**New Proposal: Evaluating the After-Effects of the Deepwater Horizon Oil Spill on Adult and Juvenile Estuarine Fishes**

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Collaborator: Martin T. O’Connell (UNO).

Cluster: Understand

Estimated Budget: $107,000

**INTRODUCTION**

The Deepwater Horizon oil platform exploded on April 20, 2010, releasing an estimated 185 million gallons of crude oil into the northern Gulf of Mexico until the well was capped on July 15, 2010. Throughout the approximate three month duration of the oil spill, millions of gallons of the dispersant Corexit 9500 were released at the well head in an attempt to minimize the amount of oil moving into coastal areas. The marshes and barrier islands of Louisiana sustained major impacts, with oil washing up on beaches, moving into marshes and settling in sensitive seagrass beds. Although the overt effects of crude and dispersed oil on estuarine species appear to have been relatively minor, it is highly likely that exposure of critical life stages of estuarine-associated fauna to oil:dispersant mixtures will produce ecological effects that may not be observed until the following reproductive season.

Resident estuarine fishes, such as spotted seatrout (*Cynoscion nebulosus*), and silver perch (*Bairdiella chrysoura*) were likely impacted at multiple phases of their life cycle by the DWH incident. These economically and ecologically important Sciaenid species spawn in estuaries during the spring and summer (Brown-Peterson and Warren 2001; Grammer et al. 2009) and complete their entire life cycle in estuarine waters. **Therefore, fish spawned during the spring and summer of 2010 were exposed to weathered and dispersed oil as larvae and juveniles, which are known to be highly sensitive life stages.** Further, modulation of the Cytochrome p4501A (CYP1A) pathway is associated with impaired reproduction, implying that simultaneous exposure to chronic hypoxia and dispersed oil may have serious implications for reproductive success in estuarine organisms. Previous research has shown a direct relationship between increased CYP1A induction and decreased vitellogenin synthesis in rainbow trout liver cells [20], and indicated a link between CYP1A expression and genes associated with reproduction in largemouth bass [11]. As exposure to crude and dispersed oil would result in induction of CYP1A, there is a clear need to investigate the effects of dispersed oil/hypoxia exposure on reproductive dynamics in organisms exposed to dispersed oil. All female silver perch reach sexual maturity in their first year of life (Grammer et al. 2009), and 80% of spotted seatrout in both Mississippi and Louisiana are sexually mature at age 1 (Brown-Peterson and Warren 2001; Nieland et al. 2002). Thus, individuals that were exposed to oil as juveniles will spawn during spring and summer 2011.

**OBJECTIVES**: **Our hypothesis is that larval/juvenile spotted seatrout and silver perch were exposed to transient concentrations of dispersed oil at a critical phase of their development, and that this will result in an identifiable delay in gonadal development in the young of the year individuals in the spring of 2011.** We propose to test this hypothesis by evaluating the reproductive condition (gonadal development, vitellogenin levels) of reproductively active spotted seatrout and/or silver perch captured from the Chandeleur Islands, Louisiana, an area that received heavy oil impacts. Fish from several age classes will be evaluated to determine if age 1 fish ( i.e., those that were juveniles during the oil incursion) show reproductive impacts compared to older fish. Gonadal histology data will also be compared to baseline data from Mississippi collections. Additionally, juvenile fishes will be collected from the Chandeleur Islands seagrass beds to document the abundance and occurrence of the target species one year post-spill. These metrics will be compared to baseline data from the area, provided through collaboration with Dr. Martin T. O’Connell, University of New Orleans.

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**APPROACH:**

*Fish collections*

Adult silver perch and/or spotted seatrout will be collected at the Chandeleur Islands during three dedicated sampling trips in May 2011, a peak reproductive time for both species. Fish will be collected in 2.5” stretch mesh gill nets. Nets will be set for 8 hour periods and checked every hour to remove and sample captured fish. Fish will also be captured by hook and line sampling, if possible. Concurrently with adult sampling, shallow seagrass areas will be sampled using a bag seine to collect juvenile fishes. The area will be seined exhaustively during each sampling trip.

Immediately upon collection, adult fish will be bled, weighed measured, and the gonadal tissue will be removed, weighed, and fixed in 10% neutral buffered formalin for histological analysis. The blood samples will be centrifuged and the plasma will be frozen for analysis. Additionally, brain and liver tissue will be removed and flash frozen in liquid nitrogen for subsequent molecular analysis. Fish will be bled, and whole blood stored in heparininzed tubes on ice for transport to the lab. Juvenile fishes will be preserved in 10% neutral buffered formalin, or flash frozen in liquid nitrogen, as appropriate for endpoint analysis.

*Laboratory analysis*

The gonadosomatic index (GSI) will be calculated for all adult fish to provide an index of spawning preparedness. Gonadal tissue will be processed following standard histological techniques and evaluated for reproductive phase (immature, developing, spawning capable, regressing, regenerating following Brown-Peterson et al. 2011) and gamete development. Abnormal gonadal or gamete development will be noted.

Blood samples will be analyzed for vitellogenin (VTG) and 11 keto testosterone (11KT) using an ELISA assay. RNA will be extracted from brain and liver tissues using standard techniques and analyzed for expression of a suite of genes expected to be implicated in reproductive and oil exposure effects (e.g., CYP1A, VTG, CYP19 Aromatase). Gene expression will be quantified via qPCR, and normalized to expression of 18S in each individual. Prior to qPCR analysis, these genes will be cloned and sequenced, in itself an important advance in understanding reproductive dynamics in these species.

Juvenile fishes will be sorted, identified to species, and density (#/m2) will be determined based on the area seined or using data from throw traps. These results will be compared with historical, pre-spill data from the area collected by Dr. Martin T. O’Connell (University of New Orleans, LA). Dr. O’Connell will collaborate with no cost to this project, and will be included as a co-author in any resulting presentations and publications. Data will be compared between pre-spill historical data sets (O’Connell) with post-spill data sets (Peterson) with PRIMER multivariate statistics (MDS, ANOSIM, SIMPER, etc., Clarke 1993; Clark and Green 1988; Clarke and Gorley 2006).

**Expected Results**

Spotted seatrout are an economically important commercial and recreational species, and silver perch are a vital component of the estuarine community, and serve as a forage species for a number of larger economically important species. Impacts on the reproductive health and success of these vital species as a result of exposure to crude or dispersed oil in critical life may presage important population and community level effects. Molecular analysis can identify specific oil-related impacts that affect growth and reproduction, while reproductive analysis provides information on the current reproductive condition of fish affected by the oil spill as both juveniles and adults. A reduction in gonadal development leading to impaired spawning success will result in changes in the juvenile fish community as well as impact future year classes of these species.

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| **BUDGET**  |  |  |  |  |
|  **CATEGORIES**  |  |  |  |  |
|  *A. Personnel*  |  |  *Monthly salary*  | *Months* |  |
|  Robert Griffitt  |  | $6,444  | 0.5 | $3,222 |
|  Mark Peterson  |  | $9,180  | 0.5 | $4,590 |
|  Nancy B Peterson  |  | $3,548  | 1.5 | $5,322 |
|  Graduate student 1  |  | $1,700  | 12 | $20,400 |
|  Graduate student 2  |  | $1,700  | 12 | $20,400 |
|  **TOTAL SALARIES**  |  |  |  | **$53,934** |
|  |  |  |  |  |
|  *B. Fringe Benefirts*  |  |  |  |  |
|  Robert Griffitt  | 29.62% | $1,909  |  | $954 |
|  Mark Peterson  | 25.48% | $2,339  |  | $1,170 |
|  Nancy B Peterson  | 32.45% | $1,151  |  | $1,727 |
|  Graduate student 1  | 4.53% | $77  |  | $924 |
|  Graduate student 2  | 4.53% | $77  |  | $924 |
|  **TOTAL FRINGE**  |  |  |  | **$5,699** |
|  **TOTAL PERSONNEL**  |  |  |  | **$59,633** |
|  |  |  |  |  |
| **Boat Rental** |  |  |  |  |
| Boat rental--McIllwain |  | 3 12-hour trips |  | 4200 |
| fuel surcharge |  | $2.60/gallon |  | 1638 |
| **TOTAL BOAT** |  |  |  | **5838** |
|  |  |  |  |  |
|  Consumables  |  |  |  | $7,000 |
|  shipping  |  |  |  | $100 |
|  |  |  |  |  |
|  F. Contractual services  |  |  |  |  |
|  histology  |  |  |  | $500 |
|  |  |  |  |  |
|  **TOTAL DIRECT**  |  |  |  | $73,071 |
|  Indirect Costs (46.5%)  |  |  |  | $33,978 |
|  **TOTAL**  |  |  |  | $107,049 |

**Literature Cited**

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