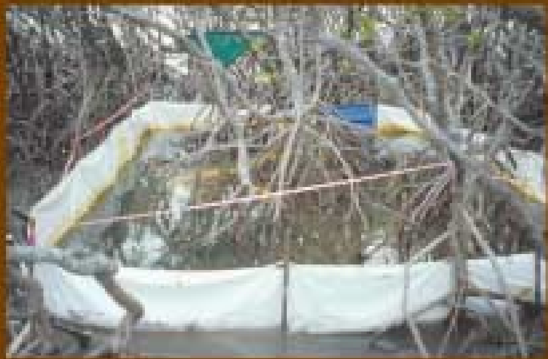
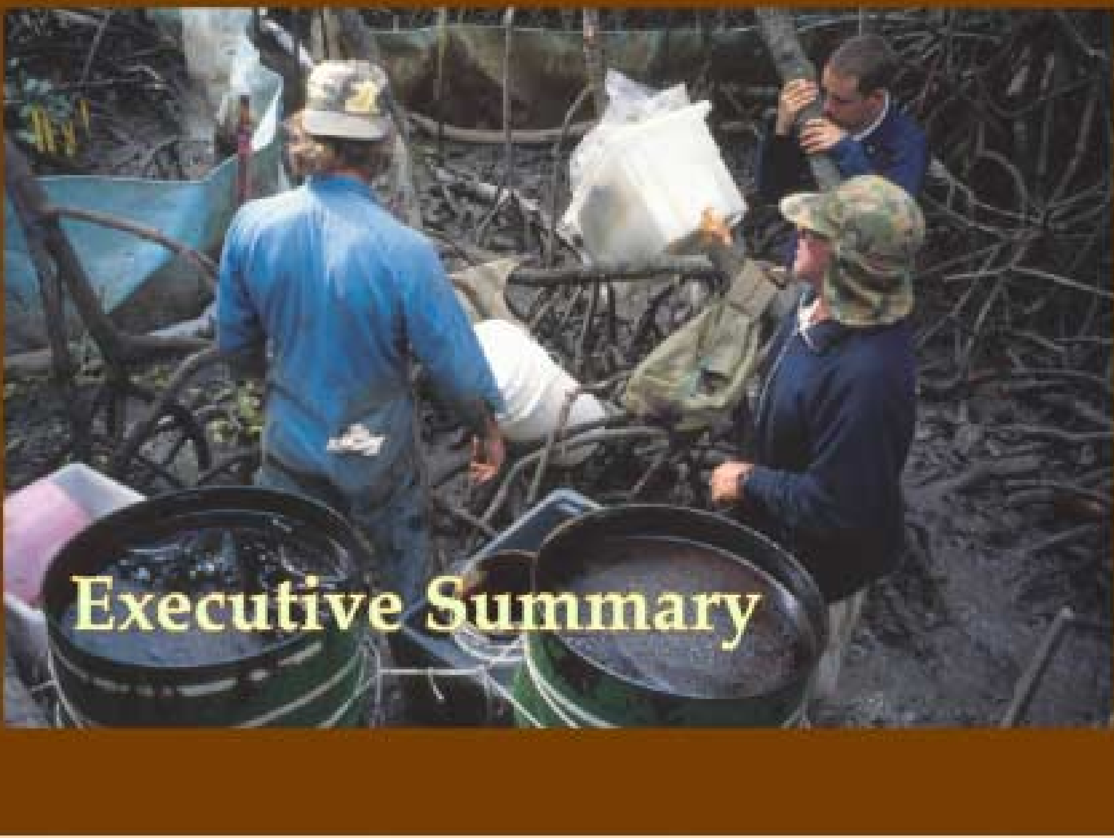


Research into the Bioremediation of Oil Spills in Tropical Australia: with particular emphasis on oiled mangrove and salt marsh habitat



Executive Summary

Research into the Bioremediation of Oil Spills in Tropical Australia: with particular emphasis on oiled mangrove and salt marsh habitat

EXECUTIVE SUMMARY

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Final Report
to the
Australian Maritime Safety Authority

21 July 1999

An environmental research project chiefly with the AUSTRALIAN MARITIME SAFETY AUTHORITY (AMSA), the GREAT BARRIER REEF MARINE PARK AUTHORITY (GBRMPA), and the ENERGY RESEARCH AND DEVELOPMENT CORPORATION (ERDC). Additional funding was received from SHELL RESEARCH LTD., UNITED KINGDOM.

Scientific research conducted under the direction of the AUSTRALIAN INSTITUTE OF MARINE SCIENCE (AIMS) and AEA TECHNOLOGY, UNITED KINGDOM (AEAT).

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Project Summary

This three-year project (1995-1998) assessed short term effects of commonly transported oils and *in situ* bioremediation on tropical Australian mangrove and salt marsh habitats. Bioremediation strategies were developed from laboratory, mesocosm and preliminary field studies. Flask experiments demonstrated the presence of hydrocarbon degrading micro-organisms in local tidal wetland habitats, the importance of oxygen in their activity, and a lack of appreciable inhibition from mangrove pore waters. Aeration trials showed forced aeration could appreciably increase the depth of the aerobic surface layer of sediments. Field trials in mangroves and salt marsh habitats gave mixed results for the effectiveness of bioremediation. There was no apparent reduction in impact on mortality of vegetation in either habitat where bioremediation strategies were applied to oiled plots. However, in mangrove plots one year after oiling, canopies had greater densities of leaves in bioremediation plots, sometimes greater than control levels. Concentrations of oil and prior condition of leafy canopies, along with levels of insect folivory and densities of grapsid crabs all influenced mortality of mangrove trees. Numbers of sipunculid worms in sediments one year after oiling appeared to have largely recovered in oiled plots treated with the bioremediation strategy. Findings from these studies form the basis for improved guidelines and recommendations for the cleanup and restoration of oiled mangrove habitat around Australia, and elsewhere.

Plate 1 (*next page*). Field trials were used to assess bioremediation of oil spills in tropical tidal habitats, specifically mangrove and salt marsh, in the Port Curtis area, near Gladstone, Queensland. Bioremediation strategies were applied immediately after treatment with oils, and monitoring of impacts and recovery continued over the next 9-13 months. **A.** Pre-weathered Bunker C fuel oil and Gippsland Light crude oil were transported to specially prepared field plots in mangrove and salt marsh areas. **B.** Depending on oil consistency, oils were hand-pumped or bucketed into mangrove plots (approximately 36 m^2 each and at three replicate sites) at a dose rate of 5 $L.m^{-2}$. Oils were applied during high water periods in order to simulate oil stranding from a large offshore spill. Mangrove plots consisted of forested stands, around 6-8 m tall, of *Rhizophora stylosa*, the common species of tropical and sub-tropical Australia. **C.** Salt marsh enclosures (approximately 1.5 m^2 each and at four replicate sites) were established in areas nearby of low-lying *Halosarcia* and associated salt marsh plants. **D.** As with mangrove plots, oils were applied during high water to simulate an offshore oil spill. The dose of oil applied to salt marsh plots was 2 $L.m^{-2}$.

Research into the Bioremediation of Oil Spills in Tropical Australia: with particular emphasis on oiled mangrove and salt marsh habitat

The occurrence of oil spills at sea, and the subsequent damage to coastlines around the world is a high profile issue that attracts a great deal of public scrutiny. As a consequence, many techniques have been developed for dealing with oil pollution both at sea and along coastlines. One approach currently being developed is bioremediation. This method aims to increase the natural degradation rate of the oil, thereby decreasing its environmental persistence. This is achieved by adding specific materials to stimulate the rate of oil degradation by indigenous micro-organisms in the contaminated environment.

The majority of work in this area has focused on beach ecosystems where the rate of degradation is frequently increased through the addition of nutrients, principally nitrogen and phosphorus-containing compounds. This has been a successful approach in a large number of situations which have included laboratory trials, experiments on artificially oiled beaches and its use after real spill incidents. In the latter case, the best examples have been: its use to treat over 74 miles of oiled shoreline following the *Exxon Valdez* oil spill in March 1989; and the *Sea Empress* incident in February 1996 when bioremediation was used to treat a contaminated beach and a large quantity of oil and oiled debris.

The development of bioremediation has resulted in an increased confidence in its use as a treatment technology for oiled sandy and cobble beaches. For other coastline areas, such as tropical salt marshes and mangroves however, the development of the technique is far less advanced, despite the fact that these shorelines are also affected by numerous oil spill events. Nonetheless, a number of workers have suggested that bioremediation could potentially be a valuable technique for remediating sediments of these intertidal habitats. But, the few reports that exist provide little evidence of the success of the tested bioremediation strategies. A number of reasons for the lack of success have been suggested which include:

- anaerobic conditions in sediment which restricts oil degradation;
- dryness of the soil, which occurs alternately through wet and dry seasons in tropical areas and results in severe limitation of biological activity;
- low pH of some mangrove soils;
- adsorption of oil onto the matrix of the sediment making it unavailable for biodegradation;

- presence of inhibitory materials derived from the mangroves; and
- the presence of other organic material present in the sediment that may be metabolised in preference to the oil.

Because the interplay of these factors is site specific, it was unclear for any particular site which factors were most likely to hamper biological activity. Thus, it was important to establish for tropical Australian salt marsh and mangrove sediments which were the key factors influencing oil biodegradation and to develop possible strategies to enhance this degradation rate.

The high degree of structure of mangrove ecosystems, their deep mud sediments, and their location in the intertidal zone, makes them highly vulnerable to oil spills. Mangrove sediments are often anaerobic, but trees flourish there because of their specialised root systems, and a close relationship with ground dwelling animals, including crabs and worms. These creatures mix and aerate deep sediments as part of normal burrowing activities. When mangroves are oiled, the animals die and their activities cease, leaving the trees which also have their above-ground breathing roots covered in oil, to suffocate.

Our literature review highlighted potential technologies developed for the bioremediation of contaminated land which had potential for application to marine wetlands. But no strategy had been developed for treating oiled tropical mangrove and salt marsh sediments. In these environments, particularly in mangroves, oxygen limitation was likely to be the most important factor. As a result, we proposed that techniques developed for dealing with similar difficulties in contaminated land bioremediation should be tested for use in these wetland sediments. It is these technologies, to a large part, which we developed and evaluated in the laboratory and mesocosm trials before proceeding to a field study with the bioremediation strategy most likely to succeed.

Laboratory Studies

We conducted a series of flask experiments to test for the presence of hydrocarbon degrading micro-organisms in representative wetland habitats. Also tested was the biodegradation of selected oils (Gippsland crude, Arabian Light crude and Bunker C fuel), that are transported along the Australian coast. We also tested for potential inhibition of biodegradation by natural organics in the mangrove pore waters and evaluated the ability of an oxygen release compound (ORC) to stimulate biodegradative processes.

Evaporation was a significant factor in removing the light alkane and aromatic hydrocarbons from air and nitrogen sparged flasks. Evaporation removed ~27 % of the Gippsland, ~37 % of the Arabian, and ~10 % of the Bunker oils. Oxygen was necessary to support biodegradation as expected. The micro-organisms were capable of biodegrading the non-volatile saturate fraction of each oil. Degradation removed another 14 % of the Gippsland, 30 % of the Arabian, and 22 % of the Bunker C oils. Normalisation of the individual aromatic hydrocarbon classes to internal triterpane biomarkers indicated some degradation of aromatics in the Arabian Light and Bunker C oils. Although alkane degradation rates were comparable in the three oils, the Gippsland oil had a higher wax content and after 14 days incubation, still contained as much as 25 % of the alkanes present in the original oil. Thus degradation of its aromatic fraction may have been delayed. Based on these results we estimate that Arabian Light Crude oil would have a shorter residence time than the other oils in mangrove sediment. It has a higher content of light hydrocarbons, which are readily removed by both physical and microbial processes. The Bunker C would be expected to have the longest residence time in mangrove sediment because it contains a larger percentage of higher molecular weight, unresolved components. Comparison of the efficiency of inoculates from three tropical intertidal habitats (*Avicennia* and *Rhizophora* mangroves, plus *Halosarcia* salt marsh sediments) indicated the presence of hydrocarbon degrading micro-organisms in all three habitats. There was no known history of oil contamination in the soil source area. There was no inhibition of degradation due to addition of mangrove pore waters. The ORC did not facilitate degradation in closed laboratory experiments. We therefore needed to investigate other means of delivering oxygen to mangrove sediments.

Aeration Experiments

The aim of the aeration experiments was to determine whether forced aeration and/or magnesium peroxide (an oxygen release compound) might provide significant increases in molecular oxygen saturation in the surface aerobic layer of sediments collected from under the common tropical mangrove trees, *Rhizophora stylosa*. It was thought that this might increase the likelihood of accelerated biodegradation of oil hydrocarbons in anaerobic sediments and help preserve trees during the critical first three to six months after oiling.

Anaerobic sediments collected from a mangrove forest were oxygenated in mesocosms using forced aeration and magnesium peroxide for forty hours. A microelectrode was used to measure oxygen concentrations along depth profiles from the surface toward the sources of oxygen buried 2-4 cm below. Both forced aeration and magnesium peroxide increased the depth of the aerobic layer, but this was considerably greater with forced aeration. The aerobic zone increased over forty hours of treatment from a mean pre-treatment depth of 600 µm to a maximum mean depth of 1930 µm with forced aeration, and 900 µm with magnesium peroxide.

The practical application of both aeration strategies in bioremediation of oiled mangrove sediments was restricted by the localised natures of their effects. Given the better results with forced aeration, and the amount, cost and toxicity of chemical additives, it was considered appropriate to further test forced aeration in mangrove forests. Results from field trials indicated that the same degree of oxygenation of sediments occurred when forced aeration was tested in sediments under living mangrove trees.

The localised influence of forced aeration however, precluded its use for application over wide areas. It was proposed that the strategy might be successful though, if it were targeted on key individuals and stands of oiled mangrove trees. This idea was based on the observation that particular trees were often responsible for maintaining stand integrity from erosion following large oil spill incidents. If such trees were kept alive, this would greatly reduce the severity of impact. The strategy may only need to be applied for 2-3 months since this was the critical period when most trees die. Based on such observations, a bioremediation strategy involving forced aeration and nutrient addition was developed for the field experiments at the Gladstone site.

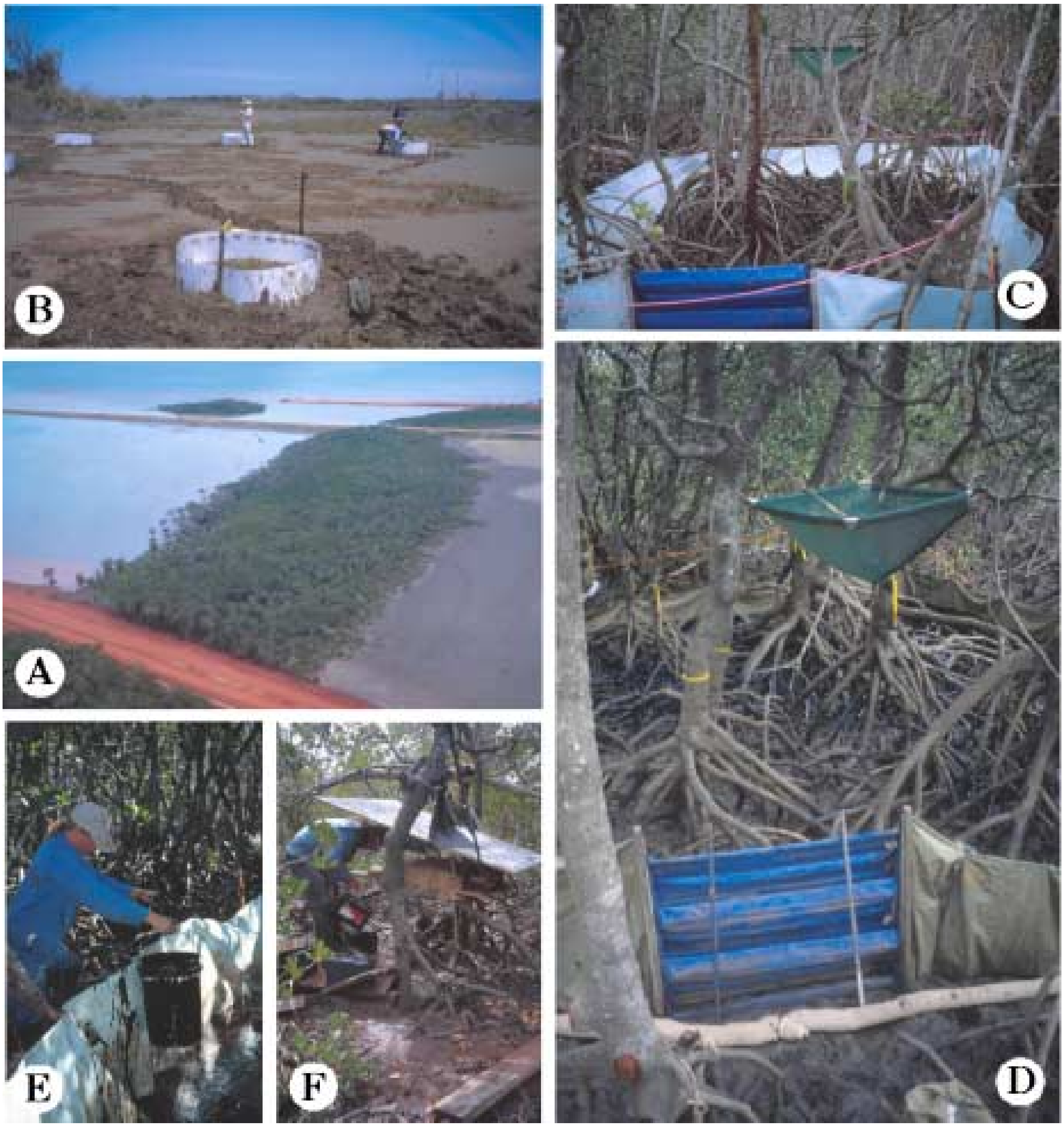
Gladstone Field Studies — Site and Setup

Approval from the Queensland Department of Primary Industries, Fisheries was obtained to conduct field experiments simulating large oil spills in mangrove and salt marsh areas bordering, Port Curtis, near Gladstone in Queensland. The same areas were already subject to reclamation by the local port authority. Salt marsh plots had low-lying vegetation, 5-20 cm high, situated on and within otherwise bare salt pans. By contrast, mangrove plots contained trees, *Rhizophora stylosa*, which were 4-9 m tall with a dense closed canopy.

Oil treatments in these field experiments included two oil types, Gippsland Light crude oil and Bunker C fuel oil. Both oils were pre-weathered in a shallow pond of seawater for 24 hours prior to application in field plots. The experimental treatments included: oil only; oil and bioremediation; and control plots with no oil. The bioremediation strategy in salt marsh involved the addition of nutrients only, while the strategy in mangroves involved forced aeration and addition of nutrients.

Approximately 2,265 L of pre-weathered oil was applied in total to, and successfully contained within, replicated field plots of salt marsh and mangrove. Successful simulation of a large oil spill incident in mangrove experimental plots was determined by: application of oils from falling high water; concentrations of hydrocarbons in sediments exceeded 30,000 $\mu g.g^{-1}$; and, notable mortality of trees 2-7 months after oiling. Enclosures allowed the free ebb and flow of tidal waters and the transference of normal wave action through plots. Enclosures were removed after two weeks without loss of oil from plots to surrounding areas.

Plate 2 (*next page*). Field trials were used to assess impacts and recovery of common tropical tidal habitats, specifically mangrove and salt marsh, in response to selected strategies applied to enhance *insitu* bioremediation of oil-contaminated sediments. **A.** Field plots were established in sea-fringing, mangrove stands of Port Curtis, as well as in higher tidal salt marsh and salt pan areas behind. The sites were each subject to future reclamation. **B.** Salt marsh enclosures consisted of thin plastic walls, around 0.5m above the sediment and 0.1m below, with two 'U-tube' pipes to allow relatively normal tidal ventilation of plots. **C.** Mangrove enclosures consisted of plastic curtains, around 1.0 m above the sediment and 0.2 m below, with a 'tide-gate' for tidal ventilation. **D.** The 'tide-gate' was 1.2 m wide and had three openings to allow relatively normal tidal flow while holding oil inside the enclosure. Litter traps were strung from trees in each plot to measure leaf production and leaf loss rates before and after treatments. **E.** Bunker C fuel oil was extremely thick and it had to be added to the plots using buckets. **F.** In mangrove plots, the bioremediation strategy included aeration of sediments. Air was supplied to treatment plots using battery-powered, large aquarium air pumps in a single accessible field station. Batteries needed to be replaced on a daily basis for approximately 4 months to maintain constant air flow.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Gladstone Field Studies —Assessment of Hydrocarbons

We investigated the relative rates of weathering and biodegradation of oil spilled in sediments and tested the influence of the bioremediation strategies. The aim of the chemistry work was to determine whether bioremediation affected the rate of penetration, dissipation or long term retention of a medium range crude oil (Gippsland) and a Bunker C fuel oil stranded in these tropical mangrove and salt marsh environments.

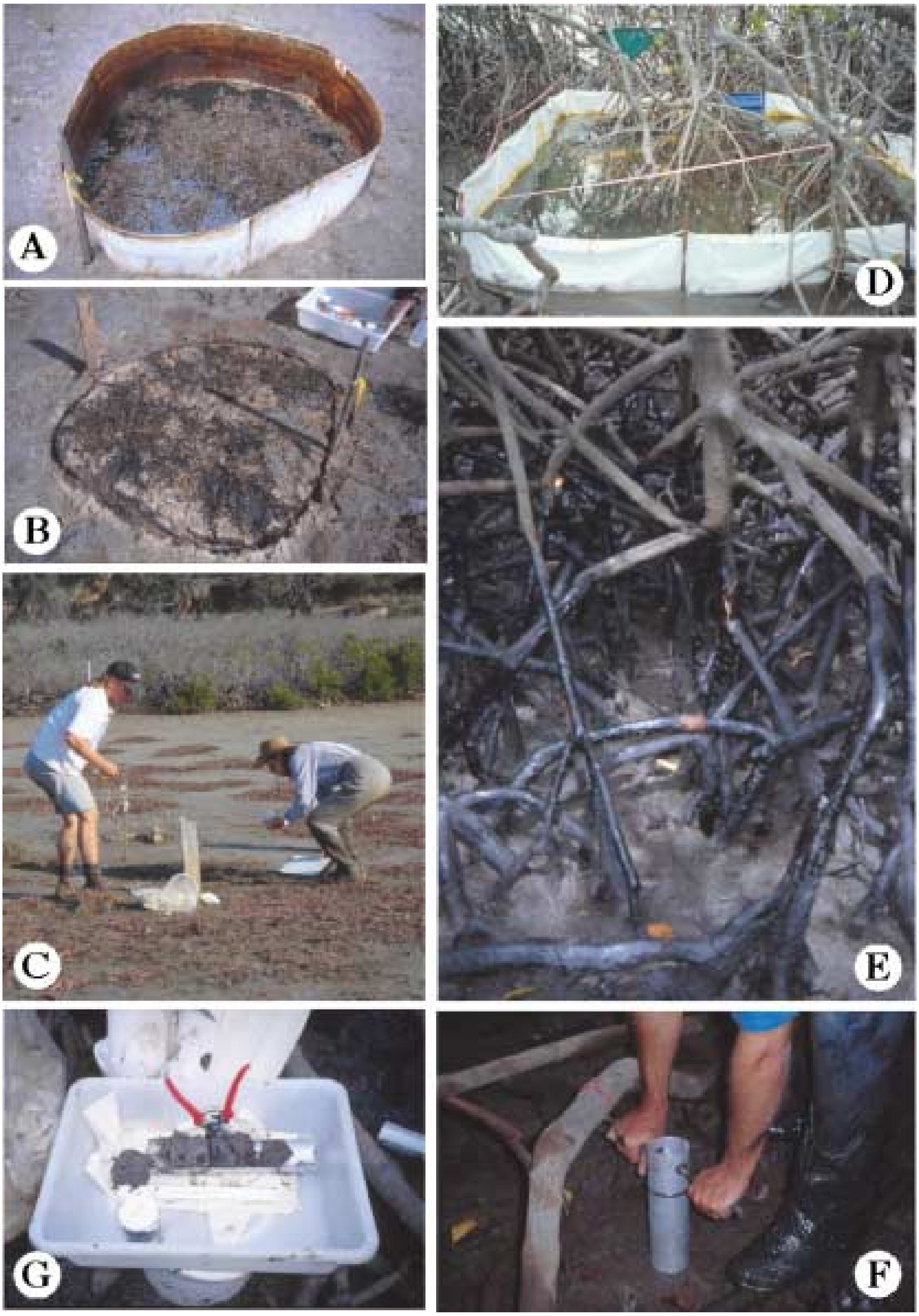
Sediment cores from three replicate plots of each treatment for mangroves and four replicate plots for the salt marsh (oil only and oil plus bioremediation) were analysed for total hydrocarbons and for individual alkane markers using gas chromatography with flame ionization detection (GC-FID). Sediments were collected at day 2 (40 hours), then approximately at 1, 2, 6 and 12 months post spill for mangroves, and day 2, then 1, 3 and 9 months post spill for salt marshes.

Over this time, oil in the oil treated plots decreased exponentially. There was no statistical difference in initial oil concentrations, penetration of oil to depth, or in the rates of oil dissipation between untreated oil and bioremediated oil in the mangrove plots. The salt marsh treated with the waxy Gippsland oil showed a faster rate of biodegradation of the oil in the bioremediated plots. In this case only, degradation rate significantly impacted the mass balance of remaining oil.

The Bunker C oil contained only minor amounts of highly degradable n-alkanes and bioremediation did not significantly impact its rate of degradation in the salt marsh sediments. At the end of each experiment, there were still n-alkanes visible in the gas chromatograms of residual oils. Thus, it was concluded that there was unlikely to be any change in the stable internal biomarkers of the oils over this time period.

The predominant removal processes in both habitats were evaporation and dissolution, with a lag-phase of 1 to 2 months before the start of microbial degradation. The chemistry data provided a context for interpretation of the biological and microbiological observations.

Plate 3 (*next page*). Field trials were used to assess hydrocarbon degradation and loss in mangrove and salt marsh sediments following addition of oils, and in response to bioremediation strategies. **A.** Saltmarsh vegetation and sediments were thickly coated in oil immediately after tidal water levels receded. **B.** After 2-4 weeks, saltmarsh enclosures were removed since oil was no longer mobile. All vegetation was dead. **C.** Sediments from 0-2 cm and 8-10 cm depths were collected regularly after application of oil treatments. **D.** As tide levels dropped after application of oils to mangrove plots, oil coated exposed roots of *Rhizophora* trees. **E.** After waters had receded from mangrove plots, oil had heavily coated both roots and sediments. **F.** Sediments in mangrove plots were sampled from 0-2, 10-12 and 20-22 cm depths. To minimise spatial variation, depth samples were pooled from 4 core samples collected randomly within each plot. **G.** Sediment cores were described and carefully sampled immediately after collection in the field to prevent contamination between samples. Bottled samples were chilled in the field and frozen prior to analysis later in the laboratory.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Gladstone Field Studies —Mangrove and Salt Marsh Vegetation

We studied the effects of the bioremediation strategy on oiled mangrove and salt marsh vegetation.

Oil treatments caused significant mortality of salt marsh vegetation, 5-20 cm tall, within one month of application. Loss of salt marsh vegetation depended on oil type, with 100% from Gippsland crude oil and 90 ± 7 % from Bunker C fuel oil after 9 months. The bioremediation strategy had no apparent effect on the loss of salt marsh vegetation or its recovery after 9 months for either oil type.

Oil treatments caused significant mortality of mature mangrove trees, *R. stylosa*, 4-9 m tall, in several plots within 3-7 months. Mortality of mangrove trees partially depended on oil type, with 0-100% for Gippsland oil, 0-40% for Bunker oil, and 0-7% for un-oiled plots after one year. The wide variation in mortality was largely unexplained by the presence and quantity of oil. The bioremediation strategy had no apparent effect on mangrove tree mortality after one year for either oil type.

Leaf loss from mangrove canopies after one year largely depended on oil type and the bioremediation strategy, with: 12-100% for Gippsland oil; 26-100% for its bioremediation; 5-44% for Bunker oil; minus 22% to plus 7% for its bioremediation; and 1-12% in un-oiled plots. Annual standing leaf biomass (dry weight $g.m^{-2}$) of surviving mangrove canopies largely depended on oil type and the bioremediation strategy, with: 636-643 for Gippsland oil; 852-980 for its bioremediation; 449-737 for Bunker oil; 735-1369 for its bioremediation; and 705-885 in un-oiled plots. Canopies were less dense in oil only plots and more dense in bioremediation plots.

Prior status of mangrove canopies, measured as leaves per shoot at the start of the study, were correlated with oil concentration at the start and leaf loss after one year. Mangrove canopies were much more sensitive to Gippsland oil than Bunker. Bioremediation enhanced leaf production of plots treated with Bunker oil. In general, canopies with less than 7 leaves per leafy shoot were more vulnerable to oiling.

Plate 4 (*next page*). Field trials were used to assess plant condition in mangrove and salt marsh plots following addition of oils, and in response to bioremediation strategies. **A.** Clumps of salt marsh vegetation were enclosed with plastic sheeting in both control and treatment plots. **B.** Oil covered all salt marsh vegetation and sediments after settling. **C.** Most salt marsh vegetation in oiled plots was dead one month after oiling. **D.** In mangrove plots, trees in several plots died 2-3 months after oiling. Tree mortality was related to several factors including leafy canopy density, and presence of fauna. **E.** Forest condition in mangrove plots was monitored using observations of leafy shoots high in the canopy. **F.** Litter fall traps were used in conjunction with shoot observations to derive measures of standing leaf densities in the canopy above each plot.


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Gladstone Field Studies —Mangrove Macro Fauna

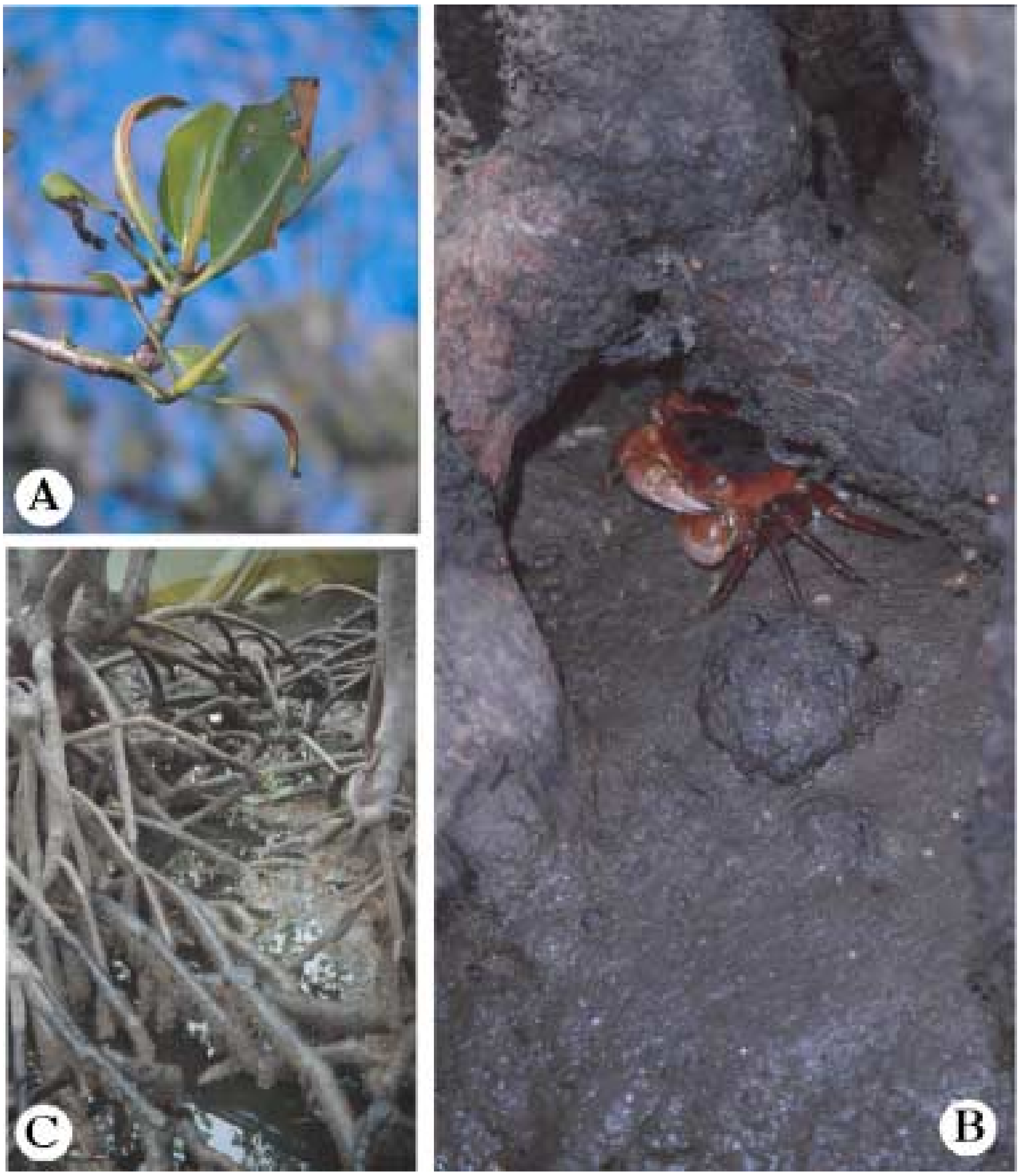
We studied the effects of the bioremediation strategy on oiled mangrove fauna. The dominant fauna included: caterpillars eating leaves in the canopy of trees; crustaceans living on and within sediments; and Sipunculid worms in the sediments.

Levels of herbivory affected the impact of oil on trees. Herbivory by caterpillars in the canopy was 3-4 times higher than normal in the study area, and tree mortality from oiling was correlated with herbivory in plots treated with Gippsland oil. Herbivores were not obviously affected by trees growing in oiled sediments. Herbivory levels after oiling were not correlated with oil concentrations in sediments.

Oil treatments caused mass mortality of both epifauna and infauna within two days of oiling. Dead macro fauna were collected from the sediment surface after oiling, to determine species diversity, density and biomass. Dominant epifauna included Alpheid pistol shrimp and Grapsid crabs. These animals died prior to the application of bioremediation strategies. Gippsland oil had the more severe impact on crabs, compared to the impact of Bunker oil. This was believed to be largely due to the patchiness of Bunker oil when it was deposited on mangrove roots and sediments. Bunker oil generally did not kill all crabs in plots, but when it did kill most crabs, tree mortality was also high.

Infauna were sampled to 22 cm sediment depth from treated and control plots one year after oiling. The dominant infauna were Sipunculid peanut worms. Sipunculids were mostly absent from oil only plots and present in bioremediation plots. The numbers of worms in bioremediation plots was greater in those with lower amounts of oil. The bioremediation strategy apparently promoted return of infauna while levels of total hydrocarbons in sediments remained comparable with oil only plots. This suggested there may have been some change in oil toxicity in bioremediation plots.

Plate 5 (*next page*). Field trials were used to assess fauna in mangrove plots following addition of oils, and in response to the bioremediation strategy. **A.** Foliage in mangrove canopies were heavily grazed by caterpillars in the study area. Plots with higher amounts of grazing were more vulnerable, and tended to have higher levels of tree mortality. **B.** Like most ground fauna, leaf-eating grapsid crabs were severely affected by oiling of mangrove plots. **C.** All fauna killed in oiled plots within 40 hours of oiling, were collected from the surface, identified, measured and weighed. Presence of below-ground Sipunculid worms were also sampled 13 months after oiling, and worms were notably present in bioremediation plots and absent in untreated, oiled plots.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Gladstone Field Studies —Microbial Biota in Mangroves

We describe the effects of the bioremediation strategy, in mangrove plots treated with Gippsland crude oil, on the indigenous populations of heterotrophic and hydrocarbon-degrading bacteria. Micro-organisms were isolated from the plots and described morphologically.

High numbers of heterotrophic micro-organisms were recorded throughout the experiment, although the numbers declined slightly in all plots with time. Oil addition stimulated the numbers of aliphatic-degrading bacteria slightly to levels of 10^4-$10^5.g^{-1}$ sediment. Bioremediation of the oiled sediment had a dramatic effect on the aliphatic-degrading population, increasing the population size by 5 orders of magnitude (to $<10^{10}.g^{-1}$) . Statistical analysis demonstrated that the effect was highly significant ($P<0.001$).

Aliphatics constitute a significant fraction of the Gippsland oil, and the results strongly suggest that the bioremediation strategy promoted the growth of micro-organisms capable of decomposing this large source of organic matter in contaminated sediments. The effect of bioremediation on the growth of aromatic-degraders was not as dramatic, possibly because aromatics constituted a smaller proportion of the oil (and therefore a smaller potential food source), and as such could not support such an abundant population. Alternatively, these organisms were unable to compete as effectively as the aliphatic-degraders for the available nutrients and oxygen in the sediment.

Morphological analysis suggested that the indigenous microbial population was diverse and comprised both aromatic and aliphatic-degrading micro-organisms. These findings confirm the conclusions of the literature review and the laboratory experiments which suggested that mangrove sediment had a competent indigenous population of hydrocarbon-degrading bacteria, and that it was low oxygen and nutrient levels which limited their growth on hydrocarbons after an oil spill. Active aeration and nutrient addition stimulated significantly the growth of hydrocarbon-degraders in oiled mangrove sediment in the field.

Plate 6 (*next page*). Field trials were used to assess micro-organisms in mangrove sediments following addition of oils, and in response to the bioremediation strategy. **A.** Mangrove sediments were sampled for presence and concentrations of micro-organisms, along with several physical parameters, using a variety of techniques. **B.** Changes in oxygen concentrations were measured with specially-designed field chambers.


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Policy guidance on the application of bioremediation to tropical tidal wetlands, mangroves and salt marshes

Bioremediation is now an established technique for the treatment of contaminated terrestrial environments and its potential to treat a range of contaminated marine shorelines in temperate environments has also been demonstrated in the last five years. The tropical shorelines that have been the subject of this study (namely mangrove and salt marsh vegetated tidal wetlands) do however pose new and different problems. We have made considerable progress in determining the potential (and limitations) of bioremediation in these areas and we summarise our main conclusions for the operational use of bioremediation as follows:

- ***In tropical salt marsh areas, application of nutrients as a bioremediation strategy had a positive effect on oil chemistry, but longer term studies are required to determine whether a biological effect may arise***

The results of the analysis of oil chemistry samples taken from the salt marsh area subjected to the bioremediation treatment, clearly demonstrated that nutrient application alone could significantly enhance oil biodegradation. However, as this experiment were carried out within a 12 month period, and no revegetation had occurred by the completion of monitoring, it was not possible to assess the effectiveness of the strategy with respect to vegetation recovery. Furthermore, it is possible that a seasonal effect may have delayed recovery. However, the finding that nutrients alone were sufficient to enhance biodegradation is important. Therefore, we recommend using bioremediation (as the application of slow release fertilizer) as a preferred option for a remedial treatment of oiled tropical salt marsh areas.

- ***In tropical mangroves, the bioremediation strategy of forced aeration plus nutrient addition had a positive biological effect, in part at least, but we were unable to determine a clear effect on sediment oil chemistry***

Using a combination of aeration and nutrient application, we were able to improve recovery in mangrove plots of surviving trees and fauna after initial tree death. Therefore, such a strategy could be used to improve longer term habitat recovery following an oil spill. However, the strategy failed as a means to protect vulnerable areas of mangrove forest since the loss of trees, which would be crucial to stand integrity in some cases, was no different in plots whether the strategy was applied or not. Therefore, there was no benefit to rapid and short term recovery. Furthermore, we were unable to demonstrate a significant increase in oil biodegradation using oil chemistry, pristane/phytane ratios. Nevertheless, it is arguable that sediment oil concentrations in this context might be less important than the effect of treatment on invertebrate re-colonisation within the surviving forested mangrove areas. It was also not determined from our investigations whether this positive effect of the treatment was due to the addition of aeration alone or the combination of nutrient addition with aeration. Clearly, additional work is needed to explore this, and other possibilities.

- ***Further work to refine the aeration system in mangroves may assist in improving the technique as a practical method to reduce the impact on particularly vulnerable sections of mangrove forests***

The bioremediation strategy tested in these investigations was in many ways the best possible option since it offered the most likely theoretical and practical method of providing a positive effect on oil-damaged mangrove forests. The basis for our preliminary understanding of natural recovery processes within mangrove ecosystems were outlined previously, and included a thorough review of relevant and current literature. Furthermore, we put a great deal of effort into making the field delivery of the strategy both affordable and practical. The authors are particularly aware of how difficult it is to work in mangrove environments, of the vulnerability of mangrove habitat to disturbance, and of the extent of oil penetration with its likely wide distribution during a spill incident.

The aeration strategy was essentially proposed based on observations of oxygen deficiency in oiled mangrove sediments, particularly within twelve months of oiling when burrowing infauna were either absent or their numbers greatly reduced. It is during this period when the larger number of trees died. We suspect that tree death was influenced by a number of factors, including the concentration and type of oil (crude oils being worst, and Bunker fuel oil was least damaging), tree condition (measured for example as leaf density), and the proportion of infauna killed by oil. Aeration of sediments was proposed to both accelerate microbial degradation of oil in sediments, as well as to replace aeration lost after burrowing and bioturbation by infauna ceased immediatley after oiling. Nutrients were added to both support the increased microbial activity, and to enhance tree condition and growth, and leaf density.

At the Port Curtis field sites, we observed a rapid decline in oil concentrations during the 12 months following oiling. Our studies, in combination with those elsewhere, demonstrated that oil was dissipated largely by tidal flushing. This important observation clearly suggests that locations at greatest long term risk are those where tidal ranges are lowest. Twelve months after oiling, oil concentrations in the field plots had dropped significantly and there was partial recovery of faunal biomass and activity. Bioremediation appeared to have assisted in this process by accelerating the degradation of oil. The important observation in this case, however, was that toxicity levels of contaminated sediments were reduced to sub-lethal levels and that natural aeration of sediments by infauna had recommenced. In this way, progress in habitat recovery was considered a matter of time.

Any strategy used in the field, was expected to be a compromise of available knowledge and resources. The strategy chosen for our field study was arrived at after considerable effort and prior experimentation, but our research efforts were understandably limited by available funding. This meant that we were unable to consider a broader range of possible methods and techniques. And, our studies do not provide sufficient evidence for discarding the strategy using aeration to assist short term recovery of mangrove trees and habitat.

For instance, there were a range of oxygen delivery systems which could have been applied in a range of different ways. The selected system to supply oxygen to the root systems of mangrove trees utilised battery operated pumps and air stone outlets to deliver air on a semi-continuous basis. Other options, or variations to the system we tested might include: varying the pulse frequency and rate of aeration; varying the number of delivery outlets; varying the depth beneath the sediment of delivery outlets; varying the placement of outlets with respect to tree roots and old crab burrows; and, altering the porosity of air stones (regards bubble size and oxygen dispersion). These things might also be expected to vary in different sediment types and different forest types. There was also the complex question regarding the type and amount of nutrients which needed to be added. We chose a mixture and amount based on the best available knowledge, but there are many alternatives including that of applying nutrients on their own.

The supply of power to the pumps was also a significant issue and it also needs further consideration. If the method were to be used in the field where it was to be applied over a wider area, and in a remote place, then there are several possibilities to make the delivery system workable. For instance, the power supply could be changed to a solar or wind driven system.

In acknowledgment of the difficulties in applying the chosen strategy over a wide area of oiled mangroves, it was proposed that the successful strategy be targeted selectively on a small number of oiled trees. The idea behind this was to protect those trees most responsible for supporting the integrity of the larger stand. These trees are recognisable as those on the exposed, often seaward margins. By comparison with inner sheltered trees, the edge trees are characteristically more stunted with wide spreading branches and numerous above-ground support roots. They collectively provide a protective barrier which if breached can lead to the erosion of the stand, and total loss of habitat in extreme cases.

In view of these observations, it is recommended that additional studies be funded to further investigate the provision of an effective aeration system coupled with nutrient addition, particularly in a strategy which might prevent trees dying during the first twelve months after oiling.

Oily Waste Disposal in Australia

The handling and disposal of oily waste is one of the chief problems facing responders at any oil spill incident. Traditionally, much of this oily waste has been disposed to local landfill sites. In many countries, landfill is now viewed as an unacceptable disposal route for oiled material since it is environmentally unsustainable.

A range of techniques involving the biological, chemical and physical processing of the waste can be used to treat and in some instances recycle the material in a more appropriate way. The first consideration in an oil spill situation is waste minimisation. The waste that is generated can be sub-divided as follows: liquid waste, oiled beach material, oil spill consumables and general shoreline debris. Our chief recommendations for the disposal of oily waste include: designating appropriate locations around Australia; and encouraging alternate strategies to landfill, such as recycling, landfarming, stabilisation, biodegradable sorbants and bioremediation.

Overall, we recommend that a dedicated and comprehensive review of oily waste, relative to Australian circumstances (policy, methods and sites), be conducted as soon as possible. We particularly identify a number of specific recommendations which arose during this review:

- ***Identify the best methods and the most appropriate locations for disposal of oily waste for specific sections along the entire Australian coastline, starting with those areas at greatest risk***

There needs to be a national approach to the prior selection of suitable techniques and places for the disposal of oily waste from large and smaller oil spills. Planning plays a crucial part in the successful management of an oil spill. The greater the amount of pre-planning that can be achieved before a spill the better. This particularly applies to waste disposal where suitable sites for techniques such as composting and land-farming need to be selected preferably prior to a spill occurring.

- ***Contingency planning should specifically discourage the use of landfill wherever possible, and encourage prior evaluation of alternative techniques such as recycling, stabilisation, incineration and bioremediation.***

  Many of the treatment techniques require some pre-planning before use (e.g. sites selection for land farming, dune disposal etc.), and may also require liaison with regulatory authorities for permits etc. (e.g. for mobile incinerators). Identification of sites where liquid oily wastes can be reclaimed also should be a priority. These items must be considered at the planning stages as during a major oil spill, time will be scarce and could mean that the only option is disposal to use landfill or other less desirable techniques.

- ***Prior consideration of the consequences of alternative techniques for disposal of oily waste must be made since these may generate large quantities of bi-products***

The distribution and utilisation of bi-products from disposal techniques (e.g. landfill, road surfacing product, bricks) needs to be considered before these measures are applied during the clean up of an actual large oil spill. The most suitable and appropriate techniques need to be encouraged within the national plan.

- ***Composting of oiled non-recyclable material should be investigated to see if it is possible to reduce oiling on, gloves, sorbent boom 'pom poms' etc., to an extent that they are no longer considered hazardous material***

We recommend that this be done in a demonstration project to prove that the technology can work and this would facilitate its use at future incidents.

- ***Investigation into the use of dune disposal; with specific reference to local conditions***

An investigation of this technique has run successfully in the UK but we have highlighted areas of concern (particularly moisture content) for its use in Australia. We feel that, in principle, this technique has a valuable role to play in the context of waste disposal and a research project looking on a small scale at the technique would answer some of the outstanding questions about its use.

- ***Investigation of the use of biodegradable sorbants***

In this report, we highlight some of the outstanding questions regarding the use of biodegradable sorbants. We recommend that work be conducted on a range of products to assess both their efficacy in relation to traditional synthetic sorbants and manufacturer’s claims with respect to biodegradability.

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Acknowledgements

This environmental research project was based at the Australian Institute of Marine Science (AIMS) in Townsville, Australia, and AEA Technology National Environmental Technology Centre in Abingdon, United Kingdom. We are grateful for the encouragement, support and assistance provided by these institutions and staff. The project was funded chiefly by the Australian Maritime Safety Authority (AMSA) and the Energy Research and Development Corporation (ERDC) with additional funding provided by the Great Barrier Reef Marine Park Authority (GBRMPA), and Shell Research Ltd., United Kingdom.

Laboratory Studies. Test oils were supplied by the Australian Petroleum Production and Exploration Association (APPEA). Students: Hilary Rainey (School for International Studies, Butler University) and Michelle Ramsey (James Cook University), assisted with the culture work and chemical extractions, Yas Tesiram (James Cook University), assisted with GC-FID analyses. This chapter was published in *Mangroves and Salt Marshes* 57: 1-18 (1998).

Aeration Experiments. Many thanks to Otto Dalhaus, Bill Laver, Michelle Ramsay, Roland Rupp and Mike Small for their generous help in these experiments.

Gladstone Field Studies. We greatly appreciate the generous assistance provided by individuals, businesses, and government officials, to all aspects of this challenging field experiment; from initial field site surveys, to permission to use the site, to establishment of enclosures in the mangroves, to donations of oil, equipment and facilities, to the conduct of monthly collections of scientific data and samples, to preparing and applying oil treatments, to cleaning up the site, to the laboratory evaluation of hydrocarbons, and more. We particularly thank: Cindy Black, Noel Bowley, Sue Codi, Mikel Duke, Trevor Gilbert, Michael Klemm, Deb Lamb, Guy Lane, Bill Laver, Judy Logan, Darren Marshall, Steve McKillup, Graham McKim-Hill, Rean Monfils, Robert Prior, Jane Rogers, Michael Small, Jamie Storrie and Emma Yates. The Gladstone Port Authority offered the use of the field site within their reclamation area at Fishermans Landing. Queensland Cement Limited, the local tenant bordering the area provided their permission also, as well as allowing free access to the site. The Ampol Refinery at Kurnell in Botany Bay donated the Gippsland Light crude oil used in the treatments. BHP Transport, Gladstone, donated the Bunker C fuel oil used in the treatments. BP Australia, Gladstone Terminal, provided the land site used for preparation and weathering of oil treatments. Enretech supplied the absorbant booms and bags of oil absorbant product. Data on rainfall and temperature for Gladstone were supplied by the Australian Bureau of Meteorology. This research project was undertaken under Fisheries Resources Permit No. 97SODB2545 granted by the Queensland Department of Primary Industries, Southern Fisheries Centre. Works 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., for helping with supplemental funding for the project.

Hydrocarbon Analyses. Laura Carolyn of AIMS assisted with sample preparation, sediment grain size analysis, and TOC analyses. The GC-FID analysis of the hydrocarbons was conducted by *Envirotest* (A.C.N 010 531 932) at Mt Gravatt Research Park in Nathan (Brisbane), Queensland. Interpretation of results was done by K.A. Burns.

Microbial Studies. This study is part of the requirements for an M.Sc. thesis at James Cook University by M.A. Ramsay, and forms part of a project conducted by the Australian Institute of Marine Science. We would particularly like to thank colleagues in the Marine Bioproducts Group at AIMS. We are grateful to the Australian Maritime Safety Authority, Australian Institute of Marine Science, Great Barrier Reef Marine Park Authority, and James Cook University for providing assistance and funding to support the research project.

Oily Waste Review. The authors are most grateful for the advice and recommendations on oil spill clean-up protocols in Australia provided by Don Blackmore, AMOSC, Peter Cochrane, APPEA, Peter Farrell, Mobil Australia, Trevor Gilbert, AMSA, Judy Logan, Gladstone Port Authority, Queensland, Greg Oliver, Woodside Petroleum, Peter Pfennig, EPA, South Australia, Jamie Storrie, GBRMPA, and Alex Willink, BP Australia.

Plate 7 (*next page*). Momentary relief after successfully deploying drums of prepared oils at the mangrove field site. Pictured from the left, Jamie Storrie, Otto Dalhaus, Michelle Ramsay, Trevor Gilbert, Michael Small, Roland Rupp, Richard Swannell and Dave Mitchell.