Assessment of the Acute Toxicity of Oil Dispersant Type 3 (4− amino−1, 2− dithiolane−4− carboxalic acid) on *Abudefduf vaigiensis* Under Laboratory Conditions

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ABSTRACT

This study was designed to determine the acute toxicity of type 3 oil dispersant (4−amino−1,2−dithiolane−4−carboxalic acid) on shallow coastal marine fish, Abudefduf vaigiensis under laboratory conditions. Two separated experimental tank series were used providing aeration to one series and other without aeration. Four replicates of each concentration value of the oil dispersant were used including one control tank for each concentration series. Eight fish of same size range of (4.2cm to 5.4cm) were put into each tank and several prior exposed tests were carried out to determine the chemical concentration range of acute toxicity on the selected fish. There was no significant difference of temperature, pH, NH_3 and salinity in both setups. Dissolve oxygen (DO) concentration decreased during 10 hours of the experiment and after that DO was constant in the aerated tank series. Several behavioral changes (slime secretion, color change, increase of operculum movement rate and surface breathing) were observed in both setups. Zig-zag swimming behavior of fish was observed in the un aerated setup. Mortality of the first fish was observed after 6 hours in the aerated tanks. Since, DO is one of the factors for fish mortality, after 9 hours of the experiment fish mortality was observed in the aerated tanks. According to the results from Probit analysis program, Version 1.5, LC_{50} values varied from 31.37-33.715 ppm in aerated tanks and these values changed between 70.702 and 71.825 ppm in not aerated tanks. Therefore, the range (70.702-71.825 ppm) of the type 3 oil dispersant could be considered as the acute toxicity level for Abudefduf vaigiensis for the natural environmental conditions.

Keywords: Oil dispersant, *Abudefduf vaigiensis, Probit Analysis,* LC_{50}

INTRODUCTION

Oil tankers, lighters, barges and off-shore operations for exploration and production of petroleum hydrocarbons can be effective sources for introduction of oil to marine environment [16]. About 75% of these oil spills were caused by ship collisions, groundings and nearly 8% were caused by oil transfer operations [9]. During past decades several small scale or large scale oil spills occur in the international seas.

The spilled oil can be accumulated in sensitive eco systems such as estuarine wetlands due to physical properties of water and transportation mechanisms like wind and ocean currents [7]. The accumulation of oil cause to kill crabs, lobsters, shell fish and the most dramatic symbol of the consequences of an oil spill is an oiled sea bird [16]. However, the effects on aquatic life can be depended on the life time of oil type. Therefore, oil spill is a main ecological and economical problem to all countries in the world and it is important to develop methods to remove oil in sea water.

There are several mechanical recovery techniques such as use of booms, use of skimmers etc for removing oil from the sea surface. However, the effectiveness of such mechanical techniques is variable and it is highly influenced by the size, nature, location and the environmental conditions in the ocean [15]. Non-mechanical techniques such as insitu burning and use of chemical dispersants have been developed to overcome some problems in the mechanical techniques [15]. Application of chemical dispersants is one of the options to accelerate the dispersion of oil from the sea surface into the water column effectively and it helps to accelerate the dilution and biodegradation of oil to reduce the economic and environmental impact from oil spill [3].

Dispersants consist of individual components of surfactants which are specific chemical compounds including oleophilic (oil liking) and hydrophilic (water liking) groups [9]. These surfactants act as a chemical bridge between oil and water phases and help to mix with each other more easily and can be reduced the viscosity of surfactant [12]. The addition of dispersant rapidly converts a much larger oil slick into small oil droplets and these small oil droplets enhances the biological degradation by increasing surface area available to microorganisms [20].

The concentrated dispersants referred as "UK Type 3" chemicals which have been recommended to use 1 part of dispersant for 20-30 parts of spilled oil [12]. Several countries have been used different types of oil dispersants according to their rules and regulations. However, there are no records for usage of oil dispersants in oil spills in Sri Lanka. Therefore, it is important to assess the toxicity of these chemicals to the organisms living in coastal ecosystems before applying the dispersants to oil spills.

Toxicity is defined as negative effects (lethal or sub lethal) on organism caused by exposing to a chemical substance[12]. In toxicological studies, Dose-Response relationship use to express the relationship between chemical exposure and toxicity. The acute toxicity of a chemical could be assessed as LC_{50} (Lethal concentration that kills 50% of the test population) and behavioral changes of the organisms can be observed.

MATERIALS AND METHODS

Selection of a Test Fish Species

Abudefduf vaigiensis (Figure 1) was selected as the test organism because, it can be easily found around the Sri Lankan coastal marine ecosystems and can be easily handled in a marine aquarium (Personal communication with aquarium owners). The characteristic features of the fish are five prominent vertical bars in which the first bar starts from dorsal fin origin to pectoral fin base, the fifth bar from the posterior margin of dorsal and anal fin [1].

Figure 1: The test species (*Abudefduf vaigiensis,* Order: Perciformes, Family: Pomacentridae)

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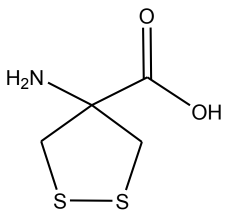
Acclimatization of Fish before the Toxicity Test

The test fish (*Abudefduf vaigiensis)* were collected from unpolluted areas in Trincomalee and Polhena and transported to the marine aquarium in aerated containers. Trial tests were carried out to determine the suitability of the fish for aquarium conditions during two weeks. 16 fish were introduced into two tanks (44×29×31 cm^3), and they were fed three times per day up to satiation with commercial Prima diet. Half of the total volume of water was removed and refilled with filtered sea water once in five days in each acclimatization tank. Commercially available Aqua zonic black night top filter (8W, 800 L/h) was fixed and aeration was provided to each tank.

Preliminary Test

Preliminary test for toxicological study is important to find the effective concentration series [8]. Two experimental setups were used respectively in the presence and absence of aeration. Twelve glass tanks (44×29×31 cm^3) were used in two series of tanks including six tanks to each series. They are labeled as (C, R1, R2, R3, R4 and R_5) in each series. The used oil dispersant in this study was Adt (4−amino−1,2–dithiolane–4 –carboxyalic acid) type 3 and the chemical formula is given below.



The concentration series of 0, 20, 40, 60, 80, 100 ppm of oil dispersant were prepared using 0, 0.60, 1.20, 1.80, 2.40 and 3.0g of the dispersant respectively. Total length and weight of all fish were measured to select the fish within the length range of 4.2cm to 5.4cm and the weight range was 3.9g to 4.8g. The length of the largest fish should not be more than twice that of the smallest fish in the same test [4]. All tanks were filled with 30L of filtered sea water and 8 fish were added into each tank and covered by a net to minimize the outside disturbances.

Percentage mortality and other morphological and functional changes such as erratic swimming, loss of relax; lethargy, discoloration, excessive mucous production etc. were observed and recorded in two experimental setups.

Acute toxicity Test

Acute toxicity test was conducted according to EPA/600/4-90/027F protocol [22]. The selected concentration ranges for the experimental set up of without aeration was 0, 28, 32, 36, 40 and 44 ppm and for the set up with aeration was 0, 65, 70, 75, 80 and 85 ppm. Each experimental setup includes four series for the decided concentration ranges.

Physico−chemical parameters were measured before and after adding the dispersant to the tanks according to the methods given in Table 1. Fish feeding was ceased 24 hr prior to the test and then the percentage mortality and other behavioral changes were observed and recorded during 24 hrs continuously. LC_{50} values were calculated using EPA probit analysis program, Version 1.5 [5].

Water temperature was measured once in two hours and other parameters were determined before adding the dispersant, after 24 hrs and at the end of the experiment after 72 hrs.

Table 1. Analytical Techniques for Physico−Chemical Parameters

Parameter	*Instrument*
Temperature	mercury bulb Thermometer
pH	salt water master test kit
Ammonia (NH_3)	Zoolek salt water NH_3 test kit
Salinity	YSI 85 meter (Japan)
Dissolve Oxygen (DO)	YSI 85 meter (Japan)

Statistical Analysis

Descriptive data analysis for calculation of mean, standard deviation, maximum and minimum values were determined using Windows excel 2010 version. One way ANOVA was used to determine the significance of the variation of physico−chemical parameters during the experimental period. Independent sample t test was performed to determine significance of the difference between LC_{50} values in two experimental setups. All statistical analysis was performed on SPSS 16.0.

RESULTS & DISCUSSION

Physico−Chemical Parameters

The Table 2 gives the mean values and the minimum and maximum values of water quality parameters in the two experimental setups. The water temperature variation was <1 ^{0}C and the salinity variation was <2 ppt in the experimental tanks and these variations were not significant.

Table 2. Mean, minimum and maximum values of the water quality parameters in aerated and not aerated experimental setups

	Without aeration	*With aeration*
Temperature		
Mean± SD	28.5 ±0.2	28.5±0.3
Min-Max	28.2-28.7	28.0-28.9
Salinity		
Mean± SD	33.0±0.1	33.0±0.2
Min-Max	32.8-33.1	32.6-33.7
pH		
Mean± SD	7.8±0	7.8±0
Min-Max	-	-
NH_3		
Mean	0	0

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Water temperature has about 70% of influence on oil and dispersant toxicity due to increase of the metabolism and then increased the uptake of potentially toxic dispersants [13]. Higher mortality of Easter oysters has been observed at warmer temperatures, in laboratory and field trials of toxicological studies [13]. The minimum and maximum temperature values in the two set ups of the present study was 28-29^{0}C. The acceptable range of water pH has been published as 7.0-7.9 for the toxicological tests with seawater and macro organisms [6]. Accordingly, the water pH was maintained at 7.8 in each tank in the present study. Since the parameters of Temperature, pH, salinity and NH_3 level in the tanks were maintained at suitable level for marine aquarium fish those parameters may not be affected on the toxicity of fish in this experiment.

The initial DO% in the experimental tanks of without aeration was 95.7% and it declined up to 48.2% and after 10 hours it increased up to 70%. DO level should not be lower than 40% in toxicological studies using warm water [6]. However, when supplying aeration the percentage DO did not change after third hour in the experimental setup.

Mortality of fish

Fish mortality was observed after 6th hour and then the mortality was the maximum at 11th hour and no fish death was observed after 13th hour in the not aerated tanks. The mortality in the aerated tanks was noticed after 9th hour and the number of dead fish increased up to the maximum level after 16th hour and the mortality was zero at the 18th hour of the experiment. After 24 hours, fish mortality did not observe in both setups can be due to the biodegradation and reduction of the effectiveness of the oil dispersant. The Figure 2 and 3 explains the variation of DO in the experimental tanks without aeration and with aeration respectively. Figure 4 explains the variation of fish mortality in the two experimental setups.

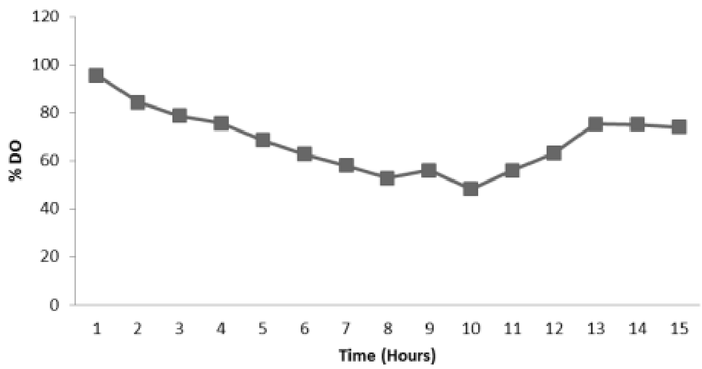

Figure 2: Variation of DO level with time in the setup without aeration

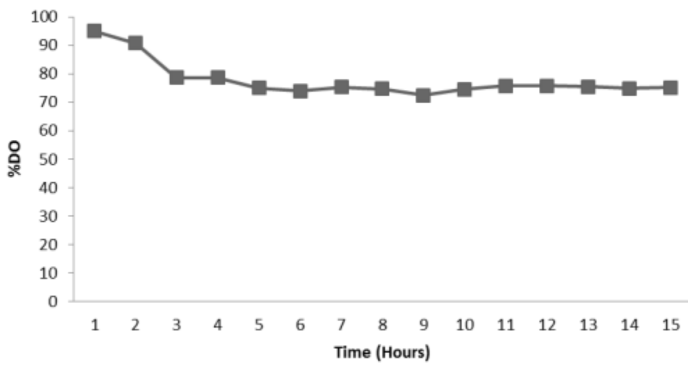

Figure 3: Variation of DO level with time in the setup with aeration

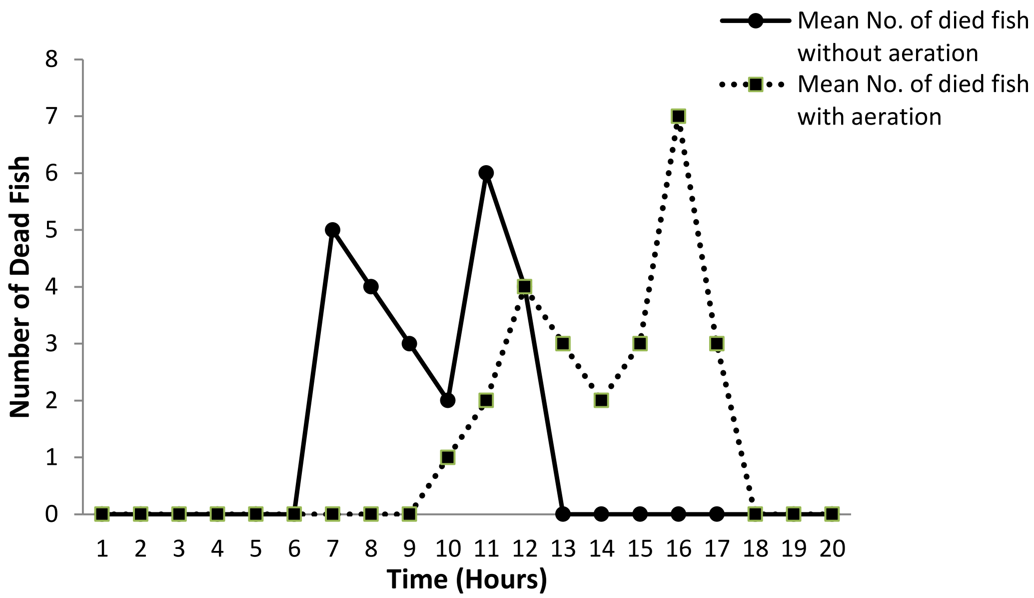

Figure 4: Variation of DO level with time in the setup with aeration

The most surfactant formulations of oil dispersants are fairly readily biodegradable under aerobic conditions by marine bacteria [11], [14], [21]. The rate of biodegradation under anaerobic conditions tends to be much lower [2], [24] and the water circulation in the experimental setup with oil dispersant did not enough to maintain the DO concentration at constant. However, no fish mortality was observed in the control tanks of the experimental setups without oil dispersant explains that the fish can live only with the water circulation in the tanks during the experimental time. Dissolved oxygen in the water can be reduced due to indirect effects of the dispersant and it is also a reason for fish death in the not aerated tanks. Therefore, the mortality of fish in the tanks with aeration was mostly due to toxic effects of the oil dispersant.

Toxicity Parameters

The calculated LC_{50} values according to Probit software in two separated experimental setups are given in Table 3. The LC_{50} values changed from 31.37 to 33.71ppm in the experimental setup without aeration and the range of LC_{50} values was 70.70 to 71.83 ppm in the tanks with aeration and these values varied significantly in the two setups ($p < 0.05$).

Table 3. Mean LC_{50} and 95% confidence limits in two experimental setups

Experimental Setup	*Series*	*LC_{50} (ppm)*	*95% confidence limit*	
			Lower	*Upper*
Without aeration	1	33.179	28.217	36.617
	2	31.370	20.701	35.362
	3	33.715	28.840	37.383
	4	33.545	26.604	38.017
With aeration	1	70.955	64.406	75.026
	2	71.796	58.673	78.128
	3	70.702	66.550	73.847
	4	71.825	66.976	75.510

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According to the five-step scale of toxicity categories [23] for the toxicity levels of chemicals in Table 4 the type 3 oil dispersant can be classified as slightly toxic chemical for *Abudefduf vaigiensis.*

Table 4. EPA five-step scale of toxicity categories used to classify chemicals based on their acute toxicity (USEPA, 2010b)

LC_{50} (ppm)	*Toxicity classification*
>100	Practically Non toxic
>10-100	Slightly Toxic
>1-10	Moderately Toxic
0.1-1.0	Highly toxic
<0.1	Very Highly Toxic

Changes Of Fish Behavior

After 2-3 hours of expose to oil dispersant the color pattern of fish changed from bright yellow color to pale yellow color. The increased breathing rate, zig- zag swimming pattern could be seen in exposed fish with no aeration. Nearly 5 minutes before the death, they fell down on to the bottom of the tank and breathed laboriously and slime secretion could be observed. The different behavioral changes in the two experimental set ups are summarized in the Table 5.

Table 5. Behavioral changes in the tanks of two set ups

Observed behavior type	*Control tank*	*With no aeration*	*With aeration*
Location of swimming	Resting at the bottom of the tank	Swimming at the upper surface of water	Swimming near the upper surface of water
Habit	Calm and quite	Active swimming	Active swimming
Operculum movement	30-35/min	45-48/min	40-48/min
Swimming movement	Horizontal and slow	Vertical Zig-zag swimming	Vertical

The exposed fish were hanging near to the surface of water column due to the narcotic effect and it is a common response to low oxygen in water. In this behavior, fish stay just below the surface, put their snout at the air-water interface, and breathe in the film of water that is in direct contact with the air [19]. The exposed fish were swimming erratically and jumping above the water column. The enzymatic as well as ionic disturbances in blood and tissues may be associated with such abnormal behavior and altered movements [10]. Operculum movement was increased up to 48/min in the exposed tanks and similar kind of changes have been observed by [18] for expose of toxins.

The dead fish were covered by a slime layer and the exposed fish lost their color patterns of vertical strips. The depigmentation is attributed to dysfunction of the endocrine (pituitary) gland under toxic stress [17].

Zigzag swimming behavior was shown by the exposed fish with no aeration. This is probably an adaptive response to escape from the danger zone [19]. According to the above explanations behavioral changes the type 3 oil dispersant may be slightly toxic to *Abudefduf vaigiensis.* Although, the laboratory experiments have been concluded the toxic level of the dispersant it can be varied at different physical conditions in the sea.

CONCLUSION

The type 3 oil dispersant can be categorized as slightly toxic chemical to the fish species, *Abudefduf vaigiensis.* It is important to consider the LC_{50} value of the Adt type 3 oil dispersant as 71.32±0.58 ppm when using the above said dispersant to oil spill in the coastal environment, Sri Lanka.

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