Effects of oil contaminants on survivorship of larvae of the scleractinian reef corals *Acropora tenuis*, *Goniastrea aspera* and *Platygyra sinensis* from the Great Barrier Reef

A. Lane¹,² and P.L. Harrison²

Abstract

Previous studies have shown that oil contaminants may seriously harm scleractinian corals, with effects ranging from sub-lethal responses including reduced reproductive success, to mortality of corals. However, information on the specific toxicity of oil and dispersed oil to coral larvae is very limited. This study examined the effects of the water accommodated fraction (WAF) of fuel oil, dispersed fuel oil, and Ardrox 6120 dispersant on the survivorship of *Acropora tenuis*, *Goniastrea aspera*, and *Platygyra sinensis* scleractinian coral larvae. The results showed that dispersed oil and dispersant are far more toxic than undispersed oil WAF to the survivorship of these larvae. The concentrations of dispersed oil and dispersant causing high levels of mortality were well within the range that may be expected in the vicinity of an oil slick that has been chemically dispersed. These results clearly demonstrate that coral reproductive success will be significantly impaired if coral larvae encounter chemically dispersed oil or dispersant. Therefore, dispersants should not be used in the vicinity of coral reefs during coral spawning episodes or the subsequent planktonic larval development phase.

Keywords Coral larvae, Toxicology, Oil, Dispersant

Introduction

Anthropogenic oil contamination on, or in the vicinity of, coral reefs may occur from a variety of sources, including exploration and mining, transfer and transportation of bulk oil, shore based refinery operations, and minor spills from smaller vessels. Even small volumes of oil spilled in the marine environment have the capacity to affect a large area, with one litre of oil capable of spreading to cover an area of up to 4 000 m² (MSPE 1988). Spilled oil may not only have acute impacts, but trapping of oil in sediments, or ongoing operational or repetitive accidental discharges, may result in chronic exposure of marine habitats to oil hydrocarbon contaminants over a prolonged period (Burns et al. 1994, Dodge et al. 1995).

Exposure of scleractinian corals to oil contamination may occur through direct contact with the oil where slicks strand on reefs, and through the oil water accommodated fraction (WAF) resulting from physical or chemical dispersion of oil. The WAF of oil contaminants will generally extend over a larger area and to greater depths than whole oil, increasing the number and type of habitats and organisms that will be exposed to the contamination (Trudel and Ross 1987, Harrison et al. 1990, Peters et al. 1997).

Previous studies have demonstrated adverse effects on scleractinian corals resulting from exposure to oil and petroleum products. Reported effects include complete or partial mortality of colonies (Johannes et al. 1975, Harrison et al. 1990), impaired growth (Johannes et al. 1975, Knap et al. 1983, Guzmán et al. 1994), behavioural effects (Lewis 1971, Knap et al. 1983, Wyers et al. 1986), damage to photosynthetic capability and mutualistic zooxanthellae (Cook and Knap 1983), and impairment of reproductive functions (Rinkevich and Loya 1977, Loya and Rinkevich 1979, Harrison 1999). Toxicity of oil to scleractinian coral larvae has also been demonstrated (Rinkevich and Loya 1977, Te 1991).

The use of chemical dispersants in oil spill management may also be harmful to scleractinian corals. Dispersed oil has greater potential to come into contact with pelagic and benthic marine organisms due to its greatly increased surface and depth distribution compared to undispersed oil (Trudel and Ross 1987). Hydrocarbon concentrations up to 50 ppm have been measured under chemically dispersed oil slicks, with elevated hydrocarbon concentrations likely up to 10 metres depth (McAuliffe et al. 1980). This is considerably higher than expected hydrocarbon concentrations under undispersed slicks, which are unlikely to exceed 10 ppm to depths of one metre, with rapid dilution below this depth (Trudel and Ross 1987). Additionally, where oil dispersants are applied to slicks that are thinly spread, the dispersant may cause extremely rapid aggregation of the oil, resulting in the application of much of the dispersant onto clean water surfaces. It is also possible that much of the applied dispersant may be washed from the surface on an oil slick by wave action (Koops 1988). Toxic effects resulting from exposure of adult corals to chemical dispersants and dispersed oil have been reported by a number of authors (reviewed in Loya and Rinkevich 1980, Grigg and Dollar 1990, Harrison et al. 1990, Peters et al. 1997). It is generally concluded that dispersant-oil combinations are
more toxic than the dispersant alone, although whether this is a consequence of the increased concentration of hydrocarbons from the dispersed oil, or from the inherent toxicity of the dispersant has not been determined.

While all of the reported effects on corals resulting from exposure to oil contaminants are important, and warrant further investigation, effects on reproductive success may potentially have a very substantial impact at a population level. This is especially true given the highly seasonal nature of sexual reproduction in the majority of reef corals, which means that an entire year’s reproductive effort by a number of species may be lost in response to a single pollution incident (Harrison and Wallace 1990, Harrison 1999). Additionally, the planktonic nature of the majority of coral larvae (Harrison and Wallace 1990, Willis and Oliver 1990), means that larval stages may be exposed to oil contaminants at considerable distances from the parent reef.

The aim of this study was to quantify the effects of exposure to the water accommodated fraction of a bunker fuel oil, Ardrox 6120™ dispersant, and dispersed fuel oil on the survival of larvae of three species of scleractinian corals from the Great Barrier Reef, Australia.

Methods

Larval Culture

Gravid colonies of the reef corals Acropora tenuis, Platygyra sinensis and Goniastrea aspera were collected from reef flat and upper reef slope habitats at Magnetic Island (Central Great Barrier Reef region), on 28 and 29 October 1999. Just prior to dusk on the evenings of 29 and 30 October 1999, individual colonies were isolated in bins of clean seawater on the beach until they spawned. Within one hour of spawning, egg-sperm bundles were collected from the surface of the water and combined in 4 L plastic pots filled with clean, unfiltered seawater. Pots were covered with plankton mesh and attached to a floating buoy on the reef flat, where they were exposed to continuous multi-directional agitation from wave action and ambient water conditions (after Babcock and Heyward 1986).

Preparation of Treatments

A bunker oil, Fuel Oil 467™ was used in the preparation of the oil water accommodated fraction (Oil WAF) and the dispersed oil treatments. This oil is characteristic of bunker fuel oils used throughout the world. Large volumes of these oils are transported throughout the world’s oceans in tankers, and up to 10,000 tonnes are carried as fuel in larger ships. The chemical dispersant used in the experiments was Ardrox 6120™ (Surechem Industries), one of the 8 chemical dispersants presently approved for use in Australian waters.

The Oil WAF treatment was prepared following the methods of Harrison et al. (1990). Fuel oil was added to unfiltered seawater at the rate of 1:10 and was stirred at high speed for 3 hours on a magnetic stirrer. The mixture was then allowed to settle for at least 2.5 hours, and the seawater-Oil WAF mixture below the remaining floating oil was siphoned off for use in the experiments.

For the Dispersed Oil treatments, 20 mLs of fuel oil were dispersed by the addition of Ardrox 6120 at the rate of 7.5% of the oil volume (in accordance with the manufacturer’s recommendations for dispersion of a fresh spill of light crude oil) prior to addition to seawater. Due to the anticipated higher concentration of hydrocarbons in the water following the chemical dispersion, the ratio of oil to seawater was diluted to 1:100. The mixture was stirred and allowed to settle before use, as for the Oil WAF treatment.

The Dispersant treatment was prepared by adding dispersant to seawater to give the same concentration of dispersant as in the Dispersed Oil treatment, giving a nominal dispersant concentration of 750 ppm. All oil and dispersant treatments were used within 24 hours of preparation to ensure that degradation of the oil, and possible increases in biodegradation by-products, were minimised.

A sample of the stock solution used in each experiment was analysed for Total Recoverable Hydrocarbons (C6 – C36 Fraction) by Gas Chromatography Mass Spectrometry. The concentrations used in the results are nominal values based on the appropriate dilution factor of the stock solution for that experiment.

Exposure of Larvae to Contaminants

In separate experiments, 3 day-old (d.o.) planula larvae of Acropora tenuis, Platygyra sinensis, and Goniastrea aspera, and 11 d.o. planula larvae of A. tenuis were exposed to a range of seven dilutions of each of the three treatments. For each concentration, ten larvae were placed in each of 5 replicate 21 mL sealed glass vials. Vials were placed in mesh bags on the reef-flat in about 2 m water depth, to provide the larvae with ambient temperatures (26.0 – 27.5°C) and multi-directional agitation throughout the exposure period.

At 12-hour intervals, the vials were retrieved from the reef flat and the number of living larvae in each vial was counted. Using methods similar to those of Reichelt-Brushett (1998) in metal toxicity studies, dead larvae were distinguished by their lack of movement and an irregular ‘fragmented’ appearance of their epidermis, or by having disintegrated completely, as occurred in some treatments. The initial 12-hour count was not undertaken for Goniastrea aspera larvae, and an additional count was done after the first 6 hours of exposure for the 11 d.o. Acropora tenuis larvae.

One-way ANOVA was used to test for significant differences in mortality rates among pollutant treatment concentrations compared to controls. Where possible, LC50 values for each contaminant were calculated using the Trimmed Spearman-Karber method (Hamilton et al. 1977, 1978), with transformation of treatment results relative to controls calculated using Abbott’s formula (Finney 1971).
Results

Fuel Oil WAF

Increased mortality was evident for both 3 d.o. and 11 d.o. Acropora tenuis larvae in the Oil WAF treatment compared to seawater controls, although this result was not statistically significant due to high variability in both treatment and control results. After 96 hours, mean mortality for A. tenuis 3 d.o. larvae was 80% at 6.8 ppm compared to 36% in seawater controls, and for A. tenuis 11 d.o. larvae was 76% at 3.7 ppm compared to 50% in seawater controls. The toxicity of the Oil WAF increased slightly with time of exposure. Hydrocarbon LC50 values for 3 d.o. A. tenuis larvae ranged from 6.1 ppm at 48 hours down to 0.7 ppm at 96 hours (Fig. 1). The LC50 for 11 d.o. A. tenuis larvae was 3.8 ppm after 96 hours, with mortality rates prior to this time being too low for LC50 values to be calculated.

Goniastrea aspera 3 d.o. larvae had significantly higher mean mortality (42%) in the highest Oil WAF concentration of 6.8 ppm, compared to 22% in controls after 96 hours exposure (F=2.45, P=<0.05). G. aspera mortality rates after 96 hours increased with increasing hydrocarbon concentration, and at all concentrations increased with time of exposure, but were too low for LC50 values to be calculated.

Platygyra sinensis 3 d.o. larvae were the least sensitive to the Oil WAF treatment, and did not show any evidence of significant toxic effects in response to any of the concentrations tested. Mean larval mortality of P. sinensis after 96 hours in this treatment varied from 28% in both the control and 6.8 ppm Oil WAF, up to 50% at 3.4 ppm Oil WAF.

Dispersed Oil

All species tested had significantly increased larval mortality rates in the Dispersed Oil treatments compared to seawater controls, and showed increased toxic effects with increased exposure time. In order to assess the relationship between the toxicity of dispersed oil and the dispersant itself, LC50 values were calculated for both the hydrocarbon concentration and the dispersant concentration in the dispersed oil treatments.

Acropora tenuis 3 d.o. larvae had significantly increased mortality at hydrocarbon concentrations \( \geq 4.9 \) ppm compared with controls (F=13.75, P=<0.001), with 96% mortality within 24 hours, and 100% mortality within 48 hours at this concentration and at all higher concentrations. At only 0.5 ppm, mean larval mortality was 66% after 96 hours, compared to 36% in the seawater controls. LC50 values ranged from 5.0 ppm at 12 hours down to 0.6 ppm at 96 hours for hydrocarbons, and 32.8 ppm at 12 hours down to 25.4 ppm at 96 hours for dispersant (Fig. 1).

Mortality of Acropora tenuis 11 d.o. larvae was significantly increased at hydrocarbon concentrations \( \geq 1.7 \) ppm compared to seawater controls after 96 hours (F=22.35, P=<0.001). Hydrocarbon LC50 values for 11 d.o. A. tenuis larvae ranged from 6.7 ppm at 6 hours down to 0.7 ppm at 96 hours. Dispersant LC50 values were 35.0 ppm at 6 hours decreasing to 8.3 ppm at 96 hours (Fig. 1).

Goniastrea aspera larvae had significantly increased mortality at hydrocarbon concentrations \( \geq 4.9 \) ppm compared to seawater controls (F=40.12, P=<0.001), with 76% mortality by 24 hours, and 100% mortality by 96 hours. LC50 values for G. aspera larvae ranged from 3.5 ppm at 24 hours to 0.8 ppm at 96 hours for hydrocarbons, and 49.2 ppm at 12 hours and 12.5 ppm at 96 hours for dispersants (Fig. 1).

Mortality of Platygyra sinensis larvae was also significantly increased at hydrocarbon concentrations \( \geq 4.9 \) ppm compared to seawater controls (F=19.95, P=<0.001), with 82% mortality within 24 hours, and 100% mortality by 96 hours. Hydrocarbon LC50 values for P. sinensis larvae were higher than for the other species tested, and ranged from 8.2 ppm at 12 hours down to 1.5 ppm at 96 hours, with dispersant LC50 values of 35.0 ppm at 12 hours decreasing to 23.7 ppm at 96 hours.

Dispersant

Acropora tenuis 3 d.o. larvae showed no apparent mortality effects from dispersant concentrations up to 15 ppm, but at \( \geq 75 \) ppm there was 100% mortality of these larvae after 12 hours of exposure. A. tenuis 11 d.o. larvae also suffered 100% mortality at dispersant concentrations \( \geq 75 \) ppm after 12 hours. Dispersant LC50 values ranged from 32.8 ppm at 12 hours down to 25.4 ppm after 96 hours for A. tenuis 3 d.o. larvae. A. tenuis 11 d.o. had a similar LC50 value to 3 d.o. larvae, of 35.0 ppm after 12 hours, but a much lower 96-hour LC50 of 8.3 ppm (Fig. 1).

Platygyra sinensis larvae showed a similar response to the 3 d.o. Acropora tenuis larvae, with 96% mean mortality by 12 hours at a dispersant concentration of 75 ppm, with 100% mortality at this concentration by 24 hours, compared to 32% mortality in the seawater controls after 96 hours. Dispersant LC50 values for P. sinensis larvae ranged from 35.0 ppm at 12 hours, down to 23.7 ppm at 96 hours (Fig. 1).

Mean mortality of Goniastrea aspera larvae was 68% at a dispersant concentration of 75 ppm after 24 hours, compared to only 8% in the control at this time, with 100% mortality at dispersant concentrations \( \geq 75 \) ppm by 48 hours. Dispersant LC50 values for G. aspera ranged from 49.2 ppm at 24 hours, down to 12.5 ppm at 96 hours (Fig. 1).

Discussion

The least toxic contaminant tested in this study was the water-accommodated-fraction of fuel oil, which caused increased mortality of Acropora tenuis and Goniastrea aspera larvae only at the higher concentrations tested. The concentrations of fuel oil WAF causing toxicity in these experiments are in the upper range of concentrations that might be expected in the vicinity of an undispersed
slick (Trudel and Ross 1987). Additionally, no toxic effects in Oil WAF treatments were observed until at least 24 hours of exposure. However, it must be considered that the buoyant nature of larvae of most broadcast spawning coral species (reviewed in Harrison and Wallace 1990) is likely to bring them into direct contact with floating oil, which may result in extremely high mortality rates (Lane and Harrison in prep).

Dispersed oil and dispersant both proved to be highly toxic to planula larvae of the scleractinian corals Acropora tenuis, Platygyra sinensis and Goniastrea asperea. Dispersed oil was the most toxic of the contaminants tested, with hydrocarbon concentrations of ≥ 4.9 ppm (dispersant concentrations of ≥ 75 ppm) resulting in 100% mortality of exposed larvae of all species within 96 hours, and with the majority of larvae dying at this concentration within 24 hours.

**Fig. 1** LC_{50} values of hydrocarbons and dispersant in different treatments for scleractinian coral larvae
The hydrocarbon concentrations causing high levels of larval mortality in this study are an order of magnitude lower than the 50 ppm hydrocarbon concentration that can occur where a slick has been dispersed in shallow water (McAuliffe et al. 1980). The response of the larvae to the dispersed oil was generally very rapid, with high levels of mortality at hydrocarbon concentrations of less than 5 ppm generally occurring within 6 to 12 hours of exposure.

The toxicity of the dispersed oil continued to increase with exposure period up to 96 hours. Additionally, the larvae in the higher concentrations of dispersed oil completely dissolved in the treatment solution. The toxicity of dispersed oil to coral larvae is consistent with the results of experiments on other aspects of sexual reproduction in reef corals. Fertilization in Acropora tenuis was completely blocked following exposure of gametes to dispersed oil at hydrocarbon concentrations of only 0.15 ppm (Harrison 1999), and EC50 values for fertilization success for A. valida, A. millepora and Platygryra sinensis ranged from 0.1 – 1.8 ppm (Harrison and Lane unpub. data).

As with the dispersed oil treatment, the dispersant treatment had rapid toxic effects, with very high rates of larval mortality at dispersant concentrations of ≥ 75 ppm within 12 to 24 hours. The dispersant concentrations that caused significant mortality of larvae in this study are well within those that may occur in the field where dispersant has been applied to an oil slick. Where dispersant is applied at the rate of 15% of slick volume (as recommended for many oil types), dispersal of a one cm thick slick could result in short-term dispersant concentrations up to 150 ppm to depths of 10 metres.

The high toxicity of oil dispersant observed in the present study is consistent with findings from other studies, which have reported substantial inhibition of fertilization success in Acropora tenuis (EC50 1.7 ppm) (Harrison 1999), and toxicity to adult corals (Lewis 1971, Elgershuizen and De Kruijf 1976, Harrison et al. 1990) and other marine invertebrates (e.g. Burridge and Shir 1995, Singer et al. 1995).

Dispersant as the Toxic Agent in Dispersed Oil

An important finding from this study is the similarity in the concentration of dispersant in both the Dispersed Oil and the Dispersant treatments that resulted in elevated mortality of coral larvae in all four experiments. Although, based on the dispersant concentration, the dispersed oil appeared to be less toxic in the first 12 hours of the experiment than the dispersant alone for Acropora tenuis 3 d.o. and Platygryra sinensis 3 d.o. larvae, from 24 hours onwards, the dispersant LC50 values were closely aligned in the two treatments (Fig. 1).

The consistency in toxicity values for dispersant in the Dispersed Oil and the Dispersant treatments, combined with the far lower LC50 values for hydrocarbons in the Dispersed Oil than in the Oil WAF treatments, strongly suggests that the dispersant itself may be responsible for the high toxicity of dispersed oil. This finding contrasts with previous suggestions that the toxicity of dispersed oil is due to elevated levels of oil hydrocarbons in the water, or to some change in the structure or bioavailability of the oil hydrocarbons resulting from chemical dispersion (Harris 1992).

Conclusions

While oil WAF can result in some mortality of coral larvae, the concentrations at which this may occur are in the upper range that could occur in the vicinity of an undispersed oil slick. However, the likelihood of contamination and coral larval mortality increases considerably where the use of chemical dispersion raises both the concentration and spatial extent of hydrocarbons in the marine environment. Additionally, the chemical dispersants themselves will enter the water column, either in conjunction with oil or where the dispersant contacts a clean water surface. This study clearly shows that substantial impacts on coral reproductive success through high levels of larval mortality may result from contact with dispersed oil and dispersant. Additionally, this larval mortality will occur within a short time period, and at concentrations of dispersed oil and dispersant far lower than may reasonably be expected to occur in a reef environment where an oil slick has been chemically dispersed. Therefore, dispersants should not be used in the vicinity of coral reefs during coral spawning periods, or subsequent planktonic larval development periods.

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