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Gladstone, Australia Field Studies: Weathering and Degradation of Hydrocarbons in Oiled Mangrove and Salt Marsh Sediments With and Without the Application of an Experimental Bioremediation Protocol

K. A. BURNS†*, S. CODI† and N. C. DUKE‡
†*Australian Institute of Marine Science, P.O. Box 3, Townsville, Qld. 4810, Australia*
‡*Marine Botany Department, University of Queensland, St. Lucia, Qld. 4072, Australia*

This field study was a combined chemical and biological investigation of the relative rates of weathering and biodegradation of oil spilled in sediments and testing the influence of a bioremediation protocol. The aim of the chemistry work presented here was to determine whether the bioremediation protocol affected the rate of penetration, dissipation or long-term retention of a medium range crude oil (Gippsland) and a Bunker C oil stranded in tropical *Rhizophora* sp. mangrove and *Halosarcia* sp. salt marsh environments. Permission for the planned oil spills was granted in the Port Authority area of Gladstone, Queensland (Australia). 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 (THC) and for individual alkane markers using gas chromatography with flame ionization detection (GC–FID). Sediments were collected at day 2, then 1, 2, 5 or 6 and 12 or 13 months post-spill for mangroves and day 2, 1, 3 and 9 months post-spill for salt marshes. Over this time, hydrocarbons in all of 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 plots treated with the waxy Gippsland oil showed a faster rate of biodegradation of the oil in the bioremediated plots. In this case only, the 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 loss 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 1–2 months before the start of microbial degradation. **© 2000 Elsevier Science Ltd. All rights reserved.**

Keywords: bioremediation; oil spills; mangroves; salt marshes; hydrocarbon weathering.

Introduction

Oil spills are known to cause severe and long-term damage to mangrove and salt marsh ecosystems (e.g., Burns and Teal, 1979; Wardrop, 1987; Corredor *et al.*, 1990; Teal *et al.*, 1992; Burns *et al.*, 1993; Vandermeulen and Singh, 1994; Duke *et al.*, 1997; Mille *et al.*, 1998 and others). Mangroves and salt marshes are important in coastal estuaries and bays on all sides of the Australian continent. As shipping terminals, industries and municipalities are also concentrated in the estuaries, these important nursery habitats for many commercially important species of fish and prawns are particularly vulnerable to oil spills (Volkman *et al.*, 1994). Oil spill response scenarios are designed to prevent, when possible, spilled oil from entering these ecologically sensitive habitats (Gundlach and Hayes, 1978; NRC, 1985). When mangrove trees die, the very structure and cohesion of the mangrove habitat becomes unstable (Nadeau

*Corresponding author. Tel.: +61-747-534211; fax: +61-747-725852.
E-mail address: kburns@aims.gov.au (K.A. Burns).

and Berquist, 1977; Duke and Pinzon, 1993; Garrity *et al*., 1994). Similarly, the death of heavily oiled salt marsh and sea grasses causes sediment erosion. The chain reaction of habitat loss and biological effects of the *Panama* oil spill were documented in detail (Jackson *et al*., 1989; Keller and Jackson, 1993). As quoted from the executive summary of the *Panama* report: 'Dead trees rotted and fell, logs and storms battered the shore, sea grass rhizome mats entirely disappeared, and sediments from all these environments eroded at rates up to several centimeters per year. The eroded sediments, and unknown amounts of oil in various stages of degradation, were deposited in neighboring habitats including sea grass beds and coral reefs, which had not been contaminated in the original spill.' In many instances, the residence times of oil in these deep mud habitats have stretched to decades, which prolonged ecosystem recovery. Thus, methods to reduce the impact of oil spills on mangroves and salt marshes are of strategic importance to spill response efforts. The aim of this study was to trial a bioremediation strategy for treating two types of oil commonly transported along the Australian coast if stranded in mangrove and salt marsh habitats.

Bioremediation technologies developed for soils and coastal beaches include tilling, nutrient and microbial additions; reviewed by Prince (1993), Atlas (1995), and Swannell *et al.* (1996). Tilling is unsuitable for mangroves because the preservation of the dense complex root systems is crucial to the survival of the trees. Oil biodegrading micro-organisms have been isolated from marine wetland environments worldwide, therefore it is unlikely that the absence of oil-degrading microbes would limit biodegradation in these areas (e.g., Scherrer, 1988; McKee and Mendolssohn, 1994; Lee *et al*., 1995). Review of the literature (Holtom *et al*., 1996; Swannell *et al*., 1994) and previous field evaluations (e.g., Scherrer and Mille, 1989; Oudot and Dutrieux, 1989; Swannell *et al*., 1995) highlighted several potentially limiting factors to oil biodegradation in mangrove mud. One major factor was anoxia. Preliminary laboratory-based flask experiments demonstrated that oil-degrading micro-organisms were present in all three common types of tropical Australia's coastal wetlands (*Rhizophora* and *Avicennia* mangrove and *Halosarcia* salt marsh sediments). These experiments also confirmed that oxygen was required to promote a significant rate of biodegradation of petroleum oils (Burns *et al*., 1999a).

Field trials showed that forced aeration could increase the layer of oxygenated surface mud in a mangrove swamp (Duke *et al*., 1999). Based on our literature review and our experimental trials, we developed a bioremediation strategy for oil spills in mangrove sediments that included a practical method for aeration of contaminated sediments. This was not intended as a strategy to be used over large areas. Some concern has been expressed about potential damage resulting from forced aeration, which may lead to changes in critical redox potential (McKee and Mendolssohn, 1994). The subsurface respiration of the roots of mangrove trees and the actions of burrowing animals are effective mechanisms for providing oxygen naturally to anoxic wet land sediment. Thus the plan for the field remediation study was based on selective aeration as a means to promote the survival of the mangrove trees. Trees are vital to maintaining the structural integrity of the forest and provide the necessary habitat for the return of burrowing animals to impacted sediments. Aeration was not tested in the salt marsh experiments because the soil was not anoxic. Nutrient additions were applied to both mangrove and salt marsh plots. Details of the field sites, bioremediation treatments and the biological studies are given in the companion paper in this issue (Duke *et al*., 2000). The microbiological studies are presented in Ramsay *et al.* (2000). This paper focuses on sediment chemistry.

The objectives of the chemistry studies were to determine if the bioremediation strategies reduced the persistence of the spilled oils over time or the rate of biodegradation. The study was designed to mimic a catastrophic oil spill approaching the mangroves and salt marshes from seaward. Two different oils were tested. Gippsland oil is a medium range crude from the Bass Strait Basin in southern Australia. Bunker C oil is commonly used in coastal shipping. The study site was several hundred hectares of *Rhizophora stylosa* forest and contiguous *Halosarcia* sp. salt marsh habitat that were designated for destruction in an extension area of the Port of Gladstone in southeastern Queensland.

This research was solicited and sponsored by the Australian Maritime Safety Authority, who defined the scope of the project and the oils to be tested.

Methods

All necessary permits from the regulatory and conservation agencies were obtained in order to conduct these field studies. The plan used a replicated design to facilitate statistically valid interpretation of the chemical data and biological data. Experimental plots were constructed in three mangrove areas of *R. stylosa* forest in the mid-intertidal zone and four areas of the high-intertidal zone salt marsh above the mangroves. Moore and McLaughlin (1978) demonstrated the need for careful experimental design in field studies. The design was to add enough oil that the surface sediments would reach an oil content of approximately 10% of the sediment dry weight. These were the initial oil concentrations in mangrove mud after the catastrophic Bahia las Minas oil spill in *Panama* (Burns *et al*., 1994). The mangrove study sites were in mature stands of 4–6 m tall *R. stylosa*. The prop roots of the trees on the edges of 6 m^2 plots were cut in a path 0.5 m wide to install experimental enclosures. Plastic retaining walls were dug down into the mud to a depth of 20 cm and supported to a height of 1 m. A floating gate was installed that

allowed tidal waters to move in and out but retained any floating oil. A litter trap was suspended in the trees. Three sets of plots were established, each with an uncut control plot delineated with marking tape stretched between the trees, a cut un-oiled control plot, an oil plot and a bioremediated oil plot. Salt marsh plots had low-lying vegetation, 5–20 cm tall situated on and within the otherwise bare salt pans behind the mangroves. The salt marsh experiment was conducted with replicates of four (1.2 m diameter or 1.13 m^2) plots for each treatment. A site location map and a detail of the mangrove plot positions are shown diagrammatically in Fig. 1. The study site was at 23°50′S and 151°13′E.

Before oiling, each plot was sampled for sediment grain size, total organic carbon (TOC) and background hydrocarbon content. The oils were pre-weathered in outdoor ponds for 24 h to simulate the type of oil as might wash ashore from a coastal ocean spill. The oils were then pumped back into steel drums and delivered to the experimental plots via helicopter. The oils were pumped into the mangrove plots at a targeted dosing rate of 5 l m^{-2}. The oil was added at high tide and the pumps were used to distribute the oil over the water surface and produce as 'even' coating of oil on roots and sediments as possible as the tide fell. The target dose was 2 l m^{-2} for salt marsh plots. The strategy of adding less oil to the salt marsh plots was that there was no complex mass of above ground roots to intercept the oil, as was the case in the mangrove plots. The bioremediation treatments consisted of sprinkling Osmocote Tropical fertilizer at 0.15 kg m^{-2} at 40 h post-oiling in both mangrove and salt marsh plots and then again at three months post-spill only in mangrove plots. Aeration of mangrove plots commenced at 40 h and continued for 111 days for the Gippsland oil plots and 140 days for the Bunker C oil plots. Further details of the plots and treatments are given in Duke *et al.* (1999, 2000). The enclosures were removed after two weeks when the oil appeared to have adhered to the sediments and above ground vegetation.

Sediments were collected at day 2, then 1, 2, 5 or 6 and 12 or 13 months post-spill for mangroves and day 2, then 1, 3 and 9 months post-spill for salt marshes. At 40 h, only surface sediments (0–2 cm) were collected using stainless steel spoons. Four samples from each plot were pooled to establish the average rate of dosing in each plot. For all subsequent sampling times, sediments were collected using a hand driven corer with 7 cm diameter aluminium core tubes. Four cores were taken at random positions within each plot. After examination, the unused portions of the cores were pushed back into the holes and a small rock placed on top to mark the spot, so that subsequent samples would not be from the exact same place. Cores were extruded onto solvent cleaned aluminium foil and sectioned. In the mangroves, the 0–2, 10–12 and 20–22 cm sections of the four replicate cores were pooled from each plot and packaged in solvent cleaned glass jars. For the salt marsh, cores were collected with 2 cm diameter plastic tubes. Four replicate 0–1 cm and the 9–10 cm core sections from each plot were combined for analysis. This composite sampling was designed to obtain adequate spatial coverage within plots while keeping the number of analyses to a minimum. Observations on the sliced cores included the presence of roots, animals, and burrows as well as oil. Samples were frozen within 1–2 h after collection. All sampling gear were cleaned thoroughly before and between handling every sample.

Analysis

Sediments were defrosted, homogenized and subsampled for wet/dry weight determination. Other subsamples were refrozen and sent to a commercial laboratory (Envirotest, Brisbane) for analysis of total hydrocarbons (THC) and individual alkanes by gas chromatography with flame ionization detection (GC–FID). The laboratory reported that approximately 2–5 g wet sediment was weighed and mixed with 2–3 times its weight with sodium sulphate (Na_2SO_4) to bind water. This mixture was extracted with dichloromethane (CH_2Cl_2) by sonication according to protocol 3550A of the US EPA. Extracts were reduced in volume using rotary evaporation and concentrated to small volumes with a gentle stream of dry nitrogen gas. When reduced to a few ml, the extracts were filtered through a Pasteur pipette containing glass wool and 1 g of Na_2SO_4 into glass vials. The oven temperature programme for the GC–FID was initially 60°C for 4 min, then 60–290°C at 6° min^{-1}, with a final hold for 25 min. The BP1 fused silica column was 0.22 mm i.d., 25 m long, with 0.25 μm film thickness. Calibration and quantification were done against an external standard mixture of *n*-alkanes in the C_{10}–C_{36} elution range plus pristane and phytane.

Sub-samples of the sediments before oiling were analysed for grain size by shaking dried sediments on a rotary shaker in a set of stainless steel geological sieves and weighing the sediment retained on each sieve. Sieves were 63, 125 and 250 μm mesh sizes. Other sub-samples were analysed for %TOC content by a high-temperature combustion process corrected for inorganic carbonate (Sandstrom *et al.*, 1986). The time zero (T_0) samples were also analysed at AIMS by GC–FID to determine if any pre-existing petroleum was present in the surface sediments.

Summary data are presented here and the entire data set is in Duke *et al.* (1999).

Results

Initial

Study plots were reasonably well matched for grain size distribution and %TOC. The average TOC content of 0–2 cm sediments in the mangrove sites ranged from 5.42% to 5.82%. The average TOC content of 0–1 cm sediments at the salt marsh sites ranged from 0.79% to 1.51%. The average amount of sand size fractions (>125

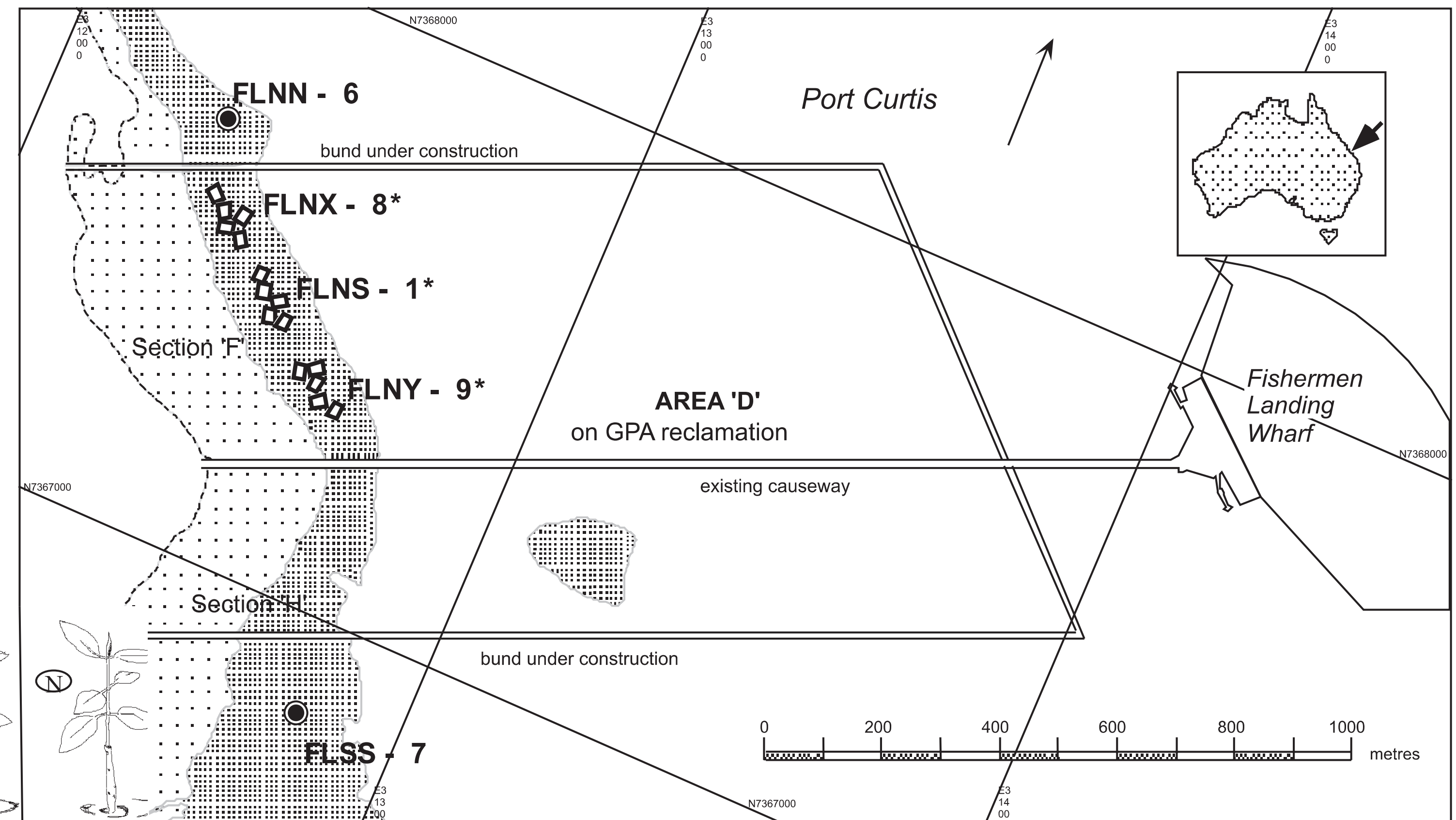

Fig. 1 Study sites around Fishermens Landing, Port Curtis, noting: three treatment sites (marked with *) within the reclamation area, AREA 'D', of the Gladstone Port Authority; and two sites of no disturbance, i.e., further control sites, outside of the reclamation area. At each site in the mangroves, five experimental plots were established for the study (plots not drawn to scale). Mangrove (darkly shaded) and saltpan (lightly shaded) areas are shown in relation to an approximate contour of the highest astronomical tides (dashed line). Bund walls constructed during the study extend across the intertidal zone and beyond the mangrove seaward margin. (23°50″S, 151°13″E).

μm) in mangroves was 20 ± 5% ($n = 16$) 0–2 cm cores, and 29 ± 12% ($n = 23$) in salt marsh 0–1 cm cores.

Initial analyses of surface sediments before oil was applied showed no evidence of previous contamination by petroleum. Extractable organic matter ranged from 0.2 to 0.3 mg g^{-1} dry wt. THCs ranged from 34 to 57 μg g^{-1} dry wt by GC–FID. The chromatograms consisted of biogenic hydrocarbons most likely from plant waxes (n-C_{27}, n-C_{29}) and from marine sources (squalene, C_{25-30} highly branched isoprenoids) based upon retention indices (e.g., Requejo and Quinn, 1983, Shinninghe Damaste *et al*., 1998). Concentrations of biogenic hydrocarbons ranged from ~50 to 500 ng g^{-1} dry wt for individual compounds. No oil was observed in control plots at any time. Chemical analysis of control site sediments in another study in the same area showed no oil spread into control sites through out the 13 months of study (Burns *et al*., 1999b).

Mangrove sediments

Most of the oil remained in the top two layers sampled in both Gippsland and Bunker C oil treatments. Table 1 shows the total oil in each plot as the sum of the 0–2 and 10–12 cm depth slices for plots treated with Gippsland oil. Also shown for the 0–2 cm slices, are the biodegradation parameters including the fraction unresolved hydrocarbons (UCM/THC) and the ratios of the isoprenoid marker compound (phytane) to its adjacent alkane (C_{18}). As biodegradation occurs the phytane/C_{18} ratio and the UCM/THC increase. Based on t-tests, no significant change in the degradation ratios occurred until after two months post-spill. Yet over this time, 90% of the THCs was lost from the sediments by evaporation and dissolution. By five months post-spill, there appeared to be a divergence in the treatments based on the phytane/C_{18} ratios, however, scatter in the data overshadowed any such trend in later samples. Based on the chemistry data, it appeared that the remediation strategy did not impact the chemistry of the residual oil or the mass balance of the amount of Gippsland oil remaining in the mangrove sediments.

Similar data for the plots oiled with Bunker C are given in Table 2. Bunker C is a heavier oil than the Gippsland and contains a very small amount of degradable alkanes. Again based on t-tests, no significant change in the degradation ratios occurred until after two months post-spill. Yet over this time, 80–90% of the THCs were lost from the sediments by evaporation and dissolution. By six months post-spill, there appeared to be a divergence in the treatments based on the phytane/C_{18} ratios, however, again scatter in the data overshadowed any such trend in the 13-month samples. Based on this chemistry data, it again appeared that the remediation strategy did not significantly impact the chemistry of the residual oil or the mass balance of the amount of oil remaining in mangrove sediments.

Salt marsh

In both oil treatments, the majority of oil absorbed to sediments stayed in the upper cm of the salt marsh. Table 3 shows the total oil in each Gippsland plot as the sum of the 0–1 and 9–10 cm depth slices. Also shown are the UCM/THC and the ratios of the isoprenoid marker compound to the adjacent alkane. Fig. 2 shows graphs of the degradation ratios and the THC loss over time for the four replicate plots. In contrast to the mangrove study, there was a significant

TABLE 1

Summary GC–FID data for mangrove experiment: phytane/C18 ratios and UCM/THC for 0–2 cm surface sediments plus THCs remaining as the sum of 0–2 and 10–12 cm slices (mg/g dry wt).

Parameter		Plot	Untreated Gippsland oil					Plot	Bioremediated Gippsland oil				
	Months		0.1	1	2	5	12		0.1	1	2	5	12
Phytane/C_{18}		1B	0.23	0.36	0.48	2.00	2.21	1G	0.30	0.34	0.40	0.94	6.44
		8E	0.20	0.29	0.34	1.48	1.79	8B	0.15	0.21	0.62	0.77	2.25
		9D	0.42	0.35	0.22	2.36	1.53	9C	0.37	0.33	0.43	1.31	1.06
	AVG		0.28	0.33	0.35	1.95	1.84		0.27	0.29	0.48	1.01	3.25
	STD		0.10	0.03	0.11	0.36	0.28		0.09	0.06	0.10	0.23	2.31
UCM/THC		1B	0.09	0.42	0.31	0.82	0.89	1G	0.27	0.34	0.62	0.96	0.77
		8E	0.06	0.21	0.29	0.96	0.68	8B	0.22	0.24	0.49	0.90	0.76
		9D	0.02	0.20	0.34	0.81	0.49	9C	0.02	0.46	0.33	0.80	0.71
	AVG		0.06	0.28	0.31	0.86	0.69		0.17	0.35	0.48	0.89	0.75
	STD		0.03	0.10	0.02	0.07	0.16		0.11	0.09	0.12	0.07	0.03
THC–GC/FID		1B	70.0	10.4	5.4	1.1	6.5	1G	135.1	29.3	33.3	1.3	0.8
sum of depths		8E	107.6	29.5	9.4	2.7	3.5	8B	228.9	17.1	14.1	2.6	4.1
(mg/g dry wt)		9D	41.7	8.0	6.6	2.0	2.4	9C	40.8	8.2	3.4	5.2	1.8
	AVG		73.1	16.0	7.1	1.9	4.1		134.9	18.2	16.9	3.0	2.2
	STD		33.1	11.8	2.1	0.8	2.1		9.41	1.06	1.52	2.0	1.7

TABLE 2

Summary GC–FID data for mangrove experiment: phytane/C_{18} ratios and UCM/THC for 0–2 cm surface sediments plus THCs remaining as the sum of 0–2 and 10–12 cm slices (mg/g dry wt)[a].

Parameter		Plot	Untreated Bunker C oil					Plot	Bioremediated Bunker C oil				
	Months		0.1	1	2	6	13		0.1	1	2	6	13
Phytane/C_{18}		1F	0.13	0.37	0.19	1.75	5.00	1E	Off scale	0.24	0.40	3.00	8.00
		8A	0.12	0.24	0.26	2.50	6.00	8D	Off scale	0.20	0.31	3.00	3.00
		9A	0.18	0.32	0.48	4.00	4.00	9B	0.26	0.32	0.18	~ND	4.00
	AVG		0.14	0.31	0.31	2.75	5.00		0.26	0.25	0.30	3.00	5.00
	STD		0.03	0.05	0.12	0.94	0.82			0.05	0.09	0.00	2.16
UCM/THC		1F	0.61	0.70	0.58	0.98	0.96	1E	0.61	0.74	0.68	0.99	0.81
		8A	0.60	0.56	0.77	0.40	0.94	8D	0.61	0.23	0.65	0.88	0.94
		9A	0.62	0.87	0.17	ND	0.96	9B	0.61	0.38	0.84	0.74	0.94
	AVG		0.61	0.71	0.69	0.86	0.95		0.61	0.45	0.74	0.87	0.90
	STD		0.01	0.13	0.29	0.07	0.01		0.00	0.21	0.11	0.12	0.06
THC–GC/FID		1F	42.6	17.1	8.9	3.0	2.2	1E	52.9	30.1	10.0	3.8	1.3
sum of depths		8A	41.9	7.9	8.0	4.7	1.2	8D	72.0	2.8	12.6	0.5	2.3
(mg/g dry wt)		9A	54.3	5.2	9.2	0.1	2.8	9B	42.9	7.4	2.2	0.5	1.5
	AVG		46.3	10.1	8.7	2.6	2.1		55.9	13.4	8.3	1.6	1.7
	STD		7.0	6.2	0.6	2.3	0.8		1.48	14.6	5.4	1.9	0.5

[a] ‘off scale’ means printer attenuation set too low. ‘NR’ means not resolved. ‘ND’ below detection level of ~10–20 μg/g dry wt.

TABLE 3

Summary GC–FID data for salt marsh experiment: phytane/C_{18} ratios and UCM/THC in 0–1 cm surface sediments plus THCs remaining as the sum of 0–1 and 9–10 cm slices (mg/g dry wt)[a].

Parameter		Plot	Untreated Gippsland oil				Plot	Bioremediated Gippsland oil			
	Months		0.1	1	3	9		0.1	1	3	9
Phytane/C_{18}		1B	0.27	0.13	0.48	0.16	1C	0.34	0.36	3.00	0.37
		2C	0.16	0.19	0.22	0.20	2D	0.10	0.33	1.33	0.86
		3D	0.34	0.13	0.19	0.20	3E	0.53	0.31	1.00	1.09
		4E	0.49	0.24	0.16	0.16	4B	0.35	0.14	0.71	0.59
	AVG		0.32	0.17	0.26	0.18		0.33	0.29	1.51	0.73
	STD		0.12	0.05	0.13	0.02		0.15	0.09	0.89	0.27
UCM/THC		1B	0.59	0.54	0.64	0.57	1C	0.61	0.57	0.82	0.50
		2C	0.51	0.54	0.59	0.61	2D	0.73	0.60	0.90	0.51
		3D	0.53	0.50	0.48	0.54	3E	0.57	0.68	0.67	0.72
		4E	0.67	0.45	0.45	0.52	4B	0.66	0.68	0.73	0.51
	AVG		0.58	0.51	0.54	0.56		0.64	0.64	0.78	0.56
	STD		0.06	0.04	0.08	0.03		0.06	0.05	0.10	0.09
THC–GC/FID		1B	6.4	1.4	3.7	1.4	1C	7.8	2.4	5.8	0.4
sum of depths		2C	4.6	3.2	2.4	2.9	2D	13.2	4.9	1.3	0.3
(mg/g dry wt)		3D	6.2	1.6	2.0	2.0	3E	8.6	3.3	NEOC	0.7
		4E	13.3	7.3	4.0	1.9	4B	8.2	5.5	1.1	1.1
	AVG		7.6	3.4	3.0	2.0		9.4	4.0	2.7	0.6
	STD		3.3	2.4	0.9	0.6		2.2	1.4	2.2	0.4

[a] ‘NEOC’ means not enough on-column for quantification.

divergence in all the parameters measured. ($p < 0.05$, paired *t*-test). Biodegradation of the Gippsland oil was enhanced in the bioremediated plots within the third month after oiling. Due to the higher elevation of the salt marsh plots compared to mangrove plots, the loss of oil due to dissolution was slightly less over the first month and ranged from ~60% to 80%. By the end of the experiment, biodegradation had contributed an additional 20–30% to the loss rate as compared with the untreated Gippsland oil.

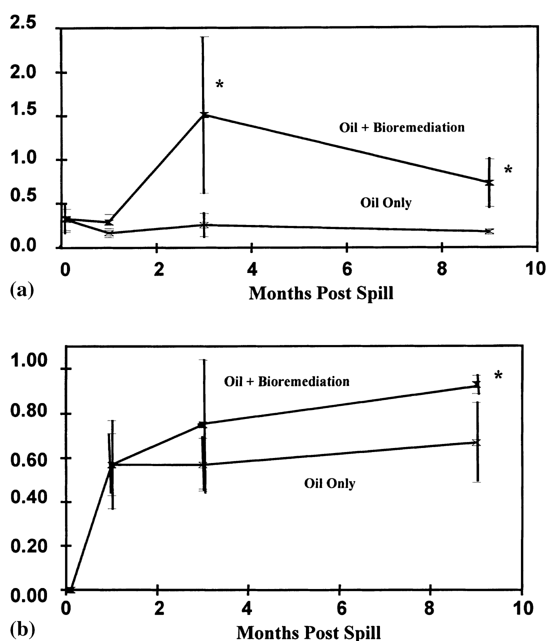

Fig. 2 Change in phytane/C_{18} ratios and total loss of hydrocarbons over time in salt marsh sediments oiled with Gippsland crude. Shown are the averages of four treatment plots ±1 S.D. * means significantly different as determined by paired *t*-test with $p < 0.05$. T_{mos} is the time in months shown on the *x*-axis: (a) Phytane/C_{18} ratio; (b) loss [$T_{40}-T_{mos}/T_{40}$ h].

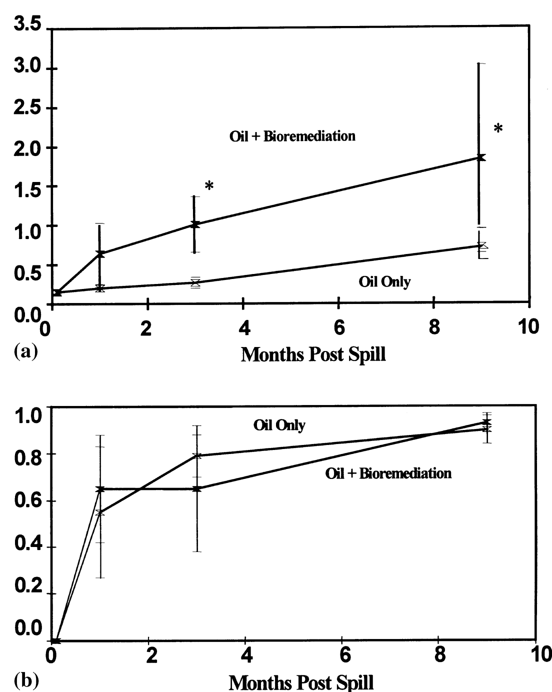

Fig. 3 Change in phytane/C_{18} ratios and total loss of hydrocarbons over time in salt marsh sediments oiled with Bunker C. Shown are the averages of four treatment plots ±1 S.D. * means significantly different as determined by paired *t*-test with $p < 0.05$. T_{mos} is the time in months shown on the *x*-axis: (a) Phytane/C_{18} ratio; (b) loss [$T_{40}-T_{mos}/T_{40}$ h].

Similar data for the salt marsh treated with Bunker C oil, are shown in Table 4. Fig. 3 shows a plot of the degradation ratios and the total loss over time for the four replicate plots. After one month, there was a divergence in the isoprenoid/*n*-alkane ratios indicating that nutrient addition had increased the rate of biodegradation. The variance was too great to show statistical difference in some of the time points, however, the trend was visually consistent when graphed. Since

TABLE 4

Summary GC–FID data for salt marsh experiment: phytane/C_{18} ratios and UCM/THC in 0–1 cm surface sediments plus THC remaining as the sum of 0–1 and 9–10 cm slices (mg/g dry wt).

Parameter		Plot	Untreated Bunker C oil				Plot	Bioremediated Bunker C oil			
	Month		0.1	1	3	9		0.1	1	3	9
Phytane/C_{18}		1D	0.11	0.21	0.21	0.52	1E	0.15	0.54	0.46	~ND
		2E	0.17	0.20	0.26	0.56	2B	0.14	0.22	1.00	3.50
		3B	0.16	0.22	0.23	1.10	3C	0.16	1.16	1.17	1.30
		4C	0.14	0.18	0.38	0.71	4D	0.16	no C18	1.40	0.73
	AVG		0.15	0.20	0.27	0.72		0.15	0.64	1.01	1.84
	STD		0.02	0.01	0.07	0.23		0.01	0.39	0.35	1.19
UCM/THC		1D	0.80	0.79	0.79	0.50	1E	0.82	0.91	1.00	1.00
		2E	0.78	0.71	0.86	0.91	2B	0.78	0.83	0.98	0.93
		3B	0.81	0.79	0.94	0.52	3C	0.80	0.88	0.95	0.94
		4C	0.71	0.85	0.88	0.55	4D	0.76	0.93	0.93	0.95
	AVG		0.78	0.79	0.87	0.62		0.79	0.89	0.97	0.96
	STD		0.04	0.05	0.05	0.17		0.02	0.04	0.03	0.03
THC–GC/FID		1D	7.8	4.6	1.1	0.3	1E	13.3	3.4	0.4	0.2
sum of depths		2E	19.2	5.2	3.7	3.8	2B	7.3	2.2	4.6	0.9
(mg/g dry wt)		3B	9.8	8.7	4.0	0.5	3C	15.1	12.2	2.2	0.6
		4C	2.3	0.3	0.3	0.2	4D	4.8	0.6	3.4	0.4
	AVG		9.8	4.7	2.3	1.2		10.1	4.6	2.6	0.5
	STD		7.0	3.0	1.6	1.5		4.2	4.5	1.5	0.2

the easily degradable alkanes are a minor fraction of the total oil, there was no difference between treatments in the total amounts of Bunker C oil remaining in the sediments at the end of the experiment.

Discussion

Initial oil levels

Mangrove plots treated with the Gippsland oil received doses that varied between 4% and 22% of the dry weight. The variation was due to the extremely waxy nature of the oil that made it difficult to deliver the same amount to each plot. Better consistency in dosing was achieved for the Bunker C plots, where initial oil levels ranged from 4.2% to 7.2% of the dry weight. The salt marsh plots received 0.5–1.3% of the dry weight for Gippsland oil and 0.2–1.9% of dry weight for the Bunker C oil. Thus despite efforts to achieve replicate dosing, the difference in the amount actually delivered to the plots was an additional factor for the interpretation of the biological data.

Exponential loss rates

The oil in the mangrove sediments was lost at an exponential rate for both oils. By combining the loss rate of all the mangrove plots in this study, a linear regression of time in months versus the log THC–GC/FID was significant. ($Y = -0.098X + 4.36$, $n = 20$, $R^2 = 0.60$, $p < 0.01$). This loss was predominantly due to evaporation and dissolution. The lag time of nearly two months to detect a change in composition due to biodegradation correlates with the microbiology studies (Ramsay *et al.*, 2000), which showed an increase in the numbers of *n*-alkane oxidizing bacteria after the first two months post-spill. Oudot and Dutrieux (1989) observed a delay in biodegradation of 1–3 months in mangrove mud oiled in Kalimantan. This delay time depends both on the time for selection of hydrocarbon degrading microbes and on the level of oil decreasing enough to support the microbial population (Fusey and Oudot, 1984). Preliminary mesocosm trials for the forced aeration system used in this project, showed that the sediments of the *Rhizophora* forest had an oxygen penetration depth of ~600 μm prior to treatment. The oxygenated zone from forced aeration extended to ~1900 μm (Duke *et al.*, 1999). Thus, most of the area of the mangrove plots was anoxic below 1–2 mm. From these results, it was reasonable to conclude that the reducing conditions of the mangrove mud prohibited the microbes from significantly affecting the mass of the remaining oil during the first six months of this study. By 12–13 months, the micro-organisms had removed most of the resolved alkanes from the oil in the surface sediments. The residual oil averaged 69% and 75% UCM for Gippsland plots, and 95% and 90% UCM for the Bunker C plots. No further observations were possible after this time because the site was completely cut off from the sea by port construction immediately following our final sampling efforts. In a parallel set of experiments conducted earlier at the same site, it took approximately two years for the oil levels to drop below 1000 μg g^{-1} dry wt (Burns *et al.*, 1999b; Duke and Burns, 1999b). Below this concentration is when we estimate that the benthic community could begin recovery (Krebs and Burns, 1977; Gilbert *et al.*, 1996).

The salt marsh plots were higher in the intertidal zone and consisted of slightly coarser, sandier sediments with significantly less organic carbon content. The salt marsh sediments were less anoxic and facilitated microbial degradation of the oils. This is most dramatic in the plots treated with Gippsland oil, where degradation appeared to reduce the amount of residual oil after only three months. There was too much of variation in the data to show statistical significance in the remaining THC data until nine months post-spill. Biodegradation of the Bunker oil was evident at three months from the change in the isoprenoid-to-alkane ratios. However, the alkanes are such minor components of the oil that degradation does not appear to affect the amount of residual Bunker oil. By nine months, oil level in most of the salt marsh plots treated with Bunker C had dropped below ~1000 μg g^{-1} dry wt. This level had been reached in the salt marsh plots treated with Gippsland oil and fertiliser. However, the unfertilised plots with Gippsland oil still contained an average hydrocarbon concentration of ~2000 μg g^{-1} at nine months.

As oil weathers, it congeals and forms tar masses on the surface sediments and roots. Oil on the surface of these particles would degrade faster than that on the inside of such globs. Therefore, high variability in the hydrocarbon concentrations and in the degradation parameters is expected in plots that are undergoing degradation. Thus, Figs. 2 and 3 show that the scatter in the isoprenoid/alkane ratios was greater in the bioremediated than the oiled only salt marsh plots. Fusey and Oudot (1984) established an inverse relationship between the rate of oil weathering and the concentration of oil in marsh sediments. In this study, the more rapid dissipation in the salt marsh plots compared to the mangrove plots could have been due to both the lower dose received and the more oxygenated condition of the salt marsh sediments.

Our other studies conducted in North Queensland mangroves have shown a rapid decline in oil levels after an accidental and a controlled spill (Burns and Codi, 1998; Burns *et al.*, 1999b). This rapid decline in hydrocarbon concentration was also observed in studies in Kalimantan (Oudot and Dutrieux, 1989). The rapid loss rate in the Queensland and Kalimantan mangrove sediments contrasts with observations made in the Caribbean. Scherrer and Mille (1989) reported very little loss of oil after 11 months, from mangrove sediments in Guadeluope that were experimentally dosed with 5 l m^{-2} of light Arabian crude oil. Munoz *et al.* (1997) observed continued high levels at this site after eight years of weathering. The chemistry studies of the catastrophic

spill of crude oil in Bahia las Minas (*Panama*) showed high levels of oil in mangrove sediments persisted for at least six years (Burns *et al.*, 1994). Fig. 4 is a summary of the loss rates of oil from these reported studies. The major physical difference in the two global regions is the average tidal height, which is only about 1 m in the Caribbean and up to 6 m along the Queensland coast and in Kalimantan. Thus, we conclude that dissolution by tidal washing is the major mechanism for removing the oil from mangrove mud on the East Coast of Queensland.

Qualitative changes

Gippsland oil is a waxy crude compared to the Bunker C oil as shown by the gas chromatograms in Fig. 5. Microbial degradation had become significant after about two months post-spill in both mangrove and salt marsh habitats, as evident by the change in isoprenoid to alkane ratios. However, even after 12–13 months, the surface sediments still contained some *n*-alkanes from the spilled oils. This means that the residual oils had only reached stage 3–4 in the degradation criteria summarized by Volkman *et al.* (1984) and again by Peters and Moldowan (1993). At this stage, alteration of the aromatic hydrocarbons or triterpane biomarkers by microbial processes would not be expected. Mille *et al.* (1988) observed sterane, diasterane and triterpane biomarkers, which were still present in 1991 samples from Brittany marshes contaminated 13 yr earlier during the *Amoco Cadiz* disaster. In this study, subtle changes in the internal biomarker patterns were consistent with the geochemical literature on oil degradation (e.g., Chosson *et al.*, 1991, 1992). The stability of the triterpane biomarkers is important to environmental forensic work and is consistent with efforts to identify the sources of coastal bitumens found around the Australian coasts (eg., Volkman *et al.*, 1992; McKirdy *et al.*, 1994). In a companion field experiment where the use of dispersant was tested, we showed that the internal triterpane and sterane biomarkers in Gippsland crude oil were unchanged after 13 months of weathering (Burns *et al.*, 1999b). Thus, the internal biomarkers would not have been altered over the short time period in this study.

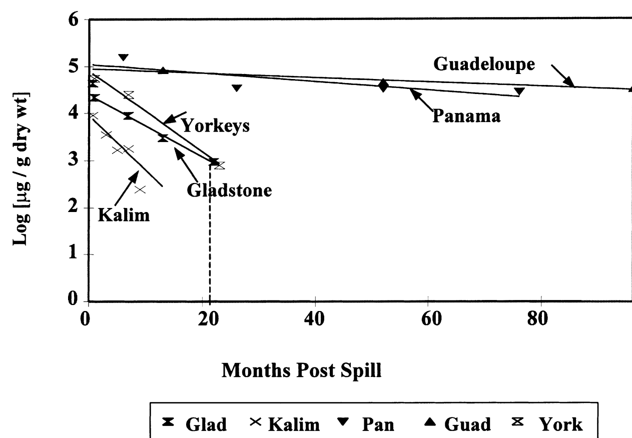

Fig. 4 Loss of oil from Pacific Region mangrove sediments (Gladstone: Burns *et al.*, 1999b; Yorkeys; Burns and Codi, 1998) compared to other oil spills in Panama (Burns *et al.*, 1994), Guadeloupe (Munoz *et al.*, 1997), and Kalimantan (Oudot and Dutrieux, 1989). Dotted line indicates time predicted for beginning of recovery of benthic fauna at Gladstone.

Unfortunately, funding was not available in this study to investigate the loss of individual aromatic hydrocarbons or saturated biomarkers by selected ion monitoring gas chromatography–mass spectrometry (SIM–GC/MS). In companion experiment, after 13 months of weathering the Gippsland oil had lost 73–81% of the individual aromatic hydrocarbons when ratioed against the internal triterpane biomarkers (Burns *et al.*, 1999b). The ratios of degradable to stable isomers in the alkylphenanthrene series showed that some biodegradation had commenced. However, most of the loss of the aromatics was due to dissolution. Fayed and Overton (1995) noted that fertilizer addition could retard the degradation of the aromatic hydrocarbons in an oil/water system. The Australian Institute of Marine Sciences has retained the extracts from the contract laboratory and a selection of the frozen sediment samples for further study.

Conclusions

The use of the bioremediation protocol in the mangroves treated with either Gippsland or Bunker C oil made no difference in the amount of oil absorbed by the sediments, the penetration of oil to depth, or the weathering patterns of the oil over time. After one year of weathering in mangrove sediments, the oils had weathered to only stage 3–4 in the petroleum geochemical scale. Changes in the hydrocarbon patterns were mostly due to losses from evaporation and dissolution from water washing with no significant difference noted between treatments. Microbial degradation became important in modifying the alkane fractions after two months. However, this did not appear to influence the mass balance of oil remaining in the mangrove sediments over this time. Thus, evaluation of the effectiveness of using the bioremediation protocol in mangroves rested on the biological assessments. The biological studies described in Duke *et al.* (2000) discuss the benefits of the remediation strategy by assisting the recovery of the mangrove trees.

The major mechanism of removal of oil from the salt marsh sediments was dissolution from tidal washing. The addition of fertilizer to the salt marsh plots did facilitate a more rapid removal of the very waxy Gippsland crude oil. Enhanced biodegradation accounted for an additional 20% less Gippsland oil remaining in salt marsh sediments after nine months. The addition of fertiliser also increased the degradation rate of alkanes in the salt marsh plots treated with Bunker C oil. However, since alkanes are a minor component of the Bunker C, biodegradation did not appear to affect the

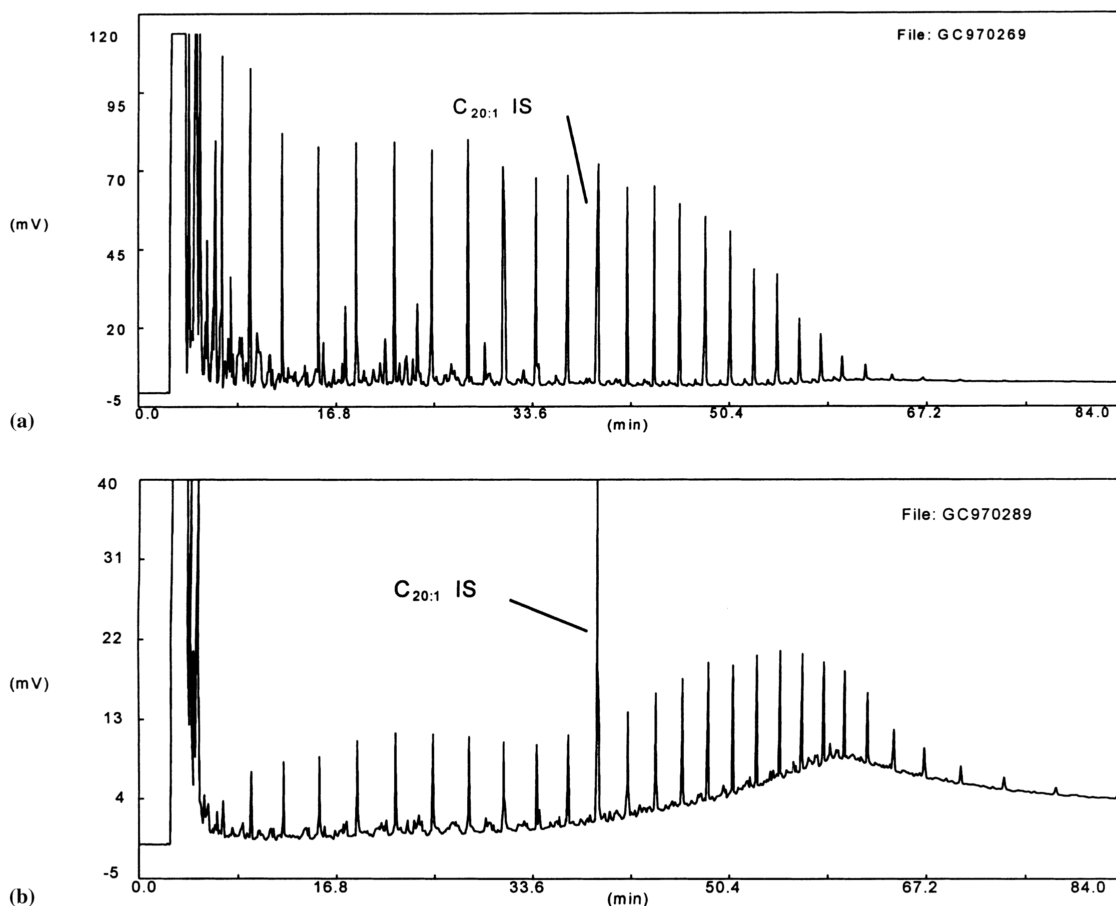

Fig. 5 Gas chromatograms of the oils used in the Gladstone field experiments after pre-weathering for 24 h. (a) Gippsland crude, (b) Bunker C oil. *Note*: $C_{20:1}$ is the internal standard eluting immediately before C_{20}. Other alkanes can be located by counting the peaks. These images do not show the same resolution as achieved with the Fisons Chromcard software. Thus the pristane/C_{17}, phytane/C_{18} and C_{20}/$C_{20:1}$ pairs are not resolved in these images. IS is the internal standard $C_{20:1}$.

total amount of oil remaining in salt marsh sediments after nine months. Thus, the use of fertilizer on oils that are biodegradable would also appear to be beneficial in contaminated *Halosarcia* marshes.

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