RUNNING HEAD: MODELING THE TOXICITY OF DISSOLVED OIL EXPOSURES

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Modeling the toxicity of dissolved crude oil exposures to characterize the sensitivity of cod (*Gadhus morhua*) larvae and role of individual and unresolved hydrocarbons

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ABSTRACT

Limited toxicity data are available to characterize the sensitivity of deep sea species to crude oil. In this study, the toxicity of an artificially weathered oil was investigated using Atlantic cod (*Gadus morhua)* larvae. A novel exposure system was applied to differentiate potential effects associated with dissolved and droplet oil with and without Corexit 9500 dispersant. After a 4 d exposure and subsequent 4 d recovery period, larval survival and growth were determined. Analytical data characterizing test oil composition including individual polyaromatic hydrocarbons (PAHs) based on GC/MS and unresolved hydrocarbon classes obtained by two-dimensional chromatography coupled with flame ionization detection (2d-GC) was used as input to an oil solubility model to calculate toxic units (TUs) of dissolved

PAHs and whole oil, respectively. Critical target lipid body burdens (CTLBBs) derived from modeling to characterize the sensitivity of the effect endpoints investigated were found to be consistent across treatments and within the range previously reported for pelagic species. TUs calculated based on PAHs captured only 3-11% of the TUs associated with the whole oil highlighting the limitations of traditional total PAH exposure metrics for expressing oil toxicity data.

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Key words: Atlantic cod, weathered crude oil, toxicity, droplets, target lipid model, toxic units

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Highlights

- Novel exposure system applied to generate crude oil exposures with and without droplets and dispersant
- Coupled oil solubility and toxicity model applied to characterize cod larvae sensitivity and role of analytically resolved and unresolved oil exposures
- Observed effects on survival and growth dictated by dissolved, not droplet oil
- Sensitivity of Atlantic cod to oil within range reported previously for pelagic species
- Unresolved oil components predicted to contribute most to observed toxicity
- Framework can be applied to improve design and interpretation of future studies

1. INTRODUCTION

Past oil spill research has largely focused on the fate of surface spills and effects on shorelines and pelagic and nektonic species. However, following the Deepwater Horizon tragedy, increased attention has been directed to deep sea oil releases (Murphy et al., 2016). While systems for incident prevention serve as the principal defense to avoid such spills, a key strategy for mitigating the safety and environmental impacts associated with a subsea well release of oil is use of subsea dispersants (John et al., 2016). When applied subsea, dispersants reduce the size of oil droplets, increasing the dissolution rate and reducing the droplet rise velocity thereby increasing the residence time for degradation in the water column. The creation of these small droplets limits the formation of slicks, reduces emissions from volatile oil components that can pose safety concerns to responders, and decreases impacts to wildlife and shoreline habitats (Brakstad et al., 2015; Johansen et al., 2013; Prince, 2015). However, an important risk trade-off when applying subsea dispersants is the increase in oil exposure to the deep ocean environment (NRC, 2005). Thus, understanding the relative sensitivity of deep sea species to dispersed oil is a current research priority for response decision-making (DeLeo et al., 2016).

Atlantic cod (*Gadus morhua*) inhabit the deeper, colder regions of the Northern Atlantic Ocean. Adults are usually found in deeper waters at depths up to 600 m, while embryos and larvae reside in coastal pelagic environments and juveniles prefer sublittoral waters (Froese and Pauly, 2017). This species has been one of the most commercially important fish species in the Northern Hemisphere for centuries (Ottera, 2004). Currently this species is labelled vulnerable on the IUCN Red List of Threatened Species, a likely result of overfishing (Sobel, 1996), although promising signs of population recovery of western population stocks have recently been reported (Rose and Rowe, 2015). Atlantic cod engages in diel vertical migrations off the sea bottom and into the water column at night to prey on invertebrates and

fish (Froese and Pauly, 2017). The commercial relevance, recognized vulnerability and barotolerance, which facilitates field collection and laboratory testing under ambient pressure, provide the rationale for investigating the sensitivity of this species to oil exposures.

Several studies have investigated the effects of dispersed oil on this species. In a screening study by Khan & Payne (2005), mortality of adult Atlantic cod and three other mature fish species were investigated following a single 4 day declining exposure to Hibernia crude oil with and without dispersant (1:1 dispersant to oil ratio (DOR) (Khan and Payne, 2005)). Cod tended to be among the most sensitive of the fish species investigated. At the nominal loading of 0.25 mL/L for the dispersant or oil alone, reported mortality was 53% and 40%, respectively. For the dispersed oil WAF prepared at the same nominal loading with both dispersant and oil (i.e. total loading of 0.5 mL/L) mortality increased to 70%. This observed toxicity is consistent with predictions assuming dispersant and oil act independently. Lyons et al. (Lyons et al., 2011) exposed juvenile Atlantic cod to two dilutions (0.2% and 1.0% v/v) of water-accommodated fraction (WAF) of weathered Mediterranean South American crude oil prepared with and without dispersant (1:25 DOR) at three test temperatures. Total polyaromatic hydrocarbons (PAHs) concentrations in test exposures were determined using fluorescence spectrophotometry and found to be significantly higher in chemically dispersed treatments. While no mortality was reported, higher levels of Ethoxyresorufin O-deethylase induction were observed in chemically than mechanically dispersed treatments, suggesting chemically dispersed oil to be more bioavailable.

Nordtug et al. 2011 (Nordtug et al., 2011b) applied a continuous dosing system to expose Atlantic cod larvae to five concentrations of artificially weathered Troll crude oil for 4 days followed by 4 day recovery period in clean seawater. Oil was pumped through a series of nozzles with seawater to create different oil dispersion treatments with a uniform droplet size distribution. Oil-dosed seawater was then delivered directly or first filtered through glass wool

to exposure chambers so comparative effects of dissolved + droplet oil versus dissolved oil could be inferred. Results indicated that food assimilation rate and survival likely decreased in a concentration-dependent manner with reported EC50s of 2 and 40 µg/L, respectively, based on the sum of parent and alkylated PAHs and analyzed using GC/MS. No consistent differences were found between the unfiltered and filtered treatments indicating oil droplets did not modulate toxicity. In a follow-up study, larval gene expression was not significantly altered by the presence of oil droplets (Olsvik et al., 2011). For three weeks, Holth et al. 2014 (Holth et al., 2014) exposed juvenile Atlantic cod to three weathered oils (Arabian Light crude oil, North Sea crude oil and ship-diesel) using a continuous flow-through system using columns containing gravel with test oils for three weeks. For each oil, two doses were investigated (2 and 6 g oil/kg gravel). While no mortality was reported, dose-related increases in hepatic CYP1A gene expression were observed for all oils. Oxidative stress biomarkers appeared to be induced in the presence of diesel, but not in the presence of crude oil. In a different experiment, cod larvae were exposed to dispersions of chemically and mechanically dispersed Troll oil of similar droplet size at three nominal oil concentrations (0.25, 0.79, or 2.5 mg oil/L) for 4 days (Hansen et al., 2016). Total PAH concentrations determined by GC/MS in the highest mechanically and chemically dispersed treatments were 8.7 and 8.4 µg/L, respectively. Approximately 50% of larvae (at first feeding stage) exposed to these concentrations survived and comparable survival was observed for larvae subjected to food deprivation alone. A significant concentration-dependent reduction in dry weight was noted for oil exposures compared to controls. The results of Hansen et al (Hansen et al., 2016) and Nordtug et al (Nordtug et al., 2011b) suggest that dissolved oil exposures may reduce survival and growth due to inadequate nutrition from impaired larval feeding.

The results of these studies indicate that oil droplets serve a limited role in contributing to cod larvae toxicity and are consistent with earlier work (Carls et al., 2008; Redman et al., 2016).

Available data also support the generalization that dispersants can enhance oil bioavailability (i.e. dissolved hydrocarbons), as reported in previous studies (Couillard et al., 2005; Mu et al., 2014; Ramachandran et al., 2004; Schein et al., 2009; Van Scoy et al., 2012; Wu et al., 2012). However, given the different test oils, dosing and analysis methods, exposure metrics, life stages and effect endpoints, comparison of toxicity results across the different studies summarized above is impossible. Further, it is unclear how sensitive Atlantic cod are to crude oil exposures relative to other test species. A model that predicts the concentration and composition of dissolved oil exposures can facilitate analysis and interpretation of such datasets by combining improved assessment of test exposures with observed dose response relationships.

The PETROTOX model was developed to predict the aquatic toxicity of petroleum substances for a given organism/effect endpoint based on oil composition and test-specific design considerations (Redman et al., 2012b). Composition is determined by analysis of the component masses of different hydrocarbon classes and carbon numbers that comprise the test oil using two-dimensional gas chromatography coupled to flame ionization detection (2d-GC). Based on the physiochemical properties of these components, the test specific oil dissolution is simulated in the dosing system used to assess toxicity. A key advantage of this approach is that a more complete compositional characterization of dissolved oil can be simulated than traditional analysis which quantitates only a subset of hydrocarbons present in the oil (e.g. BTEX, targeted parent and alkylated PAHs) and fails to differentiate dissolved from droplet oil phases (Redman, 2015). The dissolved component concentrations calculated by PETROTOX are normalized by the predicted toxicity of each component to compute toxic units (TUs). Assuming toxicity of the components can be described using concentration addition, the TUs for each component are then summed as a preferred exposure metric for evaluating concentration-response relationships and predicting toxicity for a given

organism/endpoint (Redman and Parkerton, 2015). The toxicity of the individual components is estimated using the Target Lipid Model (TLM) and hydrocarbon class-specific adjustment factors (McGrath and Di Toro, 2009). The TLM is based on an organism-specific critical target lipid body burden (CTLBB) and estimated component partition coefficients to target lipid, which is estimated from the Log octanol-water partition coefficient (Log Kow). Organism specific CTLBBs for a defined endpoint are estimated by fitting the TLM to toxicity datasets for individual hydrocarbons or related substances. CTLBBs for acute *in-vivo* endpoints (i.e. survival, immobilization) across different aquatic species range from 8.8 to 360 µmol/g octanol (n= 54 species) with chronic effects spanning from 0.4 to 137 µmol/g octanol (n = 36 species) (McGrath and Di Toro, 2009). The utility of using TUs (derived using this approach) to successfully describe toxicity of different oils and dosing methods across species has been demonstrated (Kang et al., 2014; Redman et al., 2016; Redman et al., 2014). However, based on a recent review by Klok et al. (2014), toxicity data for Atlantic cod on individual hydrocarbons are not available to derive endpoint-specific CTLBBs for this species.

The main objective of this study is to illustrate an alternative approach using the TU concept for deriving Atlantic cod survival and growth CTLBB estimates based on analysis of empirical toxicity data for dispersed crude oil with and without dispersant. The estimated CTLBBs from this analysis are then compared to CLTBBs reported for other species to determine the relative sensitivity of this species to oil. A secondary goal is to determine the extent to which the subset of speciated PAHs that were quantified in the aqueous test media understates the TUs associated with the unresolved components of the whole test oil and identify what unresolved oil components are predicted to be the most important contributors to adverse effects. The potential opportunities and advantages of extending this strategy in

169 analyzing and interpreting additional toxicity studies with different oils and test species is also
170 discussed.

171 ## 2. MATERIALS AND METHODS

172 ### 2.1. Test Oil

173 A naphthenic crude oil (Troll) from the North Sea was obtained. The oil was weathered in a
174 one-distillation step at 200°C (Stiver and Mackay, 1984), and the residue was used for the
175 experiments. This weathering process removes a substantial amount of the most volatile, but
176 also water-soluble and biodegradable, components, including benzene, toluene, ethylbenzene
177 and xylenes (BTEX). This oil and weathering degree have been used in a series of previous
178 experiments where detailed compositional analyses were available. Two-dimensional
179 chromatography coupled with flame ionization detection (2d-GG) was used to characterize
180 the mass distribution of the hydrocarbon classes that comprise the test oil as a function of
181 carbon number. Detailed analysis of individual PAHs 2–5 ring polycyclic aromatic
182 hydrocarbons in the oil was also performed by gas chromatography–mass spectrometry
183 (GC/MS) operated in selected ion monitoring (SIM) mode.

184 ### 2.2. Preparation of exposure solutions

185 The full description and validation of the experimental set-up has been previously reported by
186 Nordtug et al. 2012 (Nordtug et al., 2011a), and has been used for toxicity experiments with
187 early life stages of fish (Hansen et al., 2016; Nordtug et al., 2011b; Olsvik et al., 2011; Olsvik
188 et al., 2012; Olsvik et al., 2010). Briefly, the weathered test oil [with chemical dispersant
189 (CD) or without (MD)] was dispersed into filtered seawater (5 μm) through a series of nozzles
190 yielding a constant flow of dispersion with homogenous droplet size. The objective of the
191 exposure system is to directly compare the effects of test solutions with and without the
192 presence of oil droplets. A dilution series is created from the original dispersion and the

193 water-soluble fraction (WSF) of each dilution is separated from particulate oil by filtration.
194 Thus, the experiment consisted of two parallel exposure series, one with diluted dispersion
195 (unfiltered) and one with the corresponding water-soluble fraction (filtered). The
196 concentration gradient used for the dispersion was logarithmic with a spacing of 0.5 log-units
197 between concentrations. The filter units consisted of fine glass wool on top of a Watman glass
198 fiber filter. The exposure containers consisted of 5 L Schott borosilicate glass bottles (Schott
199 AG) with their base removed and placed upside down in a water bath. Exposure solutions and
200 clean seawater (controls) were added in the lower part of the exposure container through
201 Teflon tubing (bore 1 mm). Water was drained from the surface through a 300 μm plankton
202 mesh.

203 A peristaltic pump (Watson–Marlow) equipped with Marphrene® tubing was used to draw
204 the dispersion through the glass filters and into the WSF exposure containers. Dispersions
205 were added passively to the exposure containers through inlet Teflon resistance tubes with an
206 inner diameter of 1 mm and flow was adjusted by the height of the inlet water column. Three
207 identical exposure systems were used in order to achieve three biological replicates for every
208 exposure concentration. To characterize actual exposure concentrations in unfiltered and
209 filtered treatments samples were collected and analyzed for a suite of PAHs (see *Chemical*
210 *analyses using GC-MS)* that were quantified in the test oil.

211 **2.3. Larval toxicity tests**

212 In May 2009, a test was performed without dispersant (mechanically dispersed oil, MD). In
213 November 2009, a second test was performed, but this test included dispersant (Dasic
214 Slickgone NS, Dasic international Ltd) and the dispersant was premixed into the oil at a DOR
215 of 1:25 (chemically dispersed oil, CD). The tests were run in sequence rather than in parallel
216 due to logistic and resource constraints. A dispersant only treatment was not included since at

the oil concentrations applied (see below) the dispersant would be present at ≤ 0.1 mg/L which is well below concentrations posing toxicity concerns (Hansen et al., 2014). To maintain similar mixing conditions in the two experiments, the energy introduced during the creation of the droplets was limited by reducing the water flow through the dispersion generator. This caused the mean volumetric droplet size distributions in the two experiments as recorded by a Coulter Counter Multisizer (Beckman Inc.) to be approximately similar at 12.8 (± SD=0.12) and 10.4 (± SD=0.11) microns for the dispersions generated without and with dispersant, respectively.

Fertilized cod eggs (*Gadus morhua*; Marine Harvest Cod, Norway) were transported to the SINTEF Sealab laboratory, where they hatched and the larvae was maintained. At 9 days post hatch (dph), cod larvae were exposed to five different nominal concentrations of dispersions (25-2500 µg oil/L) and corresponding filtered dispersions, i.e., Water Soluble Fractions (WSFs) until 13 dph. At 13 dph, a four-day recovery period started in clean sea water and continued for four days until the experiment ended at 17 dph. All treatments were done in triplicates, except the control treatment, which consisted of 12 replicates. The approximate initial number of larvae per replicate was 240. During the experiment, the larvae were fed rotifers in green water (*Isochrysis galbana*). Larval survival was monitored daily during the whole period whereas dry weights were measured at the start (9 dph) and end of exposure (13 dph), and after the recovery period (17 dph). The experiment was conducted with natural seawater collected at 70 meter depth in the Trondheim Fjord. The water was sand filtered, matured for approximately 24 hours and temperature adjusted in heat exchangers before being equilibrated with oxygen. The acclimated seawater was then filtered by a 2 µm in-line cartridge filter (Cuno). The salinity was approximately 35.5 ppt and the oxygen saturation in the exposure containers was between 95 and 98% throughout the experiments. The

experiments were conducted under constant temperature (12 ± 1 °C) and dim light conditions with individual LEDSs illuminating a diffusing cover on top of each exposure container.

2.4. Chemical analyses using GC-MS

Water samples for chemical analysis (approximately 900 mL each) were collected one and three days into the exposure period from all exposures and groups. The water samples were acidified with diluted hydrochloric acid, extracted with dichloromethane, dried over Na_2SO_4 and concentrated to 1 mL. Analysis for the same suite of 44 PAHs measured in the oil were also targeted in water samples using GC/MS–SIM. The system comprised of a HP6890N gas chromatograph fitted with a Hewlett- Packard HP7683B Series auto-sampler and a HP5975B quadrupole mass selective detector. The column was a Phenomenex ZB-5MS fused silica capillary column (30 m×0.25 mm id×0.25 mm film thickness). The carrier gas was helium at a constant flow of 1.0 mL/min. A 1.0 µL sample was injected into a 310 °C splitless injector. The oven temperature was programmed from 40 °C for 1 min, then to 315 °C at 6 °C/min and held for 15 min. Data and chromatograms were monitored and recorded using MSD ChemStation (version D.03.00.611) software. The quadrupole mass spectrometer ion source temperature was 230 °C. The exposure concentrations were stable over time and concentrations for each PAH were reported as the mean value of results obtained on day 1 and day 3 of the exposure period.

2.5. Statistical analysis

To compare survival and dry weights of larvae between controls and exposed larvae and between parallel filtered and unfiltered treatments, one-way ANOVA followed by Dunnett's multiple comparisons test was performed using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com. Traditional dose response analysis of oil toxicity data was performed by evaluating observed effects on cod larvae

survival and growth as a function of measured total PAH concentrations in MD and CD unfiltered and filtered treatments. LC50s and EC20s for growth impairment were computed using the *drc* package in R (Ritz et al., 2015).

2.6. Modelling dissolved hydrocarbon exposures and toxic units

The modeling analysis applied in this study is outlined in Figure 1. First, PAH concentrations in the oil were used as inputs into an oil solubility model (Redman et al., 2012a) to predict dissolved concentrations of PAH components in each filtered treatment (where it is assumed droplet oil was excluded). This model was run iteratively to determine the oil loading that successfully fit mean measured concentrations of individual PAHs determined in the collected water samples. In step 2, the estimated oil loading from step 1 was used as input into the solubility model to match mean concentrations of individual PAHs observed in collected water samples from the unfiltered treatment where droplets were present. This step involved iteratively selecting droplet oil concentrations that minimized differences between predicted and measured PAH concentrations in each unfiltered treatment. This analysis assumes that oil and water are at equilibrium so droplet size does not alter the predicted dissolved exposures and dissolved exposures are the same in filtered and unfiltered exposure systems.

Oil composition based on 2d-GC analysis and estimated oil loadings derived in Step 1 can be input to the PETROTOX model (Redman et al., 2012b) to compute test exposures in terms of dissolved whole oil toxic units (TUs) in both filtered and unfiltered treatments providing the CTLBB for the specific organism/effect endpoint is available. However, in the case of Atlantic Cod, a CTLBB is not available. Thus, in step 3 we used the PETROTOX model to estimate the CTLBB for survival such that the observed TU-response relationship that was fit using log-logistic regression exhibited 50% cod larvae mortality at a predicted dissolved acute TU=1. The acute CTLBB derived from this analysis was then compared to CTLBBs reported

for various pelagic species to gain insights on the relative sensitivity of this deep-sea species to oil exposure. A similar analysis was repeated by selecting an a CTLBB to predict chronic TUs that were fitted to the growth effects data such that a 20% effect on cod larval growth corresponded to a predicted dissolved chronic TU=1. Given the estimated CTLBBs, the ∑TUs associated with the dissolved exposures for the resolved PAHs at each loading from Step 1 was also calculated for comparison to the ∑TUs for the whole oil derived from the 2d-GC composition in Step 3. This analysis allows the contributing role of resolved PAHs to toxicity to be quantified relative to that of the unresolved components that comprise the whole oil. Modeling in step 3 also provides insights on the importance of different unresolved hydrocarbon classes in accounting for predicted oil toxicity.

3. RESULTS AND DISCUSSION

3.1. Characterization of the test oil

Detailed 2d-GC analytical characterization data are provided in Table S1 and indicated that the test oil was comprised of 11% normal and iso-paraffins, 45% naphthenics (i.e. cycloalkanes), and 26% aromatic hydrocarbon classes with carbon numbers up to C30 with the remaining 18% representing a residual fraction of higher carbon number components. This latter fraction is assumed not contribute to aquatic toxicity (Redman et al., 2012b). Concentrations of individual PAHs determined using GC-MS are provided in Table S2, which collectively amounted to concentration of 16.4 g/kg oil or 1.64 % of the oil mass.

3.2. Characterization and modeling of exposure solutions

Individual analyte and total PAH measurements for the MD and CD experiments are summarized in Tables S3 and S4, respectively. For the MD experiment without dispersant, total mean measured PAH concentrations in unfiltered treatments ranged from 0.1 to 28.8 µg/L whereas filtered treatments ranged from 0.2 to 15.1 µg/L. In the case of the CD

313 experiment with dispersant, total measured concentrations in unfiltered and filtered treatments
314 ranged from 0.05 to 27.9 and 0.07 to 12.5 µg/L, respectively. Thus, similar total dispersed and
315 filtered PAH exposures were achieved in these separate tests.

316 Oil concentrations of individual PAHs (Table S2) were used as input to the oil solubility
317 model to estimate oil loadings and droplet oil concentrations that matched observed PAH
318 concentrations in filtered and unfiltered treatments (Figure 1). Table 1 summarizes the
319 estimated oil loadings and droplet concentrations for each test treatment based on this
320 modeling analysis. Results indicate that estimated oil loading corresponding to the observed
321 measured PAH concentrations in filtered treatments range from 5 to 1500 µg/L. In the case of
322 the CD test, the loadings derived by fitting the filtered treatments at the two highest exposures
323 are slightly less than the oil droplet concentrations obtained by fitting the total measured
324 concentrations in the corresponding unfiltered treatments. This suggests some losses may
325 have occurred during the filtration step. The results of the calibration procedure for the
326 highest treatment exposure is illustrated in Figure 2, while further plots are provided for the
327 lower test exposures in Figure S1. Predicted concentrations of individual PAHs for unfiltered
328 WAFs appear to fall on the 1:1 line (left hand panels in Figure 2) for both MD and CD
329 experiments. Predicted concentrations are in generally good agreement with measured
330 concentrations in filtered treatments particularly for the more soluble components, but
331 predictions tend to be higher than measured concentrations for the poorly soluble components
332 that are likely more susceptible to losses during the filtration process and toxicity test
333 exposures.

334 It should be pointed out that using the dosing system applied in this study equilibrium
335 conditions are assumed and appear to be reasonably described using the solubility model
336 applied. However, during oil spills in the field where rapid dilution of droplet oil occurs over
337 short time scales dissolution may be kinetically limited by mass transfer considerations

thereby precluding equilibrium conditions. Thus, the experimental design used in this study provides a conservative basis for evaluating oil exposure and should not be directly extrapolated to infer effects in the field where disequilibrium and weathering processes can alter both the concentration and composition of dissolved oil exposures.

3.3. Toxicity results

Larval cod survival and growth data for both tests are summarized in Table 2. Control survival in CD and MD test were 89.8±4.3 and 83.8±7.5% (N=12), which reflects acceptable survival for lab toxicity tests. Larval weights for controls were 0.29±0.05 mg dry (N=144) and 0.17±0.05 mg dry (N=142) in the MD and CD experiments, respectively. The weight difference between the two groups may be related to the fact that the eggs from the CD test were light manipulated to spawn in the autumn, whereas the eggs from the MD test were obtained after a slightly delayed natural spawning. Since the egg batch that produced the smaller larvae used in the CD treatment had slightly better mean control survival larvae were judged healthy despite the smaller size.

Statistically significant reductions in survival were observed only at the highest oil exposures for the CD test and at the two highest exposures in the MD test. Differences in observed survival between parallel filtered and un-filtered treatments were not significant for the MD treatment, and significant only for the highest exposure in the CD treatment (D5 vs. W5, $p<0.01$). Detectable effects on growth relative to the controls were for the MD exposure observed for the D2 ($p<0.0001$), D3 ($p<0.001$), D4 ($p<0.001$), F3 ($p<0.01$), F4 ($p<0.0001$) and F5 ($p<0.0001$) and for the CD exposure for D2 ($p<0.01$), D4 ($p<0.0001$), D5 ($p<0.0001$), F4 ($p<0.0001$) and F5 ($p<0.0001$). Comparing parallel filtered and un-filtered treatments, significant differences were only observed between the D2 and F2 treatments ($p<0.01$) for the

MD exposure, and for none of the parallel CD treatments. These results indicate that dissolved phase oil primarily dictates observed toxicity on either survival or growth of cod larvae, not dispersant or droplet oil, consistent with earlier studies (Carls et al., 2008; Gardiner et al., 2013; Nordtug et al., 2011b; Olsvik et al., 2011; Olsvik et al., 2010).

Predicted 4 d LC_{50} values and EC_{50} estimates for larval growth inhibition following 4 d exposure and a subsequent 4 d recovery period in clean water using total PAH measurements (Table 1) as the exposure metric are reported in Table 3. Results indicate mechanically dispersed oil exhibits LC50s that are about 3 fold lower than chemically dispersed oil. For sublethal effects on larval growth estimated EC50s are more uncertain and exhibit greater differences between MD and CD treatments.

3.4. Estimating CTLBB from observed toxicity and predicted TUs for the whole oil

Dissolved oil exposures derived using the 2d-GC compositional input were combined with the estimated oil loadings provided in Table 1 to calculate dissolved TUs for different CTLBB values. This calculation was performed iteratively across all treatments in both experiments to determine a CTLBB estimate corresponding to a 50% acute response at TU=1 as illustrated in Figure 3A. This procedure yielded an acute 4 d CTLBB estimate for Atlantic Cod of 42 µmol/g octanol, which falls within the range reported for other pelagic species (9 to 327 µmol/g octanol, N= 54 species) based on acute effect endpoints for single hydrocarbons (McGrath and Di Toro, 2009). This value is a factor of two lower than the CTLBB of 81 µmol/g octanol derived from 5 d zebrafish embryo-larval tests with aromatic hydrocarbons [36]. This procedure was repeated to estimate a CTLBB of 14 µmol/g octanol that corresponds to a 20% growth effect at a chronic TU = 1 as shown in Figure 3B. This value is near the median sublethal/chronic CTLBBs (0.36 to 129 µmol/g octanol, N= 36 species) that have been derived using the TLM (McGrath and Di Toro, 2009). It should be noted that since

386 modeling estimates may understate dissolved phase exposures that occurs as a result of loss
387 processes in dispersed test exposures (see previous section), the resulting CTLBBs for cod
388 larvae derived in this analysis are likely biased low and are thus conservative.

389 **3.5. Comparing predicted TUs for whole oil versus total PAH**

390 Simulated dissolved exposures were used to compute TUs associated with the PAHs targeted
391 in this study for quantifying oil exposures. These calculations are summarized in Table 1 and
392 indicate that the 44 PAHs considered only comprise 3 to 11 % of the TUs associated with the
393 whole oil; a reflection of the high naphthenic content of this oil. Further, this percentage
394 changes with oil loading such that at low loadings, PAHs contribute less than the unresolved
395 dissolved oil components.

396 These results highlight the challenges of using a limited suite of PAH analytes as an exposure
397 metric for expressing toxicity data since such measurements only capture a fraction of the oil
398 components that are expected to contribute to the observed toxicity. In addition, the fractional
399 contribution of TUs associated with PAHs changes in a non-linear manner with oil loading
400 and dosing method using the same test oil (Table 1). It is important to emphasize that since
401 the concentration of individual PAHs varies widely across different crude oils (and
402 weathering states) and given different investigators often quantitate an inconsistent suites of
403 individual PAHs to characterize oil toxicity test exposures, the ratio of TUs associated with
404 measured PAHs to unresolved dissolved oil components is expected to diverge across oil
405 toxicity studies. This conclusion is supported by the recent modeling analysis provided by
406 [39] who investigated the acute toxicity of chemically dispersed Alaska North Slope Oil to
407 sablefish. These investigators found that a suite of 38 individual PAHs used to quantify total
408 PAH exposures in CEWAF treatments represented 20% of ∑TUs that were predicted based
409 on 2d-GC analysis of the test oil. The higher percentage reported compared to our findings

410 using Troll Oil underscores the limitations of expressing and comparing oil toxicity data using
411 traditional exposure metrics that only partially characterize both the concentration and
412 composition of dissolved oil exposures (Redman and Parkerton, 2015). The practical
413 implication of this work is that expressing oil toxicity data in terms of total PAH can yield
414 misleading conclusions. Using total PAHs as the basis for interpreting toxicity test results in
415 this study, it may be concluded that MD is more toxic than the CD test oil (Table 3).
416 However, when expressed in terms of predicted TUs for the whole oil, MD and CD oil exhibit
417 comparable toxicity (Figure 3A).

418 Another important insight obtained from applying the modeling approach used in this study is
419 the importance of different unresolved hydrocarbon classes that comprise the crude oil
420 investigated in contributing to predicted effects. Figure 4 summarizes the percent
421 contribution of 2d-GC classes at the predicted total oil concentration (= 0.66 mg/L) in which
422 dissolved Troll oil exposures yield a $\sum$Acute TU = 1 (i.e. corresponding to a 50% effect on
423 cod larval survival). Unresolved di and poly aromatic hydrocarbons make up about 40% of
424 the predicted TUs which implies that the speciated PAH analysis based on GC-MS analysis
425 used in this study captures less than a quarter of the unresolved constituents in these structural
426 classes. Further monoaromatic and partially saturated cyclic structures containing one or two
427 diaromatic rings (i.e. naphthenic aromatics) contribute more than half the predicted TUs. The
428 important role of naphthenic aromatics on predicted toxicity reflects the underlying high
429 naphthenic content of Troll Oil. Linear and branched alkanes and mono, di and poly
430 naphthenic classes are constrained by aqueous solubility and collectively represent less than
431 5% of the predicted TUs. This analysis highlights how 2d-GC analysis and CTLBB estimates
432 for a given organism/endpoint can be used to evaluate how oil composition influences
433 expected toxicity and which constituents dictate effects and may warrant further study.

434 **4. SUMMARY AND RECOMMENDATIONS**

A modeling framework was applied to PAH analytical measurements to deduce associated oil loadings for simulating dissolved and droplet oil exposures in marine oil toxicity tests with cod larvae. Observed effects on larval survival and growth were found to be successfully described using dissolved phase TUs in both treatments with and without droplet oil. These results highlight the limited role oil droplets served in contributing to toxicity. The estimated acute and chronic CTLBBs derived in this analysis indicates a similar sensitivity of this deep sea species to oil as compared to other pelagic species previously investigated (McGrath and Di Toro, 2009). Total PAHs were found to account for 10% or less of the predicted TUs for the artificially weathered naphthenic oil investigated with unresolved aromatic and naphthenic aromatic components contributing the majority of TUs.

This study also highlights the limitations of using total PAH measurements as a general exposure metric in oil toxicity studies since dissolved versus droplet oil phases are not differentiated and different oils will contain varying PAH concentrations that upon dissolution will contribute in uncertain proportions and vary as a function of oil loading. Thus, the current practice for expressing oil toxicity test exposures limits the comparability between studies and the extrapolation of data to different oils. Furthermore, the use of such exposure metrics can lead to erroneous conclusions even within a given study.

The modeling framework described can be applied to other existing or future data sets (with different species and acute or chronic endpoints) to provide a comprehensive characterization of dissolved oil exposures and associated TUs. In future oil toxicity studies, direct measurements of oil droplet concentrations are recommended to independently confirm modeled estimates derived using the framework applied in this study. Additional considerations that would benefit future research include: 1). toxicity tests on individual hydrocarbons for the same organism/endpoint that is to be investigated for test oils so that a CTLBB defining the species/endpoint sensitivity can be directly determined (or confirmed, in

460 the case of the estimates derived from this study for Atlantic cod larvae) using the TLM; and
461 2). passive sampling methods that provide a more complete characterization of dissolved oil
462 exposures and thereby serves as a complimentary measure that correlates to modelled TUs
463 and observed toxicity in oil contaminated media (Redman et al., 2016). Broader adoption of
464 this strategy will foster improved insights regarding relative sensitivity of different
465 organisms/endpoints and influence of oil composition on observed effects as well as inform
466 more consistent design, analysis and interpretation of oil toxicity studies.

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578

TABLES

Table 1. Summary of modeling results for each exposure treatment

Test	Treatment	Estimated Oil Loading (µg/L)	Predicted Oil Droplet (µg/L)	Total Predicted TPAH (µg/L)	Total Measured TPAH (µg/L)	Acute TUs from 2d-GC	Acute TUs from TPAH	TPAH contribution to Acute TUs (%)
MD	D1	10	0	0.2	0.1	0.08	0.003	3.3
	D2	50	0	1.0	1.0	0.25	0.012	4.6
	D3	150	0	3.0	2.5	0.47	0.030	6.4
	D4	500	150	10.6	9.2	0.89	0.073	8.2
	D5	1500	1000	36.0	28.8	1.37	0.144	10.5
	F1	10	0	0.2	0.2	0.08	0.003	
	F2	50	0	1.0	0.9	0.25	0.012	
	F3	150	0	2.9	1.9	0.47	0.030	
	F4	500	0	8.3	6.1	0.89	0.073	
	F5	1500	0	20.1	15.1	1.37	0.144	
CD	D1	5	0	0.1	0.05	0.05	0.001	2.9
	D2	15	1	0.3	0.3	0.11	0.004	3.8
	D3	70	13	1.6	1.1	0.31	0.016	5.2
	D4	200	272	8.2	8.3	0.57	0.038	6.6
	D5	1000	1237	34.9	27.9	1.19	0.114	9.7
	F1	5	0	0.1	0.07	0.05	0.001	
	F2	15	0	0.3	0.3	0.11	0.004	
	F3	70	0	1.4	1.0	0.31	0.016	
	F4	200	0	3.7	4.4	0.57	0.038	
	F5	1000	0	14.6	12.5	1.19	0.114	

D = dispersed (unfiltered); F = filtered; TPAH=total polyaromatic hydrocarbons; TUs = toxic Units

583 **Table 2 Survival and growth effects of Troll Oil on cod larvae**

Mean survival (%) 13 d post-hatch in unfiltered (D) and filter (F) treatments

Experiment	Control	D1	D2	D3	D4	D5
MD	83.8±7.5	75.0±8.0	77.8±11.3	75.8±8.8	44.9±10.1****	6.5±4.1****
CD	89.8±4.3	88.9±7.2	81.6±9.7	92.1±3.0	92.3±4.4	56.6±2.2****
		F1	F2	F3	F4	F5
MD		74.2±4.5	71.1±3.0	69.8±3.9	46.5±20.1****	13.4±7.6****
CD		89.6±2.9	90.2±2.8	92.0±1.2	86.8±3.1	72.6±9.7***

Mean weight (mg) 17 d post-hatch in unfiltered (D) and filter (F) treatments

Experiment	Control	D1	D2	D3	D4	D5
MD	0.286±0.048	0.263±0.070	0.236±0.058****	0.238±0.075***	0.136±0.068****	-
CD	0.165±0.033	0.171±0.034	0.189±0.059**	0.163±0.034	0.107±0.037****	0.078±0.014****
		F1	F2	F3	F4	F5
MD		0.285±0.063	0.279±0.041	0.246±0.063**	0.168±0.038****	0.107±0.074****
CD		0.150±0.038	0.171±0.042	0.153±0.033	0.128±0.059****	0.067±0.008****

584 MD = mechanically dispersed; CD = chemically dispersed; D=unfiltered; F=filtered; Statistically different from
585 control (**p<0.01, ***p<0.001 and ****p<0.0001)

586

587 **Table 3 Toxicity of dispersed Troll Oil on cod larvae survival and growth based on measured**
588 **total PAH exposures**

Experiment	LC_{50} (µg/L)	EC_{50} (µg/L)
MD Unfiltered	9 (7-12)	82 (CNC)
MD Filtered	6 (4-8)	54 (CNC)
CD Unfiltered	30 (5-55)	9 (CNC)
CD Filtered	28 (CNC)	6 (CNC)

589 MD = mechanically dispersed; CD = chemically dispersed;
590 CNC = could not calculate reliable confidence intervals
591

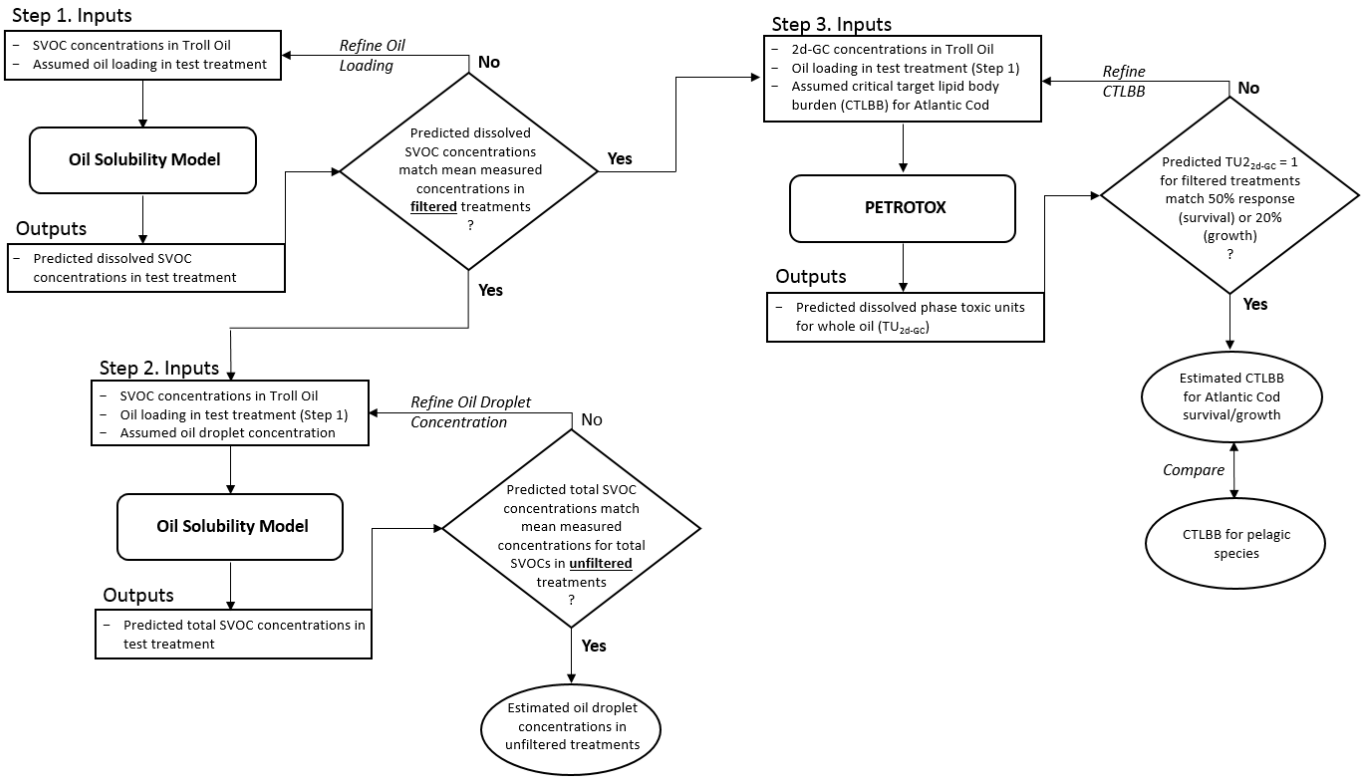
592 **FIGURE LEGENDS**

593 Figure 1: Flow chart describing modelling framework used in the analysis of oil toxicity test
594 data.

595 Figure 2: Comparison of predicted to measured concentrations of targeted PAHs in the
596 highest nominal oil test exposure. Analyte measurements below the detection limit are
597 plotted with '<' symbol. Top row shows data for the chemically dispersed and filtered
598 treatments while lower row shows data for the mechanically dispersed and filtered treatments.

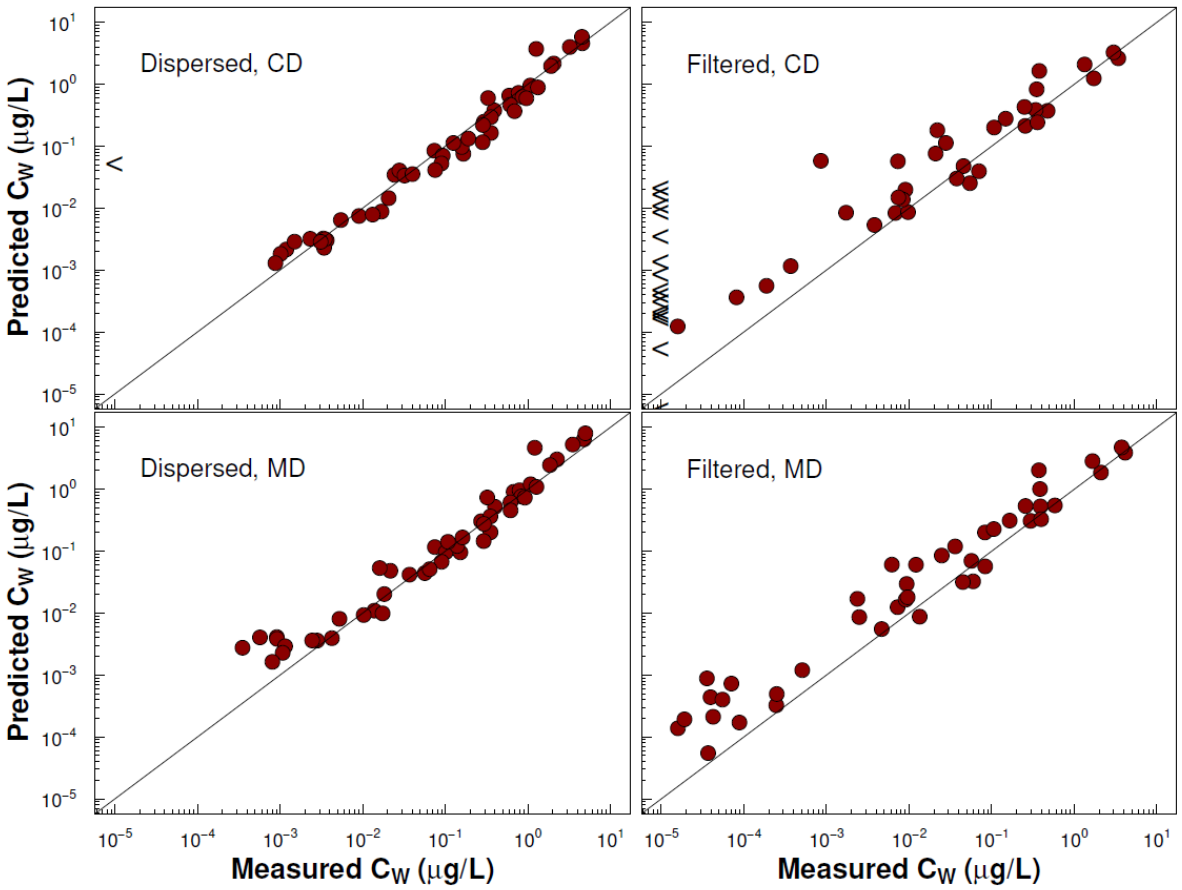
599 Figure 3: Observed effects of oil as function of TUs derived using 2d-GC oil composition. A:
600 Survival vs. Acute TU based on CTLBB fitted to effect data using logistic regression (e.g.,
601 TU=1 at 50%). B: Growth vs. Chronic TU based on CTLBB fitted to effect data using logistic
602 regression (e.g., TU chronic=1 at 20% effect). Filled and open symbols denote unfiltered and
603 filtered treatments, respectively. Purple squares and red circles represent chemically and
604 mechanically dispersed oil tests, respectively.

605 Figure 4: Percent contribution of different hydrocarbon classes comprising the test oil as
606 determined using 2d-GC analysis to predicted dissolved oil toxic units at the lethal loading.



607

608 **Figure 1**



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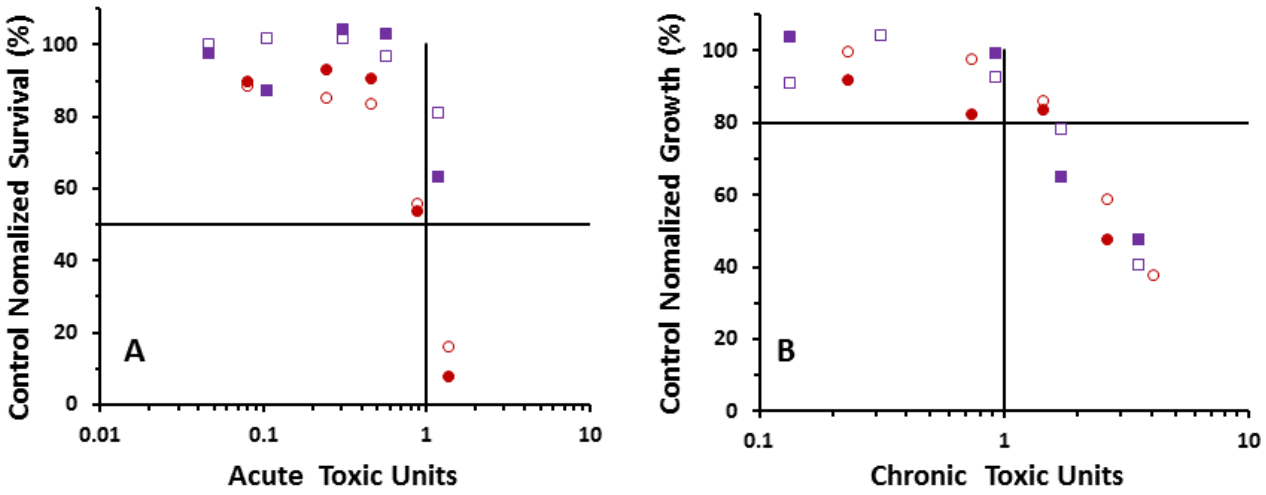
610 **Figure 2**



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612 **Figure 3**

613

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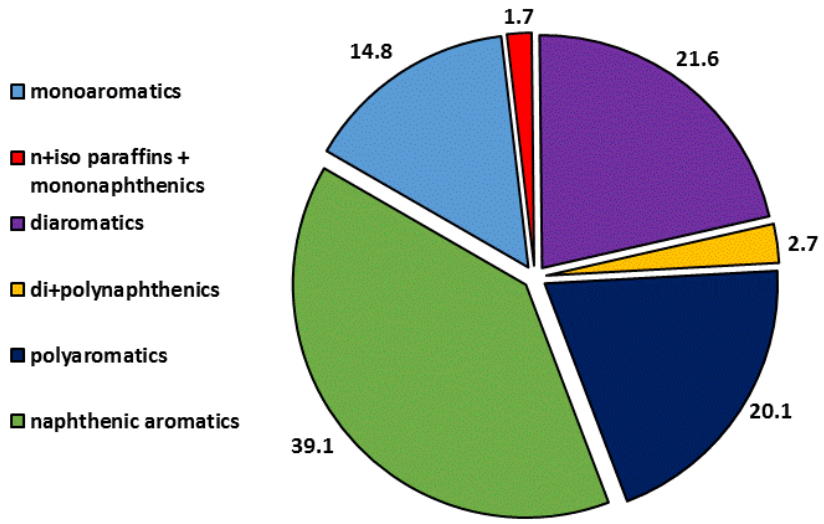

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616

617 **Figure 4**

SUPPLEMENTARY TABLES:

Table S1. 2d-GC Compositional analysis of artificially weathered troll Oil expressed as % mass of each carbon number interval

C# Initial	C# Final	nP	iP	C5	C6	iN	dN	pN	MA	nMA	DA	nDA	PA	Total
6	7	0.081	0.000	0.006	0.006	0.006	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.108
7	8	0.017	0.038	0.002	0.002	0.002	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.094
8	9	0.107	0.216	0.013	0.013	0.013	0.000	0.000	0.297	0.000	0.000	0.000	0.000	0.658
9	10	0.050	0.235	0.206	0.206	0.206	0.000	0.000	0.462	0.069	0.000	0.000	0.000	1.435
10	11	0.028	0.258	0.303	0.303	0.303	0.317	0.000	0.329	0.241	0.089	0.000	0.000	2.171
11	12	0.042	0.246	0.187	0.187	0.187	0.597	0.000	0.370	0.328	0.352	0.000	0.000	2.495
12	13	0.056	0.325	0.268	0.268	0.268	0.729	0.000	0.409	0.508	0.572	0.005	0.000	3.407
13	14	0.077	0.495	0.326	0.326	0.326	0.758	0.000	0.566	0.646	0.528	0.099	0.000	4.147
14	15	0.140	0.573	0.395	0.395	0.395	0.609	0.316	0.726	0.698	0.642	0.299	0.030	5.217
15	16	0.122	0.625	0.342	0.342	0.342	0.638	0.261	0.630	0.700	0.557	0.410	0.115	5.082
16	17	0.138	0.459	0.357	0.357	0.357	0.564	0.232	0.595	0.618	0.483	0.428	0.154	4.741
17	18	0.458	0.528	0.280	0.280	0.280	0.534	0.246	0.614	0.654	0.504	0.467	0.228	5.072
18	19	0.310	0.409	0.279	0.279	0.279	0.513	0.280	0.553	0.636	0.495	0.473	0.405	4.912
19	20	0.149	0.378	0.256	0.256	0.256	0.431	0.253	0.575	0.621	0.453	0.427	0.524	4.577
20	21	0.166	0.269	0.206	0.206	0.206	0.331	0.194	0.472	0.562	0.382	0.410	0.697	4.100
21	22	0.158	0.300	0.195	0.195	0.195	0.306	0.187	0.472	0.541	0.358	0.357	0.872	4.134
22	23	0.173	0.280	0.168	0.168	0.168	0.307	0.185	0.480	0.507	0.336	0.340	0.859	3.970
23	24	0.176	0.237	0.162	0.162	0.162	0.249	0.160	0.423	0.480	0.322	0.349	0.925	3.806
24	25	0.161	0.238	0.119	0.119	0.119	0.259	0.451	0.408	0.508	0.246	0.382	0.688	3.698
25	26	0.159	0.245	0.131	0.131	0.131	0.246	0.632	0.406	0.196	0.141	0.281	0.627	3.327
26	27	0.154	0.227	0.107	0.107	0.107	0.225	0.703	0.392	0.175	0.094	0.218	0.576	3.086
27	28	0.154	0.228	0.101	0.101	0.101	0.208	0.785	0.370	0.173	0.055	0.209	0.564	3.049
28	29	0.167	0.223	0.094	0.094	0.094	0.204	1.200	0.345	0.135	0.040	0.183	0.535	3.312
29	30	0.180	0.214	0.081	0.081	0.081	0.201	1.046	0.292	0.113	0.053	0.156	0.477	2.973
30	31	0.158	0.219	0.080	0.080	0.080	0.192	0.767	0.259	0.228	0.165	0.127	0.483	2.840
Total		3.578	7.465	4.660	4.660	4.660	8.420	7.897	10.489	9.336	6.867	5.620	8.758	82.411

623 **Table S2. GC-MS compositional analysis of artificially weathered troll oil**

Component	Concentration (g/kg)
Benzo(b)thiophene	0.006
Naphthalene	0.945
C1-naphthalenes	2.022
C2-naphthalenes	2.639
C3-naphthalenes	1.966
C4-naphthalenes	1.172
Biphenyl	0.293
Acenaphthylene	0.015
Acenaphthene	0.038
Dibenzofuran	0.032
Fluorene	0.171
C1-fluorenes	0.352
C2-fluorenes	0.540
C3-fluorenes	0.456
Phenanthrene	0.231
Anthracene	0.020
C1-phenanthrenes/anthracenes	2.144
C2-phenanthrenes/anthracenes	0.631
C3-phenanthrenes/anthracenes	0.496
C4-phenanthrenes/anthracenes	0.319
Dibenzothiophene	0.028
C1-dibenzothiophenes	0.411
C2-dibenzothiophenes	0.138
C3-dibenzothiophenes	0.111
C4-dibenzothiophenes	0.076
Fluoranthene	0.020
Pyrene	0.021
C1-fluoranthrenes/pyrenes	0.177
C2-fluoranthenes/pyrenes	0.245
C3-fluoranthenes/pyrenes	0.209
Benz(a)anthracene	0.008
Chrysene	0.037
C1-chrysenes	0.091
C2-chrysenes	0.125
C3-chrysenes	0.107
C4-chrysenes	0.051
Benzo(b)fluoranthene	0.007
Benzo(k)fluoranthene	0.003
Benzo(e)pyrene	0.008
Benzo(a)pyrene	0.003
Perylene	0.006
Indeno(1,2,3-c,d)pyrene	0.002
Dibenz(a,h)anthracene	0.001
Benzo(g,h,i)perylene	0.003
TPAH	**16.4**

624

625

Table S3a. Summary of measured PAH concentrations in dispersed (D) exposures for the MD experiment (oil with no dispersant)

Average and Stdev (N=6)	Cod-D1		Cod-D2		Cod-D3		Cod-D4		Cod-D5	
	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Benzo(b)thiophene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0402	0.0986	0.0183	0.0009
Naphthalene	0.0198	0.0066	0.0854	0.0246	0.1995	0.0111	0.7075	0.0278	2.2536	0.0834
C1-naphthalenes	0.0283	0.0124	0.1621	0.0490	0.3990	0.0305	1.4965	0.0612	4.7780	0.1907
C2-naphthalenes	0.0279	0.0174	0.1651	0.0408	0.4033	0.0414	1.5432	0.0831	4.9795	0.2138
C3-naphthalenes	0.0000	0.0000	0.1739	0.0279	0.3351	0.0312	1.1186	0.0377	3.4989	0.2225
C4-naphthalenes	0.0000	0.0000	0.0475	0.0254	0.1536	0.0203	0.5570	0.0547	1.8541	0.2421
Biphenyl	0.0049	0.0018	0.0240	0.0066	0.0562	0.0060	0.2083	0.0116	0.6735	0.0306
Acenaphthylene	0.0001	0.0001	0.0007	0.0002	0.0017	0.0002	0.0065	0.0005	0.0216	0.0019
Acenaphthene	0.0008	0.0004	0.0027	0.0005	0.0060	0.0006	0.0231	0.0011	0.0748	0.0028
Dibenzofuran	0.0010	0.0006	0.0042	0.0008	0.0089	0.0008	0.0323	0.0013	0.1015	0.0036
Fluorene	0.0029	0.0009	0.0147	0.0026	0.0334	0.0032	0.1279	0.0045	0.4003	0.0132
C1-fluorenes	0.0044	0.0015	0.0278	0.0056	0.0685	0.0086	0.2630	0.0122	0.7937	0.0453
C2-fluorenes	0.0057	0.0063	0.0393	0.0066	0.0943	0.0112	0.3290	0.0262	1.0806	0.0819
C3-fluorenes	0.0000	0.0000	0.0252	0.0047	0.0662	0.0074	0.2563	0.0311	0.8334	0.1003
Phenanthrene	0.0044	0.0026	0.0243	0.0045	0.0574	0.0066	0.2133	0.0044	0.6168	0.0221
Anthracene	0.0000	0.0000	0.0000	0.0000	0.0008	0.0012	0.0033	0.0051	0.0107	0.0167
C1-phenanthrenes/anthracenes	0.0090	0.0028	0.0445	0.0098	0.1161	0.0155	0.4284	0.0057	1.2121	0.0833
C2-phenanthrenes/anthracenes	0.0000	0.0000	0.0580	0.0174	0.1384	0.0131	0.4393	0.0351	1.2708	0.1028
C3-phenanthrenes/anthracenes	0.0038	0.0031	0.0282	0.0058	0.0759	0.0094	0.3097	0.0345	0.9202	0.1423
C4-phenanthrenes/anthracenes	0.0000	0.0000	0.0132	0.0103	0.0504	0.0065	0.1958	0.0184	0.6175	0.0679
Dibenzothiophene	0.0000	0.0000	0.0039	0.0006	0.0083	0.0008	0.0306	0.0008	0.0903	0.0032
C1-dibenzothiophenes	0.0106	0.0117	0.0312	0.0029	0.0464	0.0032	0.1202	0.0030	0.3226	0.0177
C2-dibenzothiophenes	0.0012	0.0019	0.0130	0.0029	0.0323	0.0028	0.1193	0.0105	0.3530	0.0318
C3-dibenzothiophenes	0.0003	0.0008	0.0088	0.0019	0.0247	0.0025	0.0881	0.0108	0.2919	0.0324
C4-dibenzothiophenes	0.0000	0.0000	0.0000	0.0000	0.0107	0.0054	0.0511	0.0075	0.1525	0.0163
Fluoranthene	0.0007	0.0009	0.0017	0.0012	0.0041	0.0004	0.0131	0.0005	0.0369	0.0033
Pyrene	0.0009	0.0009	0.0035	0.0024	0.0057	0.0006	0.0194	0.0011	0.0566	0.0054
C1-fluoranthrenes/pyrenes	0.0017	0.0009	0.0093	0.0019	0.0241	0.0024	0.0898	0.0104	0.2715	0.0265
C2-fluoranthenes/pyrenes	0.0010	0.0011	0.0104	0.0022	0.0275	0.0033	0.1083	0.0064	0.3520	0.0410
C3-fluoranthenes/pyrenes	0.0000	0.0000	0.0040	0.0044	0.0195	0.0033	0.0844	0.0113	0.2937	0.0432
Benz(a)anthracene	0.0002	0.0003	0.0004	0.0002	0.0011	0.0003	0.0031	0.0015	0.0139	0.0012
Chrysene	0.0003	0.0005	0.0029	0.0008	0.0061	0.0007	0.0204	0.0018	0.0649	0.0050
C1-chrysenes	0.0002	0.0005	0.0041	0.0009	0.0106	0.0016	0.0418	0.0033	0.1394	0.0160
C2-chrysenes	0.0000	0.0000	0.0007	0.0017	0.0061	0.0068	0.0483	0.0042	0.1620	0.0134
C3-chrysenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0212	0.0168	0.1080	0.0155
C4-chrysenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benzo(b)fluoranthene	0.0000	0.0000	0.0001	0.0001	0.0002	0.0004	0.0011	0.0017	0.0103	0.0011
Benzo(k)fluoranthene	0.0000	0.0000	0.0001	0.0002	0.0000	0.0001	0.0004	0.0007	0.0042	0.0007
Benzo(e)pyrene	0.0000	0.0001	0.0000	0.0001	0.0002	0.0005	0.0016	0.0026	0.0175	0.0025
Benzo(a)pyrene	0.0000	0.0000	0.0001	0.0002	0.0002	0.0005	0.0004	0.0006	0.0028	0.0015
Perylene	0.0002	0.0004	0.0000	0.0001	0.0001	0.0002	0.0004	0.0006	0.0052	0.0034
Indeno(1,2,3-c,d)pyrene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002	0.0003	0.0011	0.0012
Dibenz(a,h)anthracene	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.0001	0.0002	0.0008	0.0009
Benzo(g,h,i)perylene	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0005	0.0008	0.0024	0.0027
TPAH	**0.1**		**1.0**		**2.5**		**9.2**		**28.8**	

Table S3b. Summary of measured PAH concentrations in filtered (F) exposures for the MD experiment (oil with no dispersant)

Average and Stdev (N=6)	Cod-F1		Cod-F2		Cod-F3		Cod-F4		Cod-F5	
	Avg µg/L	Stdev µg/L	Avg µg/L	Stdev µg/L	Avg µg/L	Stdev µg/L	Avg µg/L	Stdev µg/L	Avg µg/L	Stdev µg/L
Benzo(b)thiophene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0048	0.0075
Naphthalene	0.0232	0.0066	0.0794	0.0236	0.1840	0.0232	0.6608	0.0357	2.1059	0.1871
C1-naphthalenes	0.0341	0.0098	0.1475	0.0478	0.3412	0.0333	1.3429	0.0614	4.1397	0.3421
C2-naphthalenes	0.0326	0.0090	0.1605	0.0509	0.3565	0.0419	1.3937	0.0374	3.7518	0.2198
C3-naphthalenes	0.0077	0.0119	0.1767	0.0364	0.3050	0.0227	0.8506	0.0189	1.6627	0.0804
C4-naphthalenes	0.0000	0.0000	0.0474	0.0259	0.1095	0.0092	0.2577	0.0293	0.3850	0.0305
Biphenyl	0.0063	0.0013	0.0226	0.0062	0.0514	0.0059	0.1950	0.0091	0.5831	0.0441
Acenaphthylene	0.0002	0.0002	0.0006	0.0003	0.0014	0.0002	0.0045	0.0003	0.0093	0.0006
Acenaphthene	0.0012	0.0002	0.0027	0.0006	0.0055	0.0006	0.0198	0.0001	0.0569	0.0031
Dibenzofuran	0.0018	0.0003	0.0042	0.0009	0.0083	0.0009	0.0294	0.0005	0.0834	0.0045
Fluorene	0.0039	0.0007	0.0140	0.0039	0.0297	0.0035	0.1097	0.0024	0.2961	0.0145
C1-fluorenes	0.0051	0.0019	0.0280	0.0072	0.0555	0.0064	0.1823	0.0079	0.3896	0.0177
C2-fluorenes	0.0127	0.0072	0.0399	0.0100	0.0701	0.0061	0.1752	0.0197	0.2572	0.0178
C3-fluorenes	0.0000	0.0000	0.0210	0.0118	0.0364	0.0042	0.0637	0.0057	0.0829	0.0057
Phenanthrene	0.0061	0.0014	0.0231	0.0055	0.0483	0.0060	0.1713	0.0060	0.3968	0.0196
Anthracene	0.0001	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C1-phenanthrenes/anthracenes	0.0080	0.0030	0.0427	0.0122	0.0863	0.0087	0.2290	0.0145	0.3730	0.0183
C2-phenanthrenes/anthracenes	0.0000	0.0000	0.0424	0.0092	0.0792	0.0047	0.1385	0.0128	0.1653	0.0095
C3-phenanthrenes/anthracenes	0.0021	0.0025	0.0165	0.0028	0.0239	0.0037	0.0325	0.0044	0.0361	0.0040
C4-phenanthrenes/anthracenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0043	0.0048	0.0062	0.0049
Dibenzothiophene	0.0000	0.0000	0.0038	0.0010	0.0073	0.0010	0.0248	0.0007	0.0597	0.0030
C1-dibenzothiophenes	0.0074	0.0115	0.0296	0.0023	0.0402	0.0030	0.0721	0.0027	0.1061	0.0078
C2-dibenzothiophenes	0.0007	0.0018	0.0106	0.0021	0.0182	0.0020	0.0332	0.0034	0.0448	0.0018
C3-dibenzothiophenes	0.0000	0.0000	0.0057	0.0012	0.0077	0.0014	0.0103	0.0021	0.0134	0.0011
C4-dibenzothiophenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Fluoranthene	0.0008	0.0005	0.0017	0.0007	0.0031	0.0008	0.0062	0.0005	0.0091	0.0008
Pyrene	0.0007	0.0005	0.0022	0.0006	0.0036	0.0008	0.0067	0.0008	0.0096	0.0006
C1-fluoranthrenes/pyrenes	0.0007	0.0011	0.0064	0.0009	0.0115	0.0008	0.0202	0.0024	0.0248	0.0011
C2-fluoranthenes/pyrenes	0.0000	0.0000	0.0050	0.0009	0.0074	0.0010	0.0098	0.0010	0.0121	0.0019
C3-fluoranthenes/pyrenes	0.0000	0.0000	0.0007	0.0010	0.0009	0.0014	0.0006	0.0014	0.0024	0.0026
Benz(a)anthracene	0.0000	0.0000	0.0001	0.0001	0.0007	0.0008	0.0004	0.0001	0.0005	0.0001
Chrysene	0.0003	0.0005	0.0019	0.0001	0.0030	0.0002	0.0042	0.0005	0.0047	0.0005
C1-chrysenes	0.0000	0.0000	0.0015	0.0007	0.0021	0.0002	0.0027	0.0004	0.0025	0.0008
C2-chrysenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C3-chrysenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C4-chrysenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benzo(b)fluoranthene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benzo(k)fluoranthene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002
Benzo(e)pyrene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002
Benzo(a)pyrene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
Perylene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Indeno(1,2,3-c,d)pyrene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dibenz(a,h)anthracene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benzo(g,h,i)perylene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TPAH	**0.2**		**0.9**		**1.9**		**6.1**		**15.1**	

Table S4a. Summary of measured PAH concentrations in dispersed (D) exposures for the CD experiment (oil with dispersant)

Average and Stdev (N=6)	Cod-D1		Cod-D2		Cod-D3		Cod-D4		Cod-D5	
	Avg µg/L	SDEV µg/L	Avg µg/L	SDEV µg/L	Avg µg/L	SDEV µg/L	Avg µg/L	SDEV µg/L	Avg µg/L	SDEV µg/L
Benzo(b)thiophene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0206	0.0034
Naphthalene	0.0165	0.0044	0.0683	0.0948	0.0984	0.0615	0.7435	0.3814	2.0586	0.2909
C1-naphthalenes	0.0117	0.0062	0.0521	0.0668	0.1777	0.1412	1.2640	0.1917	4.5673	0.6704
C2-naphthalenes	0.0053	0.0077	0.0456	0.0707	0.1794	0.1472	1.2975	0.1493	4.5169	0.2500
C3-naphthalenes	0.0000	0.0000	0.0274	0.0424	0.1457	0.1139	0.9721	0.1002	3.2254	0.2469
C4-naphthalenes	0.0000	0.0000	0.0157	0.0245	0.0682	0.0565	0.5338	0.0568	1.9099	0.1349
Biphenyl	0.0025	0.0014	0.0077	0.0092	0.0263	0.0194	0.1713	0.0187	0.5954	0.0344
Acenaphthylene	0.0000	0.0001	0.0003	0.0005	0.0010	0.0008	0.0069	0.0010	0.0243	0.0020
Acenaphthene	0.0001	0.0002	0.0007	0.0011	0.0028	0.0023	0.0203	0.0027	0.0738	0.0058
Dibenzofuran	0.0006	0.0005	0.0015	0.0014	0.0044	0.0030	0.0272	0.0032	0.0932	0.0056
Fluorene	0.0016	0.0003	0.0048	0.0052	0.0160	0.0126	0.1127	0.0129	0.3919	0.0212
C1-fluorenes	0.0008	0.0011	0.0070	0.0109	0.0304	0.0251	0.2306	0.0238	0.7720	0.0480
C2-fluorenes	0.0000	0.0001	0.0127	0.0197	0.0454	0.0379	0.3093	0.0413	1.0717	0.1265
C3-fluorenes	0.0003	0.0006	0.0000	0.0000	0.0104	0.0255	0.2501	0.0321	0.8626	0.1075
Phenanthrene	0.0027	0.0008	0.0082	0.0088	0.0283	0.0213	0.1916	0.0194	0.6113	0.0350
Anthracene	0.0001	0.0001	0.0002	0.0006	0.0008	0.0010	0.0362	0.0735	0.0278	0.0077
C1-phenanthrenes/anthracenes	0.0027	0.0024	0.0144	0.0192	0.0559	0.0450	0.3978	0.0334	1.2627	0.0887
C2-phenanthrenes/anthracenes	0.0013	0.0033	0.0151	0.0236	0.0593	0.0477	0.4056	0.0420	1.3222	0.1314
C3-phenanthrenes/anthracenes	0.0000	0.0001	0.0083	0.0128	0.0352	0.0298	0.2825	0.0398	0.9595	0.0916
C4-phenanthrenes/anthracenes	0.0000	0.0000	0.0050	0.0079	0.0241	0.0191	0.2062	0.0243	0.6897	0.0665
Dibenzothiophene	0.0000	0.0000	0.0005	0.0012	0.0041	0.0034	0.0282	0.0031	0.0898	0.0052
C1-dibenzothiophenes	0.0000	0.0000	0.0097	0.0151	0.0209	0.0209	0.1133	0.0089	0.3297	0.0229
C2-dibenzothiophenes	0.0000	0.0000	0.0036	0.0056	0.0159	0.0130	0.1146	0.0125	0.3574	0.0314
C3-dibenzothiophenes	0.0000	0.0000	0.0027	0.0041	0.0124	0.0101	0.0894	0.0083	0.2834	0.0425
C4-dibenzothiophenes	0.0000	0.0000	0.0000	0.0000	0.0070	0.0058	0.0498	0.0063	0.1657	0.0208
Fluoranthene	0.0005	0.0006	0.0009	0.0012	0.0019	0.0022	0.0114	0.0030	0.0322	0.0120
Pyrene	0.0004	0.0005	0.0007	0.0008	0.0022	0.0025	0.0126	0.0061	0.0401	0.0158
C1-fluoranthrenes/pyrenes	0.0000	0.0000	0.0027	0.0042	0.0125	0.0103	0.0882	0.0121	0.2939	0.0236
C2-fluoranthenes/pyrenes	0.0000	0.0000	0.0028	0.0043	0.0131	0.0108	0.1064	0.0160	0.3571	0.0267
C3-fluoranthenes/pyrenes	0.0000	0.0000	0.0009	0.0022	0.0096	0.0078	0.0814	0.0118	0.2853	0.0303
Benz(a)anthracene	0.0000	0.0000	0.0001	0.0002	0.0006	0.0005	0.0043	0.0007	0.0169	0.0025
Chrysene	0.0000	0.0000	0.0009	0.0013	0.0035	0.0028	0.0236	0.0030	0.0755	0.0068
C1-chrysenes	0.0000	0.0000	0.0007	0.0016	0.0062	0.0049	0.0480	0.0058	0.1585	0.0150
C2-chrysenes	0.0000	0.0000	0.0000	0.0000	0.0016	0.0040	0.0548	0.0076	0.1904	0.0203
C3-chrysenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0353	0.0053	0.1253	0.0172
C4-chrysenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benzo(b)fluoranthene	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0022	0.0024	0.0090	0.0072
Benzo(k)fluoranthene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0006	0.0035	0.0030
Benzo(e)pyrene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0019	0.0030	0.0132	0.0103
Benzo(a)pyrene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0006	0.0009	0.0031	0.0039
Perylene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0009	0.0013	0.0054	0.0060
Indeno(1,2,3-c,d)pyrene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0005	0.0008	0.0010	0.0025
Dibenz(a,h)anthracene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0005	0.0009	0.0009	0.0021
Benzo(g,h,i)perylene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0007	0.0011	0.0015	0.0037
TPAH	**0.05**		**0.32**		**1.12**		**8.33**		**27.90**	

Table S4a. Summary of measured PAH concentrations in dispersed (D) exposures for the CD experiment (oil with dispersant)

Average and Stdev (N=6)	Cod-F1		Cod-F2		Cod-F3		Cod-F4		Cod-F5	
	Avg µg/L	SDEV µg/L	Avg µg/L	SDEV µg/L	Avg µg/L	SDEV µg/L	Avg µg/L	SDEV µg/L	Avg µg/L	SDEV µg/L
Benzo(b)thiophene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0068	0.0076
Naphthalene	0.0211	0.0022	0.0371	0.0219	0.1045	0.0680	0.4499	0.1926	1.7247	0.2629
C1-naphthalenes	0.0165	0.0052	0.0484	0.0417	0.2696	0.3014	0.8947	0.4068	3.4164	0.5245
C2-naphthalenes	0.0091	0.0099	0.0469	0.0485	0.1551	0.1362	0.8892	0.4193	2.9941	0.4107
C3-naphthalenes	0.0000	0.0000	0.0209	0.0326	0.0981	0.0857	0.5682	0.2939	1.3348	0.3182
C4-naphthalenes	0.0000	0.0000	0.0096	0.0149	0.0486	0.0424	0.1849	0.1020	0.3507	0.0531
Biphenyl	0.0040	0.0005	0.0092	0.0069	0.0230	0.0188	0.1302	0.0584	0.4756	0.0481
Acenaphthylene	0.0001	0.0002	0.0006	0.0006	0.0010	0.0007	0.0037	0.0020	0.0090	0.0021
Acenaphthene	0.0002	0.0004	0.0012	0.0011	0.0028	0.0020	0.0130	0.0066	0.0456	0.0060
Dibenzofuran	0.0011	0.0005	0.0022	0.0010	0.0045	0.0028	0.0201	0.0100	0.0702	0.0071
Fluorene	0.0021	0.0011	0.0055	0.0037	0.0160	0.0111	0.0783	0.0335	0.2553	0.0263
C1-fluorenes	0.0014	0.0012	0.0069	0.0064	0.0284	0.0223	0.1435	0.0422	0.3416	0.0779
C2-fluorenes	0.0001	0.0002	0.0102	0.0115	0.0383	0.0307	0.1742	0.0516	0.2497	0.0288
C3-fluorenes	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0114	0.0279	0.0220	0.0341
Phenanthrene	0.0037	0.0004	0.0086	0.0057	0.0271	0.0186	0.1472	0.0114	0.3581	0.0621
Anthracene	0.0007	0.0018	0.0003	0.0006	0.0002	0.0004	0.0013	0.0026	0.0000	0.0000
C1-phenanthrenes/anthracenes	0.0037	0.0014	0.0125	0.0097	0.0470	0.0347	0.2635	0.1224	0.3775	0.0487
C2-phenanthrenes/anthracenes	0.0029	0.0046	0.0117	0.0136	0.0377	0.0302	0.1697	0.1257	0.1478	0.0080
C3-phenanthrenes/anthracenes	0.0001	0.0001	0.0022	0.0034	0.0114	0.0099	0.0386	0.0311	0.0279	0.0046
C4-phenanthrenes/anthracenes	0.0000	0.0000	0.0000	0.0000	0.0008	0.0019	0.0000	0.0000	0.0009	0.0021
Dibenzothiophene	0.0000	0.0000	0.0007	0.0012	0.0040	0.0032	0.0214	0.0033	0.0545	0.0085
C1-dibenzothiophenes	0.0001	0.0002	0.0123	0.0136	0.0249	0.0194	0.0806	0.0301	0.1070	0.0103
C2-dibenzothiophenes	0.0000	0.0000	0.0025	0.0031	0.0090	0.0073	0.0406	0.0262	0.0377	0.0044
C3-dibenzothiophenes	0.0000	0.0001	0.0004	0.0010	0.0036	0.0030	0.0117	0.0086	0.0097	0.0015
C4-dibenzothiophenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Fluoranthene	0.0004	0.0006	0.0010	0.0009	0.0015	0.0014	0.0043	0.0035	0.0084	0.0042
Pyrene	0.0003	0.0005	0.0008	0.0008	0.0015	0.0015	0.0041	0.0033	0.0074	0.0037
C1-fluoranthrenes/pyrenes	0.0000	0.0000	0.0011	0.0017	0.0058	0.0048	0.0194	0.0046	0.0210	0.0032
C2-fluoranthenes/pyrenes	0.0000	0.0000	0.0003	0.0007	0.0032	0.0027	0.0074	0.0020	0.0073	0.0020
C3-fluoranthenes/pyrenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benz(a)anthracene	0.0000	0.0000	0.0002	0.0005	0.0002	0.0002	0.0003	0.0003	0.0004	0.0002
Chrysene	0.0001	0.0002	0.0005	0.0008	0.0016	0.0013	0.0036	0.0018	0.0038	0.0019
C1-chrysenes	0.0000	0.0000	0.0000	0.0000	0.0003	0.0007	0.0014	0.0011	0.0017	0.0011
C2-chrysenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C3-chrysenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C4-chrysenes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benzo(b)fluoranthene	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benzo(k)fluoranthene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benzo(e)pyrene	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benzo(a)pyrene	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Perylene	0.0002	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Indeno(1,2,3-c,d)pyrene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dibenz(a,h)anthracene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benzo(g,h,i)perylene	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TPAH	**0.07**		**0.25**		**0.97**		**4.38**		**12.47**	

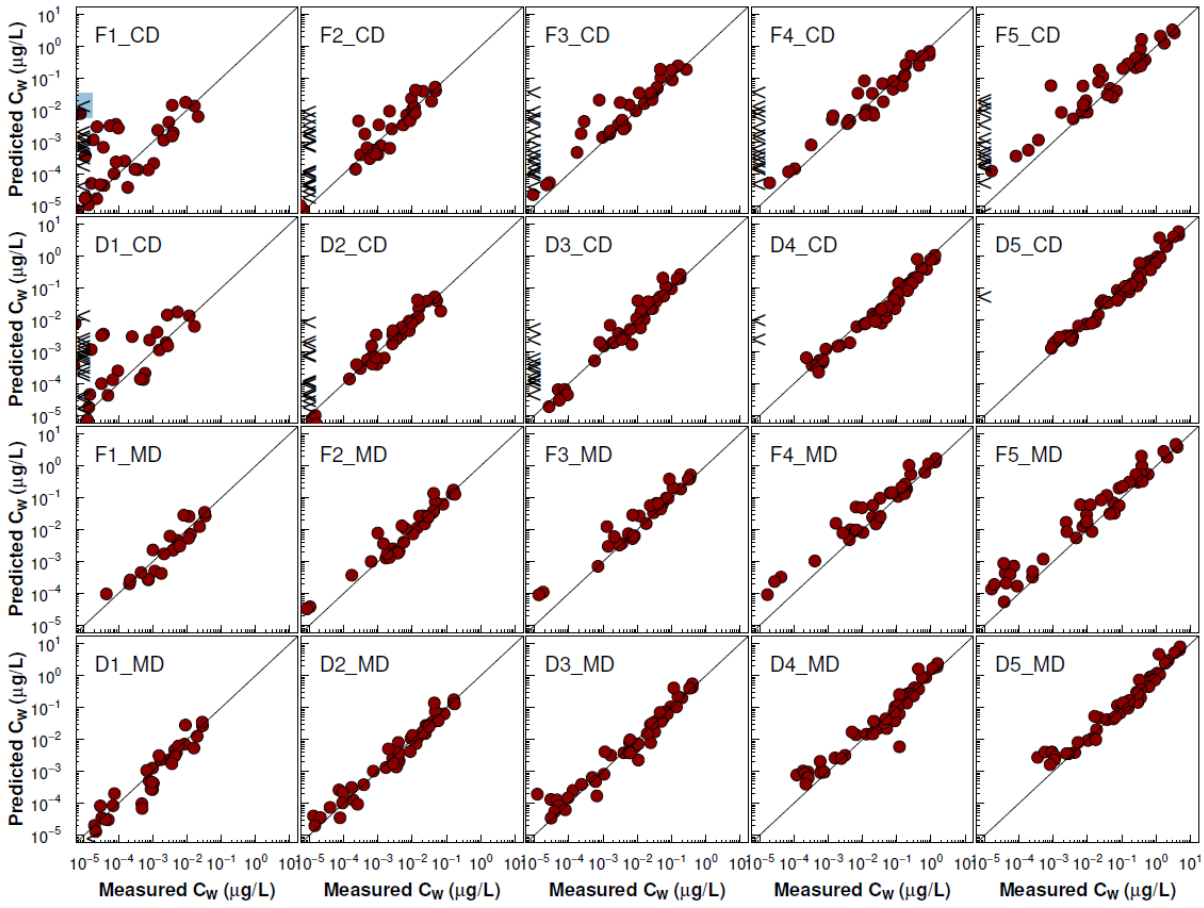
644 **SUPPLEMENTARY FIGURE LEGENDS:**

645 Figure S1: Comparison of predicted to measured concentrations of targeted PAHs in the
646 various oil test exposures. Analyte measurements below the detection limit are plotted with
647 '<' symbol. Top row shows data for the chemically dispersed treatments for each
648 loading. The second row data for the filtered chemically dispersed treatments for each
649 loading. The third row shows data for the mechanically dispersed treatments for each
650 loading. The fourth row shows data for the filtered mechanically dispersed treatments for
651 each loading.

652

653 Figure S2: Observed effects of oil as function of TUs derived using GC-MS oil composition
654 of targeted PAHs. A: Survival vs. Acute TUs B: Growth vs. Chronic TU. Filled and open
655 symbols denote unfiltered and filtered treatments, respectively. Purple squares and red circles
656 represent chemically and mechanically dispersed oil tests, respectively.

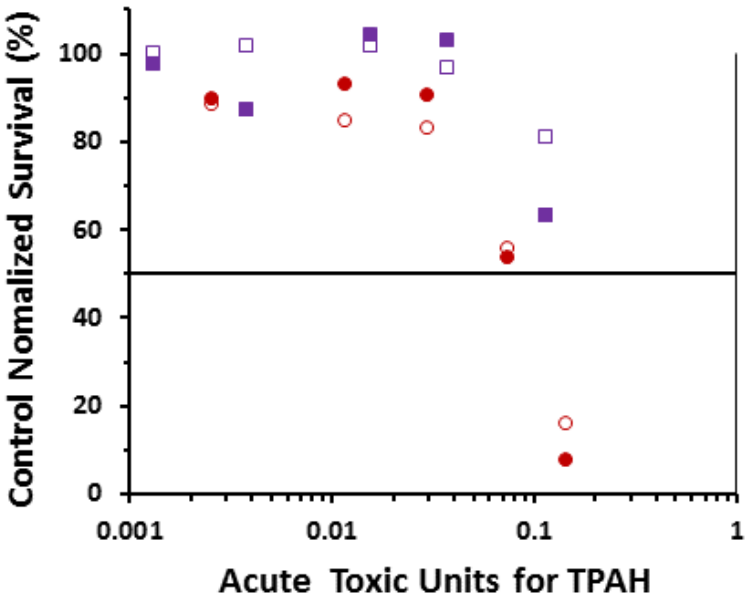
657



658

659 **Figure S1**

660



661

662 **Figure S2**

663