

## Influence of salinity and fish species on PAH uptake from dispersed crude oil

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### Abstract

The use of chemical oil dispersants to minimize spill impacts causes a transient increase in hydrocarbon concentrations in water, which increases the risk to aquatic species if toxic components become more bioavailable. The risk of effects depends on the extent to which dispersants enhance the exposure to toxic components, such as polycyclic aromatic hydrocarbons (PAH). Increased salinities can reduce the solubility of PAH and the efficiency of oil dispersants. This study measured changes in the induction of CYP1A enzymes of fish to demonstrate the effect of salinity on PAH availability. Freshwater rainbow trout and euryhaline mummichog were exposed to water accommodated fractions (WAF), and chemically-enhanced water accommodated fractions (CEWAF) at 0‰, 15‰, and 30‰ salinity. For both species, PAH exposure decreased as salinity increased whereas dispersant effectiveness decreased only at the highest salinity. Hence, risks to fish of PAH from dispersed oil will be greatest in coastal waters where salinities are low.

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### 1. Introduction

Oil spills associated with marine transport and offshore production facilities often occur in the open sea. In some cases, the oil slicks drift towards the shore into estuaries and nearshore habitats that serve as important spawning and nursing grounds for many fish species. Within these coastal areas, other environmental factors such as temperature and salinity could significantly influence the natural fate and distribution of the oil.

The effect of salinity on risks associated with an oil spill has many facets. Salinity may influence the solubility of

toxic hydrocarbons from the crude oil, the effectiveness of chemical dispersants, and the binding characteristics of residual oil fractions onto suspended particles. The accumulation of hydrocarbons by aquatic organisms in different salinity mediums could also be affected by their osmoregulatory adaptations. Therefore, the risks of toxicity from exposure to dispersed as well as undispersed crude oil may vary with salinity.

Crude oil is a mixture of several fractions of hydrocarbons, with varying solubilities depending on their octanol–water partition coefficients ( $K_{ow}$ ). Amongst them, polycyclic aromatic hydrocarbons (PAH) rank as relatively soluble, more soluble than alkanes having an equal number of carbon atoms (McAuliffe, 1987). PAH solubility is an important feature, considering that many PAH rank

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among the most toxic components of crude oil. McAuliffe (1987) found solubilities of toluene in 3.5% and 20% NaCl to be 70% and 16% of that in distilled water. For 12 aromatic hydrocarbons, Sutton and Calder (1975; reviewed in McAuliffe 1987) found that the mean reduction in solubility at 25 °C in seawater was  $68 \pm 4.4\%$ , relative to fresh water. Therefore, the concentrations of aromatic hydrocarbons in the water column after an oil spill would increase in low salinity coastal water or estuaries and have a greater impact on aquatic organisms than would be the case for spills in open ocean waters. Results of bioassays conducted with marine test organisms in salt water would not accurately predict effects on fresh- or brackish-water organisms.

Environmental factors such as salinity (Blondina et al., 1999) also modify the performance of dispersants. Much of the work on dispersant effectiveness has tested marine conditions (32–34‰ salinity), with few freshwater tests. Due to toxicity concerns, it has been assumed that dispersants would not be used in shallow waters where dispersion would be limited. Most dispersants are formulated to work within a narrow range of water salinities, close to that of seawater, and Fingas et al. (1994) reported distinct salinity interactions with the effectiveness of three commercial dispersants. With the proposed use of dispersants to treat small operational spills, and the recent development of “low toxicity” dispersant formulations, a better understanding of dispersant effects in salinities resembling near shore or coastal habitats is needed, as this can affect deployment decisions relating to location and season.

Fish can accumulate soluble petroleum hydrocarbons very rapidly (Collier et al., 1995), and a fish placed in crude oil-contaminated water will take up dissolved hydrocarbons until a steady state is established between fish and water. Gills are primary route of hydrocarbon uptake and excretion, usually by diffusion (Thomas and Rice, 1982). Highly lipid-soluble compounds will be rapidly exchanged across gills, as they have a large surface area and a thin epithelium separating blood and water. Gills, being lipid-rich and directly exposed to contaminated waters, also serve as a route for the direct uptake of hydrophobic crude oil fractions, provided there is contact between oil droplets in suspension and the gill surface.

Exposure to concentrations of petroleum hydrocarbons dissolved in water and to naturally dispersed oil droplets suspended in the water column can be greatly enhanced by the use of dispersants (Fucik, 1994). While the lighter PAH volatilize and solubilize easily, the heavier and more toxic fractions are less soluble. The hydrophobic nature of the more toxic fractions enables them to partition directly from crude oil to lipid-rich tissues coming into contact with oil droplets. This is of concern since these contaminants can bioconcentrate in tissues of organisms to factors 10–1000 times greater than in water. Fluorescing oil droplets were observed under microscope to adhere to the gills of rainbow trout (*Oncorhynchus mykiss*) exposed to the water accommodated fraction (WAF) and to the chemically-enhanced water accommodated fraction

(CEWAF, i.e., dispersed oil) of MESA crude oil (Ramachandran et al., 2004a).

Gills in teleosts are sites for osmoregulation as well. Changes in ion fluxes would take place depending on the osmotic state of the environment. Freshwater fishes, being hyperosmotic relative to fresh water, experience a net inflow of water, thereby increasing uptake of soluble compounds. In contrast, marine fish are hypoosmotic relative to salt water, and lose water by diffusion; they must drink salt water, removing excess salt via chloride cells in gill epithelium (Willmer et al., 2000). Hence, uptake of soluble PAH may be influenced by changes in osmoregulation if PAH are taken up across the gills by transport with water.

The objective of this research was to measure changes in exposure of fish to PAH when MESA crude oil was dispersed at a range of salinities. Exposure was estimated by measuring the induction of hepatic cytochrome P450 (CYP1A) activity, an indicator of PAH uptake. Induction of CYP1A is mediated by the aryl hydrocarbon (Ah) receptor, which activates the *cyp1a* gene following binding of the PAH ligand. The ligand and receptor form an activated complex which translocates to the nucleus and activates the transcription of the *cyp1a* gene to mRNA, which is subsequently translated into CYP1A protein (Di Giulio et al., 1995). The CYP1A enzyme catalyzes the hydroxylation of PAH to a more soluble and excretable form in the bile, and assays of liver CYP1A activity provide a good biomarker of PAH exposure in fish (McCarty et al., 2002). The use of CYP1A as a biomarker of exposure avoids the complications of trying to measure mixtures of hydrocarbons in small fish (2–3 g), particularly since hydrocarbon excretion limits concentrations in tissues. The CYP1A assay also focuses attention on those constituents of oil that are associated with chronic toxicity, i.e. 3–5-ringed PAH that cause embryo-larval toxicity (e.g., Brinkworth et al., 2003).

This study examined the role of salinity as a factor influencing dispersant effectiveness as indicated by changes in the uptake of PAH from dispersed crude oil by fish. The test species were rainbow trout and the estuarine mummichog (*Fundulus heteroclitus*). Rainbow trout was chosen to enable comparisons with freshwater data from previous experiments across salinities within their zone of tolerance (0–15‰). Mummichog, a more euryhaline species, enabled testing at higher salinities up to 30‰ without confounding results by osmotic stress. They are highly amenable to laboratory studies, a common and abundant species along the Atlantic coast from Florida to Labrador, and would almost certainly be affected by coastal oil spills.

## 2. Materials and methods

### 2.1. Crude oil and dispersant

The crude oil used to make up water accommodated fraction (WAF) and chemically enhanced water accommodated fraction (CEWAF) was MESA (Medium Grade South American) sour crude, an oil with a viscosity of 3–

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