Potential Ecological Effects of

Chemically Dispersed and Biodegraded Oils

Evaluation of components and concentrations relevant to policy decisions.

Mytilus edulis © K. Hiscock
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(480 Extension)

Final Report

Corophium volutator © T.Galloway

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List of Abbreviations

ANS	Alaskan North Slope crude oil
BTEX	Benzene, Toluene, Ethylbenzene and Xylenes
C9527	Corexit 9527 dispersant
DCM	Dichloromethane
DEFRA	Department for Environment, Food and Rural Affairs
DTI	Department of Trade and Industry
DWAF	Dispersed water accommodated fraction
FB	Forties Blend crude oil
GC-MS	Gas Chromatography-Mass Spectrometry
MCA	Maritime and Coastguard Agency
MMS	Minerals Management Service
PAH	Polycyclic aromatic hydrocarbons
ppm	Parts per million
SD25	Superdispersant-25
SOP	Standard Operating Procedure
TLC-FID	Thin Layer Chromatography-Flame Ionisation Detection
UVF	Ultraviolet Fluorescence Spectroscopy
WAF	Water accommodated fraction

Foreword

The Maritime & Coastguard Agency Counter Pollution Branch is responsible for responding to oil pollution occurring in the United Kingdom (U.K.) Pollution Control Zone. In sponsoring research project RP 480 (June 2002-May 2005), the MCA, along with the Department for Environment, Food and Rural Affairs and the Department of Trade and Industry, required a quantitative assessment of the ecological risks of chemically dispersing oils in waters around the U.K. The Minerals Management Service (United States (U.S.) Department of the Interior) required a similar assessment for U.S. waters. Forties Blend crude oil (FB) in conjunction with Superdispersant-25 (SD-25) was used for modelling the effects of possible U.K. spill scenarios and Alaskan North Slope (ANS) crude oil was used in conjunction with Corexit 9527 (C-9527) dispersant to simulate possible U.S. oil spill scenarios.

The assessments were made at the University of Plymouth, U.K., and the effects of dispersing oil were evaluated utilising environmentally relevant end points involving measurements of the feeding rates, growth and reproduction of two representative marine organisms. The mussel, *Mytilus edulis*, was used to determine any detrimental effects of oil in the water column, and the amphipod, *Corophium volutator*, to measure the risk posed by oil trapped in sediments. Rigorous attention was paid to quality control in all of the experiments and chemical analyses of all water, sediment and mussel tissue were carried out using standardised methods.

Overall, the experiments conducted showed that although chemically dispersed oil may initially impact mussels and amphipods to a greater extent than would untreated oil, the organisms were mostly able to recover to the same extent as control organisms or to those exposed to oil alone. The exception to this was some exposures of C-9527 dispersed water-accommodated fractions of ANS oil to mussels and amphipods where dispersion led to the highest concentrations of oil in the water and sediments.

The above project generated a large amount of additional chemical data in the form of gas chromatography-mass spectrometry (GC-MS) profiles of the hydrocarbon distributions in over 150 mussel and sediment extracts. Time and resources did not allow for processing of these data in the original study. However on securing additional funding, a proposal was made to 'data mine' this large amount of information to try to provide an insight into what individual hydrocarbons might be responsible for the toxic effects observed, and, if possible, to determine the critical 'cut off' concentrations beyond which organism recovery would no longer be observed. The aim of the present work was thus to provide identification of individual toxicants by GC-MS library matching of spectra, measurement of toxicant concentrations by comparing GC-MS mass fragmentogram peak areas with the responses of known internal standard hydrocarbons and comparison of these concentrations with the biological effects data.

Executive Summary

In previous exposure experiments, (Smith *et al*., 2006, Final Report RP 480) the toxicological impacts of crude oil/dispersant mixtures on the mussel, *Mytilus edulis* and on the amphipod *Corophium volutator* were assessed under a variety of simulation scenarios and the effects were compared with the concentrations of total oil or oil and dispersant as measured by non-specific ultraviolet fluorescence spectroscopy. In the present extension to that work (RP 480 Extension) the concentrations of individual and unresolved hydrocarbons were determined by further extensive processing of the data from over 150 GC-MS analyses obtained previously ('data-mining'). Due to the large number of files to be processed, individual hydrocarbons were assigned, or tentatively assigned, only by automated computer mass spectral library matching. Caution needs to be applied to over reliance on such automated assignments. The concentrations of compounds thus assigned were calculated *versus* the averaged response of three internal standard compounds added in known concentrations to the mussels or sediment substrates in which the amphipods were placed during testing. The responses of these internal standards were also variable and again caution should be used in applying too much credence to these averaged data. In addition, an estimate of the proportions of so-called unresolved complex mixtures (UCMs) of hydrocarbons was made. This estimate includes both unresolved non-aromatic hydrocarbons and unresolved aromatic hydrocarbons. To date only the latter have reported toxicity to mussels. The data were compiled as a series of Microsoft Excel spreadsheets to facilitate possible future use by the funding agencies or their partner laboratories.

The hydrocarbon data were compared with the toxicological data and the impact of some classes of hydrocarbons (mainly UCMs) was assessed. There was a generally poor correlation between the toxicological and chemical data, but some general trends emerged. The most consistent feature of the extracts of the mussels and of the sediments to which the amphipods were exposed (either containing oils or the dispersed oils) was the presence of elevated concentrations of components assigned by computer as alkylnaphthalenes and alkylphenanthrenes, and to UCMs of hydrocarbons, in the intoxicated mussels and adulterated sediments. Although no clear dose-response relationships emerged from most of the data, these components nonetheless appeared to be present in several of the exposures where toxic effects were observed and more were present, and more often, in animals exhibiting reduced feeding (mussels) or growth (amphipods) rates. The generally poor correlation suggests that components other than hydrocarbons also contributed to the measured toxic effects and that perhaps this contribution differed in the different oils and dispersants.

A very broad correlation was observed between UCM concentrations resulting from exposure of mussels to FB oil mixtures and reduced mussel feeding rates. It was not possible to estimate an accurate toxicity cut-off from these data but a 50% reduction in feeding rates corresponded to UCM concentrations of in the region of *very approximately* 30-100 µg g^{-1} wet weight (probably about 150-500 µg g^{-1} dry weight). This is broadly comparable to the few values available from laboratory and field studies of toxic effects of aromatic hydrocarbon UCMs on mussels (reported tissue effective concentrations (TEC_{50}) of about 120 and 500 µg g^{-1} wet weight have appeared). No such correlation was apparent for ANS oil/dispersants, suggesting other components also contributed to the observed toxicity.

An approximate correlation was found also, between decreased amphipod reproductive success and increased UCM concentrations for ANS oil mixtures, with most pronounced deleterious effects above very approximately 250 µg UCM g dry weight $sediment^{-1}$. In agreement with this, in separate experiments when amphipods were exposed to 500 µg nominal oil g sediment dry $weight^{-1}$ of more weathered ANS crude for 35 days, a slight but significant ($P \leq 0.05$) reduction in growth and a pronounced significant ($P \leq 0.05$) adverse effect on reproductive success was measured.

So far as policy recommendations are concerned, it is therefore concluded that in future oil spill monitoring following dispersant treatment, in addition to quantitation of toxic alkylnaphthalenes and alkylphenanthrenes, the quantitation of UCMs of hydrocarbons and quantitation and identification of more the polar toxicants would also be desirable.. This might provide more detailed information on the critical concentrations of these components required to produce toxicological responses. Whilst some monitoring programmes do involve determination of alkylnaphthalenes and alkylphenanthrenes, the concentrations of UCMs of (particularly aromatic) hydrocarbons and polar chemicals are more rarely reported. In addition- since it is likely that some of the non-correlation between the toxicity and hydrocarbon results was due to the toxic effects of the dispersants, the concentrations of which were not measured in the study, it is recommended that determination of dispersant concentrations also be carried out in future studies of organisms impacted by dispersant-treated spills.

1. Background

The use of oil spill dispersants as an appropriate oil spill response method in some circumstances is based on the assessment that dispersing oil into the sea is likely to be less ecologically damaging than allowing it to move into shallow water and/or to impact the shoreline. It is known that dispersing oil carries some risk of causing effects on marine organisms and critics of dispersant use suggest that dispersing oil may cause long-term effects, especially if the dispersed oil becomes incorporated into sediments.

A previous project (Smith *et al*., 2006) evaluated the effects of dispersing oil using mussels and amphipods with environmentally relevant end points such as feeding rate, growth and reproduction. The results showed that although chemically dispersing oil may initially impact these organisms to a greater extent than non-chemically dispersed oil, the organisms were mostly able to recover to the same extent as control organisms when they were then exposed to cleaner water, thus maintaining a net environmental benefit in using dispersants at the recommended concentrations.

The previous project (Smith *et al*., 2006) also generated a large amount of additional chemical data in the form of data for hydrocarbons in over 150 mussel and sediment extracts. The purpose of the present study was to 'data mine' this large amount of information to provide an insight into what individual components might be responsible for the effects observed, and to try to determine the critical 'cut off' concentrations beyond which recovery might no longer be observed.

2. Objectives and Milestones

The objective of the present study was to carry out a desk-top in depth scrutiny of the larger than expected amounts of hydrocarbon and toxicological data that emerged from the previous report (Smith *et al*., 2006). The aim was to scrutinise the data from over 150 separate analyses of mussel and sediments from laboratory studies which had attempted to assess the effects of dispersing oils on toxicity and biodegradation, to try to determine what compounds are important in the responses observed, and potentially to calculate 'cut off' concentrations for effects which may be useful in modelling of oil spill fate and effects.

<u>Objectives:</u>

1. Evaluate toxicity contributions from the hydrocarbons found in sediments and accumulated/depurated in mussel tissue.
2. Determine the depuration-recovery levels and critical cut off levels of identified hydrocarbons for recovery of the organisms.
3. Make data available in a form compatible with future oil spill modelling and monitoring exercises.

These objectives were addressed by fulfilling the following:

Milestones:

1. Quantification of hydrocarbons and toxicity contributions (data reported in March 2006)
2. Depuration-recovery levels assessed (data reported herein)
3. All data summarised and reported (data reported herein)

All the data evaluated for this project have been transformed from that provided in March 2006 (interim report) to a more user friendly format. Data from different experiments have been directly compared with the toxicological data also provided herein.

3. Methods

The experimental exposure methods and analytical methods have been extensively detailed previously (Smith *et al*., 2006). Additional experimental details can been found in Scarlett *et al*., (2005; 2006).

3.1 Data Processing.

The identities of individual chemicals were determined by comparing the mass spectra of chemicals resolved by gas chromatography with those of the NIST library of mass spectra. A percentage fit was recorded.

The concentrations of individual chemicals were determined by comparison of the integrated gas chromatographic (total ion current) peak areas with those of internal standard compounds added at known concentrations (Smith *et al*., 2006) and are expressed in $\mu g\ g^{-1}$ dry and wet weight. In addition, subtraction of the integrated area of individual resolved compounds from the area due to the resolved plus so-called unresolved complex mixtures (UCMs or 'humps: Rowland *et al*., 2001) allowed an estimation of the concentrations of the UCMs to be made.

GCMS data were transferred to Microsoft Excel datasheets (Appendices 1-3).

The data given for the analysis of the mussel tissue shows the experiment number and the exposure regime (i.e. CONtrol, WAF, DWAF) which directly relates to the feeding rate data shown herein. The compounds identified with their library match percentage (match) and retention time (RT) are shown. The results are expressed as an average over the internal standards used and only those compounds with a library fit above 80% are reported.

The data shown for the sediment analyses are given as the oil (Forties/ANS), the day and the exposure, once again relating directly to the toxicological data provided herein, and are provided in the same format as the data for the analysis of the mussel tissue thus making the data more user friendly than in the original form provided in March 2006.

4. Results

4.1 Comparison of toxic effects on *Corophium volutator* with sediment hydrocarbon concentrations.

Scrutiny of the quantification and identification GC-MS data indicated that in most samples the number and concentrations of resolved components was low compared to the background of PAH and the UCM hydrocarbons. The UCM concentrations varied however, suggesting that this fraction originated both from hydrocarbons present as background and as weathered oil added and degraded further during the assays. Where additional resolved compounds were identified by the mass spectral computer matching, a series of alkylnaphthalenes and alkylphenanthrenes was often present

In experiments with FB and SD25 overall there was no correlation between the concentrations of UCM hydrocarbons (the most common feature of the sediment hydrocarbon distributions) and growth rate, survivorship or reproduction (Figures 1-3.) When the data for experiments involving controls or dispersants were excluded, the correlation did not improve significantly.
This suggests that overall, sediment total UCM hydrocarbon concentrations resulting from FB oil are not the factor controlling these measures of toxicity in *C. volutator*. Other components of the oil and/or dispersant may be responsible. Clearly no toxicity cut-offs can be established.

Figure Legends (figures overleaf):

Figure 1. Covariation between concentration of total UCM hydrocarbons (µg g dry weight sediment^{-1} and growth rate (mg/individual/day) of *Corophium volutator* (data from studies with FB oil and SD-25).

Figure 2. Covariation between concentration of total UCM hydrocarbons (µg g dry weight sediment^{-1} and percentage survivorship of *Corophium volutator* (data from studies with FB oil and SD-25).

Figure 3. Covariation between concentration of total UCM hydrocarbons (µg g dry weight sediment^{-1} and reproductive success (neonates/female) of *Corophium volutator* (data from studies with FB oil and SD-25).

Fig 1.

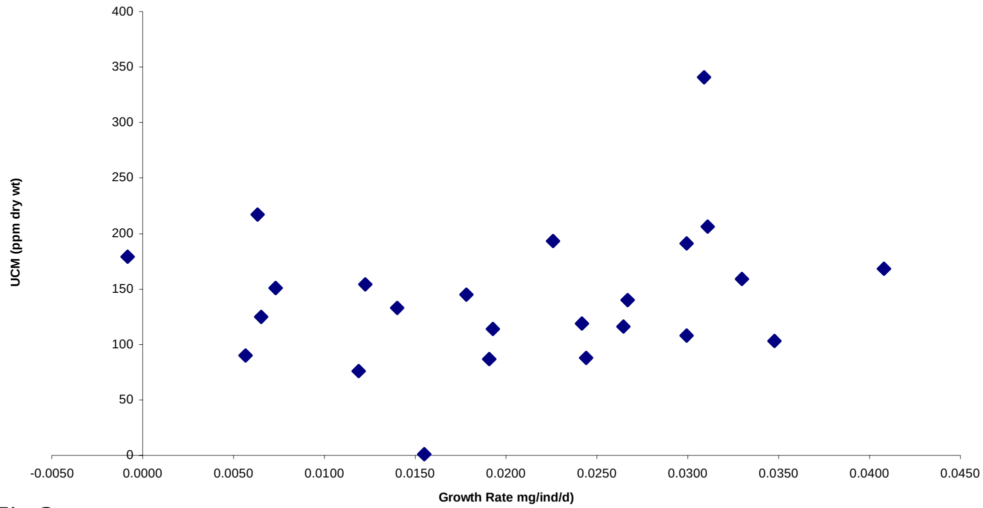


Fig 2.

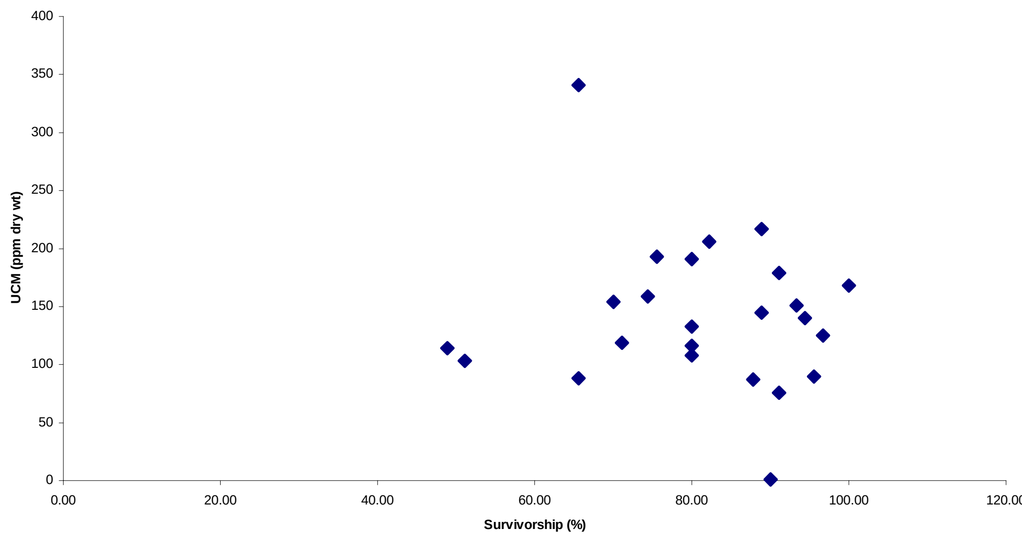
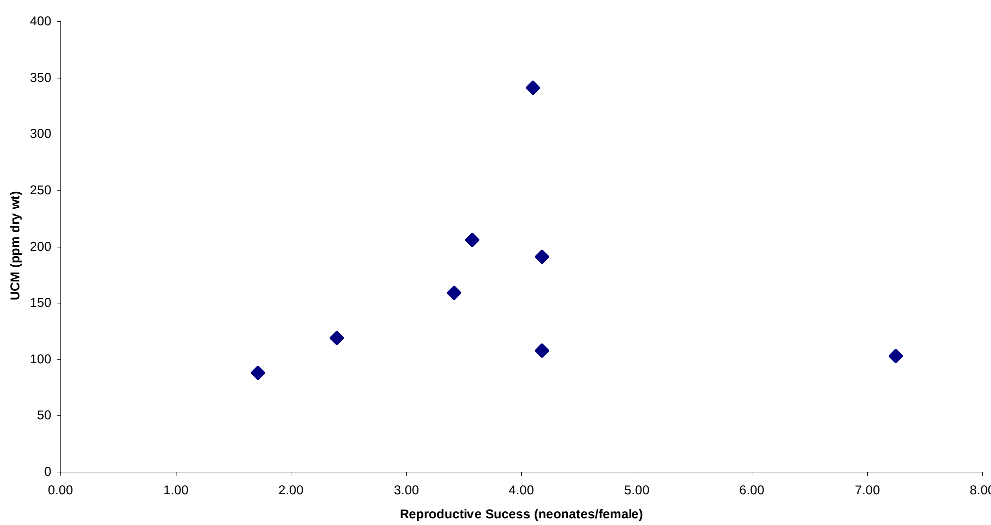


Fig 3.



In experiments with ANS and Corexit 9527, again there was no correlation between the concentrations of UCM hydrocarbons (the most common feature of the sediment hydrocarbon distributions) and growth rate or survivorship (Figures 4-5). There did appear to be some negative correlation of UCM hydrocarbon concentrations in sediments, with reproductive success (Figure 6) but the number of data points was smaller since obviously reproduction could only be assessed at the end of the life cycle. When the data for experiments involving controls or dispersants were excluded, the correlation did not improve significantly.

This suggests that overall, sediment hydrocarbon concentrations are not the factor controlling these measures of toxicity in *C.volutator*, with the possible exception of reproductive success. The reasons for the latter, if genuine (*vide infra*, Section 4.3), are unknown.

Figure Legends (figures overleaf):

Figure 4. Covariation between concentration of total UCM hydrocarbons (µg g dry weight sediment^{-1} and growth rate (mg/individual/day) of *Corophium volutator* (data from studies with ANS and C-9527).

Figure 5. Covariation between concentration of total UCM hydrocarbons (µg g dry weight sediment^{-1} and percentage survivorship of *Corophium volutator* (data from studies with ANS and C-9527).

Figure 6. Covariation between concentration of total UCM hydrocarbons (µg g dry weight sediment^{-1} and reproductive success (neonates/survivor) of *Corophium volutator* (data from studies with ANS and C-9527).

Fig 4.

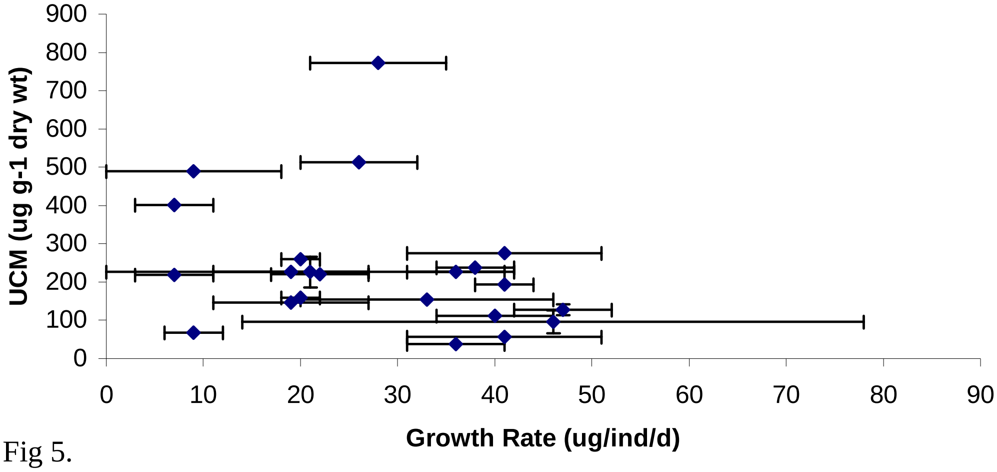


Fig 5.

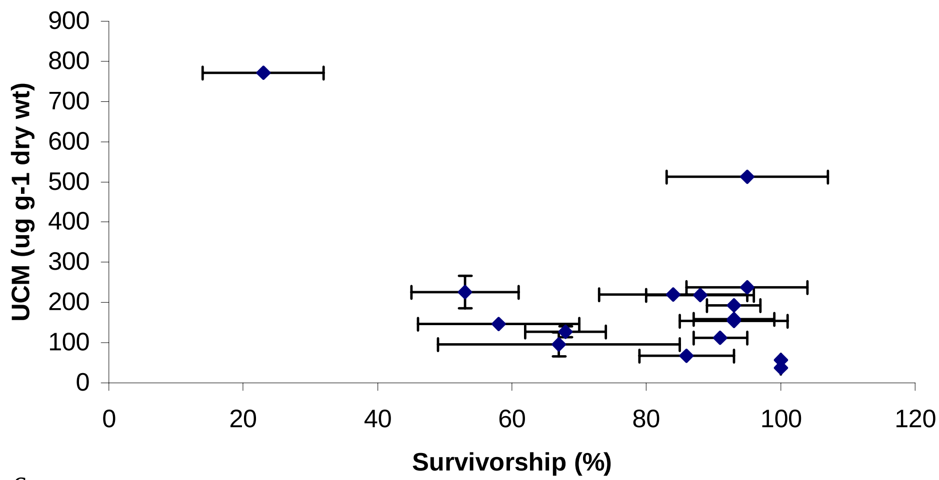
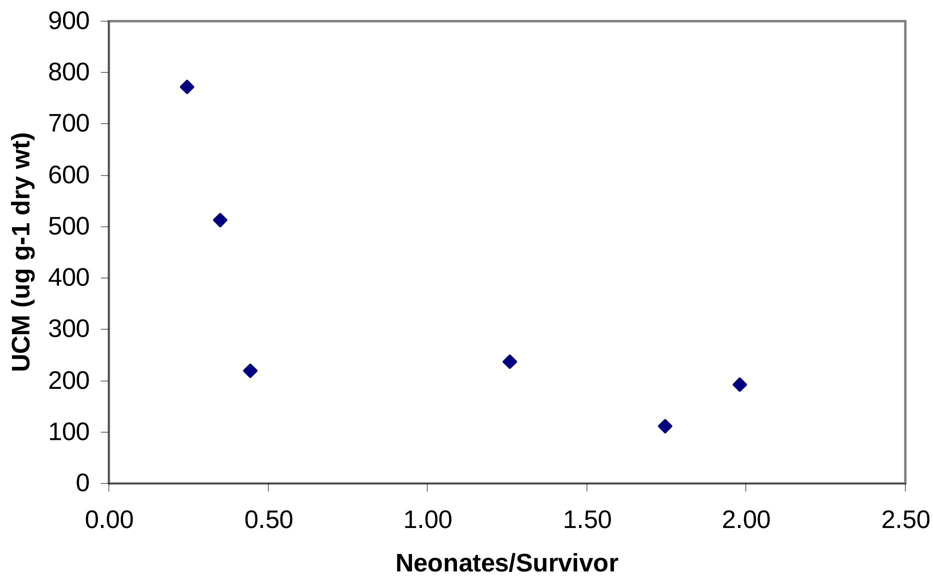


Fig 6.



A variety of factors might disrupt any putative correlation between the individual and unresolved hydrocarbon concentrations and the toxic effects:

- The sediments in which the amphipods were studied contained a background of natural and pollutant hydrocarbons. GC-MS analysis revealed these consisted of an UCM and various resolved hydrocarbons, including a series of compounds assigned as polycyclic aromatic hydrocarbons by spectral library matching. It is not known what proportion of these (if any) was bioavailable and bioaccessible to the animals. A high concentration of non-available hydrocarbons would tend to skew any relationship between toxicity and hydrocarbon concentrations.

- Any putative relationship between individual hydrocarbons and toxicity might also be skewed by the known toxicity of the dispersants (Scarlett *et al*., 2005) or the unknown toxicity of other non-hydrocarbon constituents of the oils.

- The recovery of the animals from some of the intermediate toxic effects of the oils and dispersants observed in the initial study (Smith *et al*., 2006) was attributed to the active biodegradation of some of the hydrocarbons during the life cycle bioassays. The degradation of the hydrocarbons might also skew any correlation between hydrocarbon chemistry and toxicity, particularly if biodegradation of the unresolved hydrocarbons was selective since GC-MS would not reveal the differences between a toxic and less toxic UCM.

- Amphipods feed mainly by filtering water. Thus the concentrations of hydrocarbons in the sediments might not be expected to correlate well with the toxicity of the amphipods. Rather this might be more dependent on the concentrations in the interstitial water.

4.2 Hydrocarbon concentrations and filtering rates of mussels, *Mytilus edulis* exposed to water-accommodated, dispersed and biodegraded weathered crude oils.

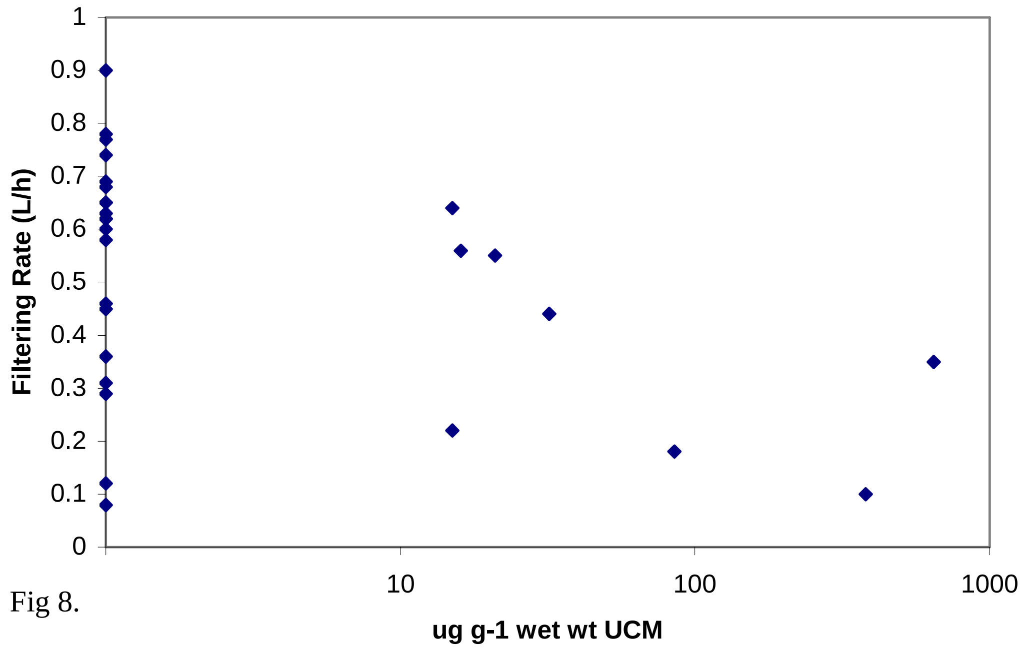
GC-MS analysis of the extracted body tissues of mussels exposed to FB oil mixtures revealed either: few hydrocarbons (controls), a few resolved hydrocarbons -mainly alkylnaphthalenes and alkylphenanthrenes (selected samples), or an unresolved complex mixture (UCM). In impacted mussels, automated gas chromatography-mass spectrometry identification of hydrocarbons versus library spectra revealed that the latter feature was most common. Appendix 5 summarises all of the data (μg g tissue wet weight^{-1}, n=1) along with the corresponding filtering rates of the control or impacted mussels (L h^{-1} ± 2 standard errors, n=9). The data for 30 separate experiments (n=270 mussels) are reported. Since the Control (unexposed) mussels were determined in each separate experiment (n=80 mussels) a revised figure (Figure 8) shows the mean of means ± 1 standard deviation for the Control mussels for improved clarity. This figure also omits the five mussels batches which had reduced filtering rates but in which the presence of high concentrations of resolved mainly alkylnaphthalenes and alkylphenanthrenes, clearly resulting from the oil, were present which depressed the measurement of the UCM by GC-MS due to normalisation on the resolved components.

Figure Legends (figures overleaf):

Figure 7. Covariation between concentration of total UCM hydrocarbons (µg g wet weight mussel tissue^{-1} and filter rate (L h^{-1}) of *Mytilus edulis* (data from all studies with FB and SD-25).

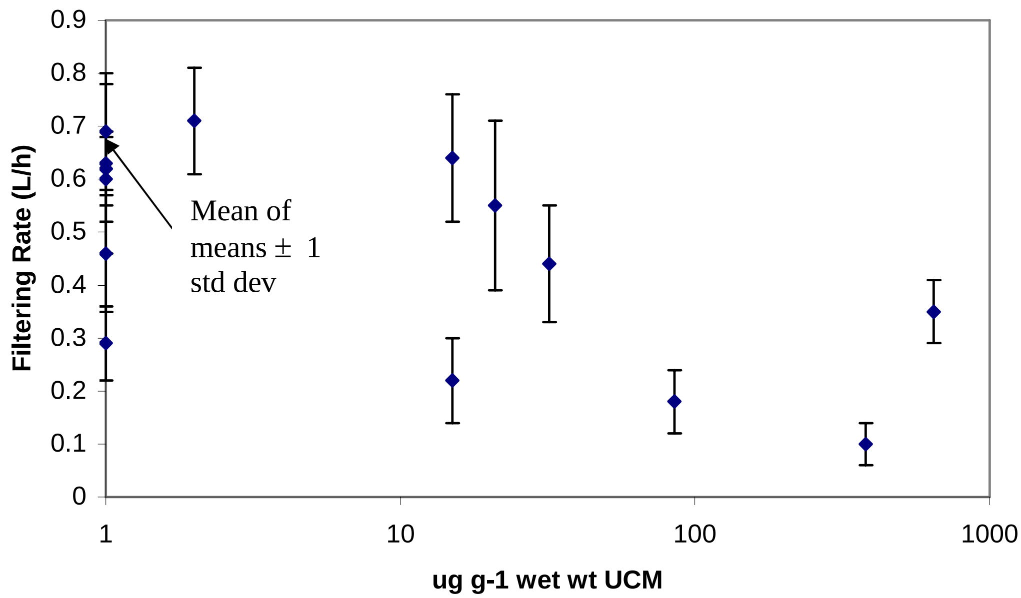
Figure 8. Covariation between concentration of total UCM hydrocarbons (µg g wet weight mussel tissue^{-1} and filter rate (L h^{-1}) of *Mytilus edulis* (data from selected studies with FB and SD-25 showing the mean of means ± 1 standard deviation for the Control mussels for improved clarity. This figure also omits the five mussels batches which had reduced filtering rates but in which the presence of high concentrations of resolved mainly alkylnaphthalenes and alkylphenanthrenes, clearly resulting from the oil, were present which depressed the measurement of the UCM by GC-MS due to normalisation on the resolved components.

Fig 7.



1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Filtering Rate (L/h)
10
100
1000
ug g-1 wet wt UCM

Fig 8.



0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Filtering Rate (L/h)
Mean of
means ± 1
std dev
1
10
100
1000
ug g-1 wet wt UCM

The data show a broad, rather vague correlation between the accumulation of oil-derived UCM hydrocarbons by the mussels and depression of their filtering rates. Inconsistencies in this correlation probably negate derivation of an accurate 'toxicity cut-off' value but suggest that a 50% reduction in feeding rate corresponds to a weathered FB residue (biodegraded or not) accumulation of very roughly a few tens to about a hundred μg g tissue wet weight^{-1}. Smith (2002) determined an estimated TEC_{50} value for biodegraded Gullfaks crude (North Sea) monoaromatic UCM of 500 μg g tissue wet weight^{-1} but noted that the value was very approximate. Reineke (2006) determined values of 119 and 116 μg g tissue wet weight^{-1} for the total aromatic and monoaromatic fractions of undegraded Monterey crude respectively, though again confidence intervals were large. Mussels with significantly reduced Scope for Growth collected from coastal U.K. sites contained about 100-125 μg g tissue dry weight^{-1} (about 500 μg g tissue wet weight^{-1}) aromatic UCMs (Booth *et al*., 2006). Thus, so far, with a limited number of studies conducted and considerable variability in the data, it appears that accumulation of hundreds of μg g tissue wet weight^{-1} of aromatic UCMs would represent a significant narcotic toxic effect to mussels. The composition of the UCMs may or may not be important (Booth *et al*., 2006). It is interesting that even in the relatively short duration of the present experiments (7 days) the UCM feature was apparent in several samples- suggesting that this may not just be a characteristic of chronic oil pollution but also a consequence of rapid oil dissolution (accommodation), biodegradation and dispersion. Obviously however, accumulation of sufficient UCM concentrations might be most important over the time course of a longer chronic exposure event(s).

GC-MS analysis of the extracted body tissues of mussels exposed to ANS oil mixtures again revealed either, few hydrocarbons (controls), a few resolved hydrocarbons -mainly alkylnaphthalenes and alkylphenanthrenes (selected samples) or an unresolved complex mixture (UCM). In impacted mussels, automated gas chromatography-mass spectrometry identification of hydrocarbons versus library spectra revealed that the latter feature was most common. Appendix 5 summarises all of the data (μg g tissue wet weight^{-1}, n=1) along with the corresponding filtering rates of the control or impacted mussels (L h^{-1} ± 2 standard errors, n=9). The data for 30 separate experiments (n=270 mussels) are reported. A revised figure (Figure 8) omits the one mussel batch which had reduced filtering rates but in which the presence of high concentrations of resolved mainly alkylnaphthalenes and alkylphenanthrenes, clearly resulting from the oil, were present which depressed the measurement of the UCM by GC-MS due to normalisation on the resolved components.

The data show no correlation between the accumulation of oil-derived hydrocarbons by the mussels and depression of their filtering rates. This lack of correlation prevents derivation of an accurate 'toxicity cut-off' value.

The observed non-correlation of the toxicity of ANS crude with hydrocarbons suggests the toxicity is not solely determined by the hydrocarbons (UCM or resolved) but is also associated with other, perhaps more polar fractions, the study of which was beyond the scope of the present project. Again this is consistent with recent findings that unresolved polar fractions are toxic to a variety of organisms in a range of bioassays (Brakstad *et al*., 2006).

Fig.9

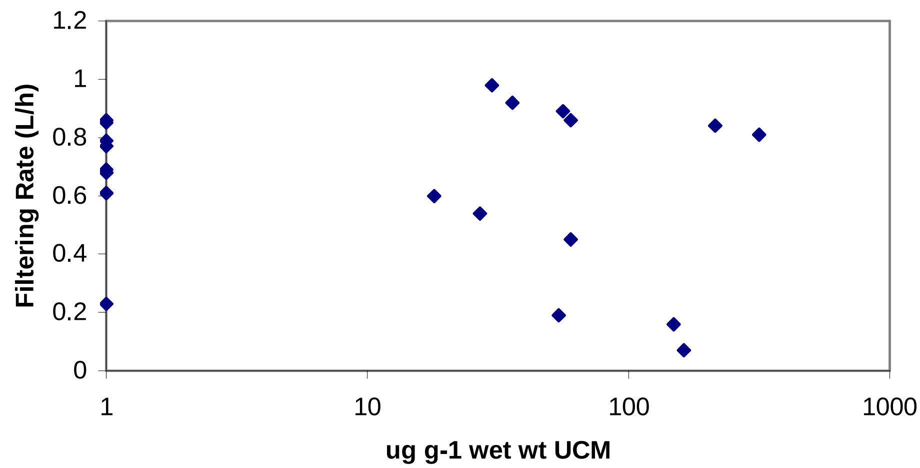


Fig.10

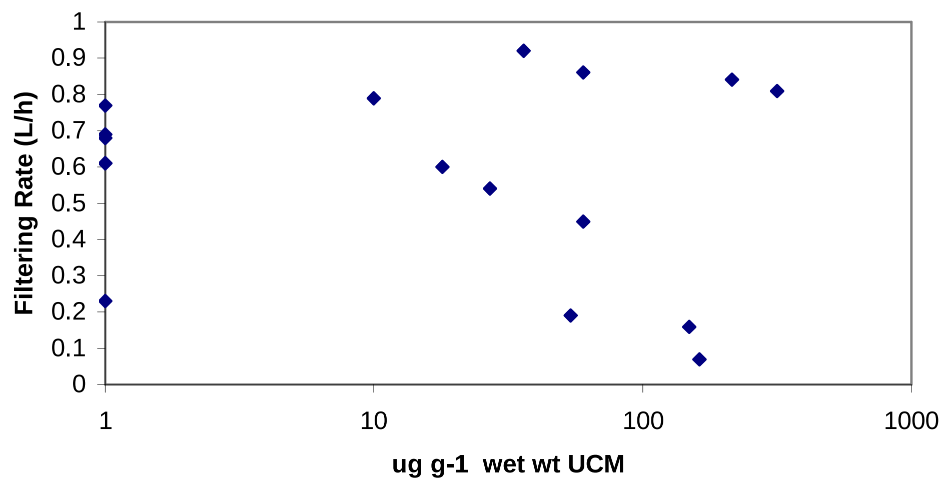


Figure 9. Covariation between concentration of total UCM hydrocarbons (µg g wet weight mussel tissue^{-1} and filter rate (L h^{-1}) of *Mytilus edulis* (data from all studies with ANS and C-9527).

Figure 10. Covariation between concentration of total UCM hydrocarbons (µg g wet weight mussel tissue^{-1} and filter rate (L h^{-1}) of *Mytilus edulis* (data from selected studies with ANS and C-9527). This figure omits mussel batches which had reduced filtering rates but in which the presence of high concentrations of resolved mainly alkylnaphthalenes and alkylphenanthrenes, clearly resulting from the oil, were present which depressed the measurement of the UCM by GC-MS due to normalisation on the resolved components.

4.3 Targeted study of the toxicity of ANS hydrocarbons to *C. volutator*.

The apparent correlation of the concentrations of ANS UCM hydrocarbons in sediments and the decreased reproductive success of *C. volutator* (Fig. 6), suggested further experiments with ANS oil should be conducted.

Therefore, *C. volutator* was exposed as previously described (Smith *et al*., 2006) but for 35 days rather than 75 days, to 500 µg g sediment dry weight^{-1} of a more extensively weathered (36% loss; *cf* Smith *et al*., 2006, 20% loss) sample of ANS oil. Whilst there were no effects on survivorship (>90%), both growth rate (Fig. 11) and reproductive success (Fig. 12) were significantly decreased relative to controls ($P \leq 0.05$; error bars ± one standard error). Reproductive success showed the strongest depletion of offspring per survivor.

Fig.11. Depression in growth rate of *C. volutator* exposed to 500 µg 35% weathered ANS Crude g sediment dry weight^{-1} for 35 days.

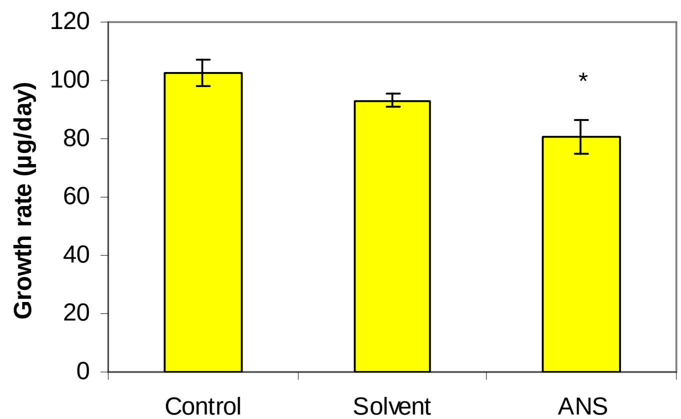
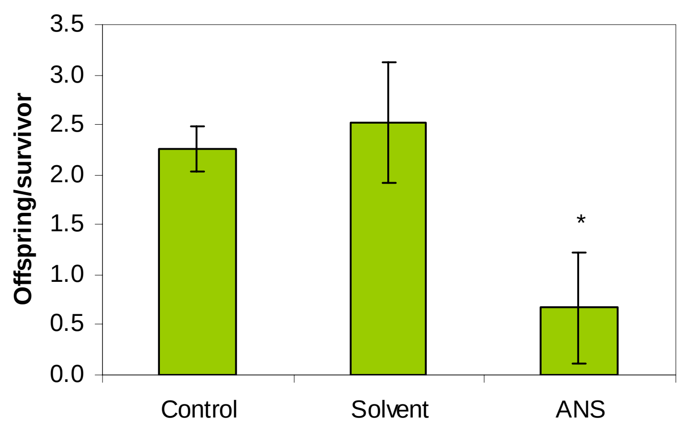


Fig.11. Depression in reproductive success of *C. volutator* exposed to 500 µg 35% weathered ANS Crude g sediment dry weight^{-1} for 35 days.



Thus it appears that ANS crude oil does adversely influence reproductive success in *C. volutator* at this nominal oil concentration.

5. Discussion & Conclusions

From the data from toxicological assays using the mussel, *Mytilus edulis* and the amphipod, *Corophium volutator* it would appear that both organisms are often able to recover after exposure to the oils and dispersed oils- provided they are then exposed to cleaner conditions due to either successive clean tides or weathering of the oil in sediments without further oil additions (Smith *et al*., 2006).

When these results are compared with the identification and quantification of individual hydrocarbons in the sediments and in the mussel tissues, similar hydrocarbons are found to be present in many of the impacted samples. These are not usually found in the non-impacted samples.

For weathered, water-accommodated oils, these hydrocarbons are mainly identified by the automated library searches of the mass spectra as methylnaphthalenes, and methylphenanthrenes and also, by manual inspection, as unresolved complex mixtures of hydrocarbons (probably mainly the more water dispersible aromatic UCMs).

Such hydrocarbons appeared to be present in virtually all exposures where effects occurred, and more of these components were present, and to a larger extent, when the greater impacts were seen (e.g. reduced feeding rate or growth rate in the organisms tested).

In experiments where mussel feeding rate was impacted, the above hydrocarbons were often present and when the organisms were able to recover, the concentrations of these components decreased in the tissues or disappeared entirely. Conversely, in the experiments where the organisms did not recover during the 7 day depuration period, these compounds were often still present in the tissues. This correlation, whilst quantitatively poor, was most evident for FB oil mixtures and was not apparent for the ANS oil mixtures, suggesting perhaps that chemicals other than, or as well as, hydrocarbons were responsible for the toxic effects observed for ANS oil/dispersant mixtures.

Indeed, a variety of other non-hydrocarbons would be expected to be present, originating from both the oils and perhaps the dispersants-but the programme of work sponsored herein was not focussed on such chemicals.

In experiments with amphipods a very similar pattern was observed in that the growth rate of the organisms was related to the appearance of hydrocarbons in the sediments and once these had decreased the organisms appeared to recover, but this may have been because other more polar compounds were degraded also.

The above effects were not often spread over clear dose-response type relationships so no accurate cut off concentrations could be calculated for most parameters.

However, a very approximate estimate was made for the concentrations of UCM hydrocarbons required to reduce feeding rate by 50% in mussels exposed to FB oil mixtures. A 50% reduction in feeding rates corresponded to UCM concentrations of in the region of very approximately 30-100 µg g^{-1} wet weight (probably about 150-500 µg g^{-1} dry weight). This is broadly comparable to the few values available from laboratory and field studies of toxic effects of aromatic hydrocarbon UCMs on mussels (reported tissue effective concentrations (TEC_{50}) of about 120 and 500 µg g^{-1} wet weight have appeared).

Also, an approximate estimate was made of the concentrations of weathered ANS crude in sediments which might appreciably affect reproductive success in *Corophium volutator*. The most pronounced deleterious concentrations which might affect reproductive success in *C. volutator* were above (very approximately) 250 µg UCM g dry weight $sediment^{-1}$. Separate experiments with more weathered ANS crude certainly confirmed this effect at 500 µg nominal oil g dry weight $sediment^{-1}$. Further experiments outwith the present study will investigate the effect of a separated aromatic hydrocarbon fraction.

These are very approximate, gross estimates, given the poor correlations from which they are derived.

So far as **policy recommendations** are concerned, it is recommended that in addition to routine monitoring of well known toxicants (such as C_{0-4} naphthalenes, and phenanthrenes), the unresolved complex mixtures and other more polar toxicants should also be quantified and reported when analyses of oiled matrices from the environment or from laboratory experiments are made following dispersed oil spill events. Both UCM hydrocarbons and the polar compounds require further identification. Appropriate methods such as GC x GC-TOF-MS are now available (e.g. Booth *et al*., 2006) and these too should be investigated further-especially their use for quantitative analyses of UCMs.

6. References

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Scarlett, A., Canty, M., Smith, E.L., Rowland S.J. & Galloway, T.S. (2006). Can amphipod behaviour help to predict chronic toxicity of sediments? *Human Ecol. Risk Assess*. (In press).

Smith E. L., Scarlett, A., Canty, M.N., Donkin, P., Galloway, T. & Rowland , S.J. (2006). Potential Ecological Effects of Chemically Dispersed and Biodegraded Oils. Final Report; RP 480. Copies available from Maritime & Coastguard Agency, Southampton, U.K.

Reineke, V. (2006). Toxic effects of aromatic hydrocarbon mixtures isolated form crude oils on the blue mussel, *Mytilus edulis*. Dissertation *Dr der Nat*., University of Oldenburg, Germany.

7. Publications

To date, two manuscripts for publication have been prepared, submitted and published or accepted for publication:

Scarlett, A., Galloway, T.S., Canty, M., Smith, E.L., Nilsson, J & Rowland S.J. (2005). Comparative toxicity of two oil dispersants, Superdispersant-25 and Corexit 9527 to a range of coastal species. *Environ. Toxicol. Chem*., 24, 1219-1227.

Scarlett, A., Canty, M., Smith, E.L., Rowland S.J. & Galloway, T.S. (2006). Can amphipod behaviour help to predict chronic toxicity of sediments? *Human Ecol. Risk Assess*. (In press).

In addition a 'spotlight' poster for presentation with a short oral introduction has been accepted for presentation at the 16th Annual SETAC Europe meeting, The Hague, The Netherlands, May 7-11, 2006.

Scarlett, A., Canty, M., Smith, E.L., Rowland S.J. & Galloway, T.S. (2006). The elephant in the room: unresolved complex mixtures of hydrocarbons (UCM) are widespread in marine sediments, but are these ignored contaminants toxic?

Appendices

Appendices containing all the processed data are supplied in the accompanying Excel data sheets.

Appendix 1. Hydrocarbon concentrations in sediments (*C.volutator*) : All data.

Appendix 2. Hydrocarbon concentrations in mussel tissues. All data.

Appendix 3. *C. volutator* bioassay data

Appendix 4. Hydrocarbon concentrations in sediments (*C.volutator*). Summary data.

Appendix 5. Hydrocarbon concentrations in mussels. Summary data.