OCS Study
MMS 2002-009

Coastal Marine Institute

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## Final Report

MMS U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

LSU Cooperative Agreement
Coastal Marine Institute
Louisiana State University

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# Effects of Simultaneous Exposure to Petroleum Hydrocarbons, Hypoxia, and Prior Exposure on the Tolerance and Sublethal Responses of Marine Animals: Blue Crabs and Killifish

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## ABSTRACT

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. The determination of 28 day tolerance and RNA:DNA ratios, EROD activity, growth condition (K) and the liver-somatic index have been used as sublethal biomarkers have been used to assess the effects of gradients of oxygen tension and the WSF of South Louisiana crude oil alone and in concert were determined for juvenile blue crabs and lesser blue crabs (tolerance and RNA:DNA ratios only) and the Gulf killifish.

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 as sensitive to the additive effects of hypoxia and the WSF of South Louisiana crude oil nor to prior exposure history. RNA:DNA ratios are not a sensitive indicator of sublethal WSF stress in the Gulf killifish , the blue crab or the lesser blue crab. EROD activity, as an indicator of Cytochrome p-450 induction increases in a dose related manner in the liver of *Fundulus grandis* from both locations on both a specific activity and total liver activity basis. *Fundulus grandis* exhibits enhanced adaptations to chronic petroleum hydrocarbon exposure but neither juvenile *Callinectes sapidus* nor *Callinectes similis* exhibit enhanced adaptation to petroleum hydrocarbons with chronic exposure.

## TABLE OF CONTENTS

Page

## LIST OF FIGURES

Page

Figure

## LIST OF TABLES

Page

Table

## 1.0 INTRODUCTION

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. These hypoxic events are known locally as dead water zones because of a paucity of fauna in the water mass during their development.

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 as illustrated by the response of coho salmon, *Oncorhynchus kisutch* (Stickle et al., 1982). All mortalities occurred during the first two days of exposure to toluene and naphthalene even though the experiment ran for 42 days. In contrast invertebrates tolerate high concentrations of the WSF for short time periods but their tolerance declines during 28 days of exposure (Stickle et al., 1987; Wang and Stickle, 1988).

The determination of long term tolerance and two sublethal biomarkers have been used to assess the effects of gradients of oxygen tension and the WSF of crude oil on marine and estuarine fauna (Das and Stickle, 1993; Kapper and Stickle, 1987; Stickle et. al., 1989; and Wang and Stickle, 1988).

This project was designed to test the following null hypotheses:

Null hypothesis 1: The WSF of crude oil and hypoxia gradients acting alone and in concert do not alter the 28 day $LC_{50}$ of the Gulf killifish, *Fundulus grandis*, or two conspecific species of portunid crabs, *Callinectes sapidus* and *C. similis*.

Null hypothesis 2: Prior exposure to sublethal concentrations of the WSF of crude oil and hypoxia acting alone and in concert do not alter the tolerance, RNA:DNA ratio or in all three species and EROD activity in the Gulf killifish. In addition, an attempt was made to determine polyamine profiles in the two species of crabs but this biomarker could not be reliably determined.

## 2.0 MATERIALS AND METHODS

Juvenile *Callinectes sapidus* and *Callinectes similis* and adult *Fundulus grandis* were collected from two sites by dip nets and fish traps and returned to LSU for experimental use. . The site at Port Fourchon, LA is impacted by produced water discharge (Rabalais et al., 1991) and the non polluted site was the beach, sea grass and local streams in the vicinity of the Florida State Marine Laboratory at Turkey Point, FL. *Callinectes similis* was not found at the Turkey Point site. GCMS aromatic hydrocarbon analysis of *Callinectes sapidus* documented that the body burden was higher at the Port Fourchon produced water site (30.6 ng.g wet weight$^{-1}$) than in those collected at Turkey Point, FL (11.9 ng.g wet weight $^{-1}$). Likewise the aromatic hydrocarbon body burden of *Callinectes similis* from the Port Fourchon produced water site was (57.1 ng.g wet weight$^{-1}$) higher than that of *C. sapidus* from Turkey Point, FL.

The constant hypoxia exposure system was described in detail in Kapper and Stickle (1987). Oxygen tensions were determined daily by injecting water samples taken anaerobically into a syringe and injected into a water jacketed Strathkelvin flow through oxygen electrode-chamber system that was connected to a Strathkelvin oxygen meter.

The flow through system used to generate the WSF of South Louisiana crude oil was designed and is in use in the Laboratory of Dr. Stanley Rice at the NMFS laboratory in Auke Bay, Alaska. Different amounts of South Louisiana crude oil and pentane were poured into a commercial cement mixer that contained a known amount of three-eighths inch pea gravel and mixed for five minutes. After five minutes of mixing the oil coated pea gravel was poured onto plywood, raked regularly and allowed to dry. Known weights of different doses of oiled gravel were put into WSF generators constructed of 12" PVC pipe that was 1 meter high. A perforated plate was placed in the bottom of each generator so sea water flowed into the bottom. Seawater (30 $^{o}/_{oo}$S) flowed through each generator by gravitational flow by entering via an inlet at the bottom and exiting at the top at a rate controlled to ~50 ml.min$^{-1}$ . Oil doses were monitored weekly by extraction into methylene chloride using standard methods established at the Auke Bay, Laboratory NMFS Laboratory (Larsen et al., 1992) and analyzed by HPLC.

The duration of all bioassays and experimental exposures was 28 days. Animals were collected at 0, 1, 7, 14 and 28 days in dosing experiments. Crabs were frozen whole in liquid nitrogen and killifish were dissected into white muscle and liver then stored into an ultracold freezer until analyzed for RNA, EROD, or polyamine profile analysis.

Two morphological indexes of fitness were utilized to determine the fitness of *Fundulus grandis* following exposure to hypoxia, the WSF of South Louisiana crude oil and a combination of the two stressors. Killifish condition factor ((K), body weight (mg) / length (mm)$^3$ ×100) was utilized to determine bioenergetic constraints on the entire fish. The liver somatic index (liver wt (mg) / body wt (mg) × 100) was determined in order to determine treatment effects on the organ in which most of the induction of aromatic hydrocarbon metabolism occurs.

RNA:DNA analysis followed procedures outlined in Das and Stickle (1993). EROD activity was determined in the liver of *Fundulus grandis* as the microsomal O-dealkylation of ethyoxyresorufin according to modification of the method of Burke et al. (1985). Protein concentration was determined by the method of Lowry et al. (1951). Activity was calculated as picomols resorufin/mg protein/min.

Statistical analyses were performed with the Statistical Analysis System (SAS Institute; 1996). Data on the $LC_{50}$ of all species (Table 1) were analyzed using probit analysis (Hamilton et al., 1977). Non-overlap of 95% fiducial limits were used as a criterion to determine significant differences between individual $LC_{50}$ values.

## 3.0 RESULTS

### 3.1 28-day Tolerance Studies

No significant difference existed in the 28 day $LC_{50}$ values of either species of crab from the less contaminated site from Turkey Point, FL and the produced water site at Port Fourchon, LA when exposed to the WSF of South Louisiana crude oil under either normoxia or hypoxia. In contrast, *Fundulus grandis* from the produced water site in Louisiana tolerated the WSF of South Louisiana crude oil better than those from Turkey Point, FL under both normoxia and hypoxia but the difference in tolerance was of small magnitude (Table 3.1).

Table 3.1. $LC_{50}$ values after 28 days exposure of *Callinectes sapidus*, *C. similis*, and *Fundulus grandis* to hypoxia, and the water soluble fraction (WSF) of South Louisiana crude oil under normoxia (156 mm Hg $O_2$) and hypoxia (75 mm Hg $O_2$ for *F. grandis* and 113 mm Hg $O_2$ for *C. sapidus* and *C. similis*). W.F. data are given in $n.l.^{-1}$ of total aromatic hydrocarbons.

| Stressor | Species | Louisiana | Florida |
|---|---|---|---|
| Hypoxia | *Callinectes sapidus* | 111 | 113 |
| | *Callinectes similis* | 45 | xxx |
| | *Fundulus grandis* | 28 | 33 |
| W.F. + Normoxia | *Callinectes sapidus* | 27 | 26 |
| | *Callinectes similis* | 26 | xxx |
| | *Fundulus grandis* | 22 | 17 |
| W.F. + Hypoxia | *Callinectes sapidus* | 28 | 29 |
| | *Callinectes similis* | 26 | xxx |
| | *Fundulus grandis* | 17 | 13 |

### 3.2 Sublethal Responses

#### 3.2.1 Blue Crab and Lesser Blue Crab RNA:DNA Ratios

There was no significant difference in the RNA:DNA ratio of either Callinectes sapidus or C. similis exposed to 0, 9, and 35ng oil. $l^{-1}$ over 28 days. In contrast, RNA:DNA ratios of C. sapidus exposed to the WSF of South Louisiana crude oil declined with WSF concentration and duration of exposure (Wang and Stickle, 1988).

#### 3.2.2 Sublethal Responses of Gulf Killifish

Several physiological indices indicated differences in the sublethal responses of Fundulus grandis from the polluted and chronically polluted sites (Szeto, 1998). The condition factor (K), liver somatic index (LSI), RNA:DNA ratios and ethoxyresorufin-O-deethylase (EROD) activity

indicated that the Gulf killifish from the Port Fourchon was better adapted to the WSF of South Louisiana crude oil than the Turkey Point Florida population.

3.2.2.1 Growth, Condition Factor and Liver-somatic Indices

Significant differences in condition factor ((K), body weight (mg) / length $(mm)^3 \times 100$) were observed among the two populations. Condition factor did not change over 28 days in the normoxia controls. Florida fish had a lower (K) than Pass Fourchon (Table 3.2). Condition factor means of hypoxia controls and hypoxia and the water soluble fraction of crude oil concentrations decreased in fish from Florida over time, whereas Pass Fourchon losses reached an asymptote by day 7. Fish from Florida gradually lost weight over time (Figure 3.1). Weight of fish exposed to hypoxia + WSF concentrations for both populations were not significantly different from their respective hypoxia-control treatment by day. Over the course of the experiment a significant decline in (K) compared to day 0 and day 1 hypoxia-control treatments were observed on days 7, 14, and 28 in both populations. Percentage differences of (K) were greatest in the Florida population at -22.6% in the day 28 hypoxia high + WSF concentration compared to the day zero fish. The decline in the day 28 high treatment in Pass Fourchon fish was -15.6%.

Table 3.2. Condition Factor (body weight (mg) / length $(mm)^3 \times 100$) $\pm$ SE in Fundulus grandis from Florida and Pass Fourchon over 28 days in normoxia (156 mm Hg $O_2$) control, hypoxia (73 mm Hg $O_2$) control, hypoxia + low and hypoxia + high water soluble treatments of South Louisiana crude oil (n = 16).

Florida

| WSF Added | Day 0 | Day 1 | Day 7 | Day 14 | Day 28 |
|---|---|---|---|---|---|
| hypoxia-control | 1.22 $\pm$ 0.02 | 1.21 $\pm$ 0.02 | 1.12 $\pm$ 0.02 | 1.02 $\pm$ 0.03 * | 0.97 $\pm$ 0.02 * |
| hypoxia + low | - | 1.12 $\pm$ 0.03 | 1.08 $\pm$0.03 * | 0.99$\pm$ 0.01 * | 0.99 $\pm$ 0.03 *a |
| hypoxia + high | - | 1.18±0.02 | 1.09 $\pm$0.03 * | 1.01 $\pm$ 0.04 * | 0.94 $\pm$ 0.02 *b |
| normoxia-control | 1.22 $\pm$ 0.02 | - | - | - | 1.36 $\pm$ 0.14 |

Pass Fourchon

| WSF Added | Day 0 | Day 1 | Day 7 | Day 14 | Day 28 |
|---|---|---|---|---|---|
| hypoxia-control | 1.33 $\pm$ 0.03 | 1.34 $\pm$ 0.03 | 1.17 $\pm$ 0.04 * | 1.21 $\pm$ 0.03 | 1.16 $\pm$ 0.03 * |
| hypoxia + low | - | 1.35 $\pm$ 0.02 | 1.07 $\pm$ 0.04 * | 1.16 $\pm$0.02 * | 1.12 $\pm$ 0.03 *a |
| hypoxia + high | - | 1.29 $\pm$ 0.03 | 1.18 $\pm$ 0.02 * | 1.17 $\pm$0.02 * | 1.12 $\pm$ 0.02 *b |
| normoxia-control | 1.33 $\pm$ 0.03 | - | - | - | 1.28 $\pm$ 0.05 |

* Significantly different from day 0 and 1 hypoxia-control and day 0 and day 28 normoxia-control
(a) n = 8; (b) n = 5

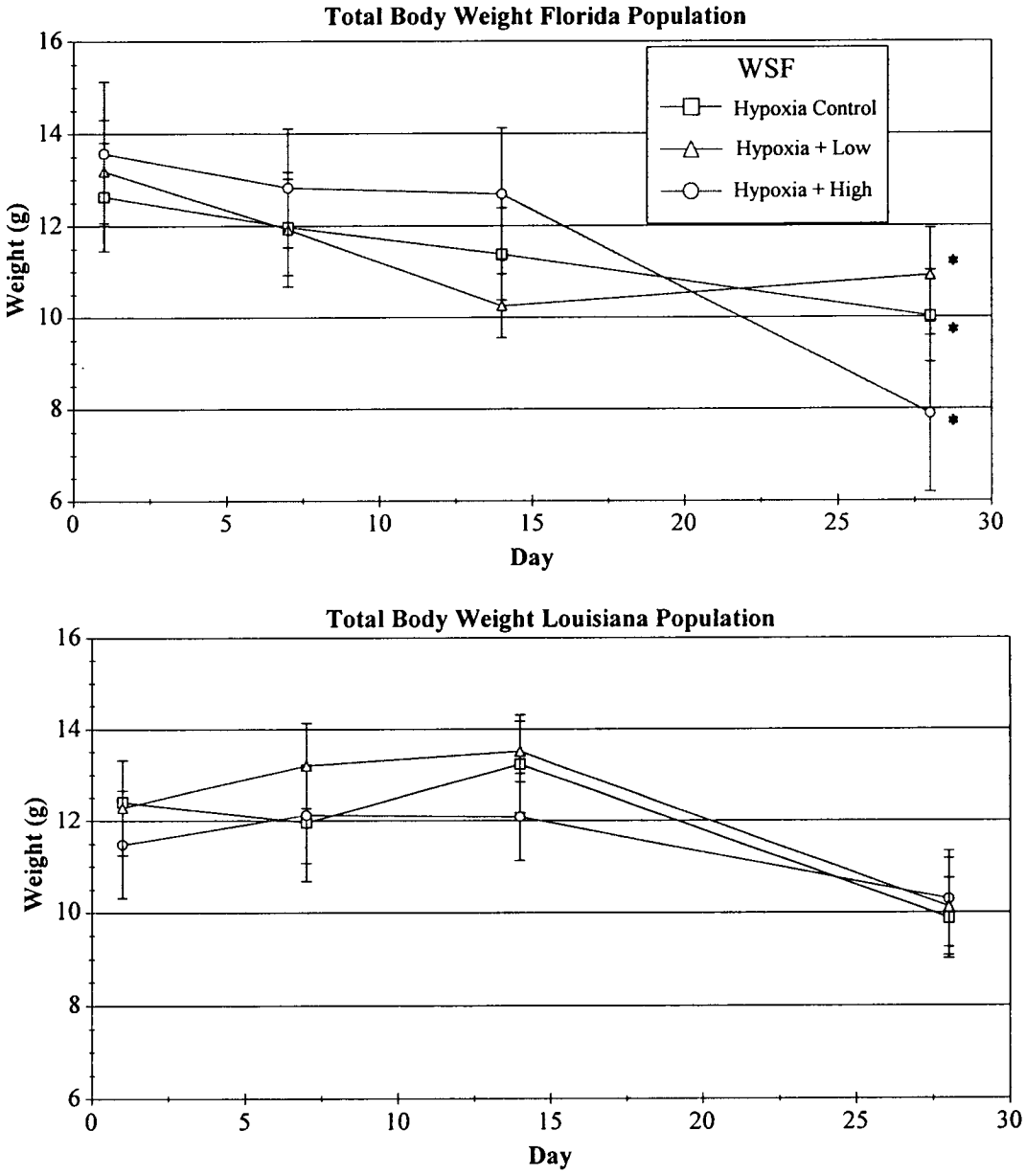


Figure 3.1. Mean total body weights ($\bar{x} \pm$ SE) of *Fundulus grandis* from Pass Fourchon and from Florida in hypoxia (73 mm Hg $O_2$) control, hypoxia + low, and hypoxia + high water soluble fraction treatments of South Louisiana crude oil. n = 16; n = 8 in Florida population day 28 hypoxia + low WSF concentration; n = 5 in Florida population day 28 hypoxia + high WSF concentration. * Significantly different from day 1 hypoxia-control.

Mean liver weights analyzed by ANCOVA with fish length as the covariate showed a trend in which weights increased with increasing WSF concentration exposure, and with days (Figure 3.2). Initial mean liver weights of both populations were similar on day 1 but final weights of the Florida population was greater. Liver weights of fish in the Florida population exposed to hypoxia + high WSF concentration increased rapidly from days 1 to 14 and reached an asymptote on days 14 to 28. The fish exposed to hypoxia + low WSF concentration increased liver weight at a slower rate, but by day 28, liver weights of fish exposed to hypoxia + low and high WSF concentration converged (Figure 3.2). Increases in liver weight of Pass Fourchon fish were not as great as in fish from Florida. Significant increases in liver weights occurred in Florida fish only on day 28 in the low WSF concentration (Figure 3.2). The high WSF concentration liver weights had high variabilty (n = 5) and were not significant.

The increase in mean liver weights in the Florida population on day 28 in the hypoxia + WSF treatments corresponded with increases in liver somatic indices (LSI) and hepatic RNA:DNA ratios (Tables 3.3, 3.5). Although RNA:DNA ratios increased in the Pass Fourchon population increases in the corresponding liver weights were not significant. Pass Fourchon RNA:DNA ratios were less than the ratios in the Florida population, and LSI and mean liver weights were not as high as those in the Florida population.

The liver somatic index (liver wt (mg) / body wt (mg) × 100) of both populations of Fundulus grandis responded in a similar manner to the WSF treatments. The LSI tended to increase with WSF concentration and with time. Increases were significant only in the Florida population (Table 3.3) on day 28 in the hypoxia and WSF concentrations which differed from the day 0 and day 1 hypoxia control and the hypoxia control on day 28. There were no difference in LSI in the Pass Fourchon treatments.

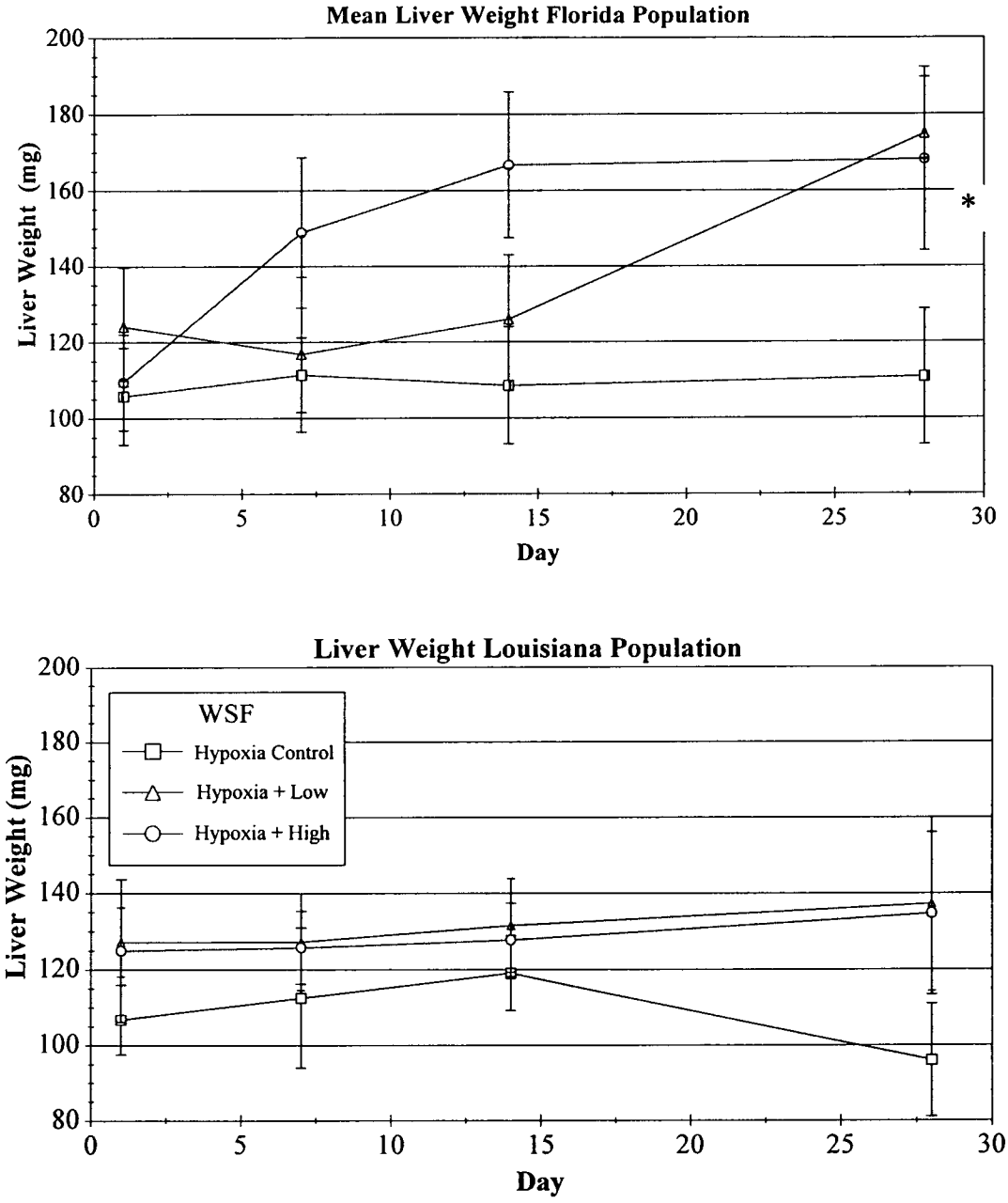


Figure 3.2. Mean liver weights ± SE of *Fundulus grandis* from Florida and Pass Fourchon over 28 days in hypoxia (73 mm Hg $O_2$) control, hypoxia + low, and hypoxia + high water soluble treatments of South Louisiana crude oil. n = 16; n = 8 in Florida population day 28 hypoxia + low WSF concentration; n = 5 in Florida population day 28 hypoxia + high WSF concentration. * Significantly different than day 1 hypoxia-control by analysis of covariance with length as covariate.

Table 3.3. Liver somatic Index (LSI, liver wt (mg) / body wt (mg) × 100) ± SE in *Fundulus grandis* from Florida and Pass Fourchon over 28 days in normoxia (156 mm Hg $O_2$) control, hypoxia (73 mm Hg $O_2$) control, hypoxia + low, and hypoxia + high water soluble treatments of South Louisiana crude oil (n =16).

Florida

| WSF Added | Day 0 | Day 1 | Day 7 | Day 14 | Day 28 |
|---|---|---|---|---|---|
| hypoxia-control | 0.84 ± 0.07 | 0.84 ± 0.07 | 1.04 ± 0.09 | 0.91 ± 0.07 | 1.21 ± 0.10 |
| hypoxia + low | - | 1.0 ± 0.08 | 1.09 ± 0.14 | 1.22 ± 0.14 | 1.61 ± 0.14 ∗ a |
| hypoxia + high | - | 0.82 ± 0.04 | 1.13 ± 0.08 | 1.25 ± 0.13 | 2.38 ± 0.41 ∗#b |
| normoxia-control | 0.84 ± 0.07 | - | - | - | 0.95 ± 0.25 |

Pass Fourchon

| WSF Added | Day 0 | Day 1 | Day 7 | Day 14 | Day 28 |
|---|---|---|---|---|---|
| hypoxia-control | 0.87 ± 0.06 | 0.88 ±0.06 | 0.86 ±0.08 | 1.06 ±0.08 | 0.88 ±0.15 |
| Hypoxia + low | - | 1.17 ±0.07 | 0.97 ±0.08 | 1.01 ±0.06 | 1.25 ±0.13 a |
| hypoxia + high | - | 1.20 ±0.11 | 1.13 ±0.07 | 1.04 ±0.08 | 1.17 ±0.13 b |
| normoxia-control | 0.87 ± 0.06 | - | - | - | 1.32 ± 0.36 |

∗ Significantly different from day 0 and day 1 hypoxia-control, and day 0 and day 28 normoxia-control.
# Significantly different from its respective control by day (a) n = 8; (b) n = 5.

There were no significant differences in LSI in the hypoxia-controls of either population over 28 days as a result of exposure to hypoxia. There was no change over 28 days in LSI in the normoxic controls. The differences seen in the hypoxia + WSF treatments were a result of exposure to the WSF of crude oil.

The Florida population LSI values differed from day zero in the day 28 hypoxia + high WSF concentration (2.38) and hypoxia + low WSF concentration (1.61). The high concentration LSI value was greater than all but the day 28 hypoxia low concentration by at least 2 fold and mean liver weights of the Florida population were significantly greater in the low WSF treatment (Figure 3.3). The day 28 high concentration had high variability (n = 5) and was not significantly increased. Increases in liver weight in the hypoxia + high WSF concentration were 58% in the Florida population and 26% in the Pass Fourchon population. Both decreases in body weight and increases in liver weight contributed to the change in LSI. Liver weights on day 28 corresponded with the significant increase in LSI of the Florida population. Liver weight and LSI did not differ in Pass Fourchon fish.

3.2.2.2 Liver RNA:DNA Ratios

Liver RNA:DNA ratios differed between populations over 28 days; ratios were higher in the Florida population due to experimental exposure. RNA:DNA ratios increased in both populations with time and with increasing WSF concentration; this was most pronounced on days 14 and 28 in the Louisiana population (Table 3.4). RNA:DNA ratios in the low and high treatments of day 28 of the Florida population were increased compared to the day 1 hypoxia-control (Table 3.4). The normoxic controls did not change over the 28 days of the experiment.

The highest increases in liver RNA:DNA ratios were observed on day 28 in the Florida population; the hypoxia + low WSF treatment ratio (2.61) and high treatment ratio (3.40) were 1.6 fold and 2.0 fold greater than the day 1 hypoxia-control ratio, respectively (Table 3.4). In the Pass Fourchon population, an increase in RNA:DNA ratios of fish exposed to the high WSF concentration compared to the day 1 hypoxia-control occurred earlier, on day 14 and remained so to day 28. Increases in Pass Fourchon were 1.6 fold greater than the day 1 hypoxia-control. RNA:DNA increases in fish from Florida occurred later (day 28), in the hypoxia and WSF treatments.

3.2.2.3 Liver EROD Activity

EROD activity differed between the populations and was greater in the Pass Fourchon population.(Figure 3.2). In both populations, peak EROD activity occurred on day 7 in the hypoxia + high WSF concentration and did not differ in the hypoxia-controls of the populations.

The highest EROD activity in the Pass Fourchon population was on day 7 in the hypoxia + high WSF concentration (301.5 $\pm$ 48.9 picomol resorufin • $min^{-1}$ • mg $protein^{-1}$) and this was the highest activity observed in either population. A secondary peak EROD activity occurred at day 14 in the hypoxia + high WSF concentration (135.8 $\pm$ 57.7 picomol resorufin • $min^{-1}$ • mg $protein^{-1}$) (Figure 3.3). EROD activity in the Pass Fourchon population decreased by day 28. EROD activity of fish exposed in the day 7 and 14 hypoxia + high WSF concentrations were significantly different from their respective hypoxia-control by day, and the day 0 hypoxia-control (8.5 $\pm$ 3.5 picomol resorufin • $min^{-1}$ • mg $protein^{-1}$). In the Florida population, EROD activity increased on day 7 (135.7$\pm$ 22.0) and day 28 in the hypoxia + high WSF concentration (128.1 $\pm$ 28.9 picomol resorufin • $min^{-1}$ • mg $protein^{-1}$) (Figure 3.3) compared to the day 0 control (8.0 $\pm$ 3.1 picomols resorufin • $min^{-1}$ • mg $protein^{-1}$).

Table 3.4. Liver RNA:DNA ratios ± SE of *Fundulus grandis* from Florida and Pass Fourchon over 28 days in normoxia (156 mm Hg $O_2$), hypoxia (73 mm Hg $O_2$), hypoxia + low, and hypoxia + high WSF treatments of South Louisiana crude oil (n = 16).

Florida

| WSF Added | Day 1 | 7 | 14 | 28 |
|---|---|---|---|---|
| hypoxia-control | 5.43 ± 0.33 | 4.54 ± 0.39 | 6.43 ± 0.32 | 4.70 ± 0.60 |
| hypoxia + low | 5.98 ± 0.27 | 5.32 ± 0.41 | 4.17 ± 0.24 | 4.64 ± 0.21 a |
| hypoxia + high | 5.49 ± 0.30 | 4.89 ± 0.40 | 5.55 ± 0.30 | 3.28 ± 0.61 b |
| normoxia-control | - | - | - | 5.62 ± 0.33 |

Pass Fourchon

| WSF Added | Day 1 | 7 | 14 | 28 |
|---|---|---|---|---|
| hypoxia-control | 6.03 ± 0.40 | 6.16 ± 0.42 | 6.47 ± 0.75 | 5.26 ± 0.65 |
| hypoxia + low | 5.62 ± 0.48 | 5.08 ± 0.48 | 4.71 ± 0.21 | 4.04 ± 0.53 a |
| hypoxia + high | 5.71 ± 0.43 | 5.28 ± 0.31 | 4.41 ± 0.51 | 3.42 ± 0.35 ✶b |
| normoxia-control | - | - | - | 6.32 ± 0.23 |

✶ Significantly different from day 1 hypoxia-control, and day 28 normoxia-control; (a) n = 8; (b) n = 5.

EROD activity of Florida fish in the day 7 hypoxia + high WSF concentration was equal to the secondary activity peak of the Pass Fourchon fish (day 14 hypoxia + high WSF concentration). EROD activity of the Pass Fourchon population on days 7 and 14 were at least 1.5 fold greater than the corresponding Florida EROD activity. Induction magnitudes for the high WSF concentrations were 9.1 ×, 11.0 ×, 4.2 ×, 6.6 × for fish from Pass Fourchon, and -1.72 ×, 4.8 ×, 2.6 ×, 4.7 ×, for fish from Florida on days 1, 7, 14, and 28 respectively compared to the hypoxia-control activities by day.

The Pass Fourchon activity in day 7 in the hypoxia + high WSF concentration was 2.2 × higher than the corresponding activity in the Florida population ($p < 0.05$).

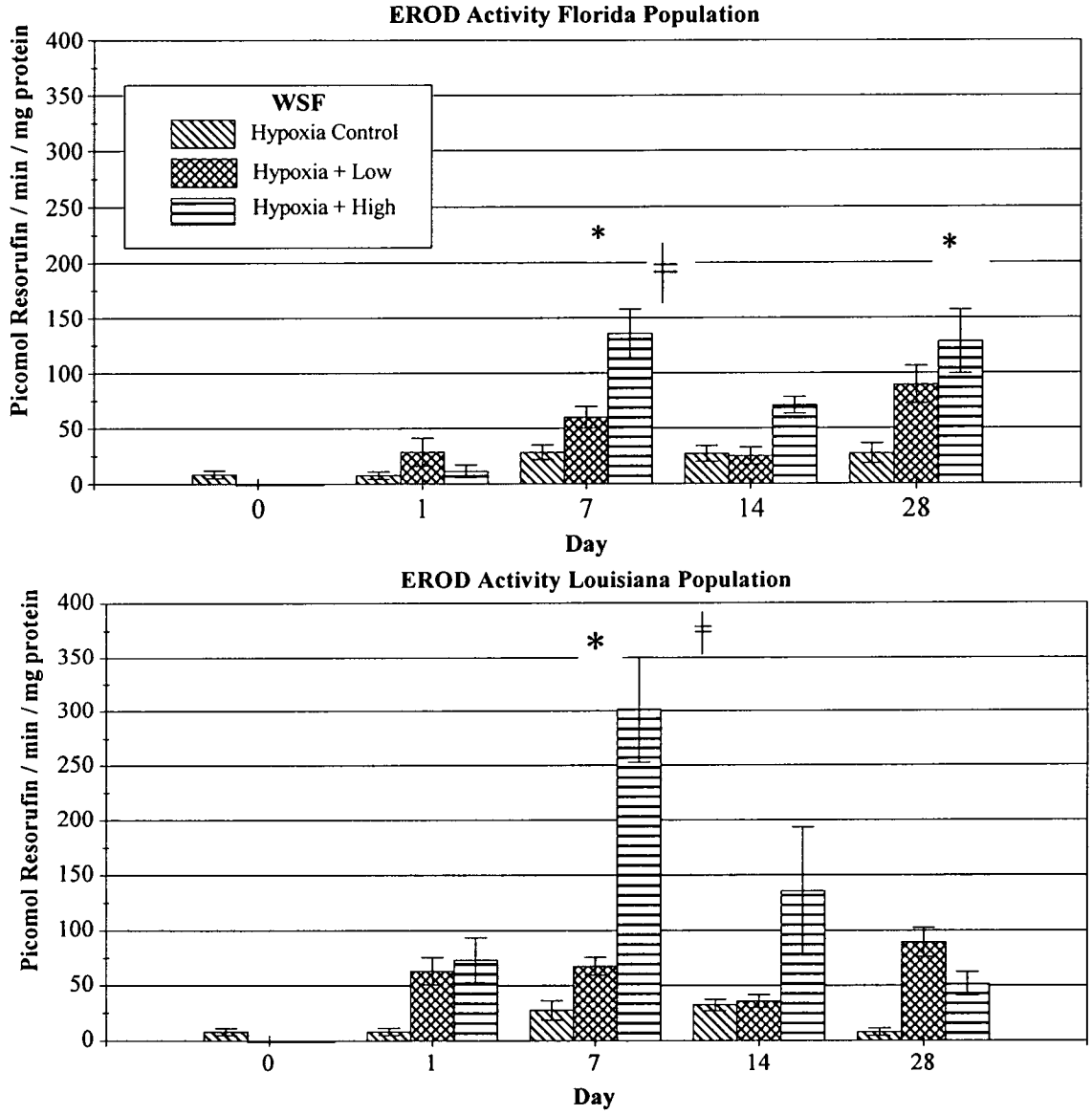


Figure 3.3. Mean EROD activity (picomol resorufin • min $^{-1}$ • mg protein $^{-1}$ ) ± SE of *Fundulus grandis* from Florida and Pass Fourchon over 28 days in hypoxia (73 mm Hg $O_2$) control, hypoxia + low, and hypoxia + high water soluble treatments of South Louisiana crude oil. n = 16; n = 8 in Florida population day 28 hypoxia + low WSF concentration; n = 5 in Florida population day 28 hypoxia + high WSF concentration; * Significantly different from Day 0; ǂ Significantly different from its respective hypoxia-control

Total liver EROD activity was calculated by multiplying EROD activity per mg of hepatic protein by the total amount of hepatic protein of each liver. Increases in total hepatic EROD activity was observed in both populations (Figure 3.4). In the Florida population, increases occurred on day 7 (8891.4 ± 3353.5 picomol resorufin • min $^{-1}$ • total hepatic protein $^{-1}$) and 28 (5453.2 ± 1766.7 picomol resorufin • min $^{-1}$ • total hepatic protein $^{-1}$) in the hypoxia + high WSF concentrations. The day 7

activity was significantly increased compared to the day 0 control and to its hypoxia control by day. The day 28 activity was greater than the day 0 hypoxia-control only. The Pass Fourchon population had increased activity on day 7 (11719.6 ± 2688.8 picomols resorufin • $min^{-1}$ • total hepatic $protein^{-1}$) in the hypoxia + high WSF concentration.

Total EROD activity in the Florida population in the hypoxia + high WSF concentration on day 7 and 28 was 4.7 ×, and 3.9 × greater than the hypoxia-control activities by day, respectively. The Day 7 hypoxia + high WSF concentration of the Pass Fourchon population was 7.6 × higher than its hypoxia-control activity by day, and 1.3 × higher than the corresponding activity in the Florida population. The day 7 hypoxia + high WSF concentration activities of the two populations were significantly different.

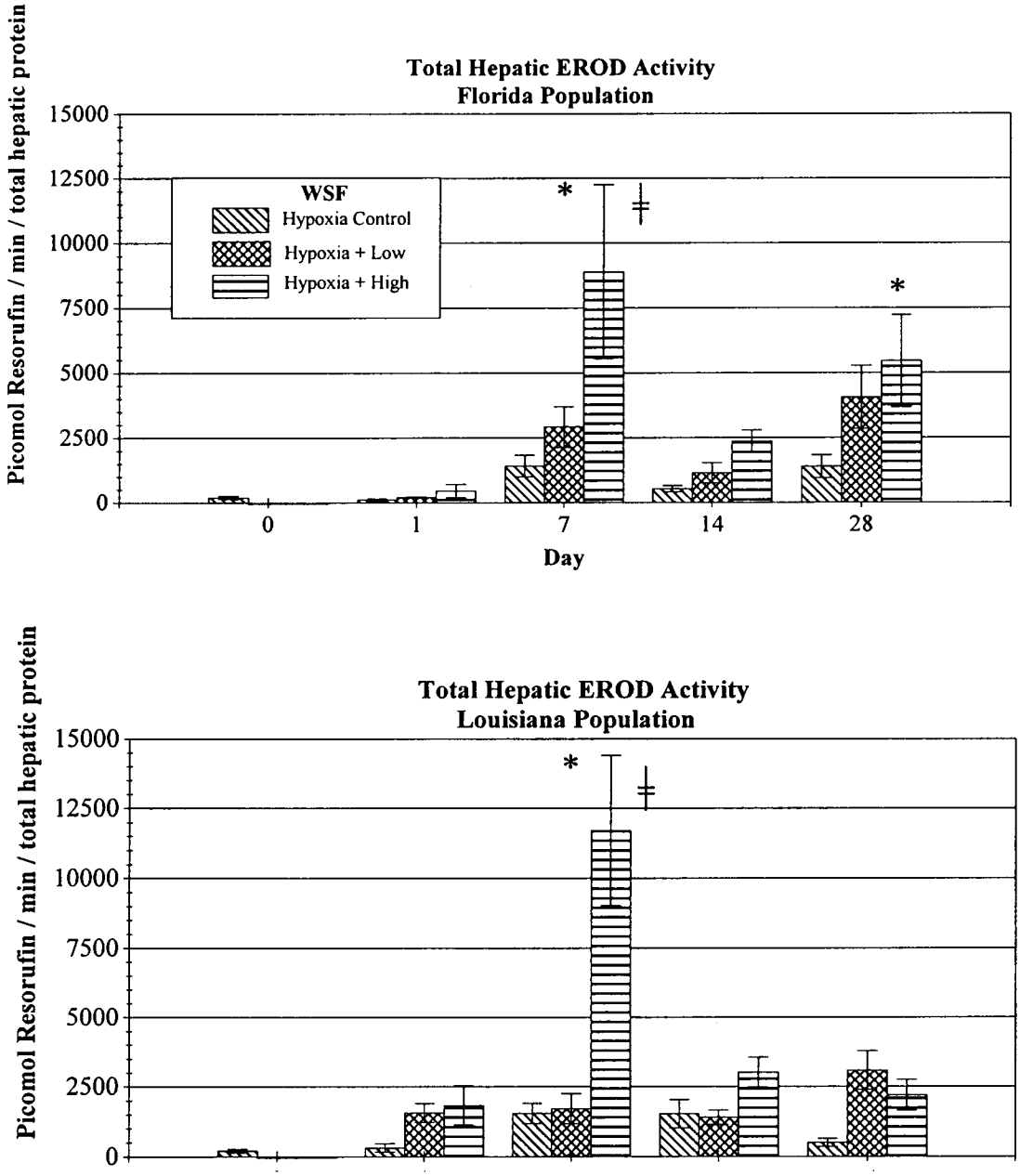


Figure 3.4. Total liver EROD activity (picomol resorufin • min $^{-1}$ • total hepatic protein $^{-1}$) $\pm$ SE of *Fundulus grandis* from Florida and Pass Fourchon over 28 days in hypoxia (73 mm Hg $O_2$) control, hypoxia + low, and hypoxia + high water soluble treatments of South Louisiana crude oil. n = 16; n = 8 in Florida population day 28 hypoxia + low WSF concentration; n = 5 in Florida population day 28 hypoxia + high WSF concentration. * Significantly different from Day 0; ǂ Significantly different from its respective hypoxia-control

3.2.2.4 White Muscle RNA:DNA Ratios

White muscle RNA:DNA ratio of Fundulus grandis from both sites varied significantly as a function of the population, day, oil concentration, and day by treatment interaction (ANOVA). Treatment means are given in Table 3.5.

Table 3.5. RNA:DNA ratios from the white muscle of *Fundulus grandis* exposed to the WSF of South Louisiana crude oil for 28 days. WSF concentrations are given as $ng.l^{-1}$ total aromatics and aliphatics. For clarity, only mean values and sample sizes (n) are presented in the table.

| Population | Day | Control | 9ng.$l^{-1}$ | 35 ng.$l^{-1}$ |
|---|---|---|---|---|
| Port Fourchon, LA | 1 | 1.335(8) | 1.335(8) | xxx |
| | 7 | 0.831(13) | 5.168(14) | 1.223(7) |
| | 14 | 1.687(16) | 4.357(13) | 1.226(16) |
| | 28 | 2.584(15) | xxx | 2.826(16) |
| Turkey Point, FL | 1 | 1.458(14) | 1.487(15) | 0.836(16) |
| | 7 | xxx | 1.468(16) | 1.031(15) |
| | 14 | 1.031(15) | 1.157(12) | xxx |
| | 28 | 1.117(13) | 1.375(8) | 1.040(5) |

## 4.0 DISCUSSION

The hypoxia tolerance of the Gulf killifish, *Fundulus grandis* (28 day $LC_{50}$ = 28 Torr $O_2$) , was significantly greater than that of the lesser blue crab, *Callinectes similis* (45 Torr) and the blue crab, *Callinectes sapidus* (111 Torr). Minimal site differences (Florida and Louisiana) existed in hypoxia tolerance. There was no difference in the 28 day tolerance of blue crabs to the WSF of South Louisiana crude oil under either normoxia or hypoxia. In contrast, Fundulus grandis from the produced water site in Louisiana were slightly more tolerant of the WSF of South Louisiana crude oil than those from Florida and fish exposed to the WSF of South Louisiana crude oil under normoxia were more tolerant than those exposed under hypoxia. Although RNA:DNA ratios are a good biomarker of bionutritional stress (starvation), this ratio is not as effective as a biomarker upon exposure of Gulf killifish or blue crabs to the WSF of South Louisiana crude oil. Induction of EROD activity occurred in a dose related manner in *Fundulus grandis* from both locations exposed to the WSF of South Louisiana crude oil under hypoxia (75 Torr O2), peaked on the seventh day of exposure and was significantly higher in fish exposed to 35µg oil/liter from the produced water site in Louisiana than in those exposed to 35µg/liter from Florida.

Statistically significant but minor differences exist in the tolerance of the Gulf killifish from a less contaminated site and a more contaminated produced water site. RNA:DNA ratios are not a particularly sensitive indicator of sublethal WSF stress in either Gulf killifish or the blue crab. EROD activity, as 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 the Gulf killifish from the produced water site. Induction of the cytochrome P-450 enzymes by xenobiotics has been documented in a number of fish species including the channel catfish *Ictalurus punctatus* (Winston et al., 1989). WSF exposure induced hepatic tissue growth in both populations; hypertrophy was the dominant cause of growth in Pass Fourchon fish and hyperplasia in Florida fish; growth 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 Pass Fourchon population. The condition factor (K) of the Florida population decreased more than Pass Fourchon, and LSI were increased in Florida fish. 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 slightly 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 appear to be minor.

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**The Department of the Interior Mission**

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.


**The Minerals Management Service Mission**

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.