



## RESEARCH

# Bioremediation of Stranded Oil on an Arctic Shoreline

ROGER C. PRINCE†\*, RICHARD E. BARE†, ROBERT M. GARRETT†, MATTHEW J. GROSSMAN†, COPPER E. HAITH†, LOIS G. KEIM†, KENNETH LEE‡<sup>1</sup>, GRAHAM J. HOLTOM§, PATRICK LAMBERT††, GARY A. SERGY‡‡<sup>2</sup>, EDWARD H. OWENS§§<sup>3</sup> & CHANTAL C. GUÉNETTE†††<sup>4</sup>

†ExxonMobil Research and Engineering Co., Annandale, NJ 08801, USA

‡Centre for Offshore Oil and Gas Environmental Research, Bedford Institute of Oceanography, Fisheries and Oceans Canada, P.O. Box 1006, Dartmouth, NS, Canada B2Y 4A2

§AEA Technology, Abingdon, Oxfordshire, OX14 3DB, UK

††Environment Canada, Ottawa, ON, Canada K1A 0H3

‡‡Environment Canada, #200, 4999 – 98th Avenue, Edmonton, AB, Canada T6B 2X3

§§Polaris Applied Sciences, #302, 755 Winslow Way East, Bainbridge Island, WA 98110, USA

†††SINTEF Applied Chemistry, Environmental Engineering, 7034 Trondheim, Norway

The application of slow-release and soluble fertilizers proved to be an effective and environmentally benign way of stimulating oil biodegradation on an Arctic shoreline. Fertilizer application to the surface of the beach delivered nutrients to the oiled sediment beneath the beach surface. There was no significant run-off of this fertilizer to either the nearshore water or to unfertilized plots, and there were no adverse toxicological effects of the fertilizer application. The fertilizer application was followed by an increase in oxygen consumption and carbon dioxide evolution from the beach, increased microbial biomass, and significantly greater biodegradation of oil on the plots that had received fertilizer. The rate of oil biodegradation was approximately doubled over the course of a year by fertilizer applications in the first two months after the spill.

Simple test kits proved adequate to monitor the fertilizer-application process in the field in a time frame that would allow the application process to be fine-tuned during treatment on a real spill. Simple test kits and portable instrumentation were useful in demonstrating the initial success of the bioremediation strategy.

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## Introduction

Bioremediation is an environmentally acceptable and cost-effective treatment of oiled shorelines in temperate climates (Prince, 1993; Bragg *et al.*, 1994; Prince & Bragg, 1997; Lee *et al.*, 1995a, 1997; Swannell *et al.*, 1996; Venosa *et al.*, 1996; Swannell *et al.*, 1999; Lee & Merlin, 1999), and there have been strong indications that similar results can be expected in the Arctic (e.g., Sendstad *et al.*, 1982, 1984; Sveum, 1987; Sveum & Ladousse, 1989). Hydrocarbons are generally biodegradable and oil-degrading microbes are

\*Corresponding author. Tel.: +1-908-730-2134.

E-mail addresses: roger.c.prince@exxonmobil.com (R.C. Prince), leek@dfo-mpo.gc.ca (K. Lee), gary.sergy@ec.gc.ca (G.A. Sergy), ehovens@polarisappliedsciences.com (E.H. Owens).

<sup>1</sup> Tel.: +1-902-426-7344.

<sup>2</sup> Tel.: +1-780-951-8855.

<sup>3</sup> Tel.: +1-206-842-2951.

<sup>4</sup> Present address: 221B Russell Avenue, Ottawa, ON, Canada K1N 7X6.

ubiquitous, but oil biodegradation is limited in most marine environments by sub-optimal levels of nutrients such as biologically available nitrogen and phosphorus. Bioremediation of oil spills has thus focused on alleviating this limitation by adding fertilizers to oiled shorelines. Part of a project entitled *In Situ Treatment of Oiled Sediment Shorelines* (Sergy *et al.*, 1998, 2003) addressed the potential that bioremediation through the addition of fertilizer to oiled shorelines would stimulate oil biodegradation in Arctic climates. The primary objective was to demonstrate that bioremediation is an appropriate option to consider during the response to an oil spill that impacts an Arctic shoreline. Thus we set out to demonstrate that fertilizer application would result in the delivery of nutrients to the interstitial water surrounding oiled sediments. This treatment action would result in an increase in microbial activity, which could be measured by monitoring oxygen consumption, carbon dioxide evolution, and an increase in microbial biomass. If this occurred, changes in the composition of the residual oil would be expected that would reflect biodegradation. We also assessed any potential toxicity that might attend the application of fertilizers.

An important second element of the experimental design was the validation of field monitoring of the success of fertilizer applications to guide modifications of bioremediation strategies. We evaluated relatively simple test kits and portable instrumentation that could be used in the field.

Both objectives were achieved. Preliminary accounts of parts of this work have been published by Garrett *et al.* (1999) and Prince *et al.* (1999).

## Experimental Methods

### Site description

As discussed in detail by Sergy *et al.* (1998, 2003) and Guénette *et al.* (2003), the experiment was conducted on shorelines near Sveagruva, Spitsbergen (approximately 78° N, 17° E) in the summers of 1997 and 1998. Air and water temperatures in August, when oil and initial treatments were applied, were 3–7 °C, although interstitial water in the shoreline was typically slightly warmer (4–9 °C). The beach was a relatively sheltered wave environment with gravel above a shallow sub-tidal mud (Site 2 in Sergy *et al.*, 2003). The shore faces approximately southeast, with an approximately 14 km fetch to the Paulabreen glacier. Winds from the southeast generated 10–30 cm waves during the summer, and on occasion deposited small icebergs near the test beaches. Winds from other directions were offshore from the beach. The head of the fjord at the coal-loading terminal near our sites

was ice covered from December 3, 1997 to June 26, 1998, and the shoreline was ice-locked during this period.

### Oil

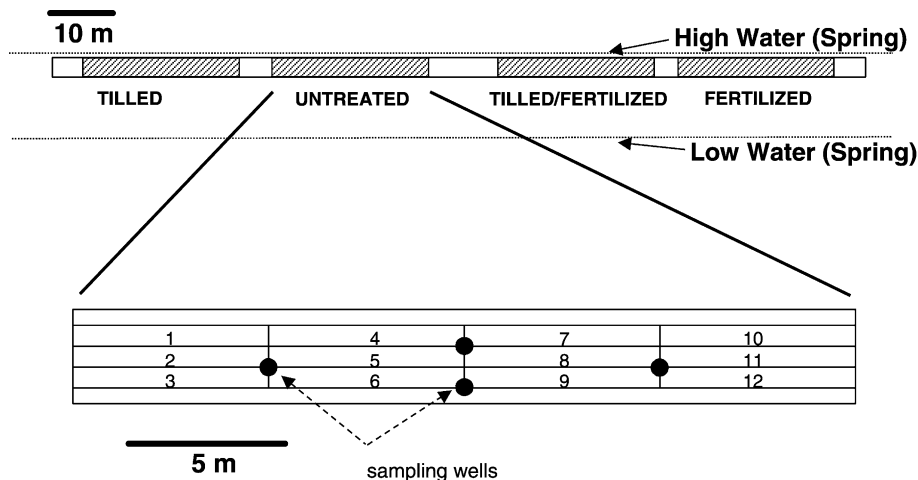
The test oil was IF-30, an intermediate fuel grade created by diluting relatively heavy distillate with lighter fractions to obtain the desired viscosity. It was applied at approximately 5 l m<sup>-2</sup> directly onto the shoreline and penetrated to a depth of approximately 15 cm. A total of 140 m of shoreline was oiled, and this was subsequently divided into four plots that were treated on a low tide one week after the oil was applied. One plot was left untreated, and two plots were tilled to a depth of approximately 20 cm by drawing tines through the plot both down the beach and across it (once in each direction) with a Bobcat front-end loader. This tilling extended to just below the depth of oil penetration. Full details of oil application and physical treatment of the plots are provided in Guénette *et al.* (2003) and Owens *et al.* (2003). Fertilizer was applied to one of these tilled plots after the first tilling, and to an untilled plot. Small sections of unoiled shoreline were treated with fertilizer as controls in the carbon dioxide evolution measurements. A schematic of the experimental plots is shown in Fig. 1. Perforated sampling wells (approximately 30 cm deep and 4 cm in diameter), in groups of three, were installed at four locations on each test plot to allow the collection of beach interstitial water from within the oiled sediment.

### Fertilizers

Both soluble (prilled ammonium nitrate and superphosphate (Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>); Hydro, Sweden) and slow-release (Inipol SP1, a slow-release formulation containing 18% N as NH<sub>4</sub>, and 1% P (as P<sub>2</sub>O<sub>5</sub>); CECA, Paris La Defense, France) forms of biologically available nitrogen and phosphorus were used. Ferrous sulfate (89% FeSO<sub>4</sub>·H<sub>2</sub>O, 5% MgSO<sub>4</sub>, 2% MnSO<sub>4</sub>; Christen Hoeg A/S, Norway) and yeast extract (Sigma, USA) were added to provide other potentially limiting nutrients. The timing and amounts of the fertilizer applications are listed in Table 1.

### On-site analytical procedures

Water samples were removed from the bottom of the wells with a 50 ml syringe, being careful not to generate any bubbles that might alter the oxygen concentration, and the water carefully placed in a small beaker. Nearshore water samples were collected with a beaker. Dissolved oxygen in the water samples



**Fig. 1** A schematic of the shoreline used in this work. The top shows the area of oiled shoreline in relation to high and low tide. The lower portion shows sampling areas and wells in one plot.

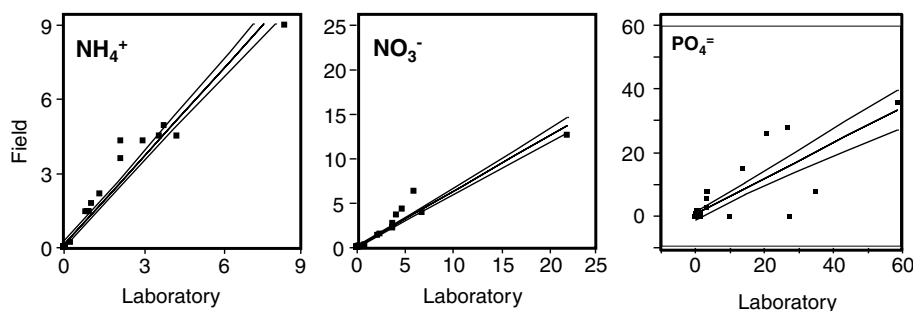
**Table 1** Fertilizer applications

Date	Experimental day	Fertilizer
Seven days after oiling [August 6, 1997]	0	100 g m <sup>-2</sup> prilled ammonium nitrate 10 g m <sup>-2</sup> superphosphate 1 g m <sup>-2</sup> ferrous sulfate 0.1 g m <sup>-2</sup> yeast extract
Fourteen days after oiling [August 13, 1997]	7	140 g m <sup>-2</sup> Inipol SP1 1 g m <sup>-2</sup> ferrous sulfate 0.1 g m <sup>-2</sup> yeast extract
Thirty days after oiling [August 29, 1997]	23	100 g m <sup>-2</sup> Inipol SP1
Sixty-six days after oiling [October 5, 1997]	58	50 g m <sup>-2</sup> prilled ammonium nitrate 5 g m <sup>-2</sup> superphosphate 1 g m <sup>-2</sup> ferrous sulfate 0.1 g m <sup>-2</sup> yeast extract 70 g m <sup>-2</sup> Inipol SP1

was measured immediately upon collection using Chemetrics K-7512 test kits (Chemetrics, Calverton, VA) which employ indigo carmine as an indicator. The salinity and temperature of water samples were measured on the beach using an Orion 130 conductivity meter (Orion Instruments, Beverly, MA). Carbon dioxide evolution from the shoreline sediment was measured *in situ* (Swannell *et al.*, 1994) with a Servomex (Crowborough, Sussex, UK) PA 404 infrared gas analyzer. The sampling head was a shallow dish, 16 cm in diameter and 5 cm deep, that was pressed into the sediment to achieve a reasonable gas seal. The CO<sub>2</sub> concentration in the head space was measured continuously, and recorded every 30 s for 3 min, or until CO<sub>2</sub> concentrations exceeded 500 vppm. The instrument was calibrated each day using an Ascarite filter to generate CO<sub>2</sub>-free air, and using ambient air levels as 340 vppm.

Other measurements were made in a small laboratory near the site within one hour of sample collection, although these measurements could have been made

on the beach if no shelter had been available. Nitrate, ammonium and phosphate levels in the interstitial water samples were measured using Chemetrics K-6902, K-1510 and K-8510 kits (Chemetrics, Calverton, VA). Nitrate is reduced to nitrite by cadmium, diazotized with a primary aromatic amine and coupled to a proprietary organic compound to produce a red azo-dye. Ammonium is detected by Nessler's reagent (alkaline mercuric iodide) yielding a yellow solution. Phosphate is measured by the reduction of ammonium molybdate by stannous chloride yielding a blue solution in the presence of phosphate. Laboratory studies confirmed that these tests accurately measured the nutrients down to a detection limit of approximately 1 μM phosphate, 6 μM ammonium and 2 μM nitrate. A representative set of samples was analyzed at the Maurice Lamontagne Institute, Fisheries and Oceans Canada, using a Technicon Autoanalyzer. The agreement of the Chemette data with this more rigorous quantitative analysis was excellent for ammonium and nitrate, as shown in Fig. 2 and Table 2, although



**Fig. 2** Comparison of nutrient analyses in the field with the Chemette systems and laboratory measurements. Ammonium and nitrate are in mM, phosphate in  $\mu\text{M}$ . The outer lines are the 95% confidence curves of the fits described in Table 2.

**Table 2** Comparison of field and laboratory measurements of nutrients in interstitial and seawater ( $n = 54$ )

Analyte	Offset ( $\mu\text{M}$ )	Slope	$r^2$
Ammonium	84	1.19	0.96
Nitrate	67	0.62	0.95
Phosphate	0.38	0.56	0.67

rather poorer for phosphate. The slope of the ammonium data is very close to 1, but it is clear that the field nitrate test kit underestimates the amount of nitrate by about 40%. This is in accord with the manufacturer's indication that there is a systematic underestimate of nitrate levels in seawater. No attempt has been made to correct for this in the data presented below. The poorer correlation for the phosphate analyses is probably a reflection of the low levels of this nutrient, and it is not clear whether the test kit is very useful as a quantitative test in the field. Nevertheless, it was useful as a qualitative indicator of the presence of phosphate on fertilized plots and not on unfertilized plots, and we present the data here.

#### Other analytical procedures

Oil was extracted from sediment samples by the large sample extraction procedure described in detail in Guénette *et al.* (2003). The extracted oil was analyzed by gas chromatography/mass spectroscopy (GC/MS) essentially following published procedures (Douglas *et al.*, 1992). Separation was performed on a Hewlett Packard HP 5890 gas chromatograph fitted with a  $30\text{ m} \times 0.25\text{ mm}$  fused silica capillary column with 5% crosslinked phenyl methyl silicone as the stationary phase. Helium was used as the carrier gas at a flow rate of 1 ml/min. Samples of 1  $\mu\text{l}$  (approximately 30  $\mu\text{l}$  oil/ml methylene chloride) were injected automatically, without splitting, by an HP 6890 Injector. The column temperature was set to 45  $^{\circ}\text{C}$  for the first 4 min, increased 8  $^{\circ}\text{C}/\text{min}$  to a temperature of 270  $^{\circ}\text{C}$  then increased 5  $^{\circ}\text{C}/\text{min}$  to 310  $^{\circ}\text{C}$  and maintained at 310  $^{\circ}\text{C}$  for 5 min. Mass spectral data were

obtained with a Hewlett Packard 5972 mass selective detector at an electron energy of 70 eV over a mass range of 35–500 atomic mass units in the total ion mode, or in selected ion monitoring mode. Spectral tuning with perfluorotributylamine followed USEPA method 8270C (USEPA, 1996). A total of 10 batches of samples were run over a period of 14 months.

Since the emphasis was on changes in the oil, we did not add external reference compounds to the samples, before or after extraction. Instead, a sample of the initial oil was run with each batch of samples, and a few samples were run in duplicate at the beginning and end of the entire sample set (some 110 beach samples). Table 3 provides a summary of some of the analytical ratios determined for the reference oil. Ratios of analytes that elute relatively closely on the column show smaller standard deviations than those to hopane, which elutes relatively late in the chromatography. This is probably due to subtle changes in mass selectivity over the period during which these samples were analyzed. Nevertheless, reproducibility was more than adequate for the data analyses performed below. Six samples were run at the beginning and middle of the analytical period, and unbiased estimates of the variance of several ratios was less than 6%, as shown in Table 4.

Sediment samples collected on day 59 (after treatment) were sent (frozen) to Microbial Insights, Inc. (Rockford, TN), where phospholipid fatty acids were extracted and analyzed as described by White *et al.* (1996).

#### Toxicity testing

Potential toxicity of beach sediments was monitored with the Microtox® Solid Phase Test protocol (Lee *et al.*, 1995b, 1997), which is a modification of that used by True and Heyward (1990). Essentially the sediment is exhaustively extracted with methylene chloride, the methylene chloride is replaced by ethanol, and dilutions are made with saline so that the final Microtox® assay contains <1% ethanol. Semi-

**Table 3** Means, standard deviations and standard errors as % of selected analyte ratios for the IF-30 standard oil measured with each set of beach samples (*n* = 10)

Ratio	Mean	Standard deviation	Standard error as %
C <sub>17</sub> :pristane	0.63	0.01	0.5
C <sub>17</sub> :hopane	13.46	2.15	6.1
C <sub>18</sub> :phytane	0.78	0.02	1.0
C <sub>18</sub> :hopane	11.22	1.81	5.4
Phenanthrene:hopane	2.53	0.46	6.1
Phenanthrene:dimethyl-phenanthrenes	0.38	0.01	0.8

**Table 4** Unbiased estimates of the variances of diagnostic ratios of six samples measured at the beginning and midway through the experiment

Ratio	Variance (%)
C <sub>17</sub> :pristane	2.95
C <sub>17</sub> :hopane	5.77
C <sub>18</sub> :phytane	1.05
C <sub>18</sub> :hopane	5.97
Phenanthrene:hopane	5.09
Phenanthrene:dimethyl-phenanthrenes	3.70

The variance was estimated as half the average absolute difference between measurements on the two dates.

permeable membrane devices (Huckins *et al.*, 1990) containing triolein were used in an attempt to concentrate nonionic organic molecules that might be washing from the sediment. The devices were low density polyethylene tubes with a wall thickness of 0.80 μm containing 1 ml of synthetic triolein (95%, Sigma), suspended in perforated stainless steel containers for protection. Triplicates were anchored in the sediment for each treatment, and at an unoiled location adjacent to Sveagruba’s coal-loading facility, in direct contact with the fine sediment. After exposure, the sampling tubes were frozen until analysis, and then washed sequentially with water, methylene chloride, acetone and isopropanol before being air dried. The lipid content was extracted with a 1:1 acetone dimethylsulfoxide mixture, which was then evaporated. Acute toxicity of the extracts was determined using the Microtox® assay (Lee *et al.*, 1995b, 1997). The toxicity data are presented in toxicity units, the reciprocal of the EC<sub>50</sub>. Data for the semi-permeable membrane devices are corrected for exposure time.

**Statistical analyses**

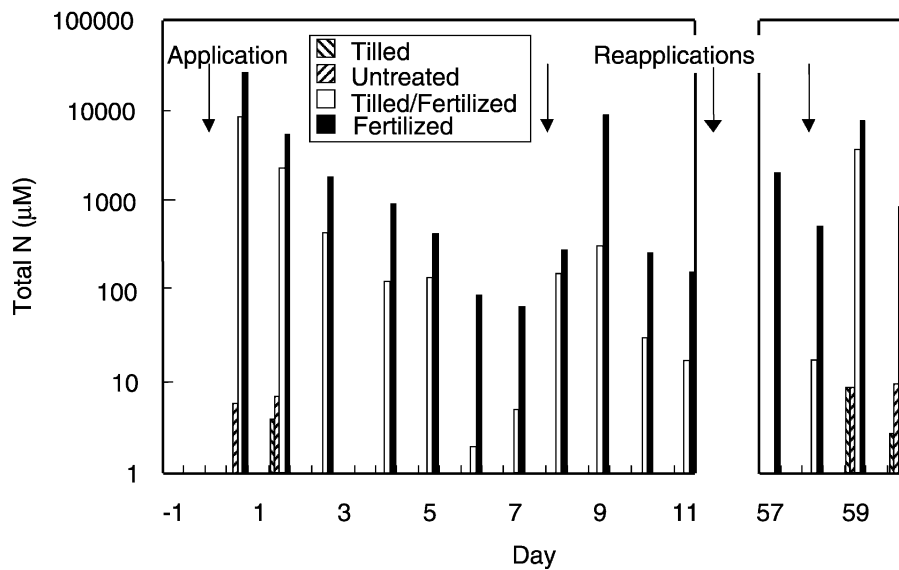
Statistical analyses relied on JMP software (v. 3.2, SAS Institute, Cary, NC). Logistical, ethical and financial constraints limited the size of the oiled shoreline available for experimentation, and we considered that 20 m was a minimum size for an experimental plot to minimize “edge effects” and wash-over of fertilizer from one plot to another (Sergy *et al.*, 2003). Thus our plots are not replicated, and in a strict

sense (see Hurlbert, 1984) we cannot be sure that any differences we see between plots are related to our treatments. But there is no doubt that the biodegradation of beached oil in most marine environments is limited by the availability of essential nutrients such as biologically useful nitrogen and phosphorus (Prince, 1993, 2002; Swannell *et al.*, 1996). The important question was whether adding fertilizer would overcome this limitation. Our experimental program, with its attendant statistical analyses, was therefore designed to measure a progression of phenomena that together would, in the words of Hurlbert (1984), allow “Common sense (and) biological knowledge (to) provide grounds for assessing whether there was a treatment effect”.

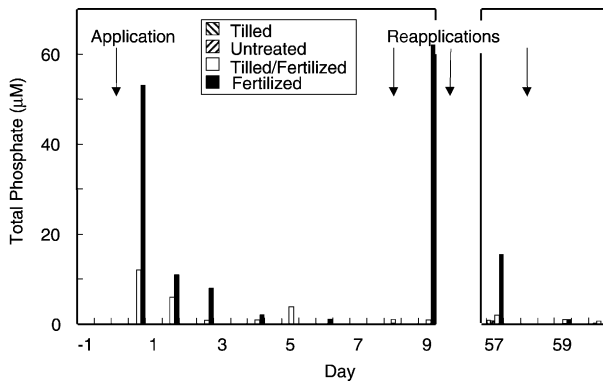
**Results and Discussion**

Both soluble and slow-release fertilizers were effective at delivering nitrogen and phosphorus nutrients throughout the oiled sediment, as shown in Figs. 3 & 4. Only very low levels of nutrient were ever detected on the unfertilized plots or in nearshore water collected just offshore, and these values were close to the detection limits of the analytical procedures. Fertilizer nutrients in the form of ammonium and phosphate were still present in water from one of the wells in October, indicating that the final application of fertilizer in August was still having at least some effect some six weeks later. The fertilizer application in October was effective at delivering nitrogenous nutrients, despite the low temperatures during this period. The different fertilizer applications (Table 1) provided different relative levels of ammonium and nitrate, and so their relative contributions were different after each fertilizer application, but both forms of nitrogen showed similar dilution kinetics after application.

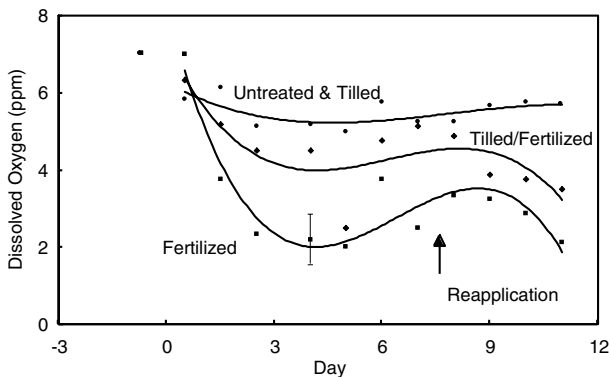
The delivery of fertilizer nutrients to the oiled sediment was followed by the consumption of oxygen, as illustrated in Fig. 5. There was an increase in carbon dioxide evolution from the fertilized oiled sediment, as shown in Fig. 6. We attribute these phenomena to an increase in microbial activity where there was both oil and fertilizer nutrients. This in turn was followed by an increase in microbial biomass on those portions of



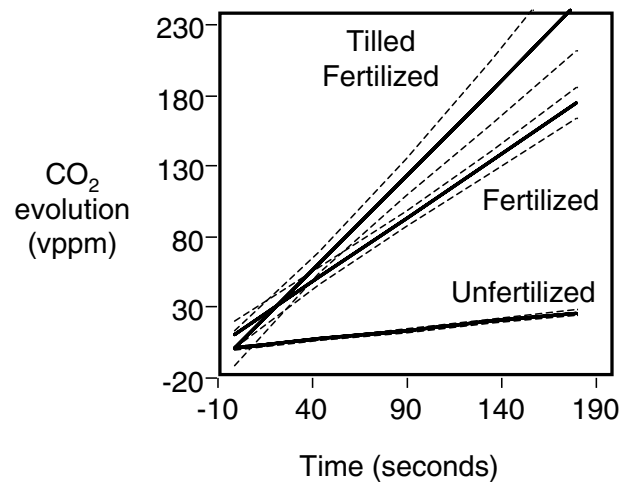
**Fig. 3** Average total fertilizer nitrogen (sum of ammonium and nitrate) in interstitial water on the plots. Note that the abscissa is a logarithmic scale. The data reflect the average of typically four samples per treatment per day, and the detection limit for an individual sample was approximately 10  $\mu\text{M}$ . The high levels on Day 57 were due to a single well, which apparently still had fertilizer nutrients from the third application in August.



**Fig. 4** Average phosphate levels in interstitial water on the plots. The data reflect the average of typically four samples per treatment per day, and the detection limit for an individual sample was approximately 1  $\mu\text{M}$ .

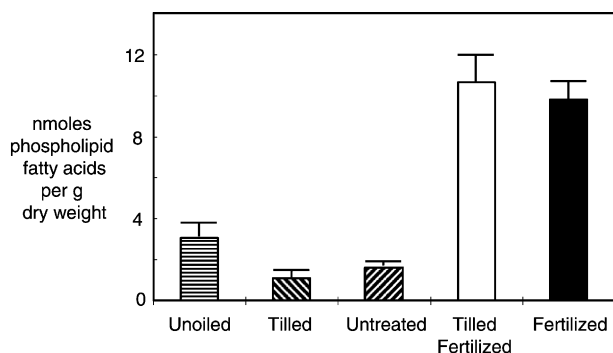


**Fig. 5** Dissolved oxygen in interstitial water from fertilized, tilled and fertilized, and untreated and tilled oiled sediments. The data reflect the average of typically four samples per treatment per day, and the one error bar reflects typical values for  $\pm 1$  standard deviation.



**Fig. 6** Average rates of  $\text{CO}_2$  evolution (in volume, vppm) on tilled/fertilized and fertilized portions of the beach were significantly greater than on the unfertilized portions of the oiled beach. The dashed lines reflect 95% confidence limits on the slopes. The unfertilized slope includes data from the untreated and tilled plots of the beach, and data collected at an adjacent unoiled beach with no treatment, and following application of the fertilizer nutrients in the absence of oil; these data sets were indistinguishable. Total number of measurements = 80.

the beach where oil and fertilizer were both present, as shown in Fig. 7. Microbial biomass was estimated by quantitative analysis of phospholipid fatty acids (White *et al.*, 1996). It is noteworthy that the oiled plots without fertilizer showed little evidence of increased microbial activity due to the presence of oil; there was no noticeable increase in  $\text{CO}_2$  evolution over unoiled controls (see Fig. 6), and no detectable increase in microbial biomass, measured as microbial

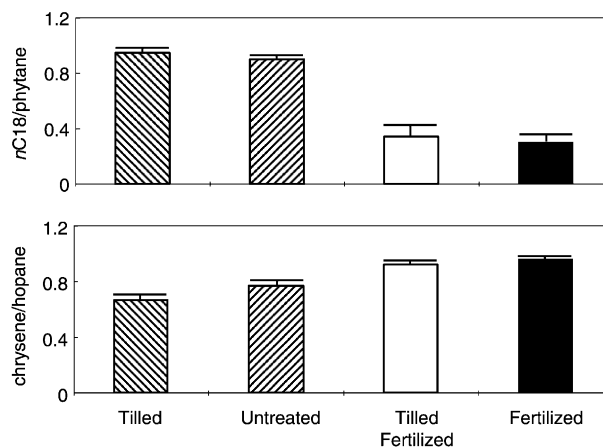


**Fig. 7** Nanomoles of phospholipid fatty acid per gram of sediment (dry weight) in October 1997, 59 days after treatment. Error bars reflect the standard error of the estimates. The amounts of phospholipid fatty acid in oiled and untreated and oiled and tilled plots were statistically indistinguishable from the unoiled remote site, while the fertilized and tilled/fertilized plots had significantly more phospholipid fatty acid (at  $p < 0.005$ ).

phospholipid fatty acids (Fig. 7). Nevertheless there was clear evidence that biodegradation was occurring on the unfertilized portions of the beach, albeit at a slower rate than on fertilized plots.

We have reported elsewhere (Grossman *et al.*, 2000) that the increase in microbial biomass on oiled and fertilized plots is accompanied by a significant change in the relative abundance of microbial taxonomic groups, particularly of those known to include hydrocarbon-degrading species.

As discussed in detail by Owens *et al.* (2003) and Lee *et al.* (2003), the major losses of oil from this shoreline were physical, most likely in association with microscopic sediment fines. Some of this oil was caught in sediment traps, and as discussed by Lee *et al.* (2003), this oil was undergoing biodegradation as it left the beach. We have addressed the biodegradation of the residual oil on the beach by looking in detail at its chemical composition using GC/MS. Aerobic biodegradation causes characteristic changes in the composition of crude oils; linear alkanes and small aromatic hydrocarbons are degraded most readily, followed by branched alkanes and polycyclic aromatic hydrocarbons, followed by alkylated polycyclic aromatic hydrocarbons and saturated ring compounds (Prince, 2002; Garrett *et al.*, 2003). Some molecules are not degraded on timescales relevant to bioremediation, and we have found that using such compounds as conserved internal markers within the oil allows a quantitative determination of the extent of biodegradation. For this approach to work, the compound of choice must have been present at a similar concentration in all the oil under study. In crude oil,  $17\alpha(H),21\beta(H)$ -hopane is particularly useful (Prince *et al.*, 1994; Venosa *et al.*, 1997; Oudot *et al.*, 1998; Brown *et al.*, 1998), but in this field trial we found that the initial concentration of hopane in the oil on dif-



**Fig. 8** The ratios of octadecane to phytane (upper) and chrysene to hopane (lower) in oils collected on Day 0 from different plots. Error bars reflect the standard error of the estimates. In both cases the ratios of the oils on the plots with fertilizer are statistically different ( $p < 0.05$ ) from those of the oils on the plots without fertilizer.

ferent parts of the experimental beach was different at the first sampling. In fact, as shown by the two ratios illustrated in Fig. 8, the oil on the plots with fertilizer appeared to be rather different from that on the plots without fertilizer. This suggests that the IF-30 oil, which is made by diluting heavy refinery fuel with lighter fractions to achieve a specified viscosity, was not well mixed prior to filling the individual drums at the refinery.

Fortunately the ratio of phenanthrene to the dimethyl- and ethyl-phenanthrenes was the same on all the plots (Fig. 9), so we used this ratio to assess biodegradation. We have previously used methylated phenanthrenes as conserved markers in following the biodegradation of diesel (Douglas *et al.*, 1992), because this refined product contains essentially no hopanes, and we discuss its use in more detail in the accompanying paper (Garrett *et al.*, 2003). Although



**Fig. 9** Changes in the ratio of phenanthrene to the dimethyl- and ethyl-phenanthrenes in oils collected on Days 0 and 399. Error bars reflect the standard error of the estimates. The samples collected on Day 0 on the different plots are statistically indistinguishable, but the differences between Day 0 and 399 are statistically significant ( $p < 0.05$ ) on each plot.

alkylated phenanthrenes are indeed biodegradable (Prince, 2002; Garrett *et al.*, 1999, 2003), their biodegradation does not usually occur until a substantial fraction of the oil has been biodegraded; in this case approximately 50% (Garrett *et al.*, 1999, 2003). At worst, using alkylated phenanthrenes as “conserved markers” will lead to an underestimate of biodegradation since the possibility exists that they are being consumed along with the more degradable components of the oil.

Figure 9 indicates that the ratio of phenanthrene to dimethyl- and ethyl-phenanthrenes changed the most on the two plots that received fertilizer. Combining the data into plots with and without fertilizer, and including data from Day 59, the last sampling date in 1997, yields Fig. 10. This figure indicates that the change in phenanthrene to dimethyl- and ethyl-phenanthrene ratio, and by inference (Garrett *et al.*, 2003) the rate of oil biodegradation, is approximately twice as rapid on the plots that received fertilizer. Tilling had no statistically beneficial effect in stimulating biodegradation.

Since Fig. 10 presents a change in ratio rather than a change in absolute concentration, it is important to bear in mind that biodegradation is both a surface phenomenon, and one limited by the availability of nutrients. Thus, a thin film of oil will likely show a greater change in ratio than a thicker one, even if more hydrocarbon is degraded in the thicker film. As discussed in detail by Sergy *et al.* (2003), the fertilized plots lost less oil to physical processes than the unfertilized ones, so in fact Fig. 10 is likely to underestimate the stimulatory effect of fertilizer.

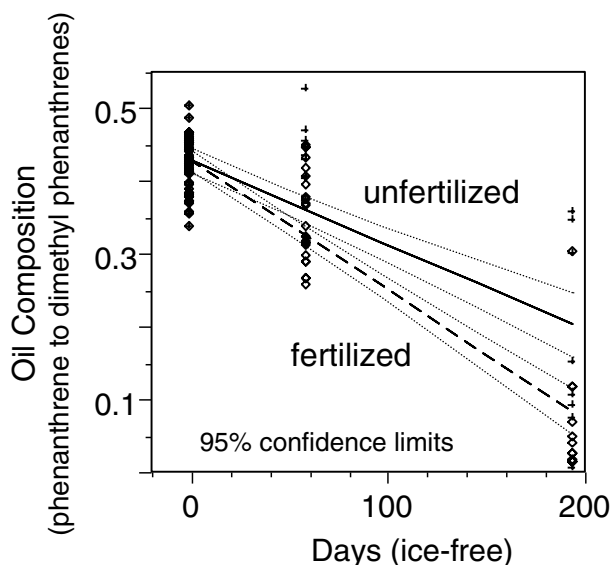


Fig. 10 Changes in the ratio of phenanthrene to dimethyl- and ethyl-phenanthrenes over time on fertilized and unfertilized portions of the beach. The pale lines reflect the 95% confidence limits of the slopes.  $n = 54$  for each slope.

The acute toxicity of the oil on the sediments was examined both by extracting the oil into methylene chloride and then ethanol, and by exposing semi-permeable membrane devices to the fine sediment so that nonionic material might be absorbed. Results of Microtox® assays of these materials are shown in Figs. 11 & 12. Acute sediment toxicity (Fig. 11) was highest on the fertilized portion of the beach on Days 0 and 1 (seven days after oil was applied to the shoreline), but this dropped substantially by Day 5. We attribute this to an effect of oil concentration rather than to an effect due, for example, to the stimulation of the generation of toxic metabolites by the bioremediation strategy because it is unlikely that these had accumulated and disappeared with these kinetics. We note that the toxicity test used here does not assess any toxicity due to the fertilizer components themselves. The semi-permeable membrane devices did not reveal any increase in toxicity over that experienced at an unoiled, although not necessarily pristine, site (Fig. 12).

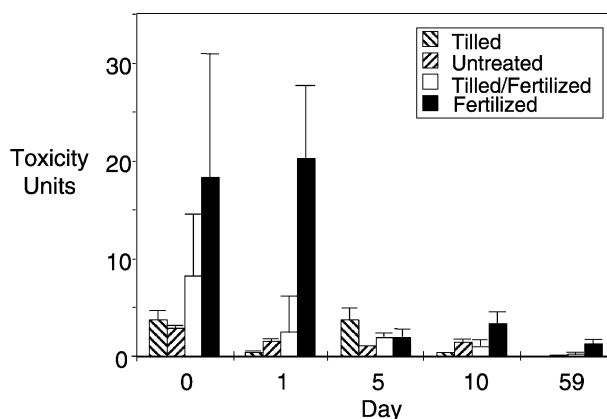


Fig. 11 Acute toxicity of shoreline sediment extracts. Units are the reciprocal of the  $EC_{50}$ , multiplied by 10,000 for convenience. Error bars reflect the standard error of the data. No toxicity could be detected at Day 399.

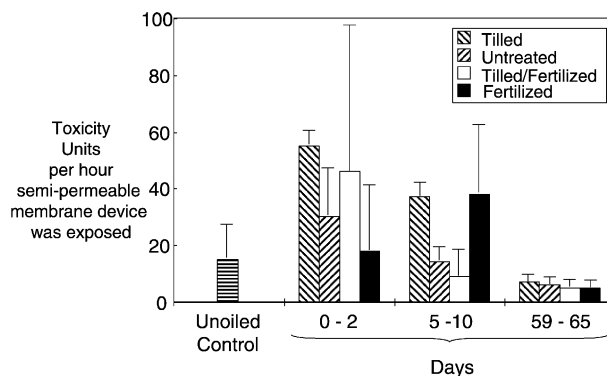


Fig. 12 Acute toxicity of material extracted into the semi-permeable membrane devices. Units are the reciprocal of the  $EC_{50}$ , multiplied by 10,000 for convenience and by the reciprocal of the exposure time for normalization. Error bars reflect the standard error of the data.

## Conclusions

The data presented here show all the characteristics expected of successful bioremediation on this Arctic shoreline:

- The application of fertilizer resulted in elevated levels of biologically available nitrogen and phosphorus in oiled sediment where fertilizer was applied, but there was no significant run-off of this fertilizer to either the nearshore water or to unfertilized plots, and there were no adverse toxicological effects of the fertilizer application.
- The delivery of nutrients was followed by an increase in oxygen consumption and carbon dioxide evolution from the beach within the first two weeks.
- This was reflected in increased microbial biomass, measured 59 days after treatment, on those plots that received fertilizer.
- This was followed by significantly greater biodegradation of oil on the plots that had received fertilizer.
- No toxicity associated with the bioremediation strategy was detected.

These logically consistent findings allow "Common sense (and) biological knowledge" (Hurlbert, 1984) to lead to the conclusion that bioremediation was successful on the mixed-sediment beaches of Spitsbergen. It is noteworthy that fertilizer applications over a relatively short period had a pronounced effect on the chemistry of the residual oil one year later.

The results allow future uses of bioremediation as a treatment option to be inexpensively "fine-tuned" during application. Simple test kits and portable instrumentation were able to demonstrate many of the phenomena expected during a bioremediation response, thus the fertilizer-application strategy could be modified within days. Simple wells installed in fertilized beaches and simple test kits would reveal whether a particular fertilizer-application rate was appropriate, and how often fertilizer should be reapplied to maintain a desired level of nutrients in a shoreline. We believe a sustained delivery of an average of 100  $\mu\text{M}$  available nitrogen to the oiled sediment is a useful target for stimulating biodegradation while minimizing adverse environmental impacts.

Simple measurements of dissolved oxygen and carbon dioxide should be able to provide confidence that a bioremediation strategy is indeed working.

Together, these should go a long way to improving the use and acceptance of bioremediation as a cost-effective and environmentally beneficial response option to oil spills that affect shorelines.

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