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## Effect of Oil and Dispersants from the Gulf of Mexico on Estuarine Fish Species

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The spill of the Deepwater Horizon oil into the Gulf of Mexico was the single largest input of oil to date into U.S. waters (4.9 million barrels; 206 million gal) (Azwell 2011) and was one of the first examples of the large-scale application of chemical dispersants to an oil spill (1.8 million gallons), an approved resource that will likely be used again. Since oil exploration and production in the GoM will continue, there will be risks of similar events potentially affecting the significant fisheries resources that exist in the area. These events will undoubtedly result in cleanup efforts similar to the ones that were performed after the DWH spill and there is consequently a pressing need to develop adequate measures to determine the sublethal effects of these types of exposures on aquatic organisms.

We obtained samples of Macondo oil and Corexit 9500 and 9527 for toxicity experiments. We weathered the oil and oil/dispersant mixtures in water by rapid stirring for 1 week in a fume hood using the methods of Hemmer et al. (2010). The water-accommodated fraction (WAF) of each test solution was diluted in artificial seawater and used to expose two species of estuarine fish (Sheepshead minnow, *Cyprinodon variegatus* and Menidia, *Menidia beryllina*). In survival tests with sheepshead minnow, we found that the addition of Corexit to the oil was more toxic than exposures to the oil by itself, decreasing the  $LC_{50}$  by 20-fold, suggesting that the presence of dispersant increased the toxicity of the mixture, presumably by making the toxic components more bioavailable. Addition of Corexit 9527 to the oil at 1/10 the concentration of the oil prior to preparing the water-accommodated fraction caused significant mortality of sheepshead minnows compared to the same concentrations of WAF from oil alone that were not lethal to the fish. The WAF from the mixture of dispersant and oil, called Chemically Enhanced Water Accommodated Fraction (CEWAF), was more toxic to fish than WAF. Columbia Analytical Services (Kelso, WA) analyzed chemical residue in each of the WAFs and found much higher PAH residue in CEWAF obtained from oil/Corexit mixtures.

Using sublethal concentrations of the WAF and CEWAFs ( $\Sigma$ PAH ~30 ng/ml) from oil, Corexits and mixtures thereof, we performed sublethal exposures of Menidia embryos and found developmental abnormalities (Fig. 1) including abnormal heart development, lower heartbeats per minute and edema, among others (Table 1). This is similar to what has been reported by Incardona et al. (2013) for other fishes and suggests a similar mechanism of action. These preliminary data suggest that exposure of sensitive estuarine species to oil and Corexit mixtures may lead to population level declines of Menidia in estuarine locations. Menidia, also known as silversides, inhabits estuaries along the Gulf coast. It has high ecological relevance to the GoM ecosystem, and is an abundant species in the salt marsh and in shallow waters. Menidia have demersal eggs, meaning that the eggs sink and are laid near shore, close to where oil was distributed near Louisiana marshes. They are widely distributed and are native to Eastern USA and the northern GoM and are important in the food chain for larger commercially valuable fish. It is an exquisitely sensitive life stage to environmental perturbations, including both natural stressors and chemical exposures. Embryonic development is particularly important for all life forms since this is the life stage during which highly complex biological processes are directing rapid growth and cellular differentiation. Our results suggest that oil plus the dispersant may have adversely affected these fish in locations that were heavily oiled.



Figure 1. Skeletal and other morphological abnormalities observed with exposures of Menidia embryos to WAF and CEWAF. (a) skeletal curvature of the tail, (b) unhatched embryos showing signs of deterioration and (c) malformations of the head and pericardial edema.

Table 1. Diminished heart beats in Menidia embryos exposed to WAF from Oil, Corexit and a mixture of the two.

Condition	Heart beats measured	Deformities
Control	82.7 beats/min	None
WAF from Oil	80.25 beats/min	Skeletal abnormalities
CEWAF	67.1beats/min	Unhatched embryo, Pericardial edema,
(Oil + Corexit 9527)		skeletal abnormalities

The DWH oil spill occurred in 2010, but there is still a lot of controversy regarding whether the damage has been contained and whether there are still long lasting effects in this ecosystem. At the population level, marine species are resilient and after time recover from repeated insults, but there is probably some cost to these occurrences in terms of the fitness of the population. Along these lines we need to consider sublethal effects from oil and dispersant exposures that may manifest themselves in later generations as permanently affecting the fitness of a particular species.

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