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Weathering of hydrocarbons in mangrove sediments: testing the effects of using dispersants to treat oil spills

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Abstract

This field study was a combined chemical and biological investigation of the relative effects of using dispersants to treat oil spills impacting mangrove habitats. The aim of the chemistry was to determine whether dispersant affected the short- or long-term composition of a medium range crude oil (Gippsland) stranded in a tropical mangrove environment in Queensland, Australia. Sediment cores from three replicate plots of each treatment (oil only and oil plus dispersant) were analyzed for total hydrocarbons and for individual molecular markers (alkanes, aromatics, triterpanes, and steranes). Sediments were collected at 2 days, then 1, 7, 13 and 22 months post-spill. Over this time, oil in the six treated plots decreased exponentially from 36.6 ± 16.5 to 1.2 ± 0.8 mg/g dry wt. There was no statistical difference in initial oil concentrations, penetration of oil to depth, or in the rates of oil dissipation between oiled or dispersed oil plots. At 13 months, alkanes were $>50\%$ degraded, aromatics were $\sim 30\%$ degraded based upon ratios of labile to resistant markers. However, there was no change in the triterpane or sterane biomarker signatures of the retained oil. This is of general forensic interest for pollution events. The predominant removal processes were evaporation ($\leq 27\%$) and dissolution ($\geq 56\%$), with a lag-phase of 1 month before the start of significant microbial degradation ($\leq 17\%$). The most resistant fraction of the oil that remained after 7 months (the higher molecular weight hydrocarbons) correlated with the initial total organic carbon content of the soil. Removal rate in the Queensland mangroves was significantly faster than that observed in the Caribbean and was related to tidal flushing.

Keywords: Oil spills; Mangroves; Weathering; Dispersants; Biomarker patterns; Degradation

1. Introduction

Oil spills are known to cause severe and long-term damage to mangrove ecosystems (e.g. Wardrup, 1987; Burns et al., 1993; Duke et al., 1997). Mangroves are important in coastal estuaries and bays on all sides of the Australian continent. Because 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). When mangrove trees die, the very structure and cohesion of the mangrove habitat becomes unstable (Nadeau and Berquist, 1977; Duke and Pinzon, 1993; Garrity et al., 1994). Therefore, the methods used to reduce the impact of oil spills on

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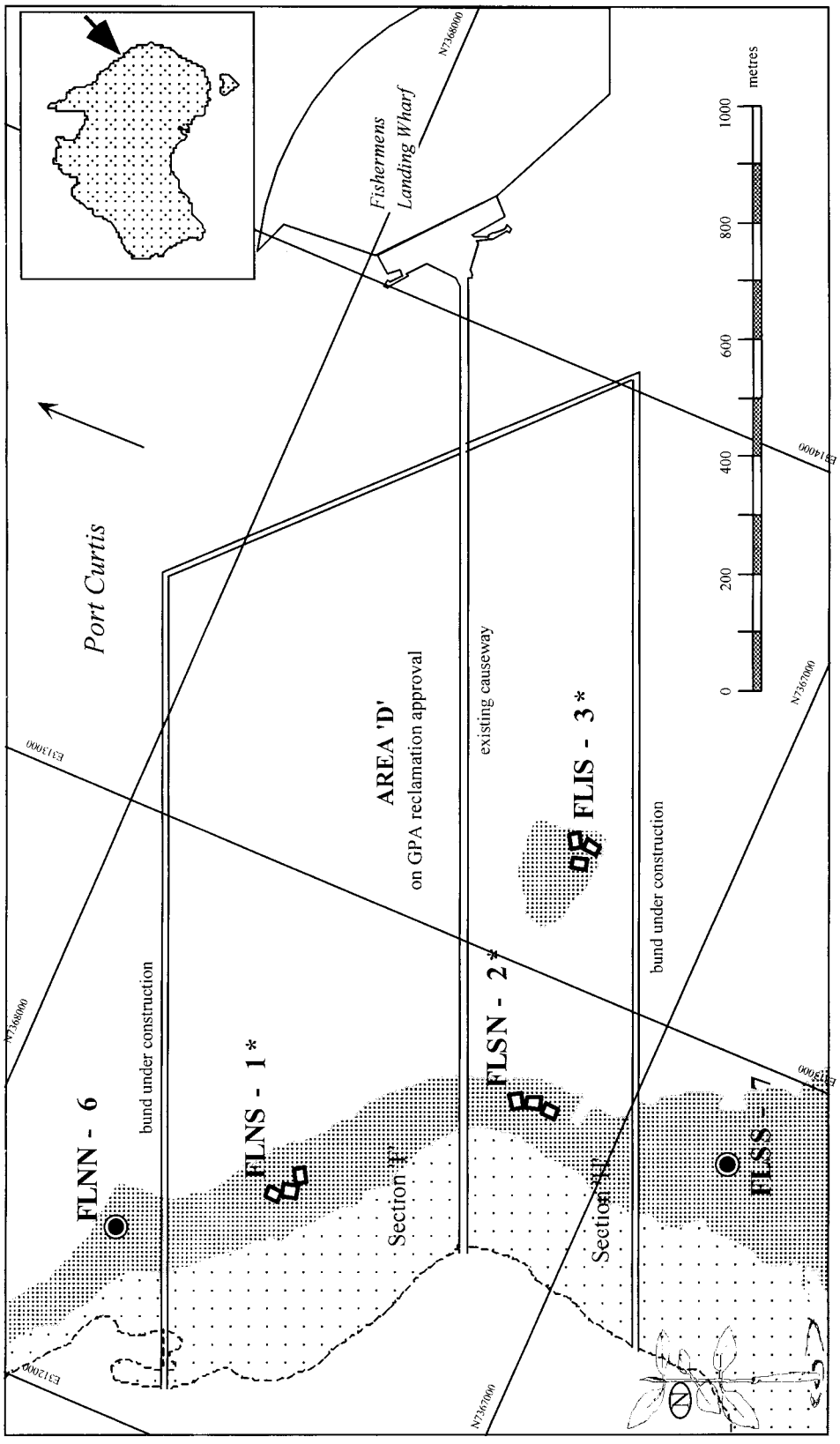

Fig. 1. Study sites around Fishermens Landing, Port Curtis. Note: three treatment sites (marked with *) within the reclamation area, AREA 'D', of the Gladstone Port Authority, and two sites of no disturbance, i.e. further control sites, outside the reclamation area. At each treatment site, three experimental 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 Highest Astronomical tides (dashed lines). Bund walls constructed during the study extend across the intertidal zone and beyond the mangrove seaward margin.

mangroves are of strategic importance to oil spill response efforts.

The use of chemical dispersants is a first order strategy in open marine waters (National Research Council, 1989). The strategy is based on a need to prevent oil spills from impacting sensitive coastlines. However, few reports have been published that address the use of dispersants in treating oil spills threatening mangrove systems. Getter et al. (1985) reported that the use of dispersant reduced the impact of oil on mangroves in an experimental spill at a site in Panama. This was a long-term study, but the experimental design had no replication (Dodge et al., 1995). Given the degree of variability observed in mangrove communities and in their responses to various treatments, it was important to gain additional data to determine whether this strategy should be used in Australian mangrove environments.

The chemistry objectives of this field study were to determine if dispersant usage to control an oil spill affects: (i) the initial concentration of oil absorbed by sediments, (ii) the depth of penetration of oil in the sediments, (iii) the persistence of the oil over time, (iv) its rate of bio-degradation, or (v) the pattern of the internal aromatic, triterpane and sterane biomarkers.

The study was designed to mimic a catastrophic oil spill approaching the mangroves from seaward. The oil was a medium range crude oil from the Bass Strait Basin in southern Australia. Samples of oil from this basin have been characterized in detail (e.g. Volkman et al., 1997) although some variability in biomarker composition exists between different wells. Oil from this basin is also included in the Oil Spill Identification Reference Kit distributed by the Australian Geological Survey Organization. The choice of oil was based on the common usage and transport of this crude oil around Australia, and on overall priorities set by the Australian Petroleum Production Exploration Association Research Working Group. The dispersant was Corexit 9527, which is stockpiled in Australia for use on this crude. The study site was several hundred hectares of *Rhizophora stylosa* forest designated for destruction in an extension area of the Port of Gladstone in southeastern Queensland. We acquired all necessary permits from the regulatory and conservation agencies to conduct a field study with a replicated design to facilitate statistically valid interpretation of the chemical data (summarized here) and biological data (Duke and Burns, 1999).

2. Methods

2.1. Experimental

Experimental plots were constructed in three areas with visually matched tidal elevation, mangrove tree composition and condition, sediment composition and benthic invertebrate populations. The plan was to add enough oil to each plot so that the surface sediments would reach an absorbed oil content of approximately 10% of the dry weight. These were the initial oil concentrations measured in mangrove mud after the catastrophic Bahia las Minas oil spill in Panama (Burns et al., 1994). The study sites were in mature stands of 6–10 m tall *R. stylosa*. The prop roots of the trees on the edges of ~6 m^2 plots were cut in a path about 0.5 m wide in order to install the 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 under it, but retained any unabsorbed and floating oil. A litter trap was suspended in the trees. Three sets of plots were established, each with an un-cut control plot delineated with marking tape stretched between the trees, a cut unoiled control plot, an oil plot and a dispersed oil plot. A site location map and a detail of the plot positions are shown in Fig. 1. The design was limited to three replicate sets of treatment and control plots due to funding and logistical constraints.

Before oiling, initial biological measurements were logged and each plot was sampled for sediment grain size, total organic carbon and background hydrocarbon content.

Pre-weathering of oil and the oil plus dispersant mixture was meant to simulate an oil spill arriving from seaward. At the local oil refinery, the oils were pumped into a shallow outdoor pond of seawater, approximately 0.1 m deep. The dispersant was added to the oil to achieve a formulation of 1:20 dispersant to oil (v/v) in accordance with industry recommended usage. Submersible pumps circulated the oil and the oil-dispersant mixture with seawater for alternate 2 h intervals over a period of 24 h. The pumps were stopped and the ponds were allowed to stratify 2 h before recovery of the oils. The floating oils were then pumped back into steel drums and delivered to the experimental plots via helicopter. The oils were then pumped into the appropriate plots at a nominal 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" a coating of oil on roots and sediments as possible as the tide fell. The enclosures were effective in retaining all visible oil with no leakage onto adjacent marshland. The oil adhered to roots and sediments within the first few days. Sampling began at 40 h post-spill. The enclosures were removed after 2 weeks when the oil had become immobilized.

At 40 h, only surface sediments (0–2 cm) were collected using stainless steel spoons. Several samples (>5

Table 1
Summary of the types of chemical measurements that were compiled for sediment samples

Abbreviation	Description
EOM	Total Extractable Organic Matter determined gravimetrically
THC-UVF	Total oil determined by UV Fluorescence analysis
THC-GC	Total Hydrocarbons determined by GC-FID
% UCM	The percentage of Unresolved hydrocarbons
[Alkanes]	Concentrations of individual isoprenoid and *n*-alkanes (μg/g dry wt)
Pristane/C_{17}; Phytane/C_{18}	Ratios of isoprenoid to *n*-alkanes as biodegradation indices
Sum [PAHs]	Sum of 218 aromatic and alkylaromatic isomers in the naphthalene/biphenyl, fluorene, phenanthrene/anthracene, benzanthracene/chrysene, fluoranthene/pyrene and benzopyrene to benzoperylene series as determined by SIM GC/MS
3 mp/2 mp; 2,7 dmp/1,7 dmp	Ratios of specific alkyl phenanthrene isomers to illustrate selective biodegradation
Sum [TTPs]	Sum of triterpane biomarkers in the hopane series as determined by SIM-GC/MS using m/z 191
Biomarkers	Selected sterane and potential demethylated hopane biomarkers as determined by SIM-GC/MS using m/z 217 and m/z 177
$Ts/(Ts+Tm)$; C29 αββ $R/(R+S)$; C29 ααα $R/(R+S)$	Ratios of specific biomarkers useful as biodegradation indices
% TOC	Percentage organic carbon in sediments before oiling
Size fractions	Percentage of sediment weights in various size fractions

per jar) from each plot were placed in four replicate jars to establish the average rate of dosing in each plot. At 1, 7, 13 and 22 months post-spill, sediments were sampled with 10 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. The 0–2, 10–12 and 20–22 cm sections of the four replicate cores had edges trimmed and were then pooled from each plot and packaged in solvent cleaned glass jars. 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 was cleaned thoroughly before and between handling every sample.

2.2. Analysis

Sediments were defrosted, homogenized and subsampled for dry weight. Approximately 10 g wet sediment was weighed and mixed with 2–3 times its weight with anhydrous sodium sulfate (Na_2SO_4) to bind water. This mixture was placed in a Teflon centrifuge tube and 20 ml of methylene chloride (CH_2Cl_2) added for extraction. Surrogate standards (*n*-alkene, $C_{22:1}$ and ortho-terphenyl, OTP) were added to each extraction to track any losses during the analysis procedures. Docos-1-ene ($C_{22:1}$) usually resolves from C_{22} in the GC-FID determinations, while ortho-terphenyl (OTP) is an aromatic hydrocarbon that can be distinguished in GC-FID analysis and does not interfere with UVF analysis. The extraction tubes were placed in a beaker of water and sonicated with the probe at maximum energy for 15 min. Extracts were filtered through pre-cleaned glass wool into a 100-ml boiling flask. Samples were extracted three times in total. Combined extracts were reduced in volume using rotary evaporation. When reduced to a few milliliters, the extracts were again filtered through a Pasteur pipette containing glass wool and 1 g of Na_2SO_4 into graduated glass centrifuge tubes with glass stoppers. If further volume reductions were required, this was achieved with a gentle stream of ultra pure nitrogen. Samples were adjusted to between 5 and 1 ml for total extractable organic matter (EOM) determinations using 10-μl aliquots gently evaporated onto the pan of a microbalance. The EOM weight provided a gravimetric determination of total oil content and was used to control lipid loading for the normal-phase chromatography cleanup procedures. Extracts were screened for their content of aromatic hydrocarbons and derivatives using ultraviolet fluorescence (UVF) spectroscopy. The cleanup procedure used 8 g of Al_2O_3 in a 1-cm diameter burette. The alumina was pre-cleaned with CH_2Cl_2 in a Soxhlet extractor, dried and then activated at 200°C for 4 h. Then 2% by weight of Milli-Q water

was added to obtain consistent activation. EOM loading was limited to 40–80 mg per column. The total hydrocarbon fraction was eluted with 10 ml of hexane, followed by 10 ml of 20% CH_2Cl_2, then with 10 ml of 50% CH_2Cl_2 in hexane. Sulfur in the extracts was removed by adsorption onto activated copper.

Cleaned extracts were then reduced with rotary evaporation and/or nitrogen and transferred into vials for analysis by gas chromatography (GC-FID) for total hydrocarbons and GC–mass spectroscopy (SIM-GC/MS) for individual aromatic hydrocarbons and biomarkers. Internal standards, added immediately before instrument injection, were used in quantifications ($C_{20:1}$ for GC-FID, and deuterated aromatic hydrocarbons for SIM-GC/MS). A composite reference oil biomarker standard distributed by the Australian Geological Survey Organization was used to identify peaks and determine retention times for triterpane and sterane biomarkers by SIM-GC/MS. Table 1 provides a summary of the chemical parameters used to measure oil losses and molecular changes of the oil over time.

For GC-FID analysis, all samples were analysed on a Carlo Erba GC8000 series gas chromatograph using cold on column injection and a DB-5MS column (J&W Scientific). For GC/MS analysis, all samples were analyzed on a HP 6890 Series II gas chromatograph/mass spectrometer equipped with a DB-5MS column (J&W Scientific). Samples were analyzed by full scan and selected ion monitoring (SIM). Similar oven temperature programs were used for both instruments as follows: 50°C for 1 min, ramp 6°C/min to 100°C, then 4°C/min to 300°C, and hold for 18 min. More specific analytical details for hydrocarbon analysis are outlined in UNEP/IOC/IAEA (1992) and are further discussed by Burns (1993a). Because of the evaporation of the most volatile hydrocarbons in the benzene series and the light alkanes during extract concentration steps, these procedures are quantitative for detecting petroleum hydrocarbons in sediments over $\sim C_{12}$–C_{36} or higher elution range.

Quality assurance/Quality Control (QA/QC) was built into the chemistry program at multiple levels. The procedures and the analyst were first tested by duplicate analysis of an international reference material (IAEA Marine Sediment 357) for EOM, UVF and GC-FID total oil content and individual aromatic hydrocarbons by SIM-GC/MS. Results were satisfactory with each component falling within one standard deviation of the reported mean from an international inter-calibration exercise. A method blank was included with each batch of 20 sediment samples. With the wide range of oil concentrations observed, it was impossible to estimate an appropriate amount of surrogate standards to add to initial extracts due to the need for extract dilution and/or co-elution on the GC.

Table 2
Percent of dry weight contained in each size fractions and TOC content of mangrove sediments (0–10 cm) before oil was added to plots

Plot	> 500 μm	> 125 μm	> 63 μm	< 63 μm	% TOC
1A	42	29	7	10	6.4
1C	45	39	14	2	4.9
1D	39	32	7	10	5.3
AVG ± STD	42 ± 3	33 ± 5	9 ± 4	7 ± 5	5.5 ± 0.8
2A	55	24	6	8	4.9
2C	39	33	8	10	5.8
2D	43	29	9	14	5.5
AVG ± STD	47 ± 11	28 ± 6	7 ± 1	9 ± 1	5.4 ± 0.6
3A	48	32	7	6	3.8
3B	52	32	6	5	3.3
3C	42	33	8	11	2.8
AVG ± STD	50 ± 3	32 ± 0	7 ± 1	6 ± 1	3.6 ± 0.4

Recovery of surrogate standards in the blanks and controls was carefully monitored and ranged from 66 to 88% for OTP and C22:1. Each extract had several independent estimates of oil content. These were used to construct linear regressions against each other and any outliers were scrutinized for sources of error in calculations. If this did not resolve an error, then another sub-sample of the sediment was analyzed. There was also an independent estimate of oil by THC-FID done by a commercial laboratory. These data were not shown to the analyst until after this data set was complete. Agreement between the laboratories was good over 4.5 orders of magnitude in oil content. Detection limits for samples analysed by AIMs were better at low concentrations because the commercial lab did only one solvent extraction and did not reduce the volume of extracts before GC analysis. Linear regression between THC-FID for the two laboratories was highly significant ($Y = 0.789\ X + 1466$, where X was the AIMS determination and Y was the contract lab determination, $n = 41$, $R^2 = 0.92$, $P < 0.001$). The QA/QC data are reported in Pratt (1997). The variation between the extractions of replicate surface samples collected at 40 h post-spill ranged from ±45 to 112%. This indicates the patchiness in the oil covering as it soaked into the sediments (Table 3).

Sub-samples of the sediments before oil was applied were analyzed 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 ground and analyzed for total organic carbon (TOC) content by a high temperature combustion process corrected for inorganic carbonate (Sandstrom et al., 1986).

Table 3
Concentrations over time of total hydrocarbons determined by GC-FID in mangrove sediments[a]

Depth (cm)	Oil only plots (mg/g)					Oil plus dispersant plots (mg/g)					Control unoiled plots (μg/g)			
	40 h	1 month	7 months	13 months	22 months	40 h	1 month	7 months	13 months	22 months	To 40 h		1 month	7 months
	1D					1A					1C			
0–2	23.8 ± 11.3	26.1	10.2	7.2	0.4	33.1 ± 17.7	28.2	7.8	0.3	0.1	57	24	57	5
10–12		0.1	5.8	1.3	0.4		2.4	9.6	0.6	1.9			69	
20–22		0.1	0.3	0.1			0.0	0.1	0.1				59	
	2A					2C					2D			
0–2	55.7 ± 22.0	25.8	6.3	2.4	0.4	23.7 ± 10.5	30.7	12.9	4.6	2.0	34	70	3	59
10–12		0.3	1.6	0.0	0.6		2.7	0.1	0.1	0.4			7	
20–22		0.0	0.0	0.3			0.1	0.1	0.1				28	
	3A					3B					3C			
0–2	24.2 ± 18.2	5.6	5.4	0.4	0.3	59.1 ± 66.2	27.6	3.1	13.3	0.4	41	47	23	3
10–12		0.0	0.2	0.1	0.1		0.0	0.1	0.0	0.1			7	
20–22		0.0	0.1	0.1			0.1	0.1	0.2				5	

[a] At 40 h only, surface sediments were sampled in the experimental plots with four replicate samples per plot. These samples were random composites from surface scoops and placed in four separate jars. All other samples were composites of the depth layers from four cores taken within each plot.

3. Results and discussion

3.1. General features

Table 2 shows the initial total organic carbon content and size fractions of surface sediments in the experimental plots. Sites 1 and 2 had similar TOC and soil texture while site 3 was slightly more porous and had a lower TOC starting value.

Initial analyses of sediments before oil was applied showed no evidence of previous contamination by petroleum. EOM ranged from 0.2 to 0.3 mg/g dry wt. Total hydrocarbons ranged from 34 to 57 μg/g 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 Damste et al., 1998). Concentrations of biogenic hydrocarbons ranged from ~50 to 500 ng/g dry wt for individual compounds. No further characterisation was conducted for background hydrocarbons. Concentration of hydrocarbons in the sediments from control sites remained low throughout the experiment (Table 3).

GC-FID analyses of hexane dilutions of the Gippsland oil collected from the cans and prior to weathering were compared to samples of the oil and dispersed oil mixtures collected after 24 h of pre-weathering. The pre-weathered oil samples (oil and dispersed oil samples) showed a loss of 2.2% ± 0.4% (n = 4) of the total hydrocarbons. This was measured by the loss of the compounds eluting between the solvent front and C_{12} on the chromatograms.

Initial oil concentrations in sediments from treatment plots collected at 40 h post-spill ranged from 3 to 6% of the dry weight. Table 3 shows the total hydrocarbon content (THC) based on GC-FID analysis of sediments in unoiled control, oil only and dispersed oil plots over time. Most of the oil stayed in the upper layers of the sediments. This is the layer most relevant to the return of burrowing organisms.

Table 4 gives the data for hydrocarbons in the surface sediments determined by EOM, UVF, GC-FID and SIM-GC/MS, the ratios of phytane to n-C_{18} as an

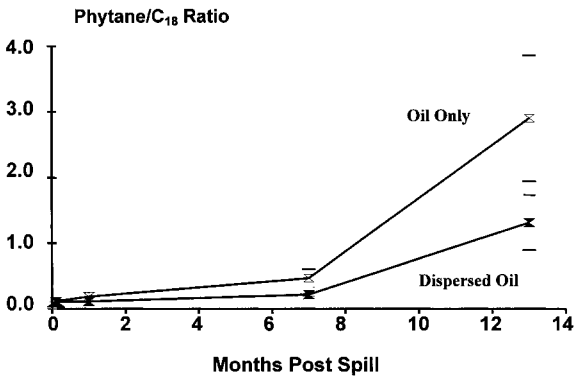

Fig. 2. Isoprenoid (phytane) to n-alkane (C_{18}) ratio used to indicate the relative stage of microbial degradation of the resolved hydrocarbons.

Table 4
Hydrocarbons in surface sediments over time determined by EOM, GC-FID, UVF and SIM GC/MS, plus phytane/*n*-C_{18} ratio and % UCM

Months post spill	Oil only			Dispersed oil		
	1D	2A	3A	1A	2C	3B
40 h						
EOM mg/g dry wt	49.7	116.4	22.9	51.7	73.1	70.6
THC-GC mg/g dry wt	41.4	126.1	49.2	55.0	75.5	96.7
UVF mg/g dry wt	69.4	130.7	34.6	56.3	88.0	130.0
PAH μg/g dry wt	1103	1428	975	1468	2120	1938
Phytane/C18	0.14	0.11	0.11	0.11	0.11	0.11
% UCM	0.57	0.50	0.38	0.60	0.52	0.56
1 month						
EOM mg/g dry wt	32.3	21.5	6.2	23.6	20.5	30.7
THC-GC mg/g dry wt	42.3	25.8	5.6	28.2	28.6	27.6
UVF mg/g dry wt	39.0	18.9	4.7	22.6	30.4	47.3
PAH μg/g dry wt	352	401	211	523	340	699
Phytane/C18	0.12	0.28	0.16	0.11	0.10	0.13
% UCM	0.75	0.72	0.54	0.70	0.55	0.50
7 months						
EOM mg/g dry wt	14.7	10.6	6.6	12.1	11.8	4.5
THC-GC mg/g dry wt	10.2	6.3	5.4	7.8	12.9	3.1
UVF mg/g dry wt	14.8	12.6	19.8	11.2	19.2	6.9
PAH μg/g dry wt	181	171	120	160	154.2	57
Phytane/C18	0.39	0.36	0.66	0.25	0.20	0.20
% UCM	0.79	0.79	0.91	0.61	0.67	0.67
13 months						
EOM mg/g dry wt	8.2	6.3	2.4	4.2	6.6	11.3
THC-GC mg/g dry wt	7.2	2.4	0.4	1.2	4.6	12.3
UVF mg/g dry wt	6.2	5.7	1.8	6.1	6.5	12.2
PAH μg/g dry wt	256	134	45	111	167	272
Phytane/C18	1.67	3.00	4.00	1.86	1.20	0.85
% UCM	0.73	0.68	0.51	0.73	0.66	0.62
22 months						
EOM mg/g dry wt	2.8	3.0	0.6	1.7	3.0	0.7
THC-GC mg/g dry wt	0.1	2.0	0.4	0.4	0.4	0.2
UVF mg/g dry wt	0.3	8.6	0.9	5.0	33.2	1.2
% UCM	0.82	0.89	0.92	0.99	0.93	0.98

indicator of the stage of degradation and the % unresolved complex mixture (UCM) in each sample. There was a sharp decline in oil concentration in most plots between 40 h and 1 month post-spill. This was a result of evaporation and removal by tidal flushing. Mean phytane/C_{18} ratios were 0.11 ± 0.00 ($n = 6$) at 40 h. The mean ratio was 0.15 ± 0.07 ($n = 6$) at 1 month post-spill, but increased dramatically over the next 13 months post-spill. There was a greater increase in this ratio in the oil only plots indicating that these plots had a slightly faster rate of change (Fig. 2). However the variance was too great to be statistically significant (Table 4). By 22 months the oil patterns were highly degraded with few resolved peaks remaining. The delay in the change in isoprenoid to alkane ratios indicated a lag time of at least 1 month before the onset of detectable rates of microbial degradation. A delay of 1–3 months has been noted in other experimental oil spills in the Caribbean (Scherrer and Mille, 1989) and Indonesia (Oudot and Dutrieux (1989). This delay relates both to the time needed for selection of hydrocarbon degrading micro-organisms and for dispersion processes to reduce oil levels enough to support the growth of the microbes (Fusey and Oudot, 1984).

After 7 months, there was no significant difference between the oil or dispersed oil plots in the amount of

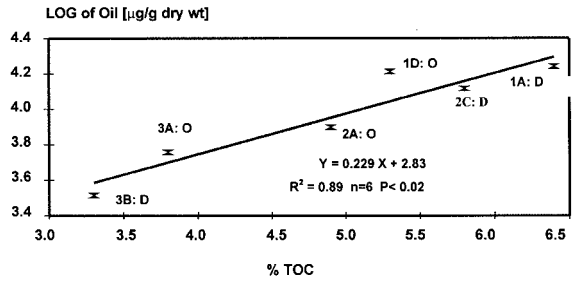

Fig. 3. Relationship between the total amount of oil remaining in sediments after 7 months as correlated with the initial total organic carbon content of the sediments. O indicates oil only plots and D indicates dispersed oil plots.

oil remaining, the depth of penetration into the sediment, or the chemical composition of the remaining oil. Most of the oil remained in the top layers (0–2 and 10–12 cm) of the sediment with very minor penetration to 20–22 cm depth (Table 3). Only one parameter was found that would account for the variation in the remaining hydrocarbon concentrations between plots. The initial % TOC of sediments in each plot was positively correlated with remaining oil (Fig. 3). Concentrations had decreased further by 13 and 22 months post-spill and a correlation between residual oil and % TOC was no longer discernable.

3.2. Exponential loss rates

The amount of oil remaining in all three depth slices were summed and plotted against time (Fig. 4). The figure presents the average ± the standard deviation of the average THC values. The data from all six of the oiled plots were pooled because there was no statistical difference related to dispersant treatment. A regression based on the first 13 months of data was extended to predict when residual hydrocarbon concentrations in the plots would drop below 1000 µg/g dry wt. Final sampling was planned to coincide with this decrease in order to study the return of benthic fauna. No further

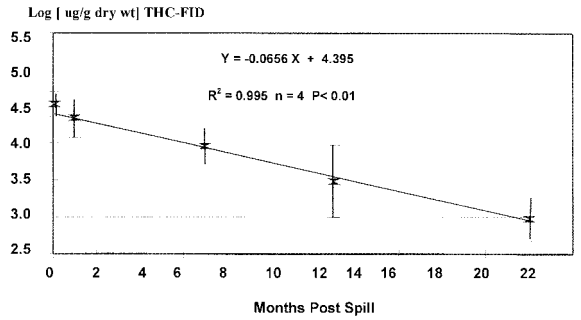

Fig. 4. The exponential decrease in THC over time.

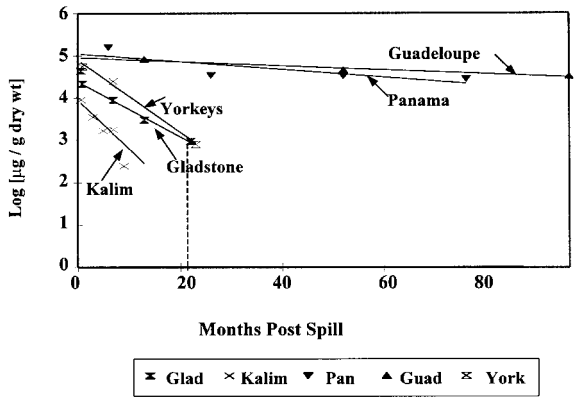

Fig. 5. Loss of oil from Queensland mangrove sediments (Gladstone: this paper; Yorkeys: Burns and Codi, 1998) compared to other oil spills in Panama (Burns et al., 1994), Guadeloupe (Munoz et al., 1997), and Kalimantan (Oudot and Dutrieux, 1989). Dotted line indicates time predicted for beginning of biological recovery at Gladstone.

observations were possible because the site was completely cut off from the sea by port construction immediately following the 22-month sampling efforts.

A second study in North Queensland showed a rapid decline in oil concentrations in mangrove sediments after a spill of mixed fuel and bunker oils (Burns and Codi, 1998). This rapid decline in hydrocarbon concentration in the Queensland 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 experimentally dosed with 5 L/m^2 of Light Arabian crude oil. The recovery time line was extended to 8 years (Munoz et al., 1997). The chemistry studies of the catastrophic spill of crude oil in Bahia las Minas (Panama) showed high levels of a medium range crude oil in mangrove sediments persisted for at least 6 years (Burns et al., 1994). For comparison, these loss rates are plotted in Fig. 5. There was large variability in individual data sets but the exponential loss rate is clear in each study. The major physical difference in the two regions is that the average tidal height is only about 1 m in the Caribbean and up to 6 m along the Queensland coast. Oudot and Dutrieux (1989) reported a rapid loss of up to 65% in 15 days when tidally driven estuarine waters spread experimentally oiled sediments outside of their experimental plots in East Kalimantan. The sediments in the Queensland studies were not redistributed outside the oiled areas as evidenced by no increase in oil content in control plots that were only a few meters from treated plots. This may relate to the fact that both Queensland studies were within the mangrove forests, while the

Kalimantan study site was along a river bank below the mangrove stands.

Fig. 5 also shows the time estimated for the benthic fauna to begin to re-colonize the oiled sediments. This time is based on when the oil concentrations would drop below 1000 μg/g dry wt, as has been observed in other studies of benthic fauna in oiled salt marshes and coastal marine sediments (e.g. Krebs and Burns, 1977; Gilbert et al., 1996). The biological studies associated with this study are detailed in Duke and Burns (1999) and confirmed that crab feeding activity had begun at 13 months but was still seriously reduced in oiled plots compared to control plots after 22 months.

3.3. Mass balance

These chemical measurements over time can be used to estimate the relative importance of various processes removing the oil from mangrove sediments. Pre-weathering had removed approximately 2.2% of this crude oil before it was added to the plots. Since microbial degradation lagged for at least 1 month, it is reasonable to assume that the exponential loss was due to water washing over the first few months. In Fig. 4, the extension of the regression line back to the *Y*-axis provided an estimate of the oil available for loss by water washing at 40 h. The estimate was ~73% of the total, with the remaining (~27%) available for loss due to evaporation. Thus for the Gippsland crude oil, tidal washing was the major mechanism for removing oil from mangrove mud. This finding is further supported by the correlation between the % TOC and remaining oil at 7 months suggesting that an equilibration of residual hydrocarbons between the organic matter in the sediments and the tidal waters had been achieved. The concepts of organic contaminants reaching equilibrium with sediment organic matter was demonstrated by many investigators and was synthesized by Schwarzenbach et al. (1993). After 13 months, microbial degradation of the alkane fractions was significant and continued, until at 22 months the alkanes were nearly entirely degraded. With respect to the mass of oil remaining, microbial degradation was not significant until after oil levels had dropped to less than ~3% of the sediment dry weight. The mean concentration of THC remaining at 13 months was 2.8 ± 2.9 mg/g dry wt. Removal of oil from sediments after this time would be related to both water washing and microbial degradation. Oudot and Dutrieux (1989) argued that the microbe resistant fraction becomes incorporated into humins for longer term preservation. Corredor et al. (1990) and Teal et al. (1992) showed that oil hydrocarbons were detectable in mangrove and temperate salt marsh sediments for 20 years. However, even if all of the remaining oil in the Gladstone plots

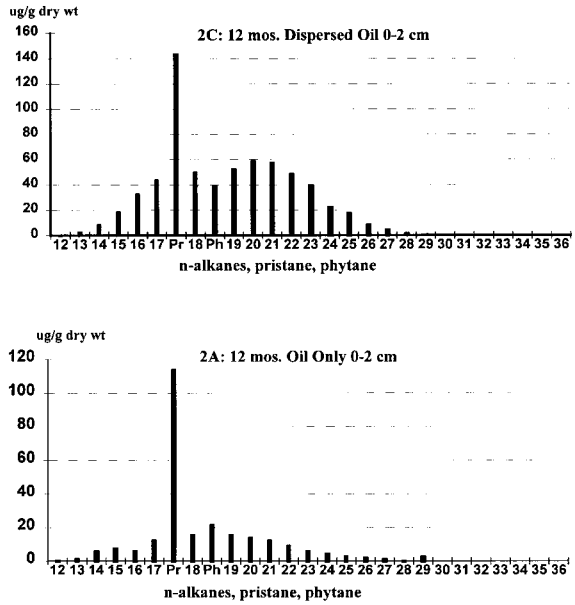

Fig. 6. Pristane, phytane and *n*-alkane content of 13-month post oil spill sediments.

at 13 months was removed by degradation, this would be a maximum of ~17% of the initial oil added. A partial mass balance estimate based on these observations would indicate that for this medium range crude oil, evaporation removed a maximum of ~27%, microbial degradation removed a maximum of ~17% and water washing removed at least ~56% of the total oil applied after the first year. These results compare well with the estimates made in Kalimantan where physical removal was the major mechanism and biodegradation rate depended on residual oil concentration over time (Oudot and Dutrieux 1989).

3.4. Changes in oil composition at 13 months

Table 4 shows there was no significant difference in the bulk parameters of oil content in surface sediments between oil only or dispersed oil plots over the first year. A two treatment "*T* test" was run on the 7- and 13-month data but the variance was too great to show differences between treatments.

After 13 months, the surface sediments still contained *n*-alkanes from the spilled oil. Fig. 6 shows the concentrations of the alkanes from station 2A (oil only) and 2C (dispersed oil) for the 13-month samples. It is clear from the figure that the oil only plots had a slightly faster degradation rate compared with the dispersed oil plots. However by 22 months, the alkanes were almost completely degraded in all the plots.

Petroleum hydrocarbons degrade through a well-documented series of stages beginning with the destruc-

Table 5
Areas of PAHs normalized to the internal area of hopane to show loss at 13 months

PAH class	To oil		Oil only		Fraction lost	Dispersed oil		Fraction lost
	AVG	STD	AVG	STD		AVG	STD	
Biphenyl	136.9	3.1	2.0	1.4	0.99	13.7	4.8	0.90
C1 BP	33.7	0.1	4.2	1.3	0.88	5.5	0.8	0.84
C2 BP	462.3	9.7	99.9	14.6	0.78	144.7	23.9	0.69
Naphthalene	422.4	10.1	0.5	0.1	1.00	10.4	6.7	0.98
C1 Naph	1229.2	26.1	23.8	10.2	0.98	87.5	35.5	0.93
C2 Naph	1746.9	52.9	239.8	111.4	0.86	299.6	96.8	0.83
C3 Naph	1419.0	37.4	293.3	39.9	0.79	401.8	85.8	0.72
C4 Naph	523.9	45.2	169.2	24.7	0.68	223.3	38.9	0.57
Acenaphthene	9.7	0.2	1.0	0.2	0.90	1.5	0.7	0.85
Fluorene	34.0	1.1	4.4	1.2	0.87	6.1	2.4	0.82
C1 FI	98.2	1.5	27.2	1.9	0.72	40.6	6.8	0.59
DBT	28.8	0.8	5.8	1.6	0.80	6.9	2.4	0.76
C1 DBT	50.5	0.1	17.1	1.5	0.66	17.7	14.9	0.65
C2 DBT	37.3	3.0	7.9	12.3	0.79	24.1	18.5	0.35
C3 DBT	26.3	6.6	9.3	7.1	0.65	21.1	5.6	0.20
Phenanthrene	94.6	2.0	12.0	4.0	0.87	22.2	9.0	0.77
Anthracene	5.1	0.7	1.1	0.1	0.78	1.7	0.5	0.66
C1 Phen/Anth	209.9	4.4	60.6	4.0	0.71	87.8	16.4	0.58
C2 Phen/Anth	290.8	11.4	149.5	10.2	0.49	215.1	8.8	0.26
C3 Phen/Anth	190.7	10.6	117.9	15.8	0.38	162.1	16.8	0.15
C4 Phen/Anth	79.0	1.1	43.6	3.0	0.45	62.1	10.1	0.21
Fluoranthene	6.4	0.1	3.0	0.3	0.53	4.6	0.4	0.29
Pyrene	6.1	0.2	3.8	0.3	0.38	5.4	0.5	0.11
C1 Pyrene	47.2	2.5	17.6	14.5	0.63	42.6	7.5	0.10
Benzo(a)anthracene	3.6	0.2	1.8	0.2	0.49	2.4	0.6	0.32
Chyrsene	6.5	0.0	4.9	0.3	0.24	6.1	0.7	0.06
C1 Chrys/BA	12.6	0.2	9.1	0.4	0.28	11.7	1.2	0.07
C2 Chrys/BA	19.4	1.0	13.4	0.6	0.31	12.3	10.1	0.37
Sum of PAH areas	7231	213	1344	197	0.81	1941	338	0.73

tion of the *n*-alkanes followed by nearly sequential destruction of more resistant classes of compounds. The degradation stages were summarized by Volkman et al. (1984), Peters and Moldowan (1993) and Munoz et al. (1997). While some structures of the aromatic hydrocarbons begin degradation during the early stages, the molecular markers in the sterane and hopane series are generally stable until all of the *n*-alkane and isoprenoid alkanes are degraded. After 13 months the Gippsland crude had only reached stage 3 in the geochemical scheme.

The composition of aromatic hydrocarbons can be a very useful tool in the chemical fingerprinting of many residual crude oils in some environments (e.g. Page et al., 1996; Boehm et al., 1997, 1998). Quantification of individual aromatic hydrocarbons showed no difference in pattern between the oil or dispersed oil treatment. Table 5 gives the content of the aromatic hydrocarbons in the 13-month surface sediments in comparison with the original oil. To illustrate the relative compositions, the areas of each congener within each alkylated series were summed and divided by the area of C_{30} 17α(h),21β(H)-hopane taken from the same SIM-GC/MS analysis. This normalization removes any possible variation from dilution factors, response factors and GC/MS injections. The data showed that the weathered oil had lost a significant fraction of the entire complement of aromatic hydrocarbons (Table 5). However, there was no detectable difference in the composition of the aromatic hydrocarbons between treatments (oil versus dispersed oil). Gippsland oil contains only minor percentages of the dibenzothiophene, pyrene/fluoranthene or benzathracene/chrysene series and no detectable content of the heavier PAHs in the benzopyrene series.

Selective degradation of individual alkyl-naphthalene, alkyl-phenanthrene and alkyl-dibenzothiophene isomers has been reported for petroleum oils by Budinski et al. (1998), Fisher et al. (1998), and others. The alteration of the predominant naphthalene series

Table 6
Ratios of peak areas of alkyl phenanthrenes and hopane to indicate biodegradation in oil, To versus 13 months post-spill sediments tested by one-way ANOVA with three levels

Ratios	To oil		Oil only		Dispersed oil	
	AVG	STD	AVG	STD	AVG	STD
Methyl phenanthrenes (4 isomers)						
3 mp/2 mp	0.98	0.00	1.33	0.12[a]	1.23	0.01[a]
2 mp/total mp	0.26	0.00	0.20	0.02[a]	0.22	0.01[a]
3 mp/total mp	0.26	0.00	0.26	0.01	0.27	0.01
2 mp/hopane	55.63	2.14	11.80	0.97[a]	19.91	4.17[a]
Total mp/hopane	101.52	2.90	31.59	1.80[a,b]	46.10	5.30[a,b]
Di-methyl phananthrenes (7 resolved isomers with baseline resolution for 1,7 and 2,7 dmp)						
1,7/2,7 dmp	3.42	0.08	2.32	0.11[a]	2.26	0.21[a]
1,7/total dmp	0.19	0.01	0.19	0.01	0.18	0.02
1,7/hopane	50.55	3.88	24.49	2.11[a,b]	35.81	3.18[a,b]
Total dmp/hopane	266.53	8.60	132.16	3.11[a,b]	204.74	7.65[a,b]

[a] Means treatment is significantly different than To ($P < 0.05$).
[b] Means oily only is significantly different than dispersed oil ($P < 0.05$).

by water washing in this study would limit their use for source discrimination after extensive weathering of the Gippsland oil. Within the di-methyl phenanthrene series, the 2,7 congener is known to be more resistant to biodegradation than other congeners. It elutes from the GC/MS before the 1,7 congener. Table 6 presents the relevant ratios from the methyl and di-methyl phenanthrene series to test for selective biodegradation. Again the ratios are derived from individual peak areas within each analysis. The loss of the phenanthrenes was significant in both degraded oils at 13 months. The oil only plots appeared to have a slightly greater loss when normalized to hopane. Since specific peak ratios changed by ~33% then it can be estimated that a maximum of one-third was lost to selective biodegradation while the majority was lost to water washing.

Another issue to address was whether dispersion caused a change in internal biomarker patterns. The triterpane hydrocarbon series was examined using the m/z 191 (hopanes) and m/z 177 (demethylated hopanes) peaks. The demethylated hopanes are generated under severe stages of petroleum degradation (e.g. Volkman et al., 1983; Peters et al., 1996). The hopane biomarker pattern and specific ratios of individual components such as the $Ts/(Ts+Tm)$ ratio are commonly used for source recognition of oils (summarized in Peters and Moldowan, 1993; Comet et al., 1993; Kvenvolden et al., 1993; Hostettler and Kvenvolden, 1994; Bence et al., 1996, Volkman et al., 1997). The $Ts/(Ts+Tm)$ ratios were computed, as were the ratios of the sum of $177/(191+177)$ peaks to look for demethylated hopanes, but no changes were found over time (Table 7). Studies on highly biodegraded oils have shown the preferential removal of the $\alpha\alpha\alpha 20R$ and the $\alpha\beta\beta 20R$ steranes compared to their S isomers (summarized in Peters and Moldowan, 1993). The ratios of the $R/(R+S)$ for the C_{29} steranes were also computed and again no changes were found between the 13-month weathered oil or dispersed oil and the initial Gippsland oil (Table 7). Munoz et al. (1997) demonstrated that the steranes and hopanes were altered faster than other classes of molecular biomarkers such as the bi, tri and tetra cyclic terpanes, diasteranes and the aromatic steriods in an 8-year study of the degradation of Arabian crude oil spilled

Table 7
Diagnostic ratios in the triterpane and sterane biomarker series[a]

	$Ts/(Ts+Tm)$	Sum $177/(177+191)$	C29 $\alpha\beta\beta$ $R/(R+S)$	C29 $\alpha\alpha\alpha$ $R/(R+S)$
Gippsland oil after 40 h	0.378 ± 0.012	0.419 ± 0.002	0.561 ± 0.001	0.520 ± 0.004
Gippsland oil after 13 months	0.361 ± 0.013	0.426 ± 0.004	0.559 ± 0.028	0.513 ± 0.016
Dispersed oil after 13 months	0.316 ± 0.016	0.426 ± 0.007	0.562 ± 0.008	0.523 ± 0.016

[a] One-way ANOVA with three-level analysis showed no difference between the weathered oils and To or between the weathered oil and dispersed oil.

in mangrove sediments. It can be concluded from these results that the decomposition of these classes of molecular markers in Gippsland oil had not yet begun by 13 months.

3.5. Further degradation at 22 months

There was not enough time or resources to continue this study for 8 years. However, there was indirect evidence that decomposition of the molecular markers in Gippsland oil was beginning at 22 months when oil concentrations had fallen below 3 mg/g dry wt and most alkanes were degraded. During the course of the study the precision of the analyses was continually evaluated by comparing the results of the independent methods of determining petroleum content in the sediments. The linear regressions calculated when comparing EOM, UVF, GC-FID and SIM-GC/MS determinations were all very highly significant for the To to 13-month samples (log [UVF] versus log [GC-FID]: $Y = 0.932\ X + 0.068$, $n = 41$, $R^2 = 0.93$, $P < 0.001$). However, for the 22 month samples, there was no correlation between the UVF and GC-FID measurements ($Y = 0.35\ X + 1.4$, $n = 12$, $R^2 = 0.52$, NS). Most of the final samples were more highly fluorescent than the GC-FID or EOM determinations would predict. A similar change in the fluorescence of residual oil was detected in the Bahia las Minas (Panama) spill after 5 years into the study (Burns and Yelle-Simmons, 1994). As oil weathers, it generally becomes more fluorescent, not only due to the loss of the relatively less fluorescent light aromatic components (e.g. naphthalenes), but also because of the formation of more fluorescent oxidation products (e.g. Literathy et al., 1991; Ehrhardt et al., 1992, 1997). The need to include aromatic hydrocarbon oxidation products in environmental assessment studies was summarized by Burns (1993b). Residual toxicity of degraded oils is increased by the presence of oxidation products (e.g. Thomas et al., 1995; Pelletier et al., 1997). Thus, these products should be included in the toxicity evaluation (e.g. Widdows et al., 1990). We suggest that the UVF measurements provide a semi-quantitative estimate of total toxicity due to residual aromatic hydrocarbons and their oxidation products. In the interpretation of residual sediment toxicity in this project, the UVF data have been considered most relevant.

3.6. Implications for treating oil spills impacting mangroves

The use of dispersant on oil spills off-shore, is intended to break up slicks and to prevent oil from entering these sensitive and vulnerable habitats. In this experiment where dispersant was added to the oil immediately before it was put onto the mangrove plots, there was no difference in sediment chemistry due to the dispersant treatment. There was no significant difference in the amount of oil absorbed by the sediments and the dispersion of absorbed oil over time. The conclusion from this study is that the decision to use dispersants to treat oil spills before they reach mangrove marshes must depend on the potential for the treatment to reduce the resultant biological impacts. The conclusion of Duke and Burns (1999) in the biological assessment work associated with this project, was that dispersed oil was significantly less toxic to the mangrove trees than oil only treatments. It would be desirable to treat oil spills threatening mangroves with non toxic dispersants provided such treatment will not cause further long term environmental damage by mixing the oil into shallow waters where coral reefs are located. As a result of this project, this strategy has already been incorporated into the oil spill contingency plans for coastal Australia.

4. Conclusions

The use of the dispersant Corexit 9527 before the Gippsland crude oil contaminated the mangroves resulted in 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 1 year of weathering in mangrove sediments, the oil had weathered to only stage 3 in the petroleum geochemical scale. The most detectable changes in the aromatic hydrocarbons were due to losses from evaporation and water washing with no difference between treatments. Ratios of specific aromatic isomers indicated some selective biodegradation of the aromatics. The diagnostic biomarkers in the triterpane and sterane groups were not changed. This is important to environmental forensic work and is consistent with efforts to identify the sources of coastal bitumens found around the Australian coasts (e.g. Volkman et al., 1992; McKirdy et al., 1994). After 22 months there was an increase in the fluorescence intensity of the residual oil which made the UVF measurements more relevant to estimating residual toxicity than either the SIM-GC/MS analysis of aromatic hydrocarbons or the total hydrocarbon content by GC-FID.

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References

Bence, A.E., Kvenvolden, K.A., Kennicutt, M.C., 1996. Organic geochemistry applied to environmental assessments of Prince William Sound Alaska, after the *Exxon Valdez* oil spill — a review. Organic Geochemistry 24, 7–42.

Boehm, P.D., Douglas, G.S., Burns, W.A., Mankiewicz, P.J., Page, D.S., Bence, A.E., 1997. Application of petroleum hydrocarbon chemical fingerprinting and allocation techniques after the *Exxon Valdez* oil spill. Marine Pollution Bulletin 34, 599–613.

Boehm, P.D., Page, D.S., Gilfillan, E.S., Bence, A.E., Burns, W.A., Mankiewicz, P.J., 1998. Study of the fates and effects of the *Exxon Valdez* oil spill on benthic sediments in two bays in Prince William Sound, Alaska 1. Study design, chemistry and source fingerprinting. Environmental Science and Technology 32, 567–576.

Budinski, H., Raymond, N., Nadalig, T., Gilewicz, M., Garrigues, P., Bertrand, J.C., Caumette, P., 1998. Aerobic biodegradation of alkylated aromatic hydrocarbons by a bacterial community. Organic Geochemistry 28, 337–348.

Burns, K.A., 1993a. Analytical methods used in oil spill studies. Marine Pollution Bulletin 26, 68–72.

Burns, K.A., 1993b. Evidence for the importance for including hydrocarbon oxidation products in environmental assessment studies. Marine Pollution Bulletin 26, 77–85.

Burns, K.A., Yelle-Simmons, L., 1994. The Galeta oil spill IV: relationship between sediment and organism hydrocarbon loads. Estuarine Coastal and Shelf Science 38, 397–412.

Burns, K.A., Codi, S., 1998. Contrasting impacts of localised versus catastrophic oil spills in mangrove sediments. Mangroves and Salt Marshes 2, 63–74.

Burns, K.A., Levings, S., Garrity, S., 1993. How many years before mangrove ecosystems recover from catastrophic oil spills? Marine Pollution Bulletin 26, 239–248.

Burns, K.A., Garrity, S., Jorissen, D., MacPherson, J., Stoelting, M., Tierney, J., Yelle-Simmons, L., 1994. The Galeta oil spill II. Unexpected persistence of oil trapped in mangrove sediments. Estuarine Coastal and Shelf Science 38, 349–364.

Comet, P.A., Rafalska, J.K., Brooks, J.M., 1993. Sterane and triterpane patterns as diagnostic tools in the mapping of oils, condensates and source rocks of the Gulf of Mexico region. Organic Geochemistry 20, 1265–1296.

Corredor, J.E., Morell, J.M., del Castillo, C.E., 1990. Persistence of spilled crude oil in a tropical intertidal environment. Marine Pollution Bulletin 21, 385–388.

Dodge, R.E., Baca, B.J., Knap, A.H., Snedaker, S.C., Sleeter, T.D., 1995. The effects of oil and chemically dispersed oil in tropical ecosystems: 10 years of monitoring experimental sites. Technical Report Series 95–014. American Petroleum Institute.

Duke, N.C., Pinzon, Z.S., 1993. Mangrove forests. In: Keller, B.D. and Jackson, J.B.C. (Eds.), Long-term Assessment of the Oil Spill at Bahia las Minas, Panama. Synthesis report, vol. II. OCS Study MMS 93-0047, US Department of Interior. Minerals Management Service, New Orleans, pp. 447–533.

Duke, N.C., Pinzon, Z.S., Prada, M.C., 1997. Large-scale damage to mangrove forests following two large oil spills in Panama. Biotropica 29, 2–14.

Duke, N.C., Burns, K.A., 1999. Fate and effects of oil and dispersed oil on mangrove ecosystems in Australia. Final project report to the Australian Petroleum Production and Exploration Association (APPEA) Box 2201, Canberra ACT. 2601.

Ehrhardt, M.G., Burns, K.A., Bicego, M.C., 1992. Sunlight induced compositional alterations in the seawater soluble fraction of crude oil. Marine Chemistry 37, 53–54.

Ehrhardt, M.G., Bicego, M.C., Weber, R.R., 1997. Photo-oxidation of 1-methylnaphthalene dissolved in seawater and exposed to sunlight under quasi-environmental conditions. Journal of Photochemistry and Photobiology A: Chemistry 108, 253–259.

Fisher, S.J., Alexander, R., Kagi, R.I., Oliver, G.A., 1998. Aromatic hydrocarbons as indicators of biodegradation in north Western Australian oil reservoirs. In: Purcell, P.G., Purcell, R.R. (Eds.), The Sedimentary Basins of Western Australia 2, Proceedings of the Petroleum Exploration Society of Australia Symposium, Perth, WA, pp. 185–194.

Fusey, P., Oudot, J., 1984. Relative influence of physical removal and biodegradation in the depuration of petroleum-contaminated seashore sediments. Marine Pollution Bulletin 4, 136–141.

Garrity, S., Levings, S., Burns, K.A., 1994. The Galeta oil spill I. Long term effects on the structure of the mangrove fringe. Estuarine Coastal and Shelf Science 38, 327–348.

Getter, C.D., Ballou, T.G., Koons, B., 1985. Effects of dispersed oil on mangroves: Synthesis of a seven year study. Marine Pollution Bulletin 16, 318–324.

Gilbert, F., Stora, G., Bertrand, J-C., 1996. *In situ* bioturbation and hydrocarbon fate in an experimental contaminated Mediterranean coastal ecosystem. Chemosphere 33, 1449–1458.

Hostettler, F.D., Kvenvolden, K.A., 1994. Geochemical changes in crude oil spilled from the *Exxon Valdez* supertanker into Prince William Sound, Alaska. Organic Geochemistry 21, 927–936.

Krebs, C.T., Burns, K.A., 1977. Long term effects of an oil

spill on populations of the salt marsh crab, *Uca pugnax*. Science 197, 484–487.

Kvenvolden, K.A., Carlson, P.R., Threlkeld, C.N., Warden, A., 1993. Hydrocarbons in oil residues on beaches of islands of Prince William Sound. Marine Pollution Bulletin 26, 24–29.

Literathy, P., Morel, G., Al-Bloushi, A., 1991. Environmental transformation, photolysis of fluorescing petroleum compounds in marine waters. Water Science Technology 23, 507–516.

McKirdy, D.M., Summons, R.E., Padley, D., Serafini, K.M., Boreham, C.J., Struckmeyer, H.I.M., 1994. Molecular fossils in coastal bitumens from southern Australia: signatures of precursor biota and source rock environments. Organic Geochemistry 21, 265–286.

Munoz, D., Guiliano, M., Doumenq, P., Jacquot, F., Scherrer, P., Mille, G., 1997. Long term evolution of petroleum biomarkers in mangrove soil (Guadeloupe). Marine Pollution Bulletin 34, 868–874.

Nadeau, R.J., Berquist, E.T., 1977. Effects of the March 18, 1973 oil spill near Cabo Rojo, Puerto Rico, on tropical marine communities. In: Proceedings of the 1977 Oil Spill Conference, American Petroleum Institute, pp. 535–538.

National Research Council, 1989. Using oil spill dispersants on the sea. Committee on Effectiveness of Oil Spill Dispersants. National Academy of Sciences Press, USA (335 pp).

Oudot, J., Dutrieux, E., 1989. Hydrocarbon weathering and biodegradation in a tropical estuarine ecosystem. Marine Environmental Research 27, 195–213.

Page, D.S., Boehm, P.D., Douglas, G.S., Bence, A.E., Burns, W.A., Mankiewicz, P.J., 1996. The natural petroleum hydrocarbon background in subtidal sediments of Prince William Sound, Alaska, USA. Environmental Toxicology and Chemistry 15, 1266–1281.

Pelletier, M.C., Burgess, R.M., Ho, K.T., Kuhn, A., McKinney, R.A., Ryba, S.A., 1997. Phototoxicity of individual polycyclic aromatic hydrocarbons and petroleum to marine invertebrate larvae and juveniles. Environmental Toxicology and Chemistry 16, 2190–2199.

Peters, K.E., Moldowan, J.M., 1993. The Biomarker Guide: Interpreting Molecular Fossils in Petroleum and Ancient Sediments. Prentice Hall, Englewood Cliffs, N. Jersey, p. 363.

Peters, K.E., Molowan, M., McCaffrey, M.A., Fago, F.J., 1996. Selective biodegradation of extended hopanes to 25-norhopanes in petroleum reservoirs. Insight from molecular mechanics. Organic Geochemistry 24, 765–783.

Pratt, C., 1997. Weathering of hydrocarbons in tropical mangrove sediments: using analytical techniques to test the effects of dispersants to treat oil spill. B.Sc. Honours Thesis, Chemistry Department, James Cook University, Townsville, Queensland.

Requejo, A.G., Quinn, J.G., 1983. Geochemistry of C_{25} and C_{30} biogenic alkenes in sediments of the Narragansett Bay estuary. Geochmica et Cosmochimica Acta 47, 1075–1090.

Sandstrom, M.W., Tirendi, F., Nott, A.L.J., 1986. Direct determination of organic carbon in modern reef sediments and calcareous organisms after dissolution of carbonate. Marine Geology 70, 321–329.

Scherrer, P., Mille, G., 1989. Biodegradation of crude oil in an experimentally polluted peaty mangrove soil. Marine Pollution Bulletin 9, 430–432.

Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M., 1993. In: Environmental Organic Chemistry. John Wiley & Sons, New York, 681pp.

Shinninghe Damste, J.S., Rijpstra, W.I.C., Schouten, S., Peltier, H., van der Maarell, M.J.E.C., Gieskes, W.W.C., 1998. A C_{25} highly branched isoprenoid alkene and C_{25} and C_{27} n-polyenes in the marine diatom *Rhizosolenia setigera*. Organic Geochemistry 30, 95–100.

Teal, J.M., Farrington, J.W., Burns, K.A., Stegeman, J.J., Tripp, B.W., Woodin, B., Phinney, C., 1992. The West Falmouth oil spill after 20 years: fate of fuel oil compounds and effects on animals. Marine Pollution Bulletin 24, 607–614.

Thomas, K.V., Donkin, P., Rowland, S.J., 1995. Toxicity enhancement of an aliphatic petrogenic unresolved complex mixture (UCM) by chemical oxidation. Water Research 29, 379–382.

UNEP/IOC/IAEA, 1992. Determination of petroleum hydrocarbons in marine sediments. (Prepared by Burns, K.A. et al.). IOC Manual and Guides No. 20, Rev. 1, (97 pp).

Volkman, J.K., Alexander, R., Kagi, R.I., Woodhouse, G.W., 1983. Demethylated hopanes in crude oils and their applications in petroleum geochemistry. Geochimica et Cosmochimica Acta 47, 785–794.

Volkman, J.K., Alexander, R., Kagi, R.I., Rowland, S.J., Sheppard, P.N., 1984. Biodegradation of aromatic hydrocarbons in crude oils from the Barrow Sub-basin of Western Australia. Organic Geochemistry 6, 619–632.

Volkman, J.K., O'Leary, T., Summons, R., Bendall, M.R., 1992. Biomarker composition of some asphaltic coastal bitumens from Tasmania, Australia. Organic Geochemistry 18, 669–682.

Volkman, J.K., Miller, G.J., Revill, A.T., Connell, D.W., 1994. Part 6. Oil spills. In: Swan, J.M., Neff, J.M., Young, P.C. (Eds.), Environmental Implications of Offshore Oil and Gas Development in Australia — Findings of an Independent Scientific Review. Australian Petroleum Production and Exploration Association, Sidney, pp. 509–696.

Volkman, J.K., Revill, A.T., Murray, A.P., 1997. Applications of biomarkers for identifying sources of natural and pollutant hydrocarbons in aquatic environments. In: Eaganhouse, R. (Ed.), Molecular Markers in Environmental Chemistry. American Chemical Society, Washington, DC, pp. 110–132 (Chapter 8).

Wardrup, J.A., 1987. The effects of oils and dispersants on mangroves: a review and bibliography. Occasional paper no. 2. Environmental Studies, University of Adelaide, South Australia, (70 pp).

Widdows, J., Burns, K.A., Menon, N.R., Page, D.S., Soria, S., 1990. Measurement of physiological energetics (Scope for Growth) and chemical contaminants in *mussel (Arca zebra)* transplanted along a pollution gradient in Bermuda. Journal of Experimental Marine Ecology 138, 99–117.