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**Method for assessing the chronic toxicity of
marine and estuarine sediment-associated contaminants
using the amphipod *Corophium volutator***

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Abstract

Acute sediment toxicity tests do not test key life stage events such as moulting and reproduction and therefore do not reveal the longer-term effects of contaminant exposure. A laboratory method is described for determining the chronic toxicity of contaminants associated with whole sediments. The test is conducted using neonates of the estuarine amphipod *Corophium volutator* at 15 °C, salinity 25 psu and a 12h light:12h dark photoperiod. The endpoints are survival and growth after 28 days and survival, growth and reproduction of amphipods upon termination of test i.e. reproduction within all control vessels (*ca*75 days). The sediment chronic toxicity test was used to investigate the effects of sediments spiked with environmentally relevant preparations of slightly weathered Alaskan North Slope crude oil, including a water-accommodated-fraction (WAF) and a chemically-dispersed (Corexit 9527) WAF. Sediment oil concentrations were quantified using Ultra-Violet Fluorescence. The amphipods exposed to chemically dispersed oil had higher mortality and lower growth rates than control-, Corexit 9527- and WAF-exposed organisms, resulting in reduced reproduction. The described method supplements the standard acute sediment test and would be particularly useful when long term ecological effects are suspected but acute tests reveal no significant mortality. The sediment chronic test reported herein has shown that sediment that was not evidently toxic during 10-day acute tests could have population-level effects on sediment-dwelling amphipods.

Keywords: Chronic Toxicity; Sediment Toxicity Tests; Oil; Sublethal effects; *Corophium volutator*

Introduction

Acute tests are very useful for identifying highly toxic chemicals but do not test key life stage events such as moulting and reproduction during which sensitivity to toxicants may be increased. Also, marine and estuarine sediments are more likely to contain moderately toxic contaminants that fail to cause significant acute mortality. In response to these issues, the USEPA have developed a 28 day life-cycle test using the estuarine amphipod *Leptocheirus plumulosus* (USEPA 2001) and this was recently used by McGee et al (2004) to compare chronic with acute toxicity of sediment from Chesapeake Bay, USA. The *L. plumulosus* test is conducted at 25°C and the organism's life-cycle is completed within the 28 day exposure period. A similar but slower growing amphipod test species in northern European waters is *Corophium volutator* Pallas, where it resides in muddy intertidal sediment with summer seawater temperatures typically around 15 °C. The species *C. volutator* is now a standard European test organism for acute sediment toxicity testing (PARCOM 1993; Roddie & Thain 2001) and has been used in many acute studies (Bat & Raffaelli 1998; Briggs et al., 2003; Ciarelli et al., 1997). *Corophium volutator* has also been used in long-term life-cycle tests (Brown et al., 1999; Conradi & Depledge 1998, 1999) but the amphipods were only exposed to aqueous toxicants and not contaminants associated with whole sediments. Peters & Ahlf (2005) have demonstrated that *C. volutator* can be successfully cultured in the laboratory and the authors recommended the use of this species for chronic exposure toxicity testing. A chronic sediment test using the amphipod *Gammarus locusta* L has recently been described which is conducted at 20°C in order to complete the life-cycle within 28 days (Costa et al., 2005). *Corophium volutator* occupies a different ecological niche to *G. locusta* (Connor et al., 2004) and therefore an additional chronic test to complement the standard acute test using *C. volutator* to evaluate the toxicity of whole sediments would be of benefit. In the test described herein endpoints are survival, growth and reproduction.

Oil contamination of marine sediments remains an on-going problem whether it originates from low-level discharge from refineries and drilling platforms or from more dramatic spills from tanker disasters. Spills such as the *Sea Empress* and *Prestige* disasters resulted in considerable contamination of marine and estuarine sediments (de la Huz et al., 2005; SEEEC 1998), but the impact of oil contamination is difficult to predict due to its complex nature. Within just hours at sea after release from a damaged tanker, weathering processes substantially change the oil composition with a subsequent loss of many of the most toxic components (Riley et al., 1980). Addition of dispersants may

result in large numbers of small oil droplets in the water column which may reach the benthos and enter the sediment, thus exposing sediment-dwelling organisms to hydrocarbon contamination (SEEEC 1998). As the oil degrades, so its composition and toxicity changes further complicating an assessment of its potential impact upon biota.

This paper describes the use of 10-day and chronic whole sediment life-cycle tests using *C. volutator* (chronic being defined as the long term sublethal effects of acute exposure (Connell & Miller 1984)). To simulate environmental exposure following an oil spill, the long-term sublethal effects of exposure to sediment initially spiked with the oil dispersant Corexit 9527, weathered Alaskan North Slope (ANS) crude oil, Water Accommodated Fraction of ANS crude oil (WAF) and chemically dispersed (using Corexit 9527) WAF (DWAF) was investigated using both the acute 10-day test and the chronic whole sediment life-cycle test using *C. volutator*.

Material and Methods

Collection and maintenance of organisms during acclimation.

Sediment and *C. volutator* were collected from an intertidal area of the Avon estuary near Aveton Gifford, south Devon UK (Ordnance Survey Grid reference: SX 683 467). Neonate amphipods were collected and separated from adults by sieving the upper 5 cm of sediment (neonates pass through a 500 μ m sieve but are retained on a 300 μ m sieve), transported back to the laboratory within one hour and placed in 5 L culture tanks lined with field-collected sieved (<300 μ m) sediment. The tanks were filled with filtered seawater 25 ± 1 psu which was aerated and maintained at 15 ± 1 °C with a 12:12 h light/dark cycle. The animals were fed weekly with 2 drops of aquarium invertebrate food (Waterlife Invert Food, Waterlife Research Industries, Longford, UK; Liquifry Marine, Interpret Ltd., Dorking, UK; Roti-Rich, Florida Aqua Farms, Dade City, FL, USA; and dried algae) per litre of overlying water and the water replaced 24 h after feeding. Amphipods were maintained under the above conditions for 7-10 days after removal from the field to acclimate them to experimental conditions. Water quality measurements: dissolved oxygen, temperature, pH and salinity were measured prior to water changes. Test conditions and acceptability requirements are given in Table 1.

Reference toxicity test

In order to test that the selected *C. volutator* population was representative of the *C. volutator* populations in general, in terms of sensitivity to a standard toxicant, the test organisms were exposed to cadmium chloride (CdCl_2) using a method described by Ciarelli et al., (1997) and their sensitivity compared with published data (Ciarelli et al., 1997). In brief, static aqueous tests of 72 h exposure were performed in the absence of sediment. Twenty adult amphipods were placed in nominal CdCl_2 concentrations ranging from 0 to 14.0 mg L^{-1} , two replicates per treatment, with a salinity of 31 psu and gentle aeration *via* a glass Pasteur pipette. The organisms were monitored daily throughout the experiment and the number surviving and deceased recorded after 72 h. A 72 h LC_{50} value was derived using the trimmed Spearman-Kärber method and compared with literature values.

Artificial weathering of oil

The fresh ANS crude oil was artificially weathered in order to simulate evaporative losses of *ca* 20% that typically occur during the first 2 – 3 h at sea following a spill; this represents the earliest optimal time that dispersants may be deployed at sea (Kevin Colcomb, Maritime & Coastguard Agency (MCA), personal communication). In brief, approximately 1000 mL of oil was placed in a 2000 mL container on a top pan balance in a fume hood with a controlled airstream and the oil allowed to evaporate. The weight of the oil at the start of the experiment was noted. Triplicate density measurements were taken to calculate the initial volume of oil. Density measurements were made by weighing known volumes of the oil at the beginning, half way through, and at the end of the experiment. Density measurements were compared to published literature (Blenkinsopp et al., 1996). The weight of the oil was noted over the duration of the experiment and adjusted to volume using the density measurements. Temperature ($18\text{--}20^\circ\text{C}$) and air flow ($\sim 0.5 \text{ ms}^{-1}$) through the fumehood was noted during the experiment. The weathering process was halted after 24h. The whole experiment was carried out three times to investigate whether the method was reproducible. The slightly ‘weathered’ oil ($19.88 \pm 0.63\%$ evaporative loss) was used to produce the starting material for all experiments. Once weathered the oil was stored in completely filled amber glass bottles at 4°C until use.

Chemical analyses of water and sediment

Water samples aliquots (100 mL) of WAF or DWAF were extracted into dichloromethane (DCM, $3 \times 25 \text{ mL} + 25 \text{ mL}$ rinse of separating funnel) for quantification of hydrocarbon concentrations by UVF

analysis. Sediment samples were extracted using an alkaline saponification method described by Kelly et al. (2000). In brief: frozen sediment samples were allowed to defrost at room temperature then mixed with a stainless steel spatula. The dry weight percentage of each sample was determined by weighing subsamples ($\times 2$) in pre-weighed foil dishes then re-weighing after drying at 105 °C for 16 h.

Approximately 50 g of wet sediment samples were digested with potassium hydroxide pellets (5 g) and methanol (100mL), under reflux for 2 hours. When cool, the digests were filtered through solvent-rinsed filter papers (Whatman 113v) into 250 mL separating funnels. The digests were extracted with *n*-pentane (2×50 mL) and the combined extract dried with anhydrous sodium sulphate then transferred to 100 mL volumetrics and made up to volume. Fluorescence values were compared to a standard curve derived from weathered ANS oil dissolved in DCM (WAFs and DWAF) or *n*-pentane (sediment). Excitation wavelengths (λ) were 254 nm (DCM) 310 nm (*n*-pentane) and emission λ 360 nm.

Preparation of Water-Accommodated-Fractions (WAFs) and chemically dispersed WAFs (DWAFs)

The apparatus was similar to that described by Ali et al. (1995). In brief, 25 mL of weathered ANS crude oil was slowly vortex mixed with 2475 mL of 25 psu seawater at a ratio of 1:99 for 24 h within a 5 L Pyrex bottle then left to re-equilibrate for one hour. DWAF was produced as above but with the addition of Corexit with an oil:dispersant ratio of 25:1 premixed with the oil. After mixing, the solution of WAF or DWAF was carefully siphoned off under low nitrogen pressure. As it was desired that the WAF should contain the soluble component of the oil with minimal presence of droplets, the number of particles present in various size categories were counted using a Beckman Z2 Coulter Particle Count and Size Analyser (Beckman Coulter, Wycombe, UK), and compared with that of seawater. WAFs were considered acceptable if particle counts were $<5\times$ that of seawater.

Spiking of sediments

Aliquots of 160 mL of sieved sediment (grain size: 33% sand, 67% silt/clay; organic carbon 3.8%) were placed in wide neck glass 500 mL bottles (Schott). Aliquots of 320 mL WAF, DWAF, Corexit and 25 psu seawater were added to the bottles and shaken at 15 °C for 3.5 h at 200 rpm on an orbital shaker. The slurry from each bottle was transferred to 2 L Pyrex beakers, allowed to settle for 16 h, the supernatant poured off and the beakers refilled to the 1200 mL mark with 25 psu seawater. Direct

spiking of weathered ANS oil was performed *via* micro-litre syringes into 160 mL aliquots of sieved wet sediment within 2 L Pyrex beakers. The oil and sediment was homogenised with 50 mL of 25 psu seawater for 60 s using a motorised hand blender to produce a slurry. The blender was rinsed into the vessel with an additional 50 mL of 25 psu seawater. The slurry was left to settle for 24 h, the supernatant poured off and the beakers refilled to the 1200 mL mark with 25 psu seawater. All beakers were left to settle for a minimum of 20 h with gentle aeration before the addition of the amphipods. An additional replicate for each treatment was produced for chemical analyses.

Acute sediment toxicity test

Acute sediment tests were based on standard 10-day sediment toxicity tests (ASTM 2000; Roddie & Thain 2001; USEPA 1994). A slight alteration to the standard protocol was made in order to give greater consistency with the chronic tests i.e. a 12:12 h light:dark regime was imposed in preference to continuous light. Adult *C. volutator* (size range 4 – 7 mm, $n = 20$) were exposed to nominal oil-spiked sediment concentrations of 0, 220, 440 and 880 $\mu\text{g g}^{-1}$ dry wt. plus 100 % WAF, DWAF and dispersant-only (400 mg L^{-1} Corexit) spiked sediment exposures (3 replicates per exposure treatment and 6 negative control replicates). The animals were not fed during the test. At the end of the test the sediment was gently sieved (300 μm) and the number of alive, dead and missing amphipods in each vessel recorded. Water quality measurements were recorded prior to commencement of the test, on day 5 or 6, and at the end of the test.

Chronic sediment toxicity test

Chronic tests were based on the acute 10-day sediment test (Roddie & Thain 2001) and the USEPA (2001) amphipod chronic sediment test. Neonate *C. volutator* (mean length 1.4 mm, SD 0.31 mm; mean weight 0.1 mg) were sorted from stock by carefully passing organisms through a 500 μm sieve to remove larger organisms. The sediment was then carefully re-sieved (300 μm) to collect the test organisms. Amphipods ($n = 30$) were transferred to 25 mL beakers *via* plastic Pasteur pipettes and then randomly allocated to exposure vessels. Care was taken to ensure that the amphipods were not trapped by the surface tension of the water. Two litre squat-form Pyrex beakers were used as exposure vessels as these provide a greater sediment surface area than the standard one litre beakers. Nominal sediment concentrations were 0, 110, 220 and 440 $\mu\text{g oil g}^{-1}$ dry weight sediment. Control treatments

were spiked with deionised water. Nine replicates for each of seven treatments were used to provide three replicates per treatment for evaluation after 28 days and the remaining six replicates for evaluation upon termination of the test. An additional set of control replicates were used to give a total of 72 exposure vessels. The animals were fed weekly with 2 drops of standard aquarium invertebrate food and the overlying water 80 % replaced 24 h after feeding. Water quality measurements were measured before addition of amphipods and prior to water exchanges during the test. Test conditions, acceptability and water quality measurements achieved are given in Table 1. The test was initiated at the beginning of June 2004.

After 28 days exposure, the amphipods from three replicates of each treatment were counted, collectively weighed to 0.1 mg (amphipods were carefully blotted on absorbent paper to remove excess water) and individually measured to the nearest 0.1 mm (excluding antennae) under a dissecting microscope. The test was terminated when reproduction was apparent in all replicates of the control treatment. Survivorship, wet weight and length of organisms were recorded. The numbers of mature adult (≥ 5.0 mm), sub-adult (< 5.0 mm), gravid females and neonates were also counted. Although the majority of neonates could be detected from their movement and separated from the debris for enumeration, the separation from the debris of the remaining organisms was facilitated by the addition of 70 % isopropanol plus a few drops of rose bengal solution (*ca* 1 g L⁻¹). All neonates were preserved in the 70 % isopropanol /rose bengal solution for recounting for quality assurance (QA) purposes.

Test chemicals and solvents

All solvents were HPLC Grade from Rathburns Ltd. (Walkerburn, Scotland). Reference estuarine sediment and CdCl₂ was obtained from Sigma-Aldrich Company Ltd, Gillingham, UK and potassium hydroxide from Fisher Scientific (Loughborough, UK. Fresh Alaskan North Slope crude oil and Corexit 9527 was obtained gratis from Minerals Management Service (U.S.)

Quality assurance (QA) and Data analyses

For QA purposes, ≥ 10 % of all counts and measurements were repeated by a second operator. Statistical analyses of results were performed using Statgraphics Plus 5.1. Following checks for variance using Bartlett's test, data were analysed by one way analysis of variance (ANOVA). Where there was a significant difference ($P \leq 0.05$) of means, the data were further analysed by the Fisher's

LSD test to determine significant differences ($P \leq 0.05$) between treatments. Non-parametric data were analysed by the Kruskal-Wallis test. Where there was a significant difference ($P \leq 0.05$) of medians, the data were further analysed by comparison of notched box-plots to determine significant differences ($P \leq 0.05$) between treatments.

Results

Reference toxicity test

No mortality was recorded within control vessels. The calculated 72 h LC_{50} for $CdCl_2$ was 7.45 mg L^{-1} (confidence limits $6.37 - 8.72 \text{ mg L}^{-1}$) which is within the reported LC_{50} value range of $2.7 - 9.9 \text{ mg L}^{-1}$ (Ciarelli et al., 1997).

Chemical analyses of water and sediment

Total Petroleum Hydrocarbon (TPH) concentrations of ANS (oil:seawater ratio 1: 99) WAF and DWAF were calculated to be 3 mg L^{-1} and 20 mg L^{-1} respectively when quantified using DCM extractions with ANS oil as a calibration standard and analysis by UVF.

Analysis of the sediment using UVF found that after correction for background fluorescence, mean measured sediment concentrations of ANS oil were 19 , 40 , and $97 \text{ } \mu\text{g g}^{-1}$ dry wt. for nominal spiked concentrations of 110 , 220 and $440 \text{ } \mu\text{g g}^{-1}$ respectively. The ANS concentrations in the sediment from spiking with WAF and DWAF were 7 and $131 \text{ } \mu\text{g g}^{-1}$ dry wt. respectively. Duplicate analyses of reference estuarine sediment gave a coefficient of variance (CV) of 0.7% .

Acute sediment toxicity test

Mortality of control organisms was low (mean = 5%). No significant mortality was exhibited at or below $440 \text{ } \mu\text{g g}^{-1}$ nominal sediment or WAF exposure (Table 2). Although 20% mortality was observed in the DWAF exposure, this was not significantly different to the Corexit 9527 treatment.

Chronic sediment toxicity test

Water quality measurements during the first 28 days of the test were well within water quality criteria as defined in Table 1. Burrows were constructed in the sediment of all exposures and no abnormal behaviour was apparent other than a possible reluctance to burrow by some oil-exposed

amphipods. After 28 days exposure, survivorship was over 90 % for control, Corexit, WAF and 220 $\mu\text{g g}^{-1}$ treatments and over 80 % for other treatments with the exception of DWAF in which there was a significant ($P \leq 0.05$) mortality of 43 %. Only organisms from the 110 $\mu\text{g g}^{-1}$ and 440 $\mu\text{g g}^{-1}$ treatments had significantly lower growth rates than control organisms but the growth rates of survivors from the DWAF-exposed sediment, 19 $\mu\text{g day}^{-1}$, were significantly less than that of both the Corexit, 41 $\mu\text{g day}^{-1}$, and WAF treatments, 36 $\mu\text{g day}^{-1}$ (Fig. 1). The lowest growth rate of 7 $\mu\text{g day}^{-1}$ was recorded within the highest nominal exposure of 440 $\mu\text{g g}^{-1}$ (Fig. 1). Differences in median lengths of *C. volutator* (Fig. 2) showed a similar trend to that of the growth rates i.e. control-, Corexit- and WAF-exposed organisms were significantly longer than all other treatments. The largest individual at 4.6 mm occurred within the Corexit treatment and smallest at 1.2 mm occurred within both the 110 $\mu\text{g g}^{-1}$ and 440 $\mu\text{g g}^{-1}$ treatments.

Water quality measurements remained well within water quality criteria during the remainder of the test (Table 1). The chronic test was terminated after 75 days when it was observed that reproduction had occurred in all of the control vessels, evidenced by the presence of tiny burrows in the sediment and greater turbidity of the overlying water. Survivorship continued to be high (>80 %) within all treatments except for DWAF-exposed amphipods in which there was a mean survivorship of only 23 %, significantly lower ($P \leq 0.05$) than all other treatments. There was also an extremely high mortality (87 %) in one replicate vessel of the Corexit treatment which also contained a single isopod, later identified as *Cyathura carinata* (Krøyer 1847). This organism was believed to be responsible for the high mortality of the amphipods within the test vessel. The results from the Corexit replicate containing the isopod were therefore omitted from statistical analyses.

The lowest mean growth rate of 16 $\mu\text{g day}^{-1}$ again occurred within the highest nominal oil concentration treatment of 440 $\mu\text{g g}^{-1}$, this was significantly ($P \leq 0.05$) less than all other treatments. Organisms exposed to, 110 $\mu\text{g g}^{-1}$, 220 $\mu\text{g g}^{-1}$ and DWAF-spiked sediment had mean growth rates significantly ($P \leq 0.05$) less than that of control, Corexit and WAF treatments. The highest mean growth rate of 41 $\mu\text{g day}^{-1}$ was achieved by amphipods exposed to WAF-spiked sediment, a rate similar to that of the controls and Corexit exposed organisms. The measured lengths of amphipods (Fig. 2) reflected that of growth rates by weight i.e. amphipods of control, Corexit and WAF treatment were significantly ($P \leq 0.05$) longer than all other treatments and 440 $\mu\text{g g}^{-1}$ exposed *Corophium* were

significantly ($P \leq 0.05$) shorter than all others. The largest individuals of 6.2 mm were found within the Corexit treatment and the smallest of 2.0 mm were found in both the DWAF and $440 \mu\text{g g}^{-1}$ treatments.

Reproduction occurred in all treatments, although three out of the six replicate vessels of both the DWAF and $440 \mu\text{g g}^{-1}$ treatments contained no offspring as did two vessels of the $220 \mu\text{g g}^{-1}$ treatment. Reproduction was greatest within the WAF-exposed organisms with a mean of 1.98 neonates per surviving adult (Table 2). The lowest mean offspring per survivor of 0.05 was recorded in the $440 \mu\text{g g}^{-1}$ treatment. Reproduction within DWAF, $110 \mu\text{g g}^{-1}$, $220 \mu\text{g g}^{-1}$ and $440 \mu\text{g g}^{-1}$ treatments were all significant less than that of Control, Corexit and WAF treatments (Table 2). No significant difference ($P > 0.05$) in sex ratio was observed, therefore the number of offspring per surviving female showed a similar trend to that of offspring per survivor (Table 2).

Discussion

Acute versus chronic endpoint sensitivity

The present study describes a method for conducting chronic whole sediment toxicity tests using the estuarine amphipod *C. volutator*. By continuing to monitor the survivorship following an initial exposure to sediment spiked with slightly weathered ANS crude oil, we have shown that sediment that might not normally be considered toxic during a standard 10-day test could significantly reduce survivorship during the life-cycle of the organism (Table 2). In addition, it was also observed that non-acutely toxic sediment could significantly depress growth rates of amphipods leading to significantly reduced reproduction (Table 2). Although only one treatment i.e. the DWAF-spiked sediment, was found to cause significant mortality during the chronic test, all other oil-exposed organisms had reduced growth rates with the exception of the WAF-spiked sediment in which only a low concentration of ANS-derived hydrocarbons was found. This is consistent with Corexit 9527 dispersing and stabilising the oil as small droplets that then became associated with the sediment. Had these contaminated sediments been collected from the environment, chemically analysed and subject to the standard acute testing test (Roddie & Thain 2001), the potential for population level effects would not have been observed.

Chronic test performance

The long-term nature of this test provides opportunities for systematic errors to amplify and it was therefore essential to keep variation in water quality parameters within strictly defined limits. During this test, the Coefficients of Variance for all water quality measurements were below 1.5 % (Table 1). Mean survivorship of amphipods within control vessels was >90 % with above 75 % in all replicates. Variation within oil-exposed organisms was larger than that of controls, a phenomena that can be used as a stress indicator but also can be problematical for statistical analysis which assumes homogeneity of variances (Forbes & Depledge 1996). The use of six replicates per treatment for the full life-cycle exposure was therefore desirable to enhance the power of the statistical analysis. One problem that did arise was the presence of an individual isopod, *C. carinata*, which presumably was mistakenly added to the vessel as a neonate whilst selecting neonate *C. volutator*. The isopod *C. carinata* resides in muddy intertidal sediments and often co-occurs with *C. volutator* (Connor et al., 2004). Although there is no literature on competition between these two species, our own tests (unpublished) suggest that when placed together within the restricted confines of an exposure vessel, *C. carinata* can eliminate *C. volutator*. The isopod has a slightly different motile action and therefore should be distinguished from the amphipods as they swim but errors are clearly possible when using large numbers of such small organisms. It is therefore important that test operators are familiar with a range of common sediment organisms as well the test species. The main limitation with the *C. volutator* chronic test is the length of time required for the test species to reach maturity and reproduce i.e. 75 days in this study compared with 28 days for both the *L. plumulosus* (USEPA 2001) and the *G. locusta* (Neuparth et al., 2005) chronic tests. Despite the relatively short test period, the *G. locusta* test suffers from very low survivorship, 50-60%, of the control organisms, probably due to cannibalistic behaviour of the test species (Dick 1995; Neuparth et al., 2005) compared to >90% control organism survivorship during the present study. As feeding behaviour is commonly altered by toxicants, cannibalism confounds the results thus making interpretation difficult. The additional costs in time and effort required for the *C. volutator* test is mitigated by the enhanced control organism survivorship. It may also prove useful to conduct partial lifecycle tests by initiating the test using juvenile *C. volutator*; thus providing a shorter test but retaining reproduction as an endpoint. Growth rate based on wet weight of organisms was used in preference to dry weight, as used by the USEPA (2001) chronic test, as it permits chemical analysis of tissue should this be required. Previous comparisons of dry and wet weight measurements showed equivalence of results (correlation significance, $P \leq 0.01$) with a dry:wet ratio of 0.228 and a strong

relationship between wet weight and body length (unpublished data), hence measurement of wet weight is an acceptable rapid means of assessing growth whilst retaining tissue for future analysis. The mean growth rate of control amphipods based on body length during the 75 days exposure was 0.07 mm day^{-1} ; this value was also reported by Peters & Ahlf (2005) for *C. volutator* grown at 15°C .

Ecological relevance

The current study was conducted during the summer (June – August) using neonates produced by amphipods that had over-wintered from the previous year. In their natural environment, *C. volutator* born at the beginning of the summer period can, if conditions are suitable, grow, mature and reproduce in the same year; individuals born later in the season grow more slowly and over-winter to reproduce the following year (Wilson 1989). Comparing the growth rates of control organisms in the present study with those from a preliminary test initiated the previous autumn, it would appear that the early summer cohort have a greater capacity for growth than the late summer/autumn cohort, i.e. despite identical laboratory conditions, the amphipods in the present study grew at a greater rate than those in the previous test (Scarlett et al., 2005). Studies conducted at different times of year may therefore not be directly comparable; this may have particular relevance to the testing of field-contaminated sediment. Sediment contaminants may have a longer period in which they can degrade during the life-cycle of late summer/autumn neonates thus allowing the organisms a greater time to recover in the absence of the toxicant. It is therefore recommended that chronic sediment tests be initiated in summer using the first generation cohort of neonate *C. volutator* or from cultured organisms, see Peters & Ahlf (2005). In terms of oil pollution, it is possible that a spill occurring in early summer will more severely affect amphipod growth rates than if it occurred later in the year.

The use of dispersants in the nearshore shallow water environment may result in surface oil being transported to the benthos leading to mortality of sensitive species; this is believed to have occurred during the Sea Empress spill off Milford haven SW Wales in 1996 where it was observed that amphipod populations disappeared following the spill (Nikitik & Robinson 2003; SEEEC 1998). In the present study the chemically dispersed oil treatment resulted in the highest concentration of hydrocarbons within the sediment and the greatest toxic effect in terms of survivorship. However, survivors of the DWAF exposure had greater mean growth rates than organisms exposed to the highest nominal whole oil-spiked sediment (Fig. 1) suggesting that oil-tolerant individuals can prosper

following the elimination of more sensitive individuals, thus creating the potential for oil-tolerant populations to develop. The concentrations of crude oil within the sediment reported in this study are consistent with subtidal concentrations following real spills e.g. Sea Empress (Nikitik & Robinson 2003; SEEEC 1998) and experimental chemically dispersed spills (Boehm et al., 1987).

Conclusions

The sediment chronic test reported here has shown that sediment that was not evidently toxic during 10-day acute tests could have population-level effects on sediment-dwelling amphipods. The amphipods exposed to chemically-dispersed weathered crude oil had higher mortality and lower growth rates than control-, Corexit 9527- and WAF-exposed organisms, resulting in reduced reproduction. It was evident from the sediment concentrations and toxicological response of the amphipods that the presence of the dispersant Corexit 9527 increased the concentration of bioavailable toxic components of the weathered ANS oil with a consequential impact on sediment-dwelling organisms. The test protocol and acceptability limits proved to be robust but the test would benefit from further development by applying the test method to field-contaminated sediments.

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Table 1.

Test conditions and acceptability limits for chronic sediment toxicity tests with *Corophium volutator*.

Water quality measurements and performance criteria achieved, mean and coefficient of variance (CV),

given in italics.

1. Test type:	Whole sediment toxicity test, static-renewal
2. Test sediment:	Mud, sandy mud or muddy sand. Sieved through 300 µm
3. Overlying water:	Filtered seawater
4. Temperature:	Salinity: daily limits: 25 psu ± 3 psu. (<i>Mean = 25.2, CV = 0.4%</i>)
5. Photoperiod:	Daily limits: 15 °C (±2 °C). (<i>Mean = 15.1 °C, CV = 1.4%</i>)
6. Test vessel:	12 h light: 12 h dark
7. Sediment volume:	2-L glass Pyrex squat-form beaker
8. Overlying water volume:	160 ml (15 mm depth)
9. Water renewal:	Fill to 1200 ml mark in test vessel (<i>ca.</i> 1000 ml)
10. Life stage and size of amphipod:	Once per week: siphon off and replace 800 ml
11. Number test organisms/vessel:	Neonates: retained between 300 µm and 500 µm mesh screens
12. Number of replicate vessels:	30
13. Feeding:	9 for toxicity test (including 3 sacrificed at 28-day stage) + dummy vessels for chemical analyses
14. Aeration:	Standard aquarium invertebrate diet: 2 mL per vessel, once per week. (mix of Waterlife Invert Food, Liquifry Marine, Roti-Rich and dried algae)
15. Dissolved oxygen (DO):	Constant 1-2 bubbles/s via glass Pasteur pipette
16. pH:	Daily limits: >3.6 mg l ⁻¹ (<i>Mean = 6.73 mg l⁻¹, CV = 1.4 %</i>)
17. Test duration:	Within 7.0 – 9.0 pH units (<i>Mean = 8.22, CV = 0.9 %</i>)
18. Endpoints:	Intermediate 28 days then when reproduction occurs in all control replicates
19. Performance criteria:	Survival and growth rate (µg individual ⁻¹ day ⁻¹) at 28 days. Survival, growth rate and reproduction upon test termination
	Control survival at test termination ≥ 70% (<i>Mean = 95%, CV = 9.0 %</i>).

Table 2.

Mean (standard error) of *Corophium volutator* acute and chronic toxicity test results on sediment spiked with weathered Alaskan North Slope crude oil (nominal concentrations $\mu\text{g g}^{-1}$ dry weight), Water-Accommodated Fraction (WAF) and Corexit 9527 dispersed WAF (DWAF) and Corexit 9527. Asterisks indicate treatments significantly less than the control ($P \leq 0.05$), NP indicates test not performed

Treatment	Acute	Chronic			
	% 10-day survival	% 28-day survival	% 75-day survival	Offspring/Survivor	Offspring/female
Control	95 (2.9)	93 (3.2)	95 (2.5)	1.3 (0.2)	2.6 (0.5)
Corexit	77 (1.7)*	100 (0.0)	91 (1.7)	1.7 (0.5)	4.2 (0.2)
WAF	100 (0.0)	100 (0.0)	98 (1.4)	2.0 (0.4)	5.7 (1.3)
DWAF	80 (2.9)*	58 (6.8)*	23 (3.5)*	0.3 (0.1)*	1.6 (1.0)*
110 $\mu\text{g g}^{-1}$	NP	86 (4.0)	84 (4.5)	0.4 (0.1)*	3.8 (1.0)*
220 $\mu\text{g g}^{-1}$	95 (2.9)	93 (3.3)	95 (5.0)	0.4 (0.1)*	2.0 (0.8)*
440 $\mu\text{g g}^{-1}$	93 (1.7)	88 (4.8)	89 (2.8)	<0.1*	0.8 (0.4)*
880 $\mu\text{g g}^{-1}$	75 (2.9)*	NP	NP	NP	NP

Figure 1 Mean growth rates ($\mu\text{g day}^{-1}$) of *Corophium volutator* exposed to sediment spiked with weathered Alaskan North Slope crude oil (nominal concentrations $\mu\text{g g}^{-1}$ dry weight), Water-Accommodated Fraction (WAF) and Corexit 9527 dispersed WAF (DWAF) and Corexit 9527 for 28 days (a) and 75 days (b). Error bars = standard error and * indicates treatments significantly less than the control ($P \leq 0.05$), ** indicates treatments significantly less than other treatments ($P \leq 0.05$).

Figure 2 Body lengths (excluding antenna) of *Corophium volutator* exposed to sediment spiked with weathered Alaskan North Slope crude oil (nominal concentrations $\mu\text{g g}^{-1}$ dry weight), Water-Accommodated Fraction (WAF) and Corexit 9527 dispersed WAF (DWAF) and Corexit 9527 for 28 days (a) and 75 days (b). Notched boxes contain inter-quartile range with + denoting mean length.

Fig 1:

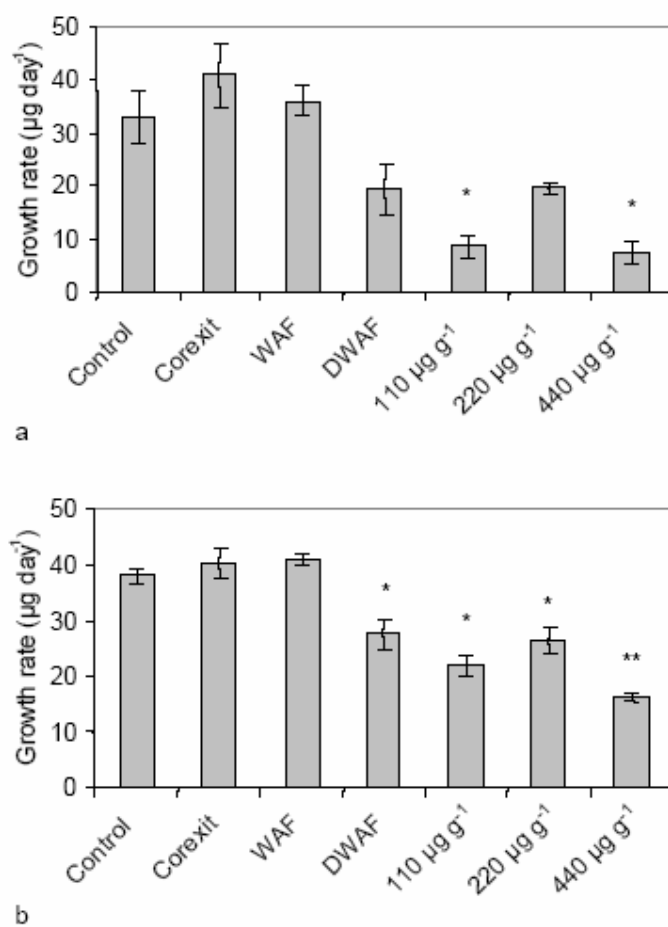
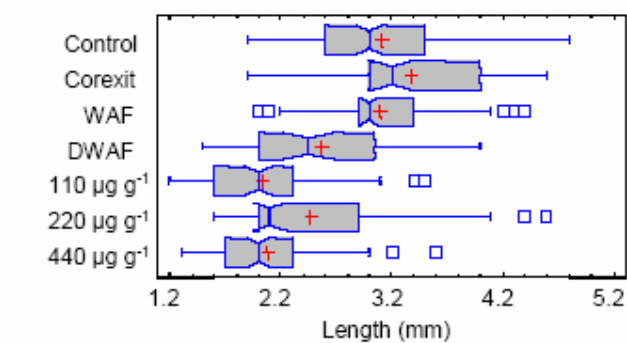
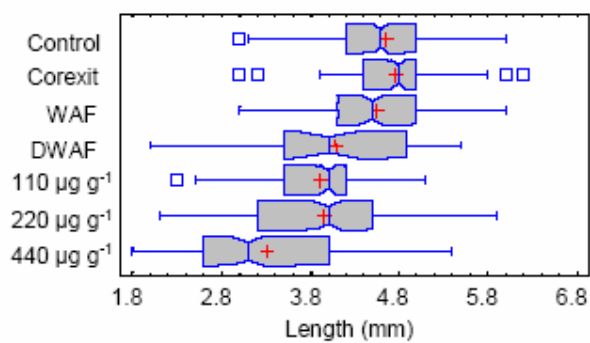


Fig 2:



a



b