

STUDY TITLE: The Effects of Simultaneous Exposure to Petroleum Hydrocarbons, Hypoxia and Prior exposure on the Tolerance and Sublethal Responses of Marine Animals

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CONTRACT NUMBER: 14-35-0001-30660-19914

SPONSORING OCS REGION: Gulf of Mexico OCS Region

APPLICABLE PLANNING AREA: Central Gulf of Mexico

FISCAL YEARS OF PROJECT FUNDING: 1993-1995

COMPLETION DATE OF REPORT: September 1998

COSTS: FY 1993-FY 1995: \$176,268

CUMULATIVE PROJECT COST: \$176,268

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KEYWORDS: Water soluble fraction, crude oil, hypoxia, Gulf killifish, blue crab, tolerance, growth, EROD, RNA:DNA ratio, synergism

BACKGROUND: Coastal Louisiana has been impacted by oil field activities for the last half century and the waters over the continental shelf west of the Mississippi River delta, are also subject to extensive periods of hypoxia during the summer months. Marine and estuarine fish are sensitive to exposure to the water-soluble fraction (WSF) of crude oil but their tolerance doesn't change much after 12-24h exposure because of the presence of an inducible cytochrome P-450 enzyme system for metabolizing aromatic hydrocarbons. In contrast, invertebrates tolerate high concentrations of the WSF for short time periods, but their tolerance declines during long-term exposure. The determination of long term tolerance and sublethal biomarkers have not been used to assess the effects of gradients of oxygen tension and the WSF of crude oil on marine and estuarine fauna.

OBJECTIVES: The intent of this project was to study the combined effects of the WSF of South Louisiana crude oil and hypoxia on the tolerance of the Gulf killifish, and juvenile blue crabs and lesser blue crabs. In addition, the affects of chronic exposure of these species to sublethal concentrations of the WSF of crude oil on the tolerance, RNA:DNA ratios and killifish condition and induction of cytochrome P-450 enzymes were compared under hypoxic conditions with the responses of these species from more pristine locales. There have been no studies investigating these interactions to date.

DESCRIPTION: Tolerance was determined over 28 days for killifish, juvenile blue crabs and juvenile lesser blue crabs exposed to five levels of hypoxia and six concentrations of the WSF of South Louisiana crude oil. Sublethal responses were determined on eight animals per time-concentration combination at three WSF concentrations on days 0, 1, 7, 14, and 28 of exposure. RNA:DNA analyses were determined on all animals and EROD analysis of cytochrome P-450 activation as well as condition index and liver-somatic index was also determined on Gulf killifish.

SIGNIFICANT CONCLUSIONS: The hypoxia tolerance of the Gulf killifish was higher than that of the lesser blue crab and the blue crab. Minimal site differences existed in hypoxia tolerance. There was no difference in the 28-day tolerance of blue crabs to the WSF of South Louisiana crude oil exposed to normoxia or hypoxia. The Gulf killifish from the produced water site in Louisiana was more tolerant of the WSF than those from Florida and fish exposed to the WSF under normoxia were more tolerant than those exposed under hypoxia. Induction of EROD activity occurred in a dose related manner in *Fundulus grandis* from both locations exposed to the WSF under hypoxia (75 Torr O₂), peaked on the seventh day of exposure and was significantly higher in fish exposed to 35?g oil/liter from the produced water site than in killifish from Florida. RNA:DNA ratios are not a particularly sensitive indicator of sublethal WSF stress in either Gulf killifish or the blue crab. EROD activity, an indicator of cytochrome p-450 induction, increases in a dose related manner in the liver of *Fundulus grandis* from both locations, peaks on the 7th day of exposure and is higher in fish from the produced water site. WSF exposure induced hepatic tissue growth in both populations. Weight loss was greatest in fish from Florida. Increases in liver size and RNA:DNA ratios were noted with EROD induction. Stimulation of growth in white muscle tissue by WSF exposure was observed in the Louisiana population. The condition factor (K) of the Florida population decreased more than the Louisiana population. Differences in (K) between hypoxia and hypoxia + WSF treatments were minor, suggesting small changes in condition would be sufficient to affect survival in *F. grandis*. Therefore, *Fundulus grandis*, from the chronically polluted site are more tolerant of exposure to the WSF of South Louisiana crude oil and exhibit an increased rate of EROD activity compared with the Florida population. However, these biological differences in response patterns are minor.

STUDY RESULTS: Chronic exposure to sublethal concentrations of the WSF of crude oil leads to increased tolerance and condition of the Gulf killifish when exposed to the WSF of South Louisiana crude oil. Exposure to the WSF under hypoxia produces slight

increases in sensitivity to crude oil in comparison with exposure under normoxia. Blue crabs and lesser blue crabs are not sensitive to the additive effects of hypoxia and the WSF of South Louisiana crude oil.

STUDY PRODUCTS: Stickle, W.B. The Effects of Simultaneous Exposure to Petroleum Hydrocarbons, Hypoxia and Prior Exposure on the Tolerance and Sublethal Responses of Marine Animals: Final Report. OCS Study MMS 2002-009. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, Louisiana, 26 pp.