



Dispersant Use and a Bioremediation Strategy as Alternate Means of Reducing Impacts of Large Oil Spills on Mangroves: The Gladstone Field Trials

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Over a three-year period (1995–1998), we studied short-term effects of dispersant use and a bioremediation strategy in two consecutive field trials in sub-tropical Australian mangroves. In each case, weathered oil was applied, and a large spill simulated, in mature *Rhizophora stylosa* trees around 4–9 m tall. In the first trial, we used Gippsland light crude oil with or without dispersant, Corexit 9527. In the second, a bioremediation strategy followed application of Gippsland oil or Bunker C fuel oil. Bioremediation involved forced aeration with supplemental application of nutrients. Dispersant use had an overall positive benefit shown as reduced tree mortality. By contrast, there was no apparent reduction in mortality of trees with bioremediation. However, one year after oiling, leaf densities of surviving trees were greater in bioremediation plots than in controls, and less in oil-only plots. These and other results have been incorporated into spill response management strategies in Australia. © 2000 Elsevier Science Ltd. All rights reserved.

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Introduction

Mangrove forests are well known for their high vulnerability to oil spills since floating oil settles with the tide and smothers both breathing and feeder roots plus a myriad of associated resident fauna (Jackson *et al.*, 1989; Volkman *et al.*, 1994). Oil deposited on tree roots

often results in the death of some trees, but it also results in depressed growth of survivors across the wider oiled area (Duke *et al.*, 1997). As such, the dramatic impact of deforestation of mangroves (when it occurs) is indicative of a much larger impacted area. The more subtle effect of sublethal damage, manifest as loss in canopy density, also weakens the forest habitat, putting remaining trees at greater risk of damage from further disturbance. The longer term effect can persist for several decades (e.g., Wardrop, 1987), and result in partial ecosystem collapse in some cases (Duke *et al.*, 1997).

Since we wish to protect and preserve tidal wetland habitat for a variety of reasons, there is an urgent need to establish techniques by which we might reduce the impact of oil spills. In this article, we briefly summarise the results of two series of field trials set up to assess the benefits of two remediation strategies. In the first, funded by the Australian Petroleum Production and Exploration Association, we investigated the benefits of dispersant use (Duke and Burns, 1999; Duke *et al.*, 1998a,b,c). In the second, funded by the Australian Maritime Safety Authority and the Great Barrier Reef Marine Park Authority, we tested a bioremediation strategy (Duke *et al.*, 1999). Field trials were designed to fill a gap between surveys of real spill incidents (e.g., Duke *et al.*, 1997; 1998c; Volkman *et al.*, 1994) and studies of seedlings in nursery conditions (Duke *et al.*, 1998a; Lai and Lim, 1984; Wardrop, 1987). This gap was considered quite wide since:

1. the great number of variables associated with an actual spill incident and the usual lateness in commencement of research activities had seriously reduced the benefits of such work;
2. by contrast, there was a lack of natural variables in potted and field seedling experiments.

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Therefore, an important consideration in these field trials was to apply oil treatments in a controlled experiment, to simulate large oil spill conditions, and to assess the impact of these treatments on mature mangroves in a natural setting. The primary goal in making this assessment was to provide practical advice and guidelines to spill responders.

Methods

Study area and sites used

Field sites were established within an area approved for reclamation by relevant Local and State authorities. The location used was close to Fishermans Landing bordering Port Curtis, just north of Gladstone, Central Queensland, Australia (Fig. 1). Gladstone has average mean daily temperatures ranging from 18.4°C minimum

to 27.5°C maximum, and an average annual rainfall of around 896 mm. During the study period, temperatures were normal but rainfall declined from 1079 mm in 1996, to 784 mm in 1997. Falls were low also in 1998 with only 573 mm having fallen by September when the field study was completed. Temperature and rainfall varied seasonally with maximal values usually reported in the summer period.

For each of the two trials, three study sites for oil treatments were chosen in mature mangrove stands of 4–9 m tall trees of *Rhizophora stylosa*; the common mangrove species of northern tropical and subtropical Australia. This species is well known for its above-ground root structure of thick and arching prop roots. The experimental design and distribution of plots and sites are shown in Table 1. In the first trial, we used 9 plots from 3 sites for enclosure treatments and their

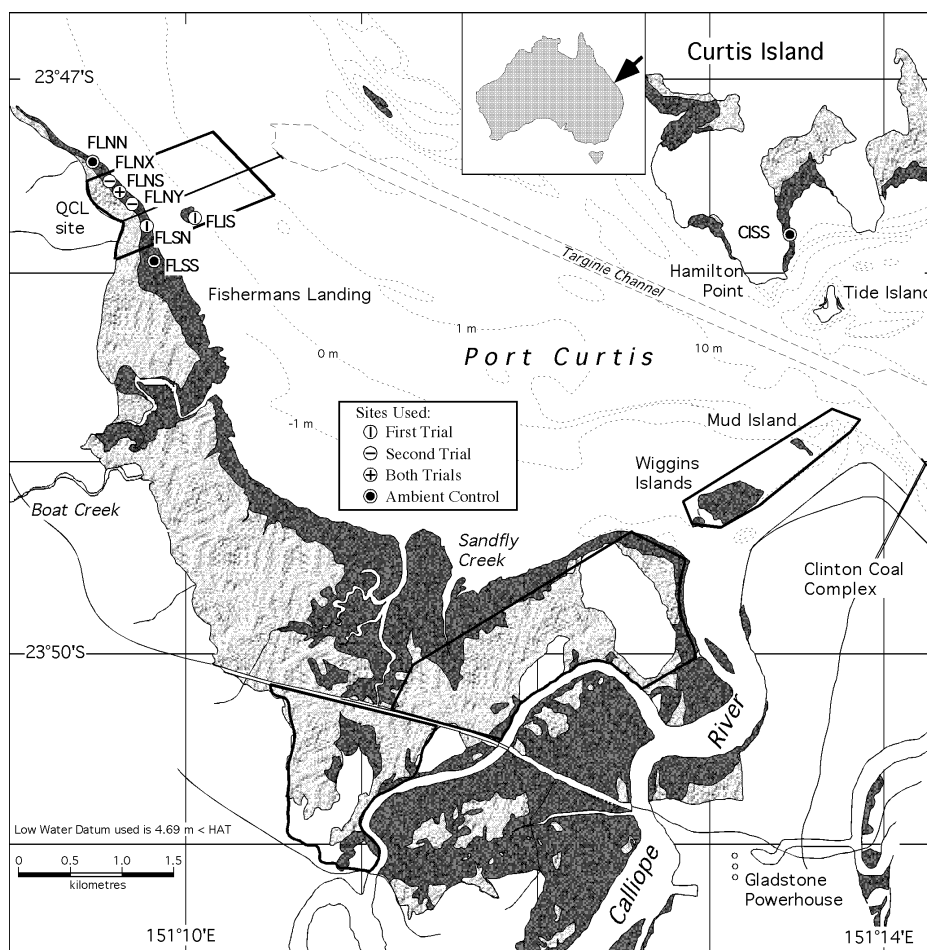


Fig. 1 Study area for the Gladstone field trials. Five oil treated sites were located within the reclamation area at Fishermans Landing (note heavily outlined area, top left). All sites had four letter code names (e.g., FLIS). Also note site code symbols used to show which sites were used in each of the two trials (see Table 1). Three ambient control sites used in both trials were located outside the reclamation area. Tidal areas above mean sea level are shaded, and include: mangroves in darker shading; and salt pan with sporadic salt marsh, in lighter shading. Depth contours in Port Curtis are shown as dashed lines. The northern edge of the city of Gladstone is shown at lower right.

TABLE 1

Experimental design for mangrove studies in two trials, showing the three replicate plots (= sites) for each of the seven treatment combinations and controls.^a

Treatments and controls	Replicate	Trial 1	Trial 2
Gippsland light crude oil	1	FLNS-D	FLNS-B
	2	FLSN-A	FLNX-E
	3	FLIS-A	FLNY-D
Gippsland plus dispersant	1	FLNS-A	
	2	FLSN-C	
	3	FLIS-B	
Gippsland with bioremediation	1		FLNS-G
	2		FLNX-B
	3		FLNY-C
Bunker C fuel oil	1		FLNS-F
	2		FLNX-A
	3		FLNY-A
Bunker with bioremediation	1		FLNS-E
	2		FLNX-D
	3		FLNY-B
Enclosure control	1	FLNS-C	FLNS-C
	2	FLSN-D	FLNX-C
	3	FLIS-C	FLNY-E
Ambient control	1	CISS-A	CISS-A
	2	FLSS-A	FLSS-A
	3	FLNN-A	FLNN-A

^a Site locations are shown in Fig. 1, using four letter site codes (e.g. FLIS), and a plot code (e.g., -E) which indicates particular plots within the respective site location. One enclosure control plot (FLNS-C) and the three ambient control plots were used in both trials (plots shown in bold text). Total numbers of enclosure plots in the first trial were nine, and there were 15 in the second trial, see text for details. The trials continued from 1996 to 1998 and 1997 to 1998, respectively.

controls, plus three additional sites with one plot each for ambient controls in locations outside the reclamation area. In this first trial, there were two oil treatments. In the second trial, we used 15 plots from three sites for enclosure treatments and controls, plus three additional sites with one plot each for ambient controls in locations outside the reclamation area, being the same as the first trial. In this second trial, there were four oil treatments. There was some overlap in sites used between the two trials (see Table 1 and Fig. 1), and one oil treatment site, FLNS, and all three ambient control plots were used in both trials. In all, eight sites were used, in which 23 enclosure plots were prepared and 18 were treated with oil.

Experimental treatments

The total amount of oil released into all treatment plots during the two trials was approximately 3500 l. The amount of oil applied to each plot depended on plot size, but the dosage rate was standardised at 5 l m⁻² for all plots treated.

The oils used in the two trials were Gippsland light crude oil and a Bunker C fuel oil; each oil is fully described by Burns *et al.* (2000). All oil treatments were pre-weathered in a pond of seawater, approximately 0.1 m deep, for 24 h prior to application. This was done to prepare oil to more closely replicate that arriving from an actual incident where oil was spilled in waters

fronting mangroves. During preparation time, the contents of the pond was stirred regularly and only allowed to settle 1 h before being pumped into drums for field deployment. Oil treatments were added to each plot as the tide rose, to simulate its arrival from a large offshore spill where oil had been floating on open water for approximately 1 day.

Treatments types were chosen at random from the three plots for each site (see Table 1), and included: Gippsland oil-only, dispersed Gippsland oil, Bunker oil-only, Gippsland oil and bioremediation, Bunker oil and bioremediation, and enclosure controls. The schedule for the trials was divided into two chief parts: the first trial was for dispersant use, and the second trial was for the bioremediation strategy. In both cases, we used specially designed experimental enclosures to contain oil treatments during the critical settling phase only.

Trial 1: the dispersant use trial

In the first trial, the dispersant Corexit 9527 was used in combination with Gippsland oil on the advice of the APPEA Research Working Group. Dispersant use with Bunker oil was not tested in these trials.

Dispersant was added to oil during weathering preparation with the intention of simulating its' application by spill responders to oil floating at sea and at some distance away from mangroves. The reasoning here was based on prior evidence of harmful effects of dispersants when in direct contact with foliage of mangrove plants (e.g., Wardrop *et al.*, 1987).

Oil treatments were applied in October 1996 for the first trial, and monitoring continued from June 1996 until August 1998.

Trial 2: the bioremediation strategy trial

In the second trial, a bioremediation strategy was evaluated. This strategy was derived after detailed consideration of a range of matters (Duke *et al.*, 1999), ranging from environmental characteristics of mangroves, presence of oil-degrading bacteria in mangrove sediments (also see Ramsay *et al.*, 2000), degradability of oils (also see Burns *et al.*, 1999), and forced aeration in mangrove sediments.

Since oil was expected to penetrate deeply into sediments via the normal network of burrows and dead root castes, the preferred bioremediation strategy for oiled mangroves involved pumping air beneath sediments and amongst below-ground tree roots. The process was referred to as 'forced aeration' in this study. Nutrients were added to supplement the strategy. This dual approach was considered best to help trees survive oiling, and to promote microbial degradation of oil in contaminated sediments. It was also considered likely that oil might be flushed out of the sediment by the upward flow of air from buried air stones. Aeration commenced at the same time as nutrients were added, around 40 h after oiling, and continued for four months.

Nutrient application involved sprinkling granules of Osmocote Tropical fertilizer over the oiled surface within 40 h of oiling. Approximately, 5.4 kg of fertilizer per plot were added; an application rate $\sim 0.15 \text{ kg m}^{-2}$. Osmocote Tropical, has a composition of: 19.0% nitrogen (9% nitrate; 10% ammonia), 2.5% phosphorus (1.9% water soluble), 10.0% potassium (water soluble, chloride free), 4.8% sulphur (sulphates) and 0.8% calcium. Additional fertilizer was applied at the same dose to plots in February 1998.

Oil treatments were applied in August 1997 for the second trial, and monitoring continued from May 1997 until August 1998.

Experimental enclosures

The aim was to simulate conditions observed during a large oil spill without losing oil to the surrounding environment. Many precautions were taken to prevent loss of oil and contamination of surrounding habitat. Experimental enclosures were constructed to contain oil treatments within the field plots (Fig. 2). To install enclosure panels, prop roots were cut along the boundary, approximately 0.5 m wide, for the four sides of each plot, approximately 6 m \times 6 m each.

Enclosures consisted of two chief parts:

1. panels of vinyl fabric, 1.2 m in height, buried 0.2 m into the sediment, and placed around most of the perimeter of approximately 30 m;
2. an oil-trapping gate of vinyl fabric, 1.2 m wide, also rising 1 m above the sediment with 0.2 m below.

Gates were designed to allow free movement of tidal waters while keeping oil inside enclosures. This was



Fig. 2 One of 18 experimental enclosures where oil treatments were applied to plots of mature *Rhizophora* forest. Plots were located within the forested area, approximately 50 m from the sea edge (in the background of the photo). The image was taken immediately after oiling so oil can be seen on the inner sides of enclosure panels. Enclosures were open to tidal flows through a 'tide gate' (the darker panel on the far side) which allowed relatively normal tidal flushing. Enclosures were removed after 2–4 weeks without loss of oil and without effecting mangroves surrounding each plot. Enclosure control plots situated within a few metres of oil treatment plots showed no indications of increased hydrocarbons during the trials, and surface activities of crabs remained normal in areas surrounding each enclosure.

considered an effective strategy since oil concentrations measured in sediments after oiling closely matched levels measured during actual large oil spill incidents (see Burns *et al.*, 2000). This experimental method supplied a consistent and known dosage of oil to each plot in the replicated sampling design (also see Duke *et al.*, 1998b).

Enclosure panels were buried 0.2 m into the sediment to prevent loss of oil as tide levels dropped. The 1 m high panels were sufficient to prevent escape of oil floating on tidal waters in plots during the critical phase for settling of oil. Oil settled and adhered to exposed roots and sediment surface mostly with the first low tide following the introduction of oil treatments to plot enclosures. With subsequent tidal flushing, very little of the oil added to enclosures was observed to float again.

Further safeguards were undertaken to ensure no oil escaped from enclosures including: installation of floating sorbant booms closely surrounding each enclosure (see Fig. 2); deployment of large bags of absorbant material within 5 m of each plot, and sufficient to soak up all oil in each plot should it escape; placement of offshore floating oil spill booms within 200 m of the site and sufficient to contain escaping oil; and, an advisory alert to the local Harbour Master to have an oil spill skimmer vessel and other equipment on standby. These measures were further backed-up with frequent and regular monitoring of plots after applying oil treatments.

These precautionary measures were successful and no oil escaped during either trial, and enclosures were removed 2–4 weeks after application of oil treatments.

Enclosure and ambient control plots

Enclosure control plots, where no oil was applied, were established at the three oil treatment sites used in each of the two trials. These plots were prepared in the same way as oil treatment plots, including cutting prop roots along the boundary. The choice of plot treatment, including enclosure control, was made by random selection from the full set of plots prepared for installation of enclosures. Only the enclosure control plot at one site (FLNS-C) was used in both trials (see Table 1).

Ambient control plots were set up outside of the designated reclamation area where oil treatments were applied and permitted (Fig. 1). These plots were subject to relatively minimal disturbance, such as observer access and non-destructive sampling. These control plots were used in both trials.

Comparison of enclosure and ambient control plots were used to assess effects of enclosure preparation and use, including root cutting and sediment disturbance. No significant differences were found between enclosure and ambient control plots for all parameters measured in each of the two trials. Furthermore, for those plots having partial tree mortality after oiling, tree death was not disproportionately greater for perimeter trees, including those with cut roots. Tree death in treated plots occurred throughout effected plots. Therefore, for this

assessment, both enclosure and ambient control plots were pooled as an overall control for comparison with oil treatments in the two trials.

Tree condition

The condition of mangrove trees was monitored before and after treatments and three principal methods are used in this assessment:

1. scores of percent tree mortality;
2. monthly collections of litter fall, used in the assessment of herbivory;
3. monthly observations of leafy shoots growing in the upper canopy of trees.

The litter collection and shoot observation methods followed in this report are described in detail by Duke *et al.* (1993). Assessment of plant condition in the first trial commenced in June 1996 and were completed in August 1998, while the second trial commenced in May 1997 ending in August 1998.

Fauna collections and activity

Dominant fauna in the mangroves of the study area were monitored during the two trials. The reason for choosing particular fauna was based chiefly on their relative importance as determined by their density and biomass in the study area. These dominant fauna were from the major structural components of the forest system, namely the foliage (herbivores), the above ground roots and sediment surface (the mangrove crabs), and the sediment (burrowing crustaceans and worms). It was reasoned that the presence and activity of these animals might reflect other impacts of oil treatments on mangrove habitat. Data reported here specifically include:

1. feeding by caterpillars on green leaves in the canopy;
2. crustaceans collected dead from the forest floor immediately after oiling;
3. leaf consumption and burial by crabs;
4. the presence and biomass of Sipunculan worms living below ground.

The study area was affected by above normal levels of herbivory (e.g., compare with Robertson and Duke, 1987). This was due almost totally to grazing by one species of leaf eating caterpillar, *Doratifera stenosa*. The role and relevance of this herbivore was determined by the conspicuous feeding scars on leaves and the number and dominance of individuals observed regularly in the canopy and moving from tree to tree. Caterpillars were bright green, 1–2 cm long, with short stinging spines over their bodies. Herbivory of leaves in the forest canopy of treatment and control plots was reliably assessed from litter fall collections during the study. This method was considered reliable since remnants of individual leaves were readily recognised in litter trap samples. Leaf area missing from fallen leaves represented the total accumulative loss to herbivory during the life of each leaf (see Robertson and Duke, 1987). Fallen leaves were sorted into four categories depending on gross amounts of leaf loss due to herbivore eating, including: 100% (full, intact) leaves; >75% leaf remaining; >50% leaf remaining; and leaves <50% complete. In each case, the number of full and remnant leaves (numbers of petioles or leaf stems) in each sample and category were counted, and total dry weights recorded. In the more extreme cases of leaf grazing, petiole stumps were recorded as leaves in the <50% complete category.

Crustaceans were monitored using two methods:

1. collection of dead animals, chiefly including Alpheids, Grapsids and Thalassinids (see Table 2), from the sediment surface of plots within 40 h of treatment application;
2. scores of percent leaf-removal by Grapsid crabs in plots before and after treatments.

Post-treatment collections of dead animals were taken to the laboratory where they were washed and sorted according to taxa. Allometric relationships of body dimensions and biomass for all dominant crustaceans collected were computed from these samples and used to calculate total dry weight biomass. Collections from plots are considered a conservative estimate of diversity

TABLE 2

Crustaceans collected dead within 40 h of oil treatment applications from two trials conducted at the Fishermans Landing site, north of Gladstone.^a

Arthropoda: Crustacea: Decapoda:	Crustacean taxa	Carapace size (mm)
Natantia Alpheidae	<i>Alpheus</i> nr. <i>pacificus</i> , Dana (1852)	8–35 <i>L</i>
	<i>Athanas japonicus</i> , Kubo (1936)	13–28 <i>L</i>
Reptantia Thalassinidae:	<i>Thalassina squamifera</i> , de Man (1915)	15–58 <i>L</i>
Grapsidae:	<i>Australoplax tridentata</i> , A. Milne Edwards (1873)	5–13 <i>W</i>
	<i>Clistocoeloma merguense</i> , de Man (1888)	5–19 <i>W</i>
	<i>Metopograpsus frontalis</i> , Meirs (1880)	9–33 <i>W</i>
	<i>Neosarmartium trispinosum</i> , Davie (1994)	10–36 <i>W</i>
	<i>Parasesarma leptosoma</i> , Hilgendorf (1869)	7–18 <i>W</i>
	<i>Perisesarma messa</i> , Campbell (1967)	7–29 <i>W</i>
	<i>Perisesarma semperi longicristatum</i> , Campbell (1967)	5–21 <i>W</i>
	<i>Sarmartium crassum</i> , Dana (1851)	8–23 <i>W</i>
	<i>Sarmartium germaini</i> , A. Milne Edwards (1869)	8–24 <i>W</i>
Ocypodidae:	<i>Uca perplexa</i> , H. Milne Edwards (1852)	–
Xanthidae:	<i>Heteropanope</i> , Stimpson (1858)	–

^a Carapace size ranges, as length (*L*) or width (*W*) depending on morphology and classification, for crustacean taxa.

and biomass for these particular crustaceans. Leaf removal assessment was made following the methods described by Robertson (1986) where the proportion of leaf material either consumed or removed down burrows was measured during a fixed low tide period of 2–3 h. In brief, this study used leaf-removal as a non-destructive measure of crab abundance and activity in plots. Leaf removal studies were conducted only during the first series of trials evaluating dispersant use.

Density and biomass of burrowing Sipunculans, the peanut worm *Phascolosoma arcuatum*, in sediment cores from treatment and control plots were measured in January 1997 and August 1998. The sampling method changed slightly from the first to the second trial. In the first, a small hand held sampler was used to take a box core, 0.024 m² area × 0.23 m depth of sediment from each plot. In the second survey, five 7 cm diameter cores were pooled, totaling 0.019 m² area × 0.22 m depth of sediment from each plot. The second method better suited sampling amongst the dense prop roots of *R. stylosa* trees while sampling approximately the same volume of sediment. For both surveys, cores of sediment were sieved and worms were scored and collected. Observations were made from the 1998 cores describing the position of Sipunculans, their burrows, holes, tree roots and other features. Sipunculans collected during the surveys were used to determine average dimensions and average dry weight biomass from which estimates of total biomass were calculated.

Results

Tree mortality

Mean estimates of tree mortality in plots after oiling are presented in Fig. 3. Plots show two-oil types, two treatments, two times after oiling, plus the mean control values. Note that control estimates were pooled from both enclosure and ambient control plots after these were shown to have nonsignificant differences. Standard

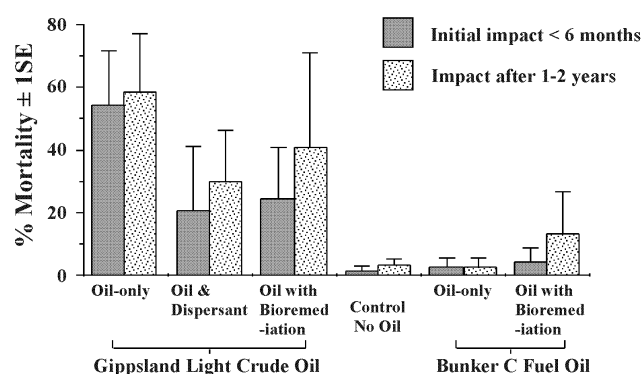


Fig. 3 Tree mortality was determined for all plots in: the two oil treatments, Gippsland crude oil and Bunker C fuel oil; the two remediation treatments, dispersed oil and the bioremediation strategy; and, pooled control plots. Error bars show standard errors for mean values of three replicate sites. Data are for two time periods, showing the initial impact within six months of oiling, and the impact or short term recovery after 1–2 years.

error bars describe the variance in data. There are several notable results. First, the no oil, control levels of tree mortality remained low over the two years of monitoring. Second, the mortality of plots treated with Bunker oil-only was no different from control levels while plots treated with Gippsland oil-only showed a significantly higher level of mortality. Third, the addition of dispersant with Gippsland oil resulted in significantly less mortality of trees than oil-only, but still much greater than control levels. Fourth, tree mortality occurred mostly in the first six months after oiling, with few additional trees dying in the 18 months afterwards. Fifth, the bioremediation strategy appeared to have had a similar effect to that shown by dispersant and Gippsland oil in the first six months. However, mortality overall increased substantially (but this was extremely variable) up to 2 years afterwards. In this context, it seems relevant that the aeration system was shut down after 4 months and no further nutrients were added in the last 18 months of study. One oddity was the slight increase in mortality noted with the Bunker bioremediation plot in the 1–2 year period after oiling. There is insufficient evidence to determine whether this was due to variability in data, or indicative of a negative effect of the bioremediation strategy.

Loss of canopy foliage

Estimates of percent leaf loss for trees in plots which survived oiling are shown in Fig. 4. Control values showed that interpretation of leaf loss with treatments must be taken in the context of a natural decline (5–10%) in foliage density during the study; probably

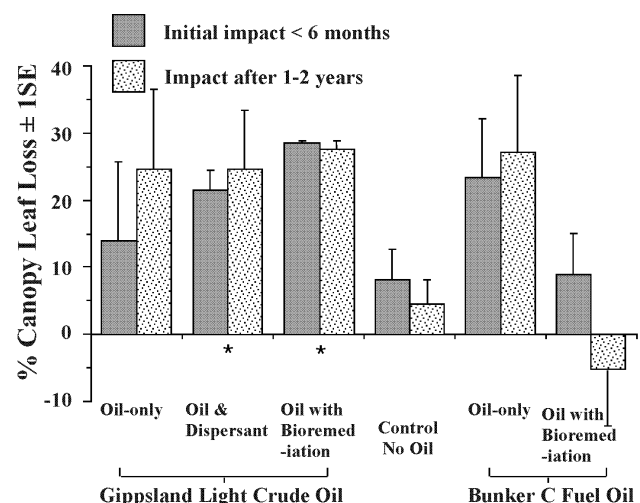


Fig. 4 Loss of canopy leaves (proportional loss of standing leaf density) in trees which survived oil treatments was determined for: the two oil treatments, Gippsland crude oil and Bunker C fuel oil; the two remediation treatments, dispersed oil and the bioremediation strategy; and, pooled control plots. Error bars show standard error for mean estimates for treatments calculated for three or more site replicates except for those marked with asterisks, which had only two replicates each. Data are for two time periods, showing the initial impact within six months of oiling, and the impact or short-term recovery after 1–2 years.

the result of lower levels of annual rainfall during the study. For Gippsland oil treatments, there were higher proportions of leaf loss, particularly in the 1–2 year period after oiling, which were unaltered by dispersant use or the bioremediation strategy. There also appeared to be an equivalent loss in leaf density in plots treated with Bunker oil-only. However, in plots treated with Bunker oil and bioremediation, there was a response, which was not appreciably different from control levels, notably in the first six months. After this, there was a tendency toward a gain in leaf density in the second year.

Leaf consumption by caterpillars

Estimates of percent leaf herbivory by caterpillars, as percent leaf loss, are presented in Fig. 5. Note that high levels in control plots were indicative of the natural prevalence of these unusually voracious eaters in the study area. Leaf loss was around 30–40% throughout the area. There are several observations to be made from these results with respect to oil treatments. First, the oil-only treatments for either oil showed no significant difference in herbivory over the two years of study. Second, the Gippsland oil and dispersant plots tended to have lower levels which were significant in the first six months but not so in the following 18 months. Third, the plots treated with the bioremediation strategy tended to have increased levels, but this was not statistically significant.

Crustacean biomass

Estimates of total biomass of benthic crustaceans, including predominantly Grapsids and Alpheids and some Thalassinids, are presented in Fig. 6. In this assessment, monitoring was based on collections made within two days of oiling, in oil-only and dispersed oil plots, and not in bioremediation plots since this strategy

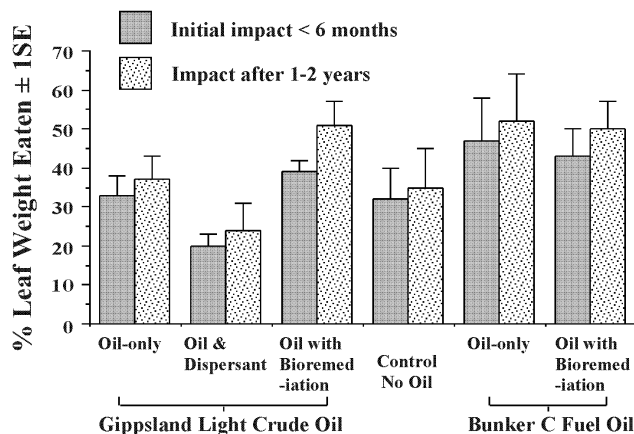


Fig. 5 Canopy herbivory levels (proportion of leaf material lost from fallen leaves) were estimated in all plots for: the two oil treatments, Gippsland crude oil and Bunker C fuel oil; the two remediation treatments, dispersed oil and the bioremediation strategy; and, the control plots. Error bars show standard errors for mean values of three replicate sites. Data are for two time periods, showing the initial impact within six months of oiling, and the impact or short-term recovery after 1–2 years.

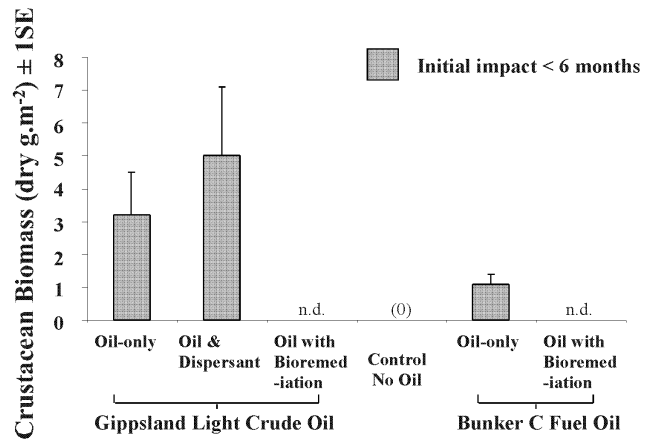


Fig. 6 Biomass and mortality of crustacean fauna, including Alpheids, Grapsids and Thalassinids, was derived from animals collected dead within two days of oil treatments being added to plots for the two oil treatments, Gippsland crude oil and Bunker C fuel oil, and the dispersed oil treatment. No dead animals were observed in control plots. Error bars show standard errors for mean values of three replicate sites. Zero value is indicated by '(0)', and treatments with no data are indicated by 'n.d.'

was not applied until after animals had died from oil treatments. No dead crustaceans were observed in control plots. The most notable point to be made from these data is that there was no significant difference between oil-only and dispersed oil plots for Gippsland oil treatments. However, there was a significant difference between oil types where mortality from Gippsland oil was significantly greater than that in Bunker oil plots. All plots had the same dosage of oil but differences in the physical and chemical characteristics of these oils may explain the differences in biotic effects. For instance, it was apparent during field trials that viscosity was notably greater in Bunker oil plots, and this oil did not spread evenly throughout the enclosures, as observed for Gippsland oil treatments. In enclosures treated with Bunker oil, crustaceans behaved relatively normal in the patches of sediment where no oil was deposited within enclosures. Only fauna in direct contact with oil were killed immediately after oiling. Estimates of biomass, as they might approximate total biomass, were therefore seen as being directly proportional to the physical coverage by oil over the sediment surface and roots, as well as being conservative because of possible animals missed during collections.

Leaf removal by crabs

The percentages of leaves removed or consumed per hour by Grapsid crabs during low tide periods after oiling are presented in Fig. 7. All treatments in this instance were with Gippsland oil-only and Gippsland oil mixed with dispersant Corexit 9527. There are several observations to be made. First, note the significantly greater levels of removal in control plots through out the study. Second, the levels were no different in oil-only and dispersed oil plots in the 1–2 year period after oil-

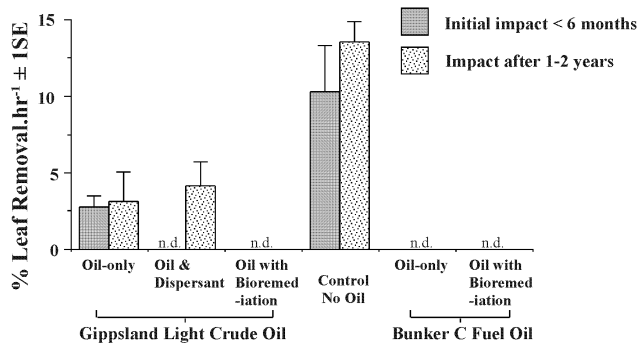


Fig. 7 Presence and relative activity of Grapsid crabs was determined by measurements of leaf removal (percentage of leaf weight taken below ground compared with the amount offered, see Methods) in plots oiled with Gippsland crude oil and the dispersed oil treatment, and enclosure control plots. Error bars show standard errors for mean values of three replicate sites. Data are for two time periods, showing the initial impact within six months of oiling, and the impact or short-term recovery after 1–2 years. Treatments with no data are indicated by 'n.d.'

ing. Third, there was no improvement or increased recovery of crab activity during the 1–2 years of monitoring post-oiling. This was curious since close scrutiny of crab activity showed there was none in the month immediately after oiling, while in the 2–6 months following, there was a steady increase which leveled off during the next 18 months. This pattern was not shown in oil dissipation which followed an exponential loss of oil over the same period (Burns *et al.*, 2000).

Biomass of sipunculan worms

Presence and total biomass of burrowing Sipunculan worms are shown in Fig. 8. There are a number of observations to be made from these results. First, note the high mean estimates for control plots overall. The slightly lower estimate for the first six months may be due to the change in sampling methodology. Note that

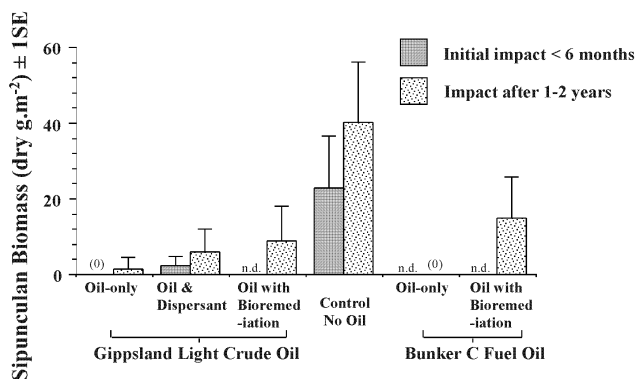


Fig. 8 Biomass of Sipunculan worms was measured in all plots for: the two oil treatments, Gippsland crude oil and Bunker C fuel oil; the two remediation treatments, dispersed oil and the bioremediation strategy; and, pooled control plots. Error bars show standard errors for mean values of three replicate sites. Data are for two time periods, showing the initial impact within six months of oiling, and the impact or short term recovery after 1–2 years. Zero values are indicated by '(0)', and treatments with no data are indicated by 'n.d.'

the latter method was considered more reliable than the earlier method (see the Methods) since the smaller core tube (7 cm diameter) more easily and efficiently sampled amongst the dense prop roots. The second point is that worms were absent in Gippsland oil-only plots during the first six months after oiling and occasionally present in dispersed oil plots. Third, there was a slight but not significant increase in Gippsland oil bioremediation plots in the 1–2 year period. This was in marked contrast with the comparative biomass samples from the Bunker oil bioremediation plot and the Bunker oil-only plots. Sipunculan worms were absent from the latter oil-only plots during the same period.

Discussion

To address the need for more relevant information on the effects of oil spills on mangroves, field experiments have been attempted in various locations and circumstances (e.g., Birkeland *et al.*, 1976; Lai and Lee, 1984; Lai and Lim, 1984; Lai *et al.*, 1984; Getter and Ballou, 1985; Getter *et al.*, 1985; Wardrop *et al.*, 1987). In only one case were the effects monitored over many years (Ballou *et al.*, 1987, 1989; Dodge *et al.*, 1995). The size and scope of these experiments varied considerably from oiling a few leaves and small saplings, to dumping oil in an open mature forest. A common problem with the larger experiments, however, was a lack of site replication. The studies reported here were planned to address uncertainties posed by unreplicated trials, and to briefly evaluate two quite different strategies for their potential benefit in reducing the impacts of large oil spills on mangrove environments. The results achieved have been informative and valuable but it is clear that greater site replication is required to gain an even better understanding of the responses of plants and animals to oil spills in natural mangrove habitat.

We studied short-term effects of dispersant use and a bioremediation strategy on sub-tropical Australian mangrove habitat over a three-year period (1995–1998). In this article, we briefly described the results of two series of field trials (Duke and Burns, 1999; Duke *et al.*, 1999). The responses of key biotic components of the particular mangrove forests studied were assessed for each of the treatments; namely, two oil types, Gippsland crude oil and Bunker C fuel oil, and two remediation treatments, dispersant use and bioremediation.

In the first trial, we used dispersant, Corexit 9527, which was pre-mixed and weathered with the oil mixture before application. There were no differences between oil-only and dispersed oil treatments on fauna. Most resident ground-dwelling macrofauna (Grapsids, Alpheids and Thalassinids) were killed by oil, particularly in Gippsland oil plots. By contrast, death of mangrove trees was significantly less in plots treated with dispersed oil. This observation was supported in concurrent trials on planthouse seedlings (Duke *et al.*, 1998a), and in field surveys of old spill sites (Duke *et al.*, 1998c; also see

Duke and Burns, 1999). Overall, dispersant use reduced tree mortality, and it possibly also enhanced foliage recovery of surviving trees and allowed for quicker recovery of Sipunculan worm numbers. Partial recovery of fauna in oiled experimental plots occurred after approximately 2 years, but there was little sign of recovery of trees and damaged forest canopy. For this reason, dispersion of spilled oil before it reaches mangroves is considered an important strategy in reducing the long-term impact of oil on mangrove habitat.

In the second series of trials, we applied a bioremediation strategy immediately after application of oil to the plots. We expanded this trial to include a second oil type, Bunker C fuel oil, for comparison. We obtained mixed results for the effectiveness of bioremediation. There was no apparent reduction in mortality of trees where bioremediation was applied. However, one year after oiling, canopy leaf densities were greater than controls in bioremediation plots, and less than controls in oil-only plots. Concentrations of oil and prior condition of leafy canopies, along with levels of insect herbivory and densities of Grapsid crabs killed by oil, all appeared to influence mortality of mangrove trees. Densities of Sipunculan worms in sediments one year after oiling appeared also to have recovered in oiled plots treated with bioremediation. These results provide an important baseline for future on-going assessment of oil-damaged sites with respect to both natural recovery and the application and testing of remedial strategies and techniques; particularly for more sensitive and badly affected areas.

The vulnerability of mangrove habitat is primarily based on the vulnerability of trees. When mangrove trees die, the habitat lacks structure and protection from erosion. Therefore, the greatest benefit in a remediation technique must be to preserve trees and ecosystem structure. Our studies have demonstrated that dispersant use, in the circumstances described in this article, had a beneficial effect in reducing mortality of mature mangrove trees in a natural setting. This finding has important implications for spill responders since it offers convenient justification for a strategy that has wider benefits. However, this does not account for potentially damaging impacts on adjacent habitats such as sub-tidal corals and seagrasses. With full consideration of such limitations, these results have been incorporated into spill response management strategies in Australia.

We greatly appreciate the generous assistance provided by individuals, businesses, and government officials, during all aspects of this challenging field experiment; from initial field site surveys, permission to use the site, establishment of enclosures in the mangroves, donations of oil, equipment and facilities, monthly collections of scientific data and samples, preparing and applying oil treatments, cleaning up the site, to the laboratory evaluation of hydrocarbons, and more.

These findings represent a brief summary of results gathered from two independent projects with the Australian Institute of Marine Science (AIMS), assessing the effects of large oil spills on mangroves and an evaluation of two selected mitigation strategies. Other aspects of these projects are reported elsewhere, including this issue. The Australian Petroleum Production and Exploration Association

(APPEA) provided chief funding for the first trial investigating oil impacts and dispersant use. The second trial, assessed bioremediation strategies as part of work tendered by the Australian Maritime Safety Authority (AMSA) and the Great Barrier Reef Marine Park Authority (GBRMPA). Both trials were fully supported by AIMS as part of the Mangrove Oil Spill Project (1995–1999). Identifications of key biota were made by: CSIRO Entomology, Canberra for the caterpillar herbivore; and Peter Davies, Queensland Museum, Brisbane for Crustaceans, notably the Grapsids. Field work would not have been possible without the generous help and support of Cindy Black, Noel Bowley, Paul Daniels, Mikel Duke, Joanna Ellison, Trevor Gilbert, Michael Klemm, Joanna Knight, Deb Lamb, Guy Lane, Bill Laver, Judy Logan, Darren Marshall, Steve McKillup, Graham McKim-Hill, Kirsty McNamara, Rean Monfils, Robert Prior, Jane Rogers, Michael Small, Jamie Storrer, Mike Walker, Andrew White and Emma Yates.

The Gladstone Port Authority made available the field site within their reclamation area at Fishermans Landing. Queensland Cement Limited, the local tenant bordering the area provided permission allowing free access to the field site. The Ampol Refinery at Kurnell in Botany Bay donated the Gippsland Light crude oil. Esso Australia donated the Corexit-9527 dispersant. BHP Transport, Gladstone, donated the Bunker C fuel oil. BP Australia, Gladstone Terminal, provided free use of the industrial site used for preparation and weathering of oil treatments. Enretech supplied the sorbant booms and bags of oil absorbant. Data on rainfall and temperature for Gladstone were supplied by the Australian Bureau of Meteorology. This research project was undertaken under Fisheries Resources Permit Nos. 96SODB0979 and 97SODB2545 granted by the Queensland Department of Primary Industries, Southern Fisheries Centre. Field studies were monitored and assisted by officers of the Queensland Department of Environment Regional Office, Queensland Department of Transport, the Great Barrier Reef Marine Park Authority and the Australian Maritime Safety Authority. We also thank Gordon Lethbridge, Shell Research Ltd., UK, for providing supplemental funding for the second trial.

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