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Short communication

Preliminary investigation of the effects of dispersed Prudhoe Bay Crude Oil on developing topsmelt embryos, *Atherinops affinis*

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Oiled seawater treated with dispersant caused cardiovascular and other abnormalities in developing topsmelt embryos.

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ABSTRACT

Static exposure experiments were conducted to assess the toxicity of dispersed Prudhoe Bay Crude Oil (PBCO) to embryos of the topsmelt (*Atherinops affinis*). Treatment with the dispersant COREXIT 9500 resulted in greater hydrocarbon concentrations in chemically enhanced water-accommodated fractions (CEWAFs) of oil, relative to the untreated water-accommodated fractions (WAFs). Topsmelt embryo development and survival to hatching was significantly inhibited in CEWAF tests while minimal effects on embryo–larval survival were observed in WAF tests. Increased hydrocarbon concentrations in the CEWAF tests caused cardiovascular and other abnormalities in developing topsmelt embryos.

1. Introduction

Topsmelt, *Atherinops affinis*, are abundant in nearshore waters from the Gulf of California to British Columbia (Allen, 1982), and their potential exposure in the event of a coastal oil spill is high. Part of the hazard assessment for a fish population exposed to an oil spill involves the potential for increased toxicity to early life stages after treatment with a dispersant.

In short-term acute toxicity tests the toxicity of untreated oil is due to dissolved hydrocarbons (e.g., Singer et al., 1998; NRC, 2005; Carls et al., 1999). Because dispersed oil contains a complex multiphase mixture of dispersant, oil droplets, dissolved hydrocarbons, and un-dispersed oil, all of which may have different modes of toxicity, understanding the specific components and mechanisms responsible for toxicity is difficult (NRC, 2005). A number of recent studies have shown that PAHs in dispersed and non-dispersed oil adversely affect fish larvae (Ramachandran et al., 2004; Couillard et al., 2005) and embryos (Barron et al., 2004; Incardona et al., 2004, 2005).

The relative responses to dispersed and un-dispersed oil vary depending on species and life stage (e.g., NRC, 2005). While Singer et al. (1998) demonstrated that topsmelt larvae are sensitive to crude and dispersed oil components, the relative effects of these mixtures on topsmelt embryo development have not been studied. Because topsmelt embryos develop in close proximity to benthic habitats in bays and estuaries where they may be affected by spill compounds, risk to this life stage is a particularly relevant topic (NRC, 2005; Incardona et al., 2005). In the current study we investigate the relative toxicity of dispersed and un-dispersed PBCO to topsmelt embryos by assessing a number of developmental endpoints. The objective was to provide preliminary information on the relative toxicity of dispersed oil using specific cardiovascular and other endpoints identified in previous studies of hydrocarbon toxicity to fish embryos. The results provide additional information on possible risk associated with oil spill response activities using the sessile embryonic stage of this ecologically important species.

2. Methods

All oil preparation methods followed standardized methods of Singer et al. (2000). All testing was conducted using PBCO and COREXIT 9500. Chemical dispersion of oil was carried out at a nominal oil:dispersant ratio of 10:1 (v:v). Untreated oil testing was performed using the water-accommodated fraction (WAF) of unweathered PBCO. WAFs were created by layering a known mass of crude oil onto a standard volume (22 L) of 0.45-μm filtered natural seawater (~33‰ salinity) in a 23-L polycarbonate carboy (resulting in a standardized 22–23% headspace, by volume), and mixing this with magnetic stirrers at a rate sufficient to provide circulation of water throughout the bottle without creating a vortex (Singer et al., 2000).

CEWAF tests were performed with solutions prepared in much the same way as WAFs. Mixing energies used to prepare CEWAFs were increased to create a vortex

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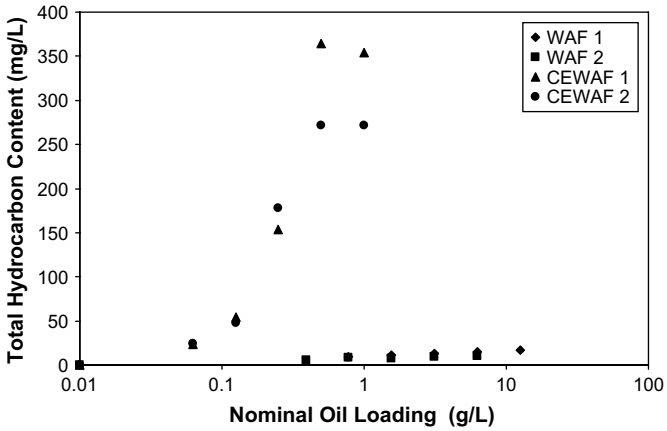

Fig. 1. Relationship between nominal loadings of Prudhoe Bay Crude Oil and total hydrocarbon content in WAF and CEWAF experiments (see text for details).

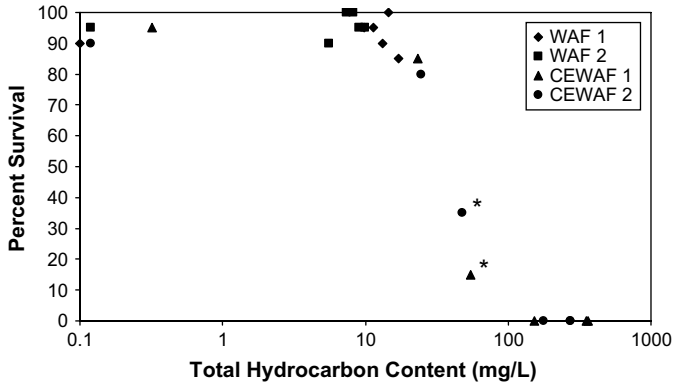

Fig. 2. Effects of total hydrocarbon content on topsmelt survival in WAF and CEWAF experiments (* denotes first concentration significantly different from the control).

20–25% of water depth to provide sufficient mixing energy for dispersion (Singer et al., 2000). Once the vortex was established, known volumes of oil and dispersant were delivered in sequence into the center of the vortex (Singer et al., 1998). The methods followed were those recommended by participants in the Chemical Response to Oil Research Forum (CROSERF – Singer et al., 2000), which are designed to allow direct comparisons among laboratories generating toxicity data usable in spill-response decision-making. Mixing lasted 18 h, followed by 6 h of settling time to allow the largest oil droplets to resurface (Singer et al., 2000) and this matched the 24-h total preparation time used for WAFs.

Chromatographic measurement of total hydrocarbon content (THC, C_6–C_{36}) was accomplished using a Hewlett–Packard 6890 gas chromatograph fitted with a flame ionization detector. Concentrations of the volatile BTEX (benzene, toluene, ethylbenzene and xylenes' fraction) were determined using HP GC/MS 6890-5973 with an HP 7695 purge and trap concentrator.

Water temperature, dissolved oxygen, and pH were monitored at the beginning and end of each test. Treatments within individual tests in this study were derived from a range of oil loadings, with replicate tests employing equivalent loading ranges.

Duplicate experiments were conducted using WAF and CEWAF and followed methods of Anderson et al. (1991). Late gastrula-stage embryos (~26-h post-fertilization) were received from Aquatic Biosystems (Fort Collins, CO) in a cooled thermos (9 °C), and single embryos were placed in glass 20-mL scintillation vials. The scintillation vials contained either WAF solutions of 0, 0.39, 0.78, 1.56, 3.13, 6.25 or 12.5 g/L oil, or CEWAF solutions of 0, 0.063, 0.13, 0.25, 0.50, or 1.0 g/L oil – all based on nominal PBCO loadings. Control and dilution water was 0.45-μm filtered (33‰ at 21 ± 1 °C). Each vial contained 20 mL of solution and vials were filled to capacity, leaving no headspace. A Teflon-lined cap sealed the tubes for the duration of each test, and each treatment was replicated 20 times. Test solutions were not renewed and vials were inverted four times daily to mix the solutions. Tests were conducted for 10 days or until all fish either hatched or died, which took up to 12 days in some replicates.

Embryo abnormalities quantified included craniofacial, cardiovascular, and skeletal abnormalities. Instances of yolk-sac edema were enumerated as yolk-sac length, measured from the pericardial sac to the posterior limit, and normalized by dividing this by fish length. Toxicity of COREXIT 9500 alone was not assessed in this study because the concentrations used were considerably lower than the lowest reported affect thresholds for fish (see National Research Council, 2005).

Data for 20 replicates at each exposure concentration were combined for statistical analysis. Data evaluation included the lowest observed effect concentrations (LOECs) and were calculated using ToxCalc (Tidepool Software, McKinleyville, CA, USA). THC (C_6–C_{36}) is the sum of BTEX (C_6–C_9) plus total petroleum hydrocarbons (C_{10}–C_{36}), quantified in Time 0 samples. Correlations between THC and larval length were investigated using Spearman Rank Correlations (Zar, 1974).

3. Results and discussion

Treating PBCO with dispersant resulted in much greater hydrocarbon concentrations in CEWAFs relative to the untreated WAFs. THC ranged from 23.41 to 364.58 mg/L in the two CEWAF tests (CEWAF average control THC = 0.234 mg/L; Fig. 1). Despite the use of ten-fold higher PBCO loading in the WAF tests, THC ranged from 5.57 to 17.02 mg/L in the two WAF tests (WAF average control THC = 0.199 mg/L). Thus, the average THC in the lowest CEWAF loadings (24.01 mg/L THC at 0.063 g/L PBCO) was greater than the THC in the highest WAF loadings (17.02 mg/L THC at 12.0 g/L PBCO).

Greater toxicity to topsmelt embryos in the CEWAF tests reflected the greater THC concentrations. No significant effects on larval hatching and survival were observed in the two WAF tests (Fig. 2), while hatching and larval survival was significantly inhibited at 54.34 and 47.77 mg/L THC in CEWAF tests 1 and 2, respectively (Fig. 2).

Because hydrocarbon chemistry was limited to characterization of THC, it is not possible to determine specific constituents responsible for the observed abnormalities in these experiments. The most volatile fractions were characterized as BTEX in the present study. We did not measure individual PAHs in these experiments, so it is not possible to quantify relative concentrations of lighter hydrocarbon fractions in the WAF and CEWAF tests. The range of total BTEX was considerably higher in the WAF tests relative to the CEWAF tests (Table 1). Based on the differences in BTEX concentrations in these experiments, it is unlikely that the cardiovascular abnormalities were due to BTEX constituents. For example, cardiovascular abnormalities were observed in embryos

Table 1
Relationships between oil (PBCO) loading and total hydrocarbon content (THC) and BTEX[a] in WAF and CEWAF tests. THC C_6–C_{36} = BTEX (C6–C9) plus total petroleum hydrocarbons (C_{10}–C_{36}; after Singer et al., 1998).

WAF 1			WAF 2			CEWAF 1			CEWAF 2		
Loading (g/L)	THC (mg/L)	BTEX (mg/L)	Loading (g/L)	THC (mg/L)	BTEX (mg/L)	Loading (g/L)	THC (mg/L)	BTEX (mg/L)	Loading (g/L)	THC (mg/L)	BTEX (mg/L)
0	0		0	0		0	0		0	0	
–	–		0.39	5.57	4930	0.063	23.41	212	0.063	24.70	99
0.78	9.78	9045	0.78	8.23	7551	0.125	54.34	1435	0.125	47.77	1570
1.56	11.38	10,540	1.56	7.38	6590	0.25	153.44	4440	0.25	177.93	2930
3.13	13.20	12,310	3.13	9.07	8040	0.50	364.58	7581	0.50	272.04	2040
6.25	14.55	13,520	6.25	9.94	8940	1.0	354.35	12348	1.0	272.03	5030
12	17.02	15,820	–	–							

[a] BTEX = total concentrations of benzene, toluene, ethylbenzene and xylene.

exposed to the lowest THC in the two CEWAF tests (23 and 25 mg/L THC) and the total Time 0 BTEX concentrations were 212 and 99 μg/L at these THC levels in CEWAF tests 1 and 2, respectively. In contrast, cardiovascular abnormalities were not observed or were minimal at 10–13 mg/L THC in the WAF tests, and the BTEX concentrations at these THC levels were 9000–12,000 μg/L. Thus, the WAF solutions were dominated by BTEX in these experiments (Table 1), and the CEWAF solutions can be assumed to have contained much higher concentrations of PAHs (e.g., Singer et al., 1998; Tjeerdema et al., 2006, 2008). Numerous other recent studies have shown that higher molecular weight PAHs in oiled water are the likely source of toxicity to fish early life stages (Carls et al., 1999; Billiard et al., 2002; Brinkworth et al., 2003; Ramachandran et al., 2004; Barron et al., 2004; Incardona et al., 2004, 2005; Couillard et al., 2005). In comparisons of WAF and CEWAF toxicity using larval topsmelt (*A. affinis*), Singer et al. (1998) found that when the results were based on total hydrocarbon content, larvae of this species were more sensitive to PBCO WAF than to CEWAF. Their studies found that WAF solutions of PBCO were composed of an average of 96% volatiles (compounds chromatographing earlier than naphthalene), whereas CEWAFs contained only 67% volatiles.

Although the majority of embryos developed and hatched in the WAF tests, some of the hatched larvae demonstrated cardiovascular abnormalities, particularly at the two highest THC concentrations in the first WAF test. Abnormal WAF-exposed larvae hatched with subtle pericardial and yolk-sac edemas and had developed (abnormal) tube hearts. Topsmelt embryos exposed to CEWAF developed cardiovascular abnormalities at all concentrations. Embryos exposed to the higher CEWAF concentrations had severe cardiovascular abnormalities within 48 h of exposure, and these fish failed to hatch. The majority of fish exposed to the lowest CEWAF concentrations hatched with pronounced pericardial and yolk-sac edemas, and had also developed tube hearts (Fig. 3). Fish with abnormal hearts also demonstrated incomplete circulation (hemostasis).

Larvae hatched after exposure to CEWAF developed cardiovascular and yolk-sac edemas and fish with these abnormalities were smaller than control fish. Length of hatched larvae in the CEWAF tests was negatively correlated with THC (Spearman Rank Rho value $= -0.557$; $\alpha = 0.001$; Fig. 4). There was no significant correlation between THC and larval size in the WAF test.

The abnormalities observed in the current study have been reported in studies using embryos of other species exposed to selected PAHs (Barron et al., 2004; Incardona et al., 2004). Incardona et al. (2005) exposed zebra fish embryos to WAF generated from weathered PBCO and determined that the observed cardiac dysfunction caused by the resulting PAH mixtures was similar to

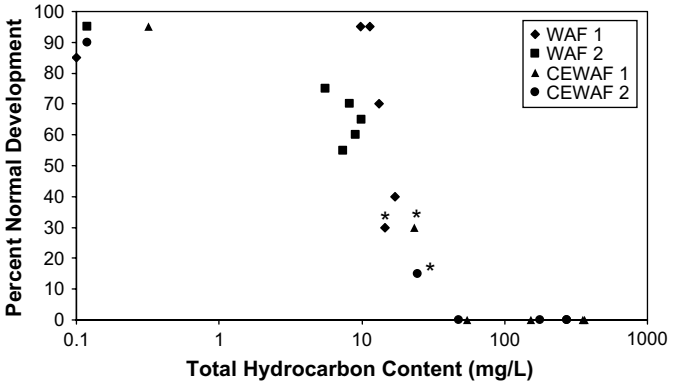

Fig. 3. Effects of total hydrocarbon content on topsmelt embryo development in WAF and CEWAF experiments (* denotes first concentration significantly different from the control).

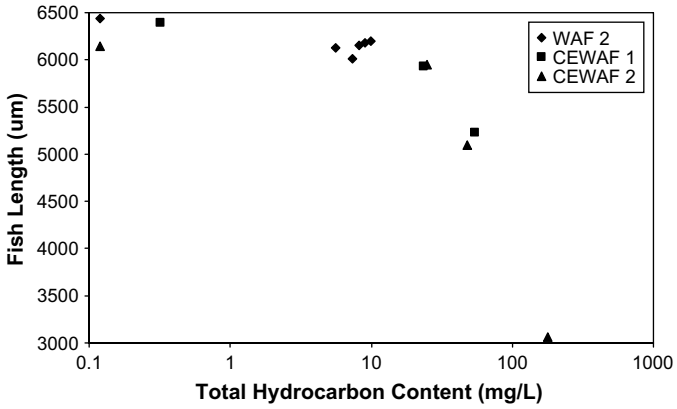

Fig. 4. Relationship between total hydrocarbon content and larval topsmelt length in WAF and CEWAF experiments (note: differences in length between treatments were not compared statistically).

effects caused by parent compounds of model tricyclic PAHs (e.g., fluorine, phenanthrene). Their research suggests that tricyclic PAHs potentially affect cardiac function by influencing several targets in developing embryo hearts, including sodium/potassium channels, as well as gap junctions.

4. Conclusions

While greater impacts on development were observed in embryos exposed to CEWAF relative those exposed to WAF, direct comparisons of relative impacts were constrained by lack of overlapping total hydrocarbon contents in the WAF and CEWAF experiments, despite the much greater oil loading in the experiments with untreated oil. Future experiments will include analyses of polynuclear aromatic hydrocarbons to investigate the role of these constituents on embryo development in treated and untreated oil exposures. It should be noted that because these experiments used unweathered oil in static exposures using closed test chambers, the results likely over-estimate toxicity of treated and untreated oil in the environment (Singer et al., 1998; McAuliffe, 1987). Future experiments with topsmelt embryos will assess toxicity of weathered oil using declining exposures to assess toxicity under more environmentally realistic conditions.

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