere Publishing Corporation.

Sajiah, L. 2003. Pengaruh Surfaktan Deterjen Linear Alkylbenzene Sulfonate (LAS) terhadap Perkembangan Stadia Larva Sampai dengan Juvenil Ikan Mas (*Cyprinus carpio* L.). Skripsi. Bogor. Fakultas Perikanan dan Ilmu Kelautan Institut Pertanian Bogor. 57 hal. (Effects of Surfactant Detergent Alkylbenzene Sulphonate (LAS) on the Growth and Development from Larval Stage until Juvenile of Common Carp (*Cyprinus carpio*). Final Project Faculty of Fisheries and Marine Sciences, Institute of Agriculture Bogor)

Saleh, S. 1973. *Patologi Umum : Dasar–Dasar Patologi. Jakarta:* Bagian Patologi Anatomik Fakultas Kedokteran Universitas Indonesia. 448 hlm. (General Pathology: Basic Pathology. Anatomic Pathology Department Faculty of Medicine Indonesia University Jakarta 448 pages)

Soetomo, M. H. A. 1997. *Teknik Budidaya Kakap Putih di Air Laut, Air Payau dan Air Tawar.* Jakarta: Trigenda Karya, 83 hlm. (Sea-Bass Culture Tecgnique in the Marinewater, Brackhishwater and Freshwater. Trigenda karya Jakarta 83 pages)

Supriharyono. 2000. *Pelestarian dan Pengelolaan Sumberdaya Alam di Wilayah Pesisir Tropis.* Jakarta: PT. Gramedia Pustaka Utama. (Sustainability and management In tropical Coastal Resources. PT. Gramedia Pustaka Raya, Jakarta)

Supriyono, E., F. Takashima and C. A. Strussman. 1998. Toxicity of Linear Alkylbenzene Sulfonate (LAS) to Juvenile Kuruma Shrimp, Penaeus japonicus: a Histopatological Study an Acute and Subcronic Level. Journal of Tokyo University of Fisheries.

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

Warren, C.E. 1971. *Biology and Water Pollution.* Philadelphia: W.B. Saunders Co.

Wedemeyer, G.A. Meyer, F.P and Smith, L. 1976. *Environmental Stress and Fish Disease.* T.F.H. Publication, Inc. Ltd. USA.

Yatim, W. 1990. *Biologi Modern Histologi.* Bandung: Penerbit Tarsito. 374 hlm. (Histology and modern Biology. Transito Publication Bandung, 374 pages)