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Analysis the Addition of Crude Oil with Different Concentration to the Mortality Clownfish (*Amphiprion percula*)

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ABSTRACT

The research is to know mortality with clownfish augment the crude oil different concentration. This research was conducted for one month in December 2018 in January 2018 at damp Laboratory, Central of Marine Aquaculture Fishery Lampung. The method used is experimental with complete random design (RAL) 4 levels of treatment and 3 levels of repeated. Treatment augment crude oil as ensuing P0 (no extras), P1 (concentration 0,013 mg/l), P2 (concentration 0,130 mg/l), P3 (concentration 1,295 mg/l). The results obtainable percentage comparison mortality and survival rate with the results respective treatment P0 MR value of 0% with SR 100%, treatment P1 MR value of 33,33% with SR 66,67%, treatment P2 MR value of 50% with SR 50%, treatmen P3 MR value of 76,67% with SR 23,33%. Statistical analysis results there indicate absolute difference between treatment augment concentration crude oil be different because F count value (4,47) > F table (4,07) standart with 5%. Mortality in research caused by hydrocarbons compound and nonhydrocarbons compound be contained in crude oil which has the quality toxic to fish.

1. INTRODUCTION

Oil is one of the sources of pollutants in the waters, which is caused due to various things ranging from petroleum exploration, oil refining, transportation accidents, pipeline leakage or engine room wastewater disposal and other ship activities (Nuryatini et al, 2010). This oil pollution can cause pollution to the waters and the sea which has an impact on the decrease in the carrying capacity of the environment which affects the disruption of the life of organisms in these waters.

Therefore, every crew must do the prevention and control of pollution originating from their ships (Setiawan et al, 2014). The negative impacts themselves include fishing that is not blocked by aquaculture, resulting in uncontrolled exploitation (Over Fishing) and also oil pollution to the aquatic and marine environment (Setiawan et al, 2014).
Previous research on the study of the impact of oil spills from oil refinery operations on water and soil quality (Sulistyono et al, 2012). Previous research on the toxicity of petroleum liquid waste on duck grouper (*Cromileptis altivetis*) seeds (Syafriadiman et al, 2009), during the acute and subacute toxicity test the concentration of heavy metal Cd toxicity was very influential ($p < 0.01$) on the mortality of duck grouper seeds and the rate of daily growth and behavior of duck grouper fish. Nofyan et al (2011), shows the effect of crude oil on the mortality and morphology of the gills of milkfish (Chanos Chanos Forskal), the higher the concentration of crude oil given for 96 hours, the higher the mortality of milkfish (Chanos chanos)

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and the morphological condition of the fish gills, milkfish contaminated with crude oil changes color to pale red, damage to the gill filament and there is a pile of crude oil in the arch and gill filament.

Previous research on the vulnerability index of coral reef ecosystems to oil spills: the case of scout islands and the Dutch islands in the Thousand Islands (Suhery et al., 2017), coral reef ecosystems in the Thousand Islands have a high susceptibility to crude oil spills, especially in the Netherlands, compared to Island Scouts are included in moderate vulnerability, the characteristics of adaptive capacity are thought to provide an important role for vulnerability. This study has the following objectives: to analyze the addition of crude oil with different concentrations of mortality in Clownfish (*Amphiprion percula*).

2. METHODOLOGY OF THE STUDY

2.1. Time and Place

The research was conducted on December 26, 2017 - January 22, 2018, at the Clownfish Laboratory, Lampung Center for Aquaculture, Jalan Yos Sudarso, Hanura Village, Padang Cermin District, Pasewaran 35454 District, Lampung Province Telf. (0721) 400139/4001380 Fax fax (0721) 4001110

2.2. Research Methods

This research was conducted with 4 treatments. Each treatment was repeated 3 (three times) so that there were 12 experimental units. The addition of oil in this study is based on previous research conducted by Syafriadiman et al (2009), on the seeds of Grouper Duck (Cromileptis altivelis). The concentration used in this study is the result of preliminary research conducted by Syafriadiman et al. (2009) with the lowest concentration of 0.685 mg / l and the highest concentration of 2.452 mg / l from the results of the preliminary study, obtained concentrations as below, which were used in this study.

P0 : Treatment without adding crude oil to the (controlled) test biota
P1 : 0.013 mg / l addition of crude oil treatment
P2 : Treatment of adding crude oil as much as 0.130 mg / l.
P3 : Treatment of adding crude oil as much as 0.130 mg / l.

The test biota used was clownfish (*Amphiprion percula*) with a size of 3 cm, 3 months old. Clownfish fish seeds are derived from the results of hatcheries conducted at the Clownfish Laboratory at the Lampung Sea Aquaculture Center, with the number of fish used as many as 120 fish and each aquarium filled with fish as much as 10 fish/aquarium. The media used in this study is the aquarium with a size of 40 cm x 40 cm x 40 cm with a volume of water 65 liters of water, the water used is seawater taken from the sea, before seawater is used, the filtering process is used so that the water used is water in accordance with the needs of life Clownfish (*Amphiprion percula*).

2.3. OBSERVED PARAMETES

In this study, research on mortality and air quality consisting of DO, temperature, salinity, pH, ammonia, and nitrite.

a. Mortality

Mortality percentage is the number of fish that die with the number of fish stocked. Calculation of the mortality of Nemo fish (*Amphiprion ocellaris*) using the formula according to Effendie (1997), namely :

$$\mathbf{MR} = \frac{\mathbf{No}-\mathbf{Nt}}{\mathbf{No}} \mathbf{X}\ \mathbf{100}\%$$

Information:

MR : Mortality (Amphiprion percula)
Nt : Final number of clownfish in the study (tail)
No : Number of clownfish stocked at the beginning of the study (tail)

b. Water Quality

Observations on air quality parameters as test media, discuss temperature, DO, pH, nitrites, nitrates and ammonia made at the beginning of the study and the last research.

c. Data Analysis

Mortality data obtained will then be analyzed using data analysis using statistics using LC50-96 hour selection probit analysis. Furthermore, the data will be tested statistically used in the study is a one-way analysis of variance (ANOVA) which compares F counts with F tables, can get F count > F table 5%> 1% then make use of appropriate research for 5% accuracy and very significantly different to 1% level. If the F count results are significantly different, it will be done with further tests to determine the significant level that occurs between those provided with the Least Significant Difference test (BNT) (Hanfiah, 2012) using the Microsoft Excel 2010 application.

3. RESULTS AND DISCUSSION

3.1. Mortality

Test results from crude oil with different concentrations of mortality for 96 hours, shown in the form of mortality of Clownfish (Amphiprion percula) are presented in Table 1 below.

Tabel 1. *Concentration Data and Amount of Mortality of Clownfish (Amphiprion percula) with Probit Analysis, during the 96-Hour Test*

Treatment	Repeat			Total Mortality	Average Mortality	Concentration log	Probit
	1	2	3				
P0	0	0	0	0	0	0	0
P1	10	10	80	100	33.33	-1,886	4,53
P2	20	60	70	150	50	-0,886	5,00
P3	70	60	100	230	76.67	0,112	5,71
Total	**100**	**130**	**250**	**480**	**160**		
Average	**25**	**32.5**	**62.5**	**120**	**40**		

(Source : Research Data, 2018)

The mortality of *Clownfish* (*Amphiprion percula*) during the addition of crude oil test conducted for 96 hours can be seen in Figure 1. Based on the data obtained during the test, the data will then be tested statistically the effect of adding crude oil with different concentrations to the mortality of Clownfish (Amphiprion percula) results from the statistical tests conducted can be seen from **Table 2** below.

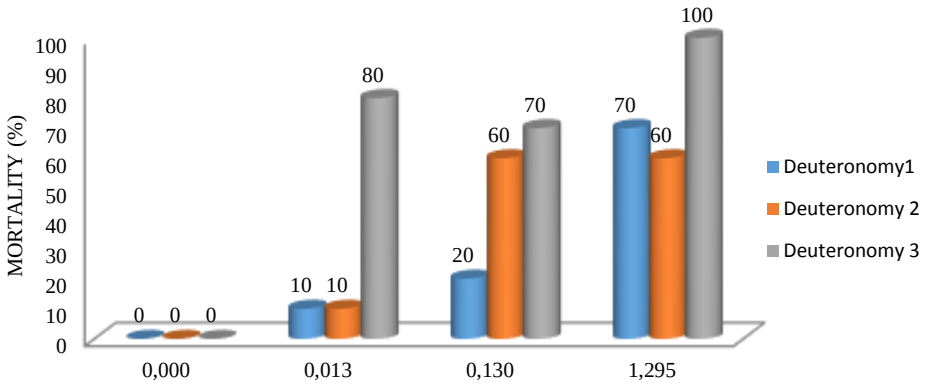

Figure 1. Mortality Addition of Crude Oil with Different Concentrations of Clownfish

Table 2. Statistical Test Results of the Effect of Addition of Crude Oil with Different Concentrations to Mortality of Clownfish (Amphiprion percula)

SK	db	JK	KT	F Count it	F table	
					5%	1%
Treatment	3	9266,67	3088,89	4,47*	4,07	7,59
Error	8	5533,33	691,67			
Total	**11**	**14800**				

(Source: Research Data, 2018)

In this study, accepting the H_1 hypothesis was significantly different. Thus it can be concluded that "the addition of crude oil with different concentrations affects the totality of Clownfish (Amphiprion percula)". Because the KK value in this study is 65.75, it is classified as large. because the F count is significantly different, the next test is the Least Significant Difference (LSD). The results of further tests of the addition of crude oil with different concentrations to the mortality of Clownfish (Amphiprion percula) according to LSD can be seen in **Table 3** below.

Tabel 3. BNT Advanced Test Results from the Statistical Tests of the Effect of Addition of Crude Oil with Different Concentrations to the Mortality of Clownfish (Amphiprion percula)

Treatment	Average	BNT 5% (2,306)	BNT 1% (3,355)
P0	0	a	A
P1	33,33	a	A
P2	50,00	b	A
P3	76,67	b	A
	D 0.05 = 49,517		**D 0.01 = 72,044**

Note: different letters indicate influence on treatment (Source: Research Data, 2018)

Based on the observations in Table 1 it can be seen that in the P0 (controlled) treatment there was no death in every observation made at a different time. In treatment P1, there was death at observation 2 hours after giving treatment at 1 test of 1 tail, death also occurred at observation 24 hours after giving treatment as much as 1 tail at 3 replications, at 48 hours observation there was an increase of 5 deaths at 3 replications and death occurred in 96 hours of observation as many as 1 animal in 2 replications and 1 animal in 3 replications. From the observation results of P1 deaths increased at 24 hours by 5 animals, total deaths in the treatment of P1 were 10 animals with an average of 3.33.

In the P2 treatment when observed at 8 hours there was 1 death in replicate 3, 1 death also occurred in the 24 hour observation on repeat 2 as much as 1 and 2 replications 3, observing at 48 hours there was an increase in mortality in 1 replication 1 as many as 1 animals, 2 tests were 5 animals and 3 questions were 1 animal. At 96 hours of observations, 1 fish died in test 1 and 3 fish in test 3, the total number of fish killed in P2 treatment was 15 fish with an average of 5. Observation of P3 treatment conducted after the addition of crude oil occurred fish mortality at an 8-hour observation where the number of fish that died 1 tail on Repetition 1, death occurred again on 24-hour observation on Repetition 1 as many as 2 and Repeat 3 as many as 10 heads. Fish mortality continued at 48 hours of observation on Repetition 1 and 2 for 2 heads, at 96 hours of observation there was a death in which the number of fish that died was 3 at Repetition 1 and 3 on Repetition 2. The total number of fish died at P3 treatment with oil concentration as much as 1.295 mg / l as much as 23 tails with an average value of 7.67.

From Table 1 and Figure 1 the highest percentage of mortality at a concentration of 1,295 mg / l and lowest at a concentration of 0,000 mg / l (control). The process of mortality in Clownfish (Amphiprion percula) for 96 hours is caused by the toxic entry of chemicals contained in crude oil into the body of Clownfish (Amphiprion percula) which makes the immune system of the fish decrease and eventually death occurs. The amount of toxicity that enters the body of an organism depends on the type of toxin, the concentration of the toxin and the length of time of contamination (Rand and Petrocelli, 1985 in Syafriadiman, 1999). According to Putra (2008), which states the high percentage of mortality of Clownfish (Amphiprion percula) because crude oil has a mixture of 50-98% hydrocarbon compounds and the remainder consists of organic substances containing sulfur, oxygen and nitrogen and inorganic compounds such as vanadium, nickel, sodium, iron, aluminum, calcium, and magnesium which can be toxic.

In Table 1, the crude oil concentration of 1.295 mg/l can cause 76% mortality of Clownfish (Amphiprion percula) during the test within 96 hours, for 50% mortality occurs in P2 treatment with a concentration of 0.130 mg / l. In previous studies conducted on duck grouper fish mortality rate of 50% occurred at a concentration of 1295 mg/l conducted by Syafriadiman et al, (2009). According to Sudarmadi (1993) in Prayitno (2000), in general, organisms will quickly experience

mortality in a short period time if given to toxics with high concentrations and vice versa will last longer if given at low concentrations of toxins.

The results of the ANOVA test from Table 2 test obtained F value calculated 4.47 with an F value of 5% (4.07) while for 1% (7.59), it can be concluded that where the calculated F value is greater than the value F table 5% and smaller than F table 1% based on the test variant of data significantly different from F table 5% and not significantly different in F table 1% between treatments. Based on the results of Table 2, the results obtained are significantly different at level F of the table 5% and based on the calculation of KK, further tests are conducted according to the Least Significant Difference test (LSD). Based on the LSD test in Table 3, it can be concluded that:

1. At the test level of 5% crude oil treatment with different concentrations of P1 (0.013 mg / l), P2 (0.130 mg / l) and P3 (1.295 mg / l) against P0 (as controlled) with other treatments significantly different.
2. At the test level of 1% crude oil treatment with different concentrations in treatments P1 (0.013 mg / l), P2 (0.130 mg / l), and P3 (1,295 mg / l) against P0 (as controlled) with other treatments not different real. So the researchers can conclude that the range of crude oil is toxic in Clownfish is 0.013 mg / l, 1.295 mg / l at a significant level of 0.05%.

Culbertson et al (2008), explained that petroleum pollution even with very low concentrations greatly affects the smell and taste of groundwater. Remains from oil spills can last for decades in coastal sediments which can affect local fauna and flora, in addition to that several studies have examined the long-term effects of oil spills also affect coastal ecosystems. In contrast to lethal impacts that can be easily quantified in the field, sublethal impacts will be accurate if proven in the laboratory. Laboratory tests show that the reproduction and behavior of fish and shellfish are influenced by the concentration of oil in water. With relatively low concentrations (<0.1 ppm), egg hatchability, survival rate, number of deformed larvae, shell closure (in shells) are significantly affected (Sulistyono, 2012).

According to Carmen et al (2009), the processing of oil and petrochemicals in refineries can produce oil sludge (Oil Sludge), which has the potential to pollute the environment. Oil mud is an oil waste formed by the process of collecting and settling oil contaminants consisting of contaminants that already exist in the oil or contaminants in the handling process. Physically, oil mud has a specific gravity between 0.93 to 1.05, colored from dark brown to black, smells of hydrocarbons and water solubility is very low. Components of oil that cannot dissolve in water will float causing seawater to be black. Some oil components sink and accumulate in the sediment as black deposits in sand and rocks on the coast. Rancid hydrocarbon components affect the reproduction, development, growth, and behavior of marine life, especially in plankton, and can even reduce fish production. The process of emulsification is a source of death in eggs, larvae and embryo development because at this stage it is very vulnerable in a polluted environment (Fakhrudin, 2004).

Short-term effects, oil hydrocarbon molecules can damage the cell membrane of marine biota, causing the discharge of cell fluids and penetration of these materials into cells. Oil directly causes death in fish due to a lack of oxygen and poisoning of carbon dioxide, direct poisoning of hazardous materials. Whereas for the term stakes more threatening young biota. Oil in the sea can be consumed by marine biota so that some compounds can be released together with dirt, while some can accumulate in fat and protein compounds (Sumadhiharga, 1995 in Misran, 2002).

3.2. Water Quality

One very determining factor in fish life is water quality. The water quality parameters measured in this study are temperature, salinity, pH, DO, and Ammonia (NH3). The results of the measurement of water quality in the study of the addition of crude oil with different concentrations of the mortality of Clownfish (Amphiprion percula) are presented in the form of Table 4 below.

Table 4. *Observation of Water Quality during Research*

No	Treatment	Salinity	Temperature (^{0}C)	DO (mg/l)	pH	Ammonia (mg/l)	Nitrite (mg/l)
1	P0	33	26,4-26,7	5,8-6,1	7,89-7,94	2,09-2,38	0,74-0,74
2	P1	33	26,4-26,5	5,7-5,9	7,94-7,96	1,92-2,15	0,68-0,72
3	P2	33	27,1-27,4	5,4-5,9	7,79-7,89	1,92-2,17	0,62-0,74
4	P3	33	26,8-27,1	5,2-5,8	7,83-7,92	1,77-2,32	0,57-0,75

(Source : Research Data, 2018)

a. Temperature

Based on observations of the temperature before and after the study, which is in the range of 26,5 - 27,5 ^{O}C, the temperature conditions are still in the optimal limit for the survival of Clownfish (Amphiprion percula). Temperature affects the metabolism of the organism, in general the growth rate increases with the increase in temperature, so that it can suppress the life of biota and can even cause death if the temperature rises to an extreme (Kordi, 2009), confirmed by Boyd in Sartika (2014), which states that the water temperature tropical ranges between 25^0 C - 32 ^{0}C are still suitable for living organisms in the waters.

Temperature changes affect the physical, chemical, and biological processes of water bodies, increased viscosity, chemical reactions, evaporation, and volatility. An increase in temperature also causes a decrease in the solubility of gases in water, for example, gas O_2, CO_2, N_2, CH_4. Besides the increase in temperature also causes an increase in the speed of metabolism and respiration of organisms, an increase in water temperature by 10°C causes an increase in oxygen consumption of aquatic biota by about 2-3 times, an increase in temperature also results in an increase in decomposition of organic matter by microbes (Effendi, 2000).

b. Salinity

Based on observations of salinity before and after the study that is 33 PSU, salinity is still in the optimum limit in Clownfish (Amphiprion percula). The Salinity of water affects the osmotic pressure of water, the higher the salinity, the greater the osmotic pressure. Biota that lives in salty water must be able to adjust to the osmotic pressure of the environment (Kordi, 2007). This statement is confirmed by Ghufran and Baso (2007) in Widiadmoko (2013), that the diversity of salinity in seawater will affect aquatic living bodies based on the ability to control specific gravity and osmotic pressure diversity.

c. Dissolved Oxygen (DO)

Dissolved oxygen in each treatment is still in the optimum limit and can be tolerated, the DO observation results showed an initial value of 6.2 mg / l and a final value of 5.2-6.1 mg / l. Low dissolved oxygen concentration is a common factor causing mortality in fish, oxygen solubility in water decreases when temperature and salt levels increase or air pressure decreases (WWF Indonesia, 2015). This is in agreement with Kordi (2007), the concentration of oxygen in water influences the growth and conversion of feed, the concentration of dissolved oxygen is one of the limiting factors for the life of farmed fish. Dissolved oxygen dissolution (DO) is largely determined by the temperature of the water, generally, if the temperature increases dissolved oxygen will decrease (Ji, 2007 in Adytama, 2015).

d. pH

The results of the pH observation before treatment showed a value of 7, 96 and after the treatment, the pH observation results were 8.3 - 7.96. The pH concentration of the water showed that the pH level in the water was still in accordance with the limits for survival of Clownfish (Amphiprion percula) concentration figures The pH of laboratory test results is in accordance with the quality standards of seawater for marine biota in accordance with Minister of the Environment Decree No. 51 of 2004.

e. Ammonia

In this study the test was conducted during the study, where the results of ammonia treatment before the study was 2.12 mg / l and the results of observations at the end of the study obtained the ammonia values ranging from 1.92 to 2.38 mg / l

results from the ammonia test conducted at the beginning and at the end of the study it was very high, exceeding the applied quality standard, in which ammonia levels were 0.3 mg / l based on Government Regulation No. 24 of 1991. Sources of ammonia in waters are the result of the breakdown of organic nitrogen (protein and urea) and inorganic nitrogen contained in stoic and water, also comes from the decomposition of organic matter (dead aquatic plants and biota) carried out by microbes and fungi that are known as ammonification (Effendi, 2003).

f. Nitrite

A Nitrite is an intermediate form between ammonia and nitrate and between nitrate and nitrogen gas which is commonly known as nitrification and denitrification processes (Effendi, 2000). In this study, a nitrite test was carried out during the study, where the results obtained at the beginning of the study were 0.84 mg / l and the test results at the end of the study were in the range of 0.57 - 0.75 mg / l. the results of the nitrite test carried out had a high value of the quality standard whose value was 0.05 mg / l based on Government Regulation No. 24 of 1991. This condition is closely related to waste originating from land containing nitrates (Simanjuntak, 2009).

4. CONCLUSION AND RECOMMENDATION

4.1. Conclusion

From the results of this study the mortality rate at P0 (0,000 mg / l) was 0%, P1 (0.013 mg / l) was 33.33%, P2 (0.130 mg / l) was 50% and at P3 (1,295 mg / l) was 76.67 The highest percentage of Clownfish fish mortality results in P3 with a concentration of 1295 mg / l, based on the results of statistical tests with ANOVA concluded that F count> F table were significantly different at the level of accuracy of 5%. From the water quality test where the addition of crude oil affect changes in water quality on ammonia and nitrite parameters which have the highest yields, this causes toxic occurrence in Clownfish (*Amphiprion percula*) which causes death.

4.2. Recommendation

The recommendations that can be given based on the results of research conducted need to be tested on oil content in the blood, stomach, and liver of clownfish (Amphiprion sp) to find out more about the level of organ damage apart from the shivering if in the long run on other marine biotas.

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