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Short and Long Term Toxicity of Crude Oil and Oil Dispersants to Two Representative Coral Species

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Oil dispersants, the tool of choice for treating oil spills in tropical marine environments, is potentially harmful to marine life, including reef corals. In a previous study, we found that dispersed oil and oil dispersants are harmful to soft and hard coral species at early life stages. In this broader study, we employed a "nubbin assay" on more than 10 000 coral fragments to evaluate the short- and long-term impacts of dispersed oil fractions (DOFs) from six commercial dispersants, the dispersants and water-soluble-fractions (WSFs) of Egyptian crude oil, on two Indo Pacific branching coral species, *Stylophora pistillata* and *Pocillopora damicornis.* Survivorship and growth of nubbins were recorded for up to 50 days following a single, short (24 h) exposure to toxicants in various concentrations. Manufacturer-recommended dispersant concentrations proved to be highly toxic and resulted in mortality for all nubbins. The dispersed oil and the dispersants were significantly more toxic than crude oil WSFs. As corals are particularly susceptible to oil detergents and dispersed oil, the results of these assays rules out the use of any oil dispersant in coral reefs and in their vicinity. The ecotoxicological impacts of the various dispersants on the corals could be rated on a scale from the least to the most harmful agent, as follows: Slickgone > Petrotech > Inipol = Biorieco > Emulgal > Dispolen.

Introduction

It is estimated that 40% of global crude oil transport is conducted offshore with much of the traffic, taking place in tropical, coral reef-rich areas (*1*, *2*). This heavy maritime traffic of crude oils and their products is prone to accidents, resulting in major or minor spillages. Although the number of major oil spills has decreased in the past decade it is still, by far, the most serious threat to the marine environment (*3*, *4*). Of the three major ways for treating marine oil spills (chemicals, mechanical containment booms, skimmers and sorbents, biological–biodegrading microorganisms), chemicals, mainly oil dispersants (*5*), are probably the most commonly used. Dispersants are chemicals that contain surfactants and/or solvent compounds that break down floating oil into small droplets within the water-column, which makes the spill less likely to reach shore (*6*). Dispersed oil is subjected to natural forces such as waves and currents that promote dissolution of oil droplets. Use of dispersants for treating oil spills is governed by local and national regulations determining, for instance, distance from shore and depth at which treatment is allowed (*7*). However, since most oil-tanker accidents occur near the shore, it is essential to evaluate the impacts of oil dispersants on organisms that live on the seabed (*8*), including sea grass populations and coral reefs.

Most information on ecotoxicological impacts of dispersants comes from studies on North American dispersants of the Corexit family (*9*). Information on other dispersants (e.g., European manufactured dispersants) is limited and their effects on corals is deficient (*4*).

Recent studies on possible toxicity of "environmentally improved" third generation oil dispersants revealed that even these improved chemicals could harm marine biota, in general, and reef corals, in particular. For example, Negri and Heyward (*1*) found that oil-dispersed Corexit 9527 inhibits fertilization of mature eggs and the metamorphosis of *Acropora millepora* larvae. Harmful effects of improved chemicals were also reported by Epstein et al. (*10*) in their study on impacts of five-third-generation dispersants (Inipol IP-90, Petrotech PTI-25, Bioreico R-93, Biosolve and Emulgal C-100) on planula larvae of a stony coral (*Stylophora pistillata*) and a soft coral (*Heteroxenia fuscesense*). The last-mentioned authors found that, compared to the toxicity of dissolved oil fractions, dispersed oil causes a dramatic increase in toxicity to larvae of both species.

In this study, we employed the nubbin bioassay (*11*) to examine the effect of dispersants, oil, and dispersed oil on mature coral colonies of two branching scleractinian corals. We examined the long-term impacts (for up to 50 days) after a single, short (24 h) administration of toxicant.

Materials and Methods

Colonies of *Stylophora pistillata* and *Pocillopora damicornis* were collected from shallow waters (4–6 m depth) in Eilat, Red Sea, and transported, immersed in seawater within insulated containers, to the National Institute of Oceanography, Haifa where they were maintained under conditions previously described in ref *12*. To include a wide species-variability, each set of assays was performed on nubbins taken from three different genotypes per species (all nubbins from a single colony were genetically identical, an outcome that reduces variation within and among tests).

Nubbins (average surface area 31.1 ± 9.7 mm^2, approximately 5–10 polyps each) were pruned from coral colonies using an electrician's wire-cutter and immediately immersed in seawater to minimize stress. The exposed skeletal surfaces of the freshly cut nubbins were dried with paper towels and glued with a drop of cyanoacrylate glue (Super Glue 3, Loctite, Ireland) onto dry glass slides, five nubbins per glass (*13*).

A stock solution of water-soluble fractions (WSFs; 10) from Egyptian crude oil was prepared by adding 5 mL of crude oil to 995 mL of filtered seawater (1:200 ratio). Then, the mixture was shaken for 24 h at 80 rpm after which only the soluble fraction was collected in a separating funnel. The dispersed oil fractions (DOF) stock solution was prepared according to the manufacturer's recommendations, usually 1:10 dispersant:oil volume ratio. For this purpose, we added 0.5 mL of the tested dispersant to the crude-oil–seawater mixture and followed the above preparation methodology. The dispersant stock solution was prepared by adding 0.5 mL of the tested dispersant (1:2000 ratio) to 995.5 mL of filtered seawater.

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TABLE 1. Average Survivorship of *S. Pistillata* and *P. Damicornis* Nubbins One Week after Administration (24 h) of Nine Graded Solutions of Crude Oil WSP and Dispersed Oil Fractions (DOF)

species	treatment	*N*	100%	75%	50%	25%	10%	5%	1%	0.5%	0%
S. pistillata	Egyptian WSF	540	92% ± 4%	92% ± 4%	94% ± 3%	95% ± 3%	98% ± 2%	93% ± 4%	93% ± 4%	100%	100%
	DOF Biorieco	540	0%	0%	0%	0%	96% ± 0%	100%	96% ± 3%	100%	100%
	DOF Dispolen	540	0%	0%	0%	0%	0%	98% ± 6%	98% ± 7%	98% ± 6%	98% ± 6%
	DOF Emulgal	540	0%	0%	0%	0%	93% ± 4%	91% ± %	100%	99% ± 1%	96% ± 3%
	DOF Inipol	540	0%	0%	0%	0%	65% ± 57%	100%	99% ± 2%	100%	100%
	DOF Petrotech	540	0%	0%	0%	0%	85% ± 3%	80% ± 28%	97% ± 6%	95% ± 5%	92% ± 11%
	DOF Slickgone	540	0%	0%	0%	88% ± 12%	98% ± 3%	100%	100%	100%	97% ± 6%
P. damicornis	Egyptian WSF	540	74% ± 13%	72% ± 5%	71% ± 9%	68% ± 3%	60% ± 52%	73% ± 16%	78% ± 8%	71% ± 12%	85% ± 5%
	DOF Biorieco	540	0%	0%	0%	0%	38% ± 48%	96% ± 7%	100%	97% ± 5%	97% ± 5%
	DOF Dispolen	540	0%	0%	0%	0%	0%	98% ± 2%	98% ± 2%	97% ± 6%	100%
	DOF Emulgal	540	0%	0%	0%	0%	29% ± 27%	95% ± 6%	93% ± 6%	92% ± 11%	95% ± 6%
	DOF Inipol	540	0%	0%	0%	0%	35% ± 28%	60% ± 8%	67% ± 10%	85% ± 5%	83% ± 7%
	DOF Petrotech	540	0%	0%	0%	0%	98% ± 3%	95% ± 9%	92% ± 6%	93% ± 3%	98% ± 3%
	DOF Slickgone	540	0%	0%	0%	97% ± 2%	92% ± 11%	100%	100%	100%	93% ± 6%

TABLE 2. Average Survivorship of *S. Pistillata* Nubbins, One Week after Administration (24 h) of Nine Graded Solutions from Each Tested Dispersant

dispersant	N	100%	75%	50%	25%	10%	5%	1%	0.5%	0%
Biorieco	540	0%	0%	0%	0%	3% 6%	98% ± 3%	100%	98% ± 3%	100%
Dispolen	540	0%	0%	0%	0%	0%	92% ± 14%	93% ± 3%	98% ± 3%	98% ± 3%
Emulgal	540	0%	0%	0%	0%	2% ± 3%	73% ± 26%	100%	97% ± 2%	94% ± 5%
Inipol	540	0%	0%	0%	0%	73% ± 28%	100%	98% ± 3%	100%	98% ± 3%
Petrotech	540	0%	0%	0%	0%	83% ± 23%	71% ± 39%	80% ± 29%	64% ± 37%	79% ± 33%
Slickgone	540	0%	0%	0%	0%	82% ± 28%	100%	97% ± 3%	98% ± 3%	100%

Six dispersants were tested: Emulgal C-100 (Amgal Chemicals, Israel), Dispolen 36S (SEPPIC), Inipol 90 (CECA S.A. France), Petrotech PTI-25 (Petrotech Emergency), Slickgone NS (Dasic Int. Ltd. UK), Bioreico R-93 (Reico France). The dispersants stock solutions were considered to be at 100% concentration. In the experiment with crude oil WSFs, nubbins were introduced into seawater with six different concentrations of crude oil WSF: 100, 75, 50, 25, 10, and as a control, 0% as control. Nine concentrations were tested in both the dispersants and DOFs experiments: 100, 75, 50, 25, 10, 5, 1, 0.5, and as a control, 0%.

The above assays were performed on short- (7 days) and long-term (50 days) acute responses to oil WSFs and dispersed oil fractions. The dispersants' impacts were tested only in short-term assays. In each experiment, 60 nubbins (20 from each genotype; 10 800 nubbins total) were subjected during a 24 h period to different toxicant concentrations. The nubbins were then washed carefully for several minutes under freshly filtered seawater and placed in new aquaria supplied with flowing filtered seawater. Survivorship was monitored daily during the first week of the experiment, and it was then monitored weekly. Horizontal tissue growth on the glass slide was evaluated weekly.

For grading the relative toxicity of the tested dispersants to corals, the results (see below) of each experiment (a) were divided by the control values of that experiment (b) and then multiplied by the concentration of the administrated toxicant (DOF, WSF, dispersant; c). The sum value (e) of all treatments of each dispersant ($e = \Sigma(a/b)xc$) was divided by the value of the most toxic dispersant in that experiment (f), providing a grade (g) for dispersant toxicity ($g = e/f$). Average quality of dispersant toxicity (q) was calculated from both coral species (*Stylophora*, *Pocillopora*). The relative toxicity (RT) of a specific dispersant was established as the average values from the survivorship after 7 days (q, 7), survivorship after 50 days (q, 50), and percentages of nubbins that developed horizontal tissues on the substrates (q, tissue), combining short- and long-term toxicity results.

Results

Two timeframes (short and extended post treatment period) assays were applied to evaluate various ecotoxicological impacts that developed following 24 h exposure to toxicants. The short time assays examined the survivorship of the nubbins up to a week after exposure to various WSF and DOF concentrations. Most results (dead vs alive) were detectable as early as the end of the 24 h treatments, but in several treatments, dead tissue lingered on the coral skeleton for a few more days, making it difficult to determine the status of the nubbins. Therefore, the endpoint of the short-term experiments was confirmed within 7 days after adminstering the toxicant.

None of the crude oil WSF concentrations had any impact on the survivorship of either *Stylophora pistillata* or *Pocillopora damicornis* nubbins ($p > 0.05$ ANOVA one-way; Table 1). However, all 100% (stock solution) dispersed oil DOFs (the recommended concentrations by the manufacturers, 1:10), as well as the 75 and 50% (1:20) concentrations of all dispersant−oil combinations, the 25% concentrations of all dispersant−oil combinations (except Slickgone), and the Dispolen DOF 10% concentration, caused 100% mortality to nubbins of both coral species. Bioreico, Emulgal and Inipol 10% DWSPs showed significant mortality only in *P. damicornis* nubbins with 38, 29, and 35% survivorship respectively, compared to 97, 95, and 83% of the controls ($p < 0.05$ t test; Table 1).

A control experiment on the affect of dispersants alone was preformed on *Stylophora pistillata* nubbins. As with the DOF solutions, all 100% (stock solution; 0.05%) of the dispersants, as well as 75, 50, and 25% concentrations of all dispersant solutions resulted in 100% nubbins mortality. Furthermore, Dispolen 10% concentration caused 100% mortality to the nubbins, and Emulgal and Biorieco 10% concentrations caused 98 and 97% mortality, respectively. On the other hand, The results of the Inipol, Slickgone, and Petrotech 10% concentrations showed high survivorship rates (73, 82, and 83%, respectively; Table 2).

In the expanded experiments, nubbins were maricultured for 50 days following acute exposure to the WSFs and DOFs concentrations. Within this period, the cultured nubbins did not show any delayed mortality effects (Table 3). Most nubbins that had survived the first week continued to live. Three weeks after toxicant administrations, nubbins attached onto glass slides started to grow flat horizontal tissues and

TABLE 3. Average Survivorship of *S. Pistillata* and *P. Damicornis* Nubbins 50 days after Administration (24 h) of Nine Graded Solutions of Crude Oil WSP and Dispersed Oil Fractions (DOF)

species	treatments	N	100%	75%	50%	25%	10%	5%	1%	0.5%	0%
S. pistillata	Egyptian WSF	540	76% ± 30%	82% ± 15%	77% ± 27%	75% ± 5%	73% ± 5%	78% ± 14%	80% ± 11%	76% ± 15%	83% ± 8%
	DOF Biorieco	540	0%	0%	0%	0%	92% ± 11%	67% ± 36%	77% ± 36%	99% ± 2%	89% ± 10%
	DOF Dispolen	540	0%	0%	0%	0%	0%	87% ± 20%	85% ± 21%	82% ± 22%	82% ± 23%
	DOF Emulgal	540	0%	0%	0%	0%	91% ± 6%	90% ± 9%	98% ± 3%	97% ± 5%	96% ± 2%
	DOF Inipol	540	0%	0%	0%	0%	59% ± 51%	96% ± 4%	96% ± 4%	99% ± 2%	88% ± 7%
	DOF Petrotech	540	0%	0%	0%	0%	53% ± 20%	53% ± 45%	34% ± 27%	43% ± 42%	31% ± 28%
	DOF Slickgone	540	0%	0%	0%	83% ± 11%	88% ± 20%	98% ± 8%	88% ± 18%	86% ± 25%	83% ± 19%
P. damicornis	Egyptian WSF	540	41% ± 41%	23% ± 24%	36% ± 36%	31% ± 24%	46% ± 40%	43% ± 38%	47% ± 41%	45% ± 37%	48% ± 44%
	DOF Biorieco	540	0%	0%	0%	0%	25% ± 40%	67% ± 41%	81% ± 20%	67% ± 16%	66% ± 12%
	DOF Dispolen	540	0%	0%	0%	0%	0%	58% ± 23%	87% ± 8%	90% ± 13%	82% ± 15%
	DOF Emulgal	540	0%	0%	0%	0%	13% ± 12%	57% ± 14%	63% ± 33%	63% ± 33%	63% ± 27%
	DOF Inipol	540	0%	0%	0%	0%	21% ± 26%	26% ± 24%	21% ± 18%	54% ± 11%	62% ± 3%
	DOF Petrotech	540	0%	0%	0%	0%	88% ± 8%	88% ± 13%	88% ± 8%	90% ± 5%	83% ± 8%
	DOF Slickgone	540	0%	0%	0%	80% ± 16%	68% ± 7%	92% ± 11%	90% ± 6%	93% ± 8%	70% ± 14%

TABLE 4. Percentages of Nubbins (of Total Surviving) That Developed, after 50 Days, Horizontal Tissue on the Substrate (NA, Not Available, All Nubbins Died)

			toxicant concentration				
species	treatments	N	10%	5%	1%	0.5%	0%
S. pistillata	Egyptian WSF	420	98% ± 2%	93% ± 4%	93% ± 4%	100%	100%
	DOF Biorieco	250	11% ± 11%	38% ± 14%	58% ± 19%	71% ± 36%	64% ± 15%
	DOF Dispolen	200	NA	49% ± 40%	47% ± 45%	53% ± 32%	45% ± 41%
	DOF Emulgal	300	77% ± 22%	73% ± 4%	66% ± 15%	64% ± 18%	91% ± 10%
	DOF Inipol	260	26% ± 23%	64% ± 18%	76% ± 3%	81% ± 10%	77% ± 7%
	DOF Petrotech	130	91% ± 4%	51% ± 21%	48% ± 17%	25% ± 11%	59% ± 34%
	DOF Slickgone	320	59% ± 13%	63% ± 18%	65% ± 18%	80% ± 17%	60% ± 29%
P. damicornis	Egyptian WSF	220	65% ± 24%	77% ± 12%	70% ± 9%	85% ± 11%	89% ± 8%
	DOF Biorieco	190	36% ± 11%	80% ± 17%	77% ± 16%	89% ± 8%	54% ± 21%
	DOF Dispolen	190	NA	88% ± 14%	90% ± 8%	91% ± 10%	94% ± 4%
	DOF Emulgal	160	0%	13% ± 26%	31% ± 18%	98% ± 3%	82% ± 17%
	DOF Inipol	190	5% ± 11%	42% ± 48%	39% ± 49%	38% ± 33%	23% ± 25%
	DOF Petrotech	260	39% ± 34%	73% ± 11%	59% ± 24%	76% ± 15%	76% ± 18%
	DOF Slickgone	300	64% ± 21%	70% ± 17%	69% ± 11%	85% ± 7%	88% ± 9%

skeletons on the slides as recorded earlier (*11*). Nubbins that started to spread on substrates were monitored and photographed digitally, as described in ref *11*. After 50 days, most surviving nubbins grew horizontal tissues but some showed delayed effects in two parameters: percentage of nubbins that developed horizontal tissue and onset for initial tissue growth.

None of the crude oil WSFs had any impact on lateral growth of either *Stylophora pistillata* or *Pocillopora damicornis* nubbins ($p > 0.05$, one-way ANOVA; Table 4). Since *S. pistillata* and *P. damicornis* nubbins exposed to 10% Dipolen WSF solution had not survived after one week, they were not taken into consideration in the growing long-term assay. However, at least, some of the experiments with dispersant administration revealed significant impacts on lateral growth. *S. pistillata* nubbins exposed to Biorieco and Inipol at 10% DOF concentration exhibited long-term effect of suppressed growth. Under the influence of these materials, only 11 and 26%, respectively, of the surviving nubbins developed horizontal tissues after 50 days from toxin exposure, compared to the 64 and 77%, respectively, of the control ($p < 0.05$ t test; Table 4). *P. damicornis* nubbins exposed to Emulgal 10% DWSP failed to grow horizontal tissues. *P. damicornis* nubbins exposed to Emulgal 5 and 1% DOFs showed suppressed development: 13 and 31%, compared to 82% of the control ($p < 0.05$ t test; Table 4). Furthermore, Biorieco 10 and 5% DOF caused three- and two-week delays in *S. pistillata* nubbins horizontal tissues development. Dispolen 5% DOF caused a one-week delay, and Inipol 10 and 5% DOF caused one- and two-week delays, respectively (Table 5). Biorieco 10%, Dispolen 5%, and Emulgal 5% DOF caused delays to growth of *P. damicornis* nubbins' horizontal tissues by one week, and Inipol 10% DOF caused a two-week delay (Table 5).

TABLE 5. Weeks Delay in Beginning of Horizontal Tissue Growth (NA= Not Available, All Nubbins Died; NT, No Horizontal Growth Developed)

		toxicant concentration				
species	treatments	10%	5%	1%	0.5%	0%
S. pistillata	Egyptian WSF	0	0	0	0	0
	DOF Biorieco	−3	−2	0	0	0
	DOF Dispolen	NA	−1	0	0	0
	DOF Emulgal	0	0	0	0	0
	DOF Inipol	−2	−1	0	0	0
	DOF Petrotech	0	0	0	0	0
	DOF Slickgone	0	0	0	0	0
P. damicornis	Egyptian WSF	0	0	0	0	0
	DOF Biorieco	−1	0	0	0	0
	DOF Dispolen	NA	−1	0	0	0
	DOF Emulgal	NT	−1	0	0	0
	DOF Inipol	−2	0	0	0	0
	DOF Petrotech	0	0	0	0	0
	DOF Silckgone	0	0	0	0	0

Discussion

Oil dispersants play an important role in environmental technologies aiming to enhance the dissolving of oil in the water column by converting oil spills into chemically dispersed droplets. Potential ecotoxicological effects of this change must be taken into consideration because benthic organisms that initially are not affected by oil may be exposed to the harmful impacts of dispersants (*9*, *10*). The fragile coral reefs, and, particularly their building blocks, the scleractinan corals, need extra care when dealing with the devastating agents of oil and oil dispersants.

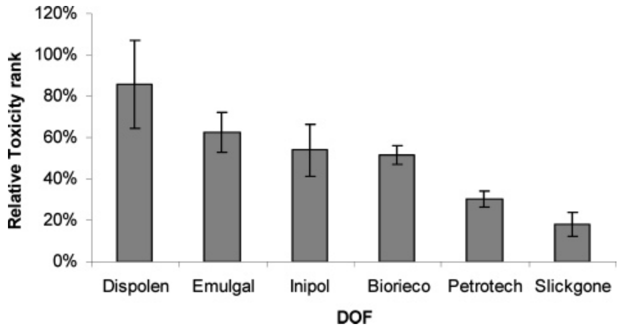

FIGURE 1. Relative toxicity (RT) of the six tested dispersants on corals (calculated form the results presented in Tables 1, 3−5). DOF, dispersed oil fractions.

As reported earlier (*10*), the results of this study have reconfirmed the increased toxicity of dispersed oil when compared to untreated oil (but there is no synergistic detrimental impacts from the dispersant and oil together). Even when dealing with improved formulas, often described as environmentally friendly dispersants (*4*), the high toxicity effects of all six approved−to-use oil dispersants on corals, strengthen the general recommendation to ban oil dispersants from the vicinity of coral reefs, if possible (when the oil slick is not stranded on the reef flat). Earlier (*10*), we found that both dispersed oil and oil dispersants are harmful to early stages of soft and hard coral species by reducing settlement and survivorship rates and by altering morphology and behavior of planulae and spats. Dispersed oil revealed synergistic detrimental impacts (*10*). In the present study, we gained knowledge on ecotoxicological impacts to corals by employing the nubbin assay (*11*), on thousands of coral fragments taken from two coral branching species. Evaluating the ecotoxicological impacts on coral nubbins during short and long periods (up to 50 days after toxicant administration), allowed us to observe the effects on the whole organism by monitoring survivorship and the physiological parameter of tissue growth on the substrate.

The results of the six dispersants examined here have revealed that the manufacturers recommended usage ratio of 1:10 dispersant:oil inflicts significant harm on coral colonies. Epstein et al.(10) rated the dispersants they tested, from the least to the most toxic compound, as follows: Petrotech < Bio-solve < Emulgal < Bioreico = Inipol. By consolidating the parameters tested in the present study on both coral species into one common scale, the relative toxicity (RT) value, we found that out of the six tested dispersants, Slickgone is the least toxic dispersant to corals and Dispolen is the most toxic (Figure 1). Rating the RT values of the tested dispersants resulted in the following order: Slickgone < Petrotech < Inipol = Biorieco < Emulgal < Dispolen (Figure 1). It is interesting to note that the four dispersants employed here and by Epstein et al. (*10*) were ranked, in both experiments, by the same toxicity hierarchy (Petrotech < Inipol = Biorieco < Emulgal). Of the two new oil dispersants used here, one (Slickgone) emerged as the least toxic to corals, whereas the second (Dispolen) was found to be the most toxic to corals.

In conclusion, corals are particularly susceptible to oil detergents and dispersed oil. Consequently, decision-making authorities should carefully consider these results when evaluating possible use of oil dispersants as a mitigation tool against oil pollution near coral reef areas. The results of the present and earlier studies (*10*, *11*) imply that the use of any oil dispersant in coral reefs and its vicinity should be avoided. Chemical dispersants should be considered only in emergencies, when oil slicks are shore-bound and threatens to smother the reef flats.

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