

## Toxicity of dispersed oil on Gold–saddle rabbitfish *Siganus guttatus* fry

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<https://doi.org/10.69721/TPS.J.2017.09.1.03>

### ABSTRACT

The acute toxicity of dispersant Mardeus-455 added to water accommodated fractions (WAF) were evaluated in Gold-saddle rabbitfish *Siganus guttatus* fry after 72 h exposure under laboratory conditions. Mortalities of fry exposed to different concentrations of dispersed oil were recorded every hour for the first 6 h, every 3 h for the next 12 h, and every 6 h thereafter. Results showed that the higher the ratio of dispersant and WAF, the higher the mortality of the rabbitfish fry which may be due to the enhanced availability of polyaromatic hydrocarbons (PAH). The LC<sub>50</sub> was computed at 3.692% of the oil volume. The application of dispersant in cleaning oil spills must be limited to reduce its harmful effect in the marine environment. Assessing toxicity of dispersed oil in fish fry may help understand the extent of environmental damage after cleaning oil spill using dispersant.

**Keywords:** acute toxicity, dispersed oil, *Siganus guttatus*, water accommodated fraction, mortality

### INTRODUCTION

Oil spill is a common sea mishap occurring worldwide that affects the marine environment and its organisms. Several concerned agencies are on full alert to mitigate the impacts of the oil spill the soonest possible time. One of the famous oil spill incidents that occurred in world history was the Exxon Valdez in 1989 that spilled 41 million liters of oil into the marine environment (Lin and Mendelssohn 2004). In the Philippines, the oil spill incident off Guimaras coasts in 2006 by MT Solar I tanker spilled an estimated 7.6 million liters of oil which brought much attention not only from the national government, research and academic institutions, but also from the private

sectors because of the impact it brought in one of the richest fishing grounds in the country (Uno et al. 2010).

To minimize the destruction caused by oil spills, different clean-up methods are being used to mitigate the effect of spilled oil in the marine environment before it reaches the shorelines and its organisms (McIntosh et al. 2010). Of these methods, dispersants are widely used. The chemical dispersants are applied to breakdown oil and move it from the top of the water, making the oil easily dissipated in the atmosphere (Fiocco and Lewis 1999). This method helps in cleaning and restoring the status of affected areas, but dispersant combined with oil slick in the water becomes more toxic to marine organisms since dispersed oil is incorporated in the water column (Page et al. 2000). Some reports indicated that the use of dispersant alone posed lower toxicity than dispersed oil when tested in marine organisms (Fingas 2008). However, the viability of dispersant as a mean to control and minimize the overall environmental damage has been proven and is cost effective.

The aftermath effects of oil spill are much anticipated in the coastal areas since after the spill, water are mixed with oil due to current and wave action. This creates the water accommodated fraction (WAF) mixed with water column (McIntosh et al. 2010). Beach, mangrove flats and seagrass beds are the first affected by oil spill since these areas serve as the nursery and feeding ground of juveniles of marine organisms. Observations during various oil spill accident, where dispersants have been used, have shown a considerable mortalities among marine animals in the intertidal zones (Rice et al. 1977). Once these areas are affected by oil spill and dispersants are applied in nearshore environment, the aquatic organisms are at risk and high mortality is observed when improperly used (Rice et al. 1977, Milinkovitch et al. 2011). The toxicity of the oil spill and dispersant will affect future food supply since recruitment will be hindered.

Despite the number of publications on the toxicity of crude oil and dispersed oil in marine organisms, most of these studies deal on long term toxicity (i.e. >10 days) (Lockhart et al. 1996, Cohen and Nugegoda 2000, Scarlett et al. 2006). The rabbitfish (family Siganidae) being a tropical fish have fry that normally inhabit seagrass beds and can easily be affected during oil spill events. It is important to determine the toxicity of dispersed oil to rabbitfish fry since they usually inhabit the upper water column at this stage and can be found in the estuaries and coastal areas where the spilled oil are usually carried over time. A study on the toxicity of crude oil and oil-dispersant mixtures to juvenile rabbitfish *Siganus rivulatus* was conducted (Eisler and Kissil 1975). Quinitio and Siladan (2013) also studied the

reproductive performance of *Siganus guttatus* exposed to dispersed bunker oil but uses mature individuals from areas affected during the 2006 Guimaras oil spill incident. No similar study was conducted for *S. guttatus* fry. To address such gap, this study aimed to determine the mortality of Gold-saddle rabbitfish *Siganus guttatus* fry exposed to dispersed oil for 72 h. Moreover, the LC<sub>50</sub> of the dispersed oil was also determined.

## MATERIALS AND METHODS

### Experimental Animal

The Gold-saddle rabbitfish (*S. guttatus*) fry with 1.8-2.5 cm total length were obtained from the Marine Fish Hatchery of Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD). The fry were stocked in basins and provided with aeration for three days acclimation prior to the experiment. These were fed to satiation with artificial feeds twice a day until they were acclimatized. The fry were not fed 24 h prior to and during exposure to the experiments.

### Experimental Treatments

**Water accommodated fraction preparation.** The water accommodated fraction (WAF) was prepared in a closed, low-energy system using filtered (0.40 µm) seawater (35 ppt). The filtered seawater (3600 ml) was placed in amber, round glass flask, and 400 ml of crude oil (Petron Bunker C oil) was added by drops on the water surface while stirring using a magnetic stirrer with speed set at 360 rpm. The stirring was carefully controlled to avoid a large vortex and formation of oil droplets or emulsions. After 18 h of stirring, the WAF was siphoned out and placed in glass jars leaving 500 ml only. The WAF (500 ml) was then diluted in 10 L water to have a final ratio of 1:300 (oil and water). This prepared WAF is effective for 2–3 days (El Samra et al. 1986).

**Preparation of treatments.** The dispersant Mardeus-455 used was obtained from the Marine and Environmental Protection Command of the Philippine Coast Guard (PCG-MEPCOM). This was applied in an aquarium filled with 10 L WAF per treatment. The rabbitfish fry were exposed to different levels of toxicity 30 min after the application of dispersant to the WAF.

The different treatments with the amount of WAF and dispersants used are shown in Table 1. The treatment used was based on the study of

Barron (2003) where they used dispersant: oil ratio of 1:10 to 1:50. Each treatment had four replicates. The fry were exposed to the different treatments and 72-h LC<sub>50</sub> (lethal concentration wherein 50% of the population died) were determined.

Each aquarium was aerated and stocked with 10 fry. The number of surviving fry was checked every hour for the first 6 h, every 3 h for the next 18 h, and every 6 h from 24 h to 72 h. The fry were considered dead if no opercular movement were observed, and/or seen floating in the water surface.

Table 1. The volume of water accommodated fraction and dispersant and the corresponding oil: water ratio used in different treatments.

Treatment	Number of test fry per replicate	Volume of dispersant (ml)	Dispersant: oil ratio (%)
*Control	10	0	WAF only
1	10	0.44	1:80 (1.25)
2	10	0.88	1:40 (2.50)
3	10	1.16	1:30 (3.33)
4	10	1.75	1:20 (5.00)
5	10	3.50	1:10 (10.00)

**Water parameters.** Water temperature and salinity were measured every time the mortality was checked which started from the start of the experiment. The salinity and water temperature range were 34 – 35 ppt and 25 – 28.5°C, respectively.

### Statistical Analyses

The LC<sub>50</sub> of the dispersant was determined using Probit Analysis of the Statistical Package for Social Sciences (SPSS ver. 15). Mortality was first log transformed and fitted with the probit values before it was analyzed. The LC<sub>50</sub> was computed using the formula:

$$y = a + bx$$

Where:

$y$  = probit value at LC<sub>50</sub> (value is 5)

$a$  = intercept

$b$  = slope

$x$  = unknown variable

In determining the significant differences between and among treatments, analysis of variance (ANOVA) was used in each period at  $\alpha = 0.05$ .

## RESULTS

The mean cumulative mortality of rabbitfish fry are shown in Figure 1. When the fish were exposed to the different treatments, it was noted that they were lurking around the aquarium and seemed to gasp for air. During the first hour of exposure (1h), the highest oil: dispersant ratio (Treatment 5) showed the highest mean mortality (100%), followed by Treatment 4 (92.5%), Treatment 3 (25.0%), Treatment 2 (7.5%), and Treatment 1 (2.5%). No mortality was observed in the Control treatment. The mean cumulative mortality in the Control and Treatments 1 and 2 were significantly different from that of Treatments 3, 4 and 5. On the other hand, the mean cumulative mortality in Treatment 3 were significantly different ( $p < 0.05$ ) with Treatments 4 and 5, while no significant differences were observed between Treatments 4 and 5 ( $p > 0.05$ ).

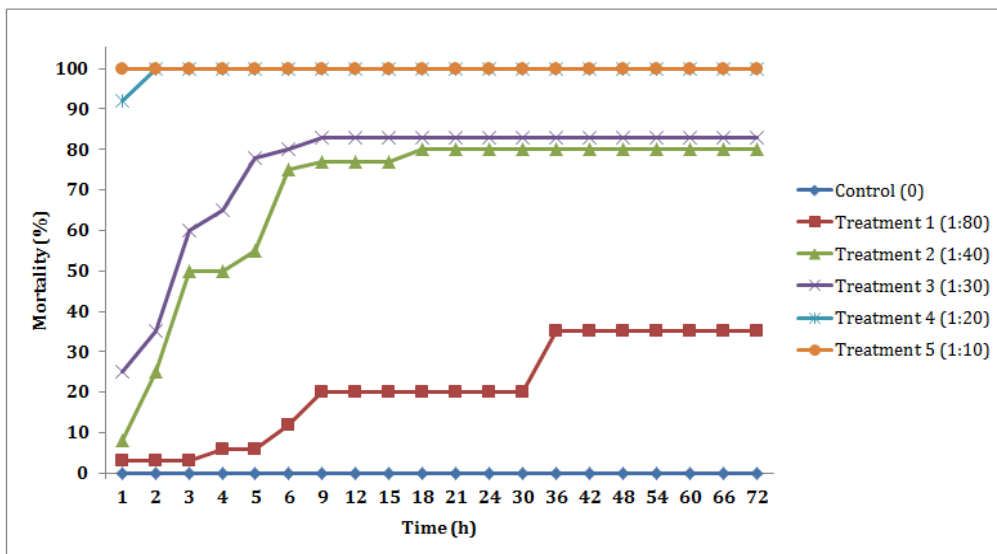


Figure 1. Mean cumulative mortality of Gold-saddle rabbitfish *Siganus guttatus* fry exposed to different concentrations of dispersed oil in different time scales.

After 2 h, all fry were dead in Treatment 4, and 25% and 35% of fry died in Treatments 2 and 3, respectively. An increase in mortality was observed in Treatment 3 with a mean of 60.0% after 3 h. Treatment 2 had 50.0% mean mortality and it was 2.5% in Treatment 1. During 3 h of exposure, mean mortality in the Control and Treatment 1 already showed significant difference ( $p < 0.05$ ) with Treatments 2 and 3. Treatments 2 and 3 showed significant difference ( $p < 0.05$ ) with Treatments 4 and 5.

In the fourth hour of exposure, 65.0% of the fry were dead in Treatment 3, 50% in Treatment 2, 7.5% in Treatment 1, and still no mortality in the Control. After 5 h, 77.5% mean cumulative mortality was observed in Treatment 3, 57.5% in Treatment 2, no increase in mortality in Treatment 1 (7.5%), and Control (0%). On the other hand, a mean of 80% had died in Treatment 3, 75.0% in Treatment 2, 12.5% in Treatment 1, and no mortality in the Control at the 6 h of exposure. During these exposure periods, the Control and Treatment 1 showed significant difference ( $p < 0.05$ ) with Treatments 2, 3, 4 and 5. On the other hand, Treatments 2 and 3 showed significant difference ( $p < 0.05$ ) with Treatments 4 and 5. The difference in the mean cumulative mortality among treatments changed after 6 h of exposure, wherein the Control and Treatment 1 were already significantly different ( $p < 0.05$ ) with Treatments 2, 3, 4, and 5 but no significant difference ( $p > 0.05$ ) were observed among the latter four treatments.

The trend of mean cumulative mortality seemed to have changed at the 9 h of exposure, wherein 80.0% occurred in Treatment 3, 77.5% in Treatment 2, 20.0% in Treatment 1, and still no mortality in the Control. The Control had significant difference ( $p < 0.05$ ) with Treatments 1, 2, 3, 4 and 5, while Treatments 1, 2 and 3 also had significant difference ( $p < 0.05$ ) with that of Treatments 4 and 5. This trend continued until the 12 h of exposure, but Treatment 3 had changed at 12 h with 82.5%, showing no significant difference ( $p > 0.05$ ) with Treatments 4 and 5. At 15 h, an increased mean cumulative mortality of 80.0% was observed in Treatment 2 and was already not significantly different ( $p > 0.05$ ) with Treatments 3, 4 and 5. From then on until 72 h, no increase in mortality had been recorded, showing a plateau in the mortality rate curve. Treatments 4 and 5 showed highest mean cumulative mortality (100.0%) at the end of the 72 h exposure period, followed by Treatment 3 (82.5%), and Treatment 2 (80.0%). Only 20% of the fry died in Treatment 1, and no mortality was observed in the Control.

The  $LC_{50}$  of all treatments was calculated to be 3.692%. This means that 3.692% of the oil volume may give a 50% mortality to rabbitfish fry.

## DISCUSSION

This study showed the toxicity of different dispersant concentrations applied to WAF using rabbitfish (*S. guttatus*) fry as test animal in a 72-h exposure period. The mortality in each treatment increased with time, except in the Control. In the 1 h after exposure, mortality in the Control and Treatments 1 and 2 (low dispersant concentrations) were lower compared to that of Treatments 3, 4 and 5 (high dispersant concentrations). The two highest concentrations (Treatments 4 and 5) showed abrupt mortalities during the first hour of exposure. It is possible that in the high dispersant concentrations, the PAHs of the oil were already available in the water, which may be the main cause of fry mortality (Singer et al. 1998) in the 1st, 2nd and 3rd hour of exposure. These results showed that when higher concentrations of dispersant were added in the WAF, higher mortalities occurred among the rabbitfish fry. The same result was observed by Cohen et al. (2005) in the 96-h toxicity test of Australian bass (*Macquaria novemaculeata*) using dispersed crude oil, where they noted that almost 90% of the test animals already died in the 6 mg/L dispersant: oil ratio. Moreover, Edwards et al. (2003) had similar results using inland silverside *Menidia beryllina* and estuarine invertebrate *Mysidopsis bahia* as test animals, wherein 80% mortality was observed in a 72-h exposure period. The recommended use of a dispersant-to-oil percentage of 20% (1:5) in the red sea bream (*Pagrus major*) yielded higher oil concentrations in the water, and resulted in a higher mortality rate than the use of lower percentages of dispersant to oil (Koyama and Kakuno 2004).

Generally, the effects of the different concentrations of the dispersant vary with brands. The effectiveness of dispersant generally increases with increasing dispersant application (Fingas 2002). In the study of DeCola (1999, as cited by Barron 2003) under laboratory conditions of the dispersant Corexit 9500 on the Alaska North Slope crude oil, the dispersant effectiveness was directly proportional to the dispersant: oil ratio, ranging from 1:10 to 1:50. Polyaromatic hydrocarbons (PAH) were accounted for much of the toxicity based on several studies (Barron et al. 2003, Gonzales et al. 2006). Dispersed droplets are also important route of exposure that causes mortality of some organisms in the marine environment either through droplet/gill interactions or through ingestion (Fuller et al. 2004). Studies showed that some organisms accumulated PAHs differently via particulate or dissolve routes. Marine organisms may also be exposed to oil by contamination of their food. Many oil constituents, such as the monoaromatics (MAHs) and PAHs are narcotic substances which cause a state of arrested activity of protoplasmic structures (Barron et al. 2003). Many studies have found that the PAH concentration is much higher in

chemically-dispersed oil than for physically-dispersed oil (Barron 2003). Moreover, several researchers have recently noted higher toxicities of chemically-dispersed oil and that the PAH bioaccumulation kinetics are increased in chemical dispersions (Committee 2006). This study proved that chemically dispersed oil is more toxic in marine organisms than crude oil alone. The result of this study also concurs to Almeda et al. (2014) where chemically dispersed oil caused greater mortality in microzooplankton than crude oil alone.

The  $LC_{50}$  of a substance is the “lethal concentration of which 50% of the test population died”. A  $LC_{50}$  of 3.692 was determined in this study which indicates that a 3.692% of the dispersant: oil ratio gave a 50% mortality in rabbitfish fry samples. This low  $LC_{50}$  might be attributed to the dispersant’s low surfactant components when it was manufactured. The study by Greco et al. (2006) calculated a 6.6%  $LC_{50}$  of Corexit 9500 when it was used in dispersing spilled oil in Alaskan waters. It is possible that different brands of dispersant would give different  $LC_{50}$ .

This study revealed that no mortality was observed in crude oil (WAF) alone and the lowest dispersant application (0.44 ml) had lower mortality compared with the other treatments. This suggests that the application of 5% or higher dispersant (1:20 dispersant: oil ratio) on WAF would give significant mortality to rabbitfish fry. Rhoton et al. (2001) found out that application of Corexit 9527 alone is less toxic compared when it was applied in crude oil in *Menidia berylina* juvenile. This is because oil treated with dispersants enhances the droplet formation of oil, increasing both its accommodation into the water column and its solute-solvent interfacial contact area (Singer et al. 1998). Bobra et al. (1989) showed that toxicity of dispersed oil on eggs and juvenile of marine fish have different sensitivity compared to mature fish. This is also in accordance to some studies where early life stages of marine fish seems to be more sensitive to PAH exposure than adult fish (McIntosh et al. 2010). Mature life stages of animals are more tolerant to exposures in different oil-dispersant mixtures compared to eggs and juvenile fish (Fingas 2002). It was also suggested that variations in PAH resistance of different life stages of marine fish may reveal the differences in the bioaccumulation of hydrocarbons by eggs and larvae (Carls and Rice 1988).

Based on the results of this study, WAF alone and WAF applied with the lowest concentrations of dispersant (1.25% dispersant to oil volume) incurred the lowest mortality while the dispersed oil concentration of 5% and 10% incurred the highest mortalities on rabbitfish *S. guttatus* fry in a 72-h exposure. The higher the dispersant: oil ratio, the higher the mortality which was attributed to increased concentration of dispersant that caused the



polyaromatic hydrocarbons (PAHs) in the WAF to become more available in the water which are known to be toxic. Even at the lowest dispersant: oil ratio, mortality occurs and may affect mortality of siganid fry.

While the purpose of dispersant is to reduce the effect of oil spill in aquatic ecosystems, it turns out that application of dispersant to mitigate the negative impact of oil spill complicates the problem. The application of dispersant in cleaning oil spills must be limited to reduce its harmful effect in the marine environment and its organisms. A similar study using adult rabbitfish must also be considered. Since there are no documented studies on the effect of dispersant Mardeus-455, it is recommended to compare its toxicity with other brands of dispersant in rabbitfish and in other marine organisms to provide more adequate information on the negative effects of dispersants in aquatic environment if not used properly.

## ACKNOWLEDGMENTS

We would like to thank the Marine Finfish Hatchery of SEAFDEC/AQD Tigbauan Main Station for providing the rabbitfish fry, and to PCG–MEPCOM - Western Visayas for providing the dispersant. The help of Manong Condring in the preparation of WAF and various aquaria is also appreciated. The senior author was a recipient of the DOST-PCAMRD scholarship for the MS Fisheries (Fisheries Biology) degree. We are also thankful to the comments and suggestions of two anonymous reviewers.

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### **ARTICLE INFO**

*Submitted: 10 March 2017*

*Revised: 5 May 2017*

*Accepted: 15 July 2017*